III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Chloroprene, CH2:C(Cl)CH:CH2 (formula weight 88.5), is a colorless, flammable, volatile liquid. It has a pungent, ethereal odor. The odor threshold has been reported to range from about 0.1 to several hundred ppm [1,2]. Additional physical and chemical properties are presented in Table XII-1 [3,4].

Chloroprene was first synthesized in 1930 by Carothers et al [5]. Acetylene was bubbled through cuprous chloride/ammonium chloride solution yielding monovinyl acetylene, CH2CHCCH. This was then chlorinated with hydrochloric acid in cupric chloride/ammonium chloride solution to form 2-chloro-1,3-butadiene, CH2:C(Cl)CH:CH2 (beta-chloroprene). Byproducts of the reactions were acetaldehyde (3-7%), methyl vinyl ketone (unknown percentage), and vinyl chloride (0.5%). Although this method is still in commercial use throughout the rest of the world, chloroprene has been synthesized from 1,3-butadiene in the United States since 1970 [6].

The butadiene process involves the chlorination of butadiene in the gas phase to 1,4-dichloro-2-butene and 3,4-dichloro-1-butene; the latter, being the precursor of chloroprene, is distilled off, and the 1,4-dichloro-2-butene is isomerized to 3,4-dichloro-1-butene by distillation over copper and cupric chloride [6-8]. The 3,4-dichloro-1-butene is dehydrohalogenated with aqueous sodium hydroxide to yield chloroprene. Chloroprene is removed from the mixture of sodium hydroxide and 3,4-dichloro-1-butene by vacuum distillation. The purified chloroprene is stored at less than -10 C in the presence of antioxidants. Although the butadiene process is cleaner and

less likely to explode than the acetylene process, it introduces a different set of impurities, mainly alpha-chloroprene [6,8].

All the chloroprene produced is subjected to polymerization, usually in an emulsion in the presence of resin, fatty acids, and alkylmercaptans [6]. Polymerization is usually initiated by the addition of a "peroxy" salt, producing neoprene or polychloroprene latex. Unreacted monomeric chloroprene is steam stripped from the latex and recycled. The stripped latex is coagulated by decreasing the pH, precipitated by decreasing the temperature, collected in large sheets, and washed to remove salts. The major worker exposure to chloroprene occurs in the manufacture of the monomer and during the polymerization to neoprene latex. The process, through the steam stripping, is completely enclosed and can be a continuous-flow operation. However, specialty latices are made in batches. Exposure to chloroprene occurs primarily through leakage or accidental spillage during its manufacture and during maintenance [3,7,9,10,11 (pp 7,40)]. The latex may contain 0.01-0.5% free monomeric chloroprene [12]. The air above the latex inside storage vessels can also be a source of worker exposure. Chloroprene vapor may remain in the tank after the latex is removed. Exposure may occur during tank entry if improper work practices are followed [12,13].

In chloroprene manufacture, workers may also be exposed to butadiene, acetylene, chlorobenzenes, calcium carbide, monovinyl acetylene, hydrogen chloride, acetic acid, dichlorobutenes, chlorine, alkyl mercaptans, alkylamines, and antioxidants; other ingredients are proprietary. The toxicity of many of these compounds is not well documented, but 1,4-dichloro-2-butene, which is also an intermediate in the manufacture of

nylon, produced local tumors after intraperitoneal (ip) or subcutaneous injections in female ICR/HA Swiss mice [14], and 3,4 dichloro-2-butene has been shown to be mutagenic in bacteria [15].

Chloroprene is used to manufacture only polychloroprene latex and neoprene rubber. The latex is used in the manufacture of some types of rubber cement. Production figures for chloroprene are not available, but the production of neoprene rubber increased from 112 million pounds in 1950 to 410 million pounds in 1974, an average production increase of about 5-6%/year. In 1974, the manufacture and use of chloroprene was reported by two companies, Du Pont and Petrotex, at four plants. NIOSH estimates that 2,500 workers are potentially exposed to chloroprene during its production and polymerization in the United States [16,17].

Historical Reports

The first study of chloroprene toxicity was reported by Von Oettingen et al [18] in 1936. Acute toxicity was determined in mice, rats, cats, rabbits, and pigeons. Exposures of mice and rats for 30-90 days to airborne chloroprene in the range of 28-98 ppm were also conducted. More detailed information is included in Animal Toxicity.

In 1942, Roubal [19] reported an investigation of the toxicologic and hygienic aspects of the Czechoslovak chloroprene rubber industry. This is believed to be the first report of human exposure. Workers involved in the washing and polymerization operations experienced loss of hair. Other workers complained of a sensation of pressure in the chest with rapid pulse, severe fatigue, and conjunctivitis and necrosis of the corneal epithelium. Albumin was reported to be present in the urine of a small

number of workers who, according to Roubal, presumably had had massive chloroprene exposure. Since blood pressure changes varied, Roubal did not believe that blood pressure change was a good indicator of the early stages of chloroprene poisoning. The exposure duration before the occurrence of symptoms was not reported. No environmental chloroprene concentrations were reported.

Nystrom [20] described a series of medical studies carried out in Swedish chloroprene plants between 1944 and 1947. These were the first and only studies to include experimental reports of human exposure to airborne chloroprene and to report a human fatality. Experimental exposure of human subjects to chloroprene at 973 ppm led to nausea and giddiness in 15 minutes in resting subjects and in 5-10 minutes in subjects performing light work. Nystrom noted anemia in pilot plant workers who were exposed at air concentrations estimated to be approximately 459 ppm. The range of concentrations of chloroprene in the air was from 56 to greater than 334 ppm in the main chloroprene plant after full operation was achieved. Nystrom stated that, in the pilot plant, air concentrations must have been much higher. Workers, especially those in the fractional distillation department, developed extreme fatigue and unbearable chest pains after exertion about 1 month after starting work. The symptoms were particularly severe by the end of the workday. Because of fatigue and severe chest pains, 90% of the workers often had great difficulty bicycling to their homes after work and had to rest repeatedly. Both pain and fatigue usually subsided by the following morning, returning during the next workday. These workers also noted changes in their personalities towards irritability and quick-tempered behavior. Contact dermatitis (25-30%) and

reversible hair loss were also noted in some workers, especially in the polymerization area, where 90% of the workers showed hair loss. Liver and kidney functions were normal; no albumin was detected in the urine. Lung function tests were normal, and 67/80 had normal lung roentgenograms, 8/80 had sinus obliteration, 2/80 had parenchymatous changes, and no data were presented for 3 workers. The basal metabolic rates of 10 workers and the electrocardiograms in 15 workers were normal. Nystrom stated that exposure at high but unspecified concentrations of chloroprene for short periods of time led to temporary unconciousness in an unknown number of men. On regaining consciousness, the men were able to resume work immediately.

Nystrom [20] also found a single fatality resulting from occupational exposure to chloroprene in 1948. A worker entered a polymerization vessel containing chloroprene vapor (concentration unspecified) for cleaning purposes without first ventilating it. After 20-30 seconds, the man became unconscious. He was rescued within 3-4 minutes, but resuscitation attempts failed. Pulmonary edema was noted at autopsy. Fluid was also found in the larynx, trachea, and bronchi as well as in the terminal bronchioles and the parenchyma of the lungs. Microscopic examination showed marked hyperemia of the lungs and blood vessels, and large amounts of thin fluid were observed throughout the lung tissue.

In 1948, Ritter and Carter [21] related that synthetically prepared dimers of chloroprene caused rapid hair loss in rodents when applied to the skin. Cyclic dimers and short-chain polymers were prepared by refluxing chloroprene with para-tertiary-butylcatechol. No information was given on the structural nature of the dimers and polymers. Two drops of the resulting solutions were put on the backs of unspecified numbers of mice

and guinea pigs. Within 4-10 days, the hair on the skin exposed to the solutions of the cyclic dimer and of the short-chain polymer fell out. After 6 weeks, the hair had regrown. The authors concluded that, based on the experimental results and their observations that human hair loss occurred exclusively in manufacturing areas where chloroprene was polymerized, chloroprene monomer did not cause hair loss. These findings might also be explained by higher monomer concentrations in the polymerization area.

Effects on Humans

Factory workers exposed to chloroprene have been the subject of extensive study in the USSR. Few studies were found on effects of occupational exposure to chloroprene in other countries.

Sanotskii [22] quoted a study by Fomenko, Katosova, and Davtian (from an unavailable report) who investigated disturbances in spermatogenesis after 6-10 years of worker exposure to chloroprene and morphologic disturbances after 11 years of exposure. In addition, spontaneous abortion was said to occur in the wives of chloroprene workers more than three times as frequently as it did in the general population. The actual data, however, were not presented in this report. Because these observations may have resulted from air contamination with agents other than chloroprene, a detailed clinical and safety inspection was made. The results showed that the main hazard to workers was chloroprene vapor, with concentrations ranging from 1 to 7 mg/cu m (0.28 to 1.94 ppm). Ammonia, in concentrations ranging from 2 to 4 mg/cu m, was the most frequently encountered other volatile substance. Such concentrations of ammonia, however, were within

the maximum permissible concentration (MPC) of 20 mg/cu m and were not considered to be a hazard to health.

In 1967, Lejhancova [23] investigated hair loss resulting from chloroprene exposure at a rubberized fabric factory in a process employing six women. The concentration of airborne chloroprene ranged from 17 to 81 ppm (61 to 292 mg/cu m) inside the factory. Chromatographic analysis of the polychloroprene latex at the end of the study showed 3.88% free chloroprene, but no dichlorobutene or small polymers. Lejhancova mentioned that the monomeric content of the fresh latex supplies may have been much higher, since polymerization continues at room temperature during storage of the latex. A few days after receiving the latex, six female workers complained of headache, nausea, and severe fatigue. After 2 weeks, one woman began to lose her scalp hair, becoming completely bald within 4 weeks. Later, three other women lost their scalp hair, but two were unaffected.

Lejhancova [23] concluded that the loss of hair resulted from exposure to free chloroprene monomer in the polychloroprene latex and that better hygiene practices in the plant should decrease the chloroprene concentrations and, subsequently, the hair loss. After unspecified plant improvements, the women's hair grew back within 6 months.

In 1969, after the opening of a polychloroprene manufacturing plant in France, Malassis and Malassis-Jouve presented, in a section of a paper by Paulet and and Malassis [24], the results of a study of a group of workers in the chloroprene industry. The authors reported a high frequency of chemical burns in 100 of 130 workers (77%) exposed to chloroprene. Conjunctivitis was also noted. Hair loss was reported in 34 of 130 workers

(26%). This occurred in the polymerization area where exposure concentrations were unknown. Sexual impotency, involving both libido and sexual dynamics, was reported by workers, but no further details were given. All such disorders disappeared after the workers were relocated. Ventilation of the plant was improved later. No deviations from normal were reported in either bound or free cholinesterase activity in blood samples collected from 54 workers segregated into 4 groups on the basis of probable exposure to chloroprene.

Avakian et al [25], from 1956 to 1958, observed and reported on the health of up to 273 persons with 7-13 years of experience working with chloroprene. No indications of the chloroprene air concentrations or the sex of the workers were given. Disorders of the cardiovascular system were stated to be of primary concern to the authors. Fifteen percent of the workers complained of heaviness in the chest, slow pulse was noted in 48%, fast pulse appeared in 19%, 6.7% showed signs of cardiac neurosis, and hypotension was observed in 15-30%. Capillary permeability was said to be significantly increased in a majority of the 136 workers tested. No frequency of occurrence of these signs and symptoms in control workers was given. Ninety-six workers were subjected to electrocardiographic examination: 27% had decreased heart rates, 33% had signs of myocardial dystrophy, and 15% showed atherosclerosis of the cardiac vessels. In a control group of mechanics (unknown number and sex), myocardial dystrophy was diagnosed in 7.3% and atherosclerosis in 4.8%. Followup examinations on the exposed workers through 1958 showed that the incidence of myocardial dystrophy increased to 39.2% and that of cardiac neurosis increased to 9.2%.

Avakian et al [25] concluded that the toxic influence of chloroprene increased with the length of exposure. Although the authors stated that there were comparatively high concentrations of chloroprene in the production areas, no actual concentrations were given. The authors mentioned the use of prophylactic measures, including periodic medical examinations, vitamin administration, vacations at health resorts, and more balanced nutrition, to increase the working capacity of the workers. The medical significance of these findings is difficult to evaluate because of the authors' imprecise description of the correlation between the abnormal conditions found in workers and cardiac neurosis and myocardial dystrophy.

Immunologic response and reactivity in chloroprene factory workers (Kirov, Armenian SSR) were the subjects of a 1965 study by Mikaelian and Frangulian [26]. Blood samples from 208 workers, said to be mostly male (number of women not given), from 25 to over 50 years of age with extended exposure to chloroprene, were examined for titer of what were called OH agglutinins and the phagocytic index. The durations and concentrations of the workers' exposures were not specified in the paper. The titer of agglutinin and the phagocytic index were determined both before and after immunization against what was described as Typhus abdominalis (perhaps Salmonella typhosa or S. typhi) and compared with the results of blood samples from 113 workers not exposed to chloroprene.

No specific method was given for determination of the titer of OH agglutinins, but the phagocytic index was determined by incubating serum with either specific (Typhus abdominalis) or nonspecific (Staphylococcus aureus) organisms [26]. One billion bacterial cells were incubated with citrated blood for 30 minutes at 37 C. Smears of the mixture were

prepared, stained, and observed microscopically. The number of phagocytized bacteria/100 leukocytes was counted, and the average number of phagocytized bacteria/leukocyte (ranging from 0.1 to greater than 10) was considered the phagocytic index.

The increase in titer against Typhus abdominalis was not marked in the exposed workers [26]. After tetravaccine vaccinations (in the United States, by definition, tetravaccine imparts immunity to typhoid, paratyphoid A and B, and cholera), the agglutinin titer did not exceed dilutions of 1:50 or 1:100 in 52% of the individuals. In unexposed workers, 89% of the vaccinated individuals had titers of 1:800 or more, and 45% had titers of 1:3,200 or more.

The phagocytic index showed a similar trend [26]. Prior to the immunizations, the index with Typhus abdominalis ranged from 0.1 to 2.0 for all workers. After vaccinations, this index ranged from 2.1 to 3.0 for only 19% of the workers; the remainder were still in the range of 0.1-2.0. Blood samples from 68 unexposed workers (60%) were in the range of 3.1-10 after immunization, while 27% ranged from 4.1 to 10. The nonspecific phagocytic index for Staphylococcus aureus was not markedly affected by the administration of tetravaccine. The authors [26] concluded that chloroprene exposure depressed the "defense mechanisms" (immune response) of humans. Since the chloroprene concentration in the plant during the study was not determined, no dose-response relationship could be established.

Another approach to studying the effect of chloroprene on human immune capacity was given by Kechek and Semerdzhian [27] in 1962. Blood samples from 39 workers in various chloroprene exposure areas of the Kirov

plant were compared with samples from 10 workers who were not exposed to chloroprene. Total proteins, total albumins, and alpha, beta, and gamma globulins were measured in the serum obtained from each worker. The protein content of each fraction was determined by precipitation with decreasing concentrations of monosubstituted potassium phosphate with subsequent determinations of the proteins in each fraction by comparison of the turbidity of the solutions with that of a calibrated turbidity standard. The authors stated that this method gave results comparable with those obtained by paper electrophoresis. There were no significant changes in total serum protein, albumin, or alpha globulin when control sera were compared with those from exposed workers; however, a significant increase in the beta globulin fraction and a significant decrease in the gamma globulin fraction were noted. The authors implied that some of the gamma globulin fractions had been converted to beta globulin, but no direct evidence for this interconversion was presented.

As part of the State Sanitary Inspection Commission's program to evaluate the Maximal Air Concentration documentation in the USSR, Mnatsakanian [1] studied the range of odor threshold for chloroprene and the effects of chloroprene on the sensitivity of the retina to light during dark adaptation. The odor threshold, based on about 700 total determinations, for 11 persons ranged from 0.11 to 0.56 ppm. The average was 0.25 ppm. The average maximal imperceptible concentration was 0.2 ppm. Three subjects (24, 29, and 30 years of age) were tested for sensitivity of their dark-adapted eyes to light. These three subjects had odor thresholds of 0.25, 0.11, and 0.14 ppm, respectively. No alteration in the sensitivity to light of the dark-adapted eyes was observed with exposure

below the individual's odor threshold. Exposure above the odor threshold decreased dark-adapted light sensitivity. The purity of the chloroprene used in this study was not reported. The neurophysiologic significance of studies of this type is not clear. The findings could indicate an effect on the innervation of the iris, on the retinal receptors, on the statistical conduction of sensory impulses in the optic nerve, or on the central mechanisms of visual perception.

Carcinogenic, mutagenic, teratogenic, and reproductive effects will be discussed in later portions of the document.

Epidemiologic Studies

Epidemiologic data available on the incidence of cancer and other effects in workers exposed to chloroprene are, again, primarily from the USSR.

Khachatrian's [28] investigation of the occurrence of skin cancer in Erevan, USSR, from 1956 to 1970, considered a total of 24,989 persons of both sexes. These people were assigned to one of five groups according to their occupational exposures. The group "chloroprene workers" consisted of 684 employees involved in the production of chloroprene and polychloroprene latex and rubber. The group "chloroprene derivative workers" comprised 2,250 employees, mainly from shoe factories, with exposure to polychloroprene cement. There was thus a total of 2,934 persons with some exposure to chloroprene. The group "chemical workers" included 4,780 employees with no chloroprene exposure but with prolonged contact with lacquers, acetone, benzene, gasoline, and acids (truck drivers and cabinet makers). The last two groups, 8,755 nonchemical workers and 8,520

nonindustrial workers, served as controls. (The text of the article referred to the fourth group as chemical workers, but the accompanying table referred to them as nonchemical.)

Khachatrian [28] diagnosed skin cancer in 137 people from the total of 24,989 persons studied. The exact numbers, percentages of total workers, average ages, and work experiences of the cancer patients in the five groups of workers are listed in Table III-1. Frequencies have been recalculated from the author's data and are also presented in the table.

TABLE III-1

OCCURRENCE OF SKIN CANCER IN RUSSIAN WORKERS FROM 1956 TO 1970

	Chloroprene Workers	Chloroprene Derivative Workers	Chemical Workers	Nonchemical Workers	Nonindustrial Workers
No. examined	684	2,250	4,780	8,755	8,520
No. of cases	21	38	32	35	11
Reported %	3.00	1.60	0.66	0.40	0.12
Corrected %*	3.07	1.69	0.67	0.40	0.13
Average age of patients	59.6	59.1	64.4	68.9	72.05
Average years of employment	9.5	8.7	13.8	15.4	16.3

*Recalculated from author's data

Adapted from reference 28

The greatest prevalence of skin cancer, 3%, was found in the chloroprene workers. In workers from the nonchemical and nonindustrial situations, the frequencies of skin cancer were 0.40% and 0.12%, respectively. Recalculation of the latter frequency from the data presented in the paper gives a value of 0.13%. Both groups were regarded as controls, but the calculations were based only on the nonindustrial workers. Corrected frequencies and ratios are given below in parentheses after those presented by Khachatrian. Khachatrian reported that, in those workers with extended (undefined) chloroprene contact during manufacture, the frequency of skin cancer was stated to have increased 25-fold (recalculated value 23.1); of of workers in contact with chloroprene derivatives (latices, adhesives, and rubber), there was a stated increase of 13.3-fold (13.1). When the chemical workers were considered, the frequency of skin cancer increased by a factor of 5.5 (5.2). This was based on a frequency of 0.12% (0.13%) in the nonindustrial workers.

The neoplasms in the three control populations were found mostly on the face, neck, and hands, and they often occurred at the sites of various birth defects on the skin (moles and birthmarks) [28]. In the case of workers with chloroprene contact, the neoplasms were most frequently located on the skin of the nose and ears. It was stated that in 90% ± 7% of the workers with tumors, the neoplasms had been preceded by skin changes characterized as chronic dystrophic or inflammatory skin conditions, such as eczema, cracks, and dyskeratoses. The progression of skin lesions in chemical workers was similar to that observed in chloroprene workers. No further information on sex, prior work histories, or durations of exposure was given in the paper. The concentrations of chloroprene and of any other

toxic compounds in the workplace were not presented.

Khachatrian [28] concluded that chloroprene was a carcinogen or cocarcinogen for human skin. The author felt that the observed chronic dystrophic and inflammatory skin ailments, which usually preceded the skin cancer, had a role in the development of eventual neoplasms.

In a second study, Khachatrian [29] observed the prevalence of lung cancer in 19,979 workers in the city of Erevan, USSR. Although there were four groups, rather than five as in the previous paper, the assignment of the workers to groups was similar, except that no distinction was made between workers exposed to chloroprene and those using only chloroprene derivatives. The total number of workers with occupational chloroprene exposure and the number of chemical workers with no chloroprene exposure (4,780) were the same as in the previous study. Chemical workers without chloroprene exposure were defined as those with any exposure to chemicals other than chloroprene (truck drivers, polishers, cabinet workers, painters, gas station attendants, and typesetters). The numbers of nonindustrial (6,045 professionals) and nonchemical (6,220) workers were smaller than those used in the skin cancer epidemiologic study [28]. A total of 87 persons suffering from lung cancer were identified (82 men and 5 women), 16 of whom had formerly been workers in a chromium plant. The individual's career progression, age at which the individual began working, work experience, evidence of contagious lung disease (before and after starting work), and smoking habits were all considered in the study, but detailed information was not provided in the paper. There was no description of the specific types of lung cancer.

Sixty-six (76%) of the workers with lung cancer also suffered from chronic bronchitis, three (3.4%) had tuberculosis, and four (4.5%) had pneumonia [29]. Fifty-seven (66%) of the cancer patients were heavy or long-term smokers. The lung cancer data are listed in Table III-2 [29].

TABLE III-2

OCCURRENCE OF LUNG CANCER IN RUSSIAN WORKERS FROM 1956 TO 1970

	Chloroprene Workers*	Chemical Workers	Nonchemical Workers	Nonindustrial Workers
No. examined	2,934	4,780	6,045	6,220
No. of cases	34	22	11	4
Reported %	1.24	0.46	0.8	0.064
Corrected %**	1.16	0.46	0.18	0.064
Average age of patients	44.5	54.9	59.3	60.2
Average years of employment	8.7	10.3	14.9	18.5

*Includes chloroprene derivative workers
**Recalculated from author's data

Adapted from reference 29

The methods used for diagnosis were not discussed in the paper. There are inconsistencies between the table and the text regarding percentage of cancer and the number of cancers tabulated as occurring in chloroprene plant workers (18) plus workers from shoe factories (17). The total number of lung cancers in workers exposed to chloroprene was stated to be 34, but

from the percentage stated in the text, it appears to be based on neither 34 nor 35 patients, but on 36 cases of cancer (2,934 x 1.24%). The incidence of lung cancer among workers with extended contact with chloroprene and its derivatives was stated to be 1.24% (actually 1.16%); the average age of these stricken workers was 44.5 years [29].

Khachatrian [29] described 16 additional cases of lung cancer in former maintenance workers, cleaning women, laundry workers, and chemical workers from the plant where chromium compounds had been used in plant processes. The total number of workers in the plant was not given, but there was stated to be a 4.2% frequency of lung cancer among them. All of the 16 former chromium plant workers were found to have perforated nasal septa, which had developed during their employment.

Khachatrian [29] concluded that extended contact with chloroprene and its derivatives led to significant increases in primary lung cancer. In addition, it was stated that chloroprene was as carcinogenic as chromium compounds. The author reported that the chemical workers without chloroprene exposure had a lung cancer frequency 0.37 times that of workers exposed to chloroprene. From the data in Table III-2, the corrected ratio is calculated to be 0.40. The author stated that the frequency in nonchemical workers was 0.16 times, and in nonindustrial workers it was 0.05 times (actually 0.06), that in workers exposed to chloroprene.

It is very difficult to assess the actual risk of lung or skin cancer for the Russian workers exposed to chloroprene. Savelev, Deputy Chief of the Administration of Foreign Relations of the Soviet Ministry of Health [8] has stated in a letter that a panel of Russian experts examined Khachatrian's investigations and found errors in her methods that led to

incorrect conclusions. He stated that the Ministry of Health planned a formal statement on this matter, but none has been forthcoming to date.

Pell [30] recently submitted to NIOSH summaries of preliminary reports of an epidemiologic survey of cancer mortality in male employees engaged in the manufacture of chloroprene and its polymerization to neoprene rubber. There were two study populations, one from the Louisville Works in Kentucky, (1,661 persons) and one from the Chambers Works in Deepwater, New Jersey (243 persons). The Louisville group consisted of those persons on the wage roll who were working at the plant on June 30, 1957. Chloroprene production began at Louisville in 1942, but the routine recording of deaths through life insurance claims did not begin until 1956. The records of the Louisville group were assessed through December 31, 1974. Workers in the Deepwater cohort were employed in the neoprene rubber manufacturing area between 1931 and 1948, and mortality data were analyzed for the period from 1956 through 1974.

Three groups from the 1,661 workers in Louisville were considered to have the highest exposure to chloroprene: 263 maintenance mechanics, 458 chemical operators, and 124 workers in other high-exposure occupations [30]. All persons in the cohort who died during the study period were identified by (1) plant personnel and medical records, (2) the corporate medical division's files of deceased employees and retirees, and (3) a followup on 240 employees missing from these two sources because of short terms of employment. Nineteen of these employees had yet to be traced at the time of reporting. Women (84 at Louisville and 19 at Deepwater) were excluded from the overall study because of little or no exposure at both plants.

The observed number of deaths for each type of cancer was compared with the number expected based on death rates for the various cancers in US men and in male employees and retirees of the company [30]. In the Louisville study, 18 deaths were caused by cancer of the respiratory system (17.1 expected based on US statistics), and 16 of these were the result of lung cancer. Four lung cancer deaths occurred in maintenance mechanics (3.8 expected), three in chemical operators (3.2 expected), and two in other high-exposure occupations (1.6 expected). The exact nature of these job classifications was not indicated. The differences between the mortalities due to lung cancer in the worker population and in the US male population were judged to be insignificant. Incidence of skin cancer was not considered.

Four maintenance mechanics with lung cancer were still alive and were so identified at the end of the study [30]. If these four cases are added to the four deaths due to lung cancer in the same group of workers, the total of eight cases of lung cancer in the maintenance mechanics would account for 40% of the reported cases of lung cancer; however, maintenance mechanics constituted only 17% of the population studied. Pell felt that this excess risk of lung cancer might "be the result of... [another] chemical carcinogen in the plant, ...cigarette smoking [smoking at work is permitted in this group], ...or a fortuitously high concentration of [cancer] cases." Seven of the eight affected maintenance mechanics were known cigarette smokers.

At Deepwater, three deaths from lung cancer were observed (3.5 expected) [30]. None of the three who died was a maintenance mechanic, but one mechanic who died from myocardial infarction had lung cancer. The

mortality from bladder cancer was 10 times the expected value (3 observed, 0.3 expected); however, these workers had also had significant exposure to beta-napthylamine, a known bladder carcinogen to which these deaths were attributed.

The reports by Khachatrian [28,29] suggest an excess of skin and lung cancer in chloroprene-exposed workers, while the report by Pell [30] concludes that there was no significant excess of cancer in chloroprene workers except in maintenance mechanics. However, limitations in methodology and study design of these investigations preclude an assessment of the carcinogenic risk.

None of these epidemiologic studies give adequate consideration to environmental concentrations, job classification, intensity or duration of exposure, or latency--all factors known to influence the risk of cancer. In each of these studies, the investigator did not analyze the data separately for chloroprene polymerization workers. (The greatest risk in the vinyl chloride industry was in polymerization workers, not in monomer production workers.) The Pell [30] and Khachatrian [28,29] studies do not mention the criteria by which the cancers were diagnosed, nor are the cell types for skin and lung cancer indicated.

In the Khachatrian reports [28,29], adjustments for age and sex also are not mentioned. The difference of 40-50 years between mean age of cancer patients and mean length of employment for these patients suggests that total employment histories appear to be lacking.

In the Pell study [30], past information indicates that some causes of death indicated on the death certificates were classified for the chloroprene-exposed workers, but not for the control group. This procedure

may have introduced bias. Pell used industrial controls that were composed of subsets of workers exposed to multiple industrial agents known or suspected to be carcinogenic. Such an approach underestimates the true carcinogenic risk. In the Pell report, cases of cancer were identified through life insurance claims. Since skin cancer may be a nonfatal disease, this method would only identify fatal skin cancer cases and result in an underestimate of the skin cancer risk.

Pell [30] also suggests an excessive risk of lung cancer among maintenance mechanics, a group expected to have high exposure to chloroprene. However, the ages of the lung cancer patients among the maintenance mechanics are not compared with those of the lung cancer patients in the other study groups. Thus, definitive evaluation of this observation of pulmonary cancer is not possible, although the observation does suggest an excess of lung cancer in maintenance mechanics exposed to chloroprene.

Two human studies carried out between 1950 and 1954 in the Kirov chloroprene plant in Soviet Armenia were described by Mkhitarian [31,32]. In the first of these, 110 workers (sex unspecified) were studied. In the second study [32], data for an additional four workers were considered. In the following discussion, whenever a range of numbers of persons is given, the smaller number refers to the earlier study [31] and the larger to the later one [32]. Three groups of workers were selected according to the shop they worked in, and five professions were identified: shop A (highest exposure), where 30-31 cleaners and loaders worked; shop B (intermediate exposure concentration), where 33-35 equipment operators worked; and shop C (negligible chloroprene exposures), where 18-19 persons worked as rollers

and packers. It was not stated directly that these workers handled the latex sheet. The actual exposure concentrations were not given. Of the 110-114 workers considered in the studies, data were presented for 81-85 of them, the rest apparently having been control groups from other shops.

Work experience was broken down in the following manner: 28 workers had more than 10 years' service, 20 had between 5 and 10 years' service, and 33-37 had 5 years' service or less [31,32]. No further breakdown into job groupings was done. Blood samples from the workers were tested for the following: glucose, cholesterol, total protein, albumin, total globulins, glutathione (oxidized and reduced), fibrinogen, carbonic anhydrase, catalase, calcium, chloride, and reserve alkalinity (the tendency toward acidosis). The assays used were not clearly defined. Blood pressure also was measured. The author stated that exposure to chloroprene led to hypoglycemia, hypocholesterolemia, decreased carbonic anhydrase activity, decreased reserve alkalinity, hypotension, and decreased blood clotting time in some or all of the worker groups. Data supporting the last two statements were not presented. There was no significant change in catalase activity, total globulins, or total glutathione. Increases were noted in total protein, albumin, calcium, oxidized glutathione, fibrinogen, and chloride. No statistical considerations were presented, but the author stated that most data had been statistically processed and were reliable. No data from control subjects were given, but normal values for several of the quantities measured were stated. Concentrations and exposure durations for individuals were also not presented.

In 1964, Mnatsakanian and Mushegian [33] studied the influence of chloroprene on porphyrin metabolism in Armenian children living near the

Kirov plant. Three groups of children of both sexes between the ages of 5 and 8 years were selected from schools located at various distances from the chloroprene plant. Average concentrations of chloroprene in the air during May and September ranged from 0.08 to 0.13, from 0.07 to 0.12, and from 0.04 to 0.05 ppm in schools located at distances of 100, 500, and 700 meters from the plant, respectively. The May through September values were averages of 10 daily assays by microcombustion [34]. Between May and September, urine was collected during each schoolday (5-6 hours), and total coproporphyrin was measured spectrophotometrically by the method of Gusev and Smirnov [35]. The authors [33] stated that the quantity of coproporphyrin varied little according to age and sex, so that the data were analyzed as functions of the distance from the chloroprene source and the air concentration, with no further breakdown.

In the first school (average exposure range of 0.08-0.13 ppm of chloroprene), 42 children excreted an average of 6.36 ± 0.46 μg of coproporphyrin [33]. In 99 children in the second school, the average coproporphyrin excretion was 5.51 ± 0.36 μg (average exposure range of 0.07-0.12 ppm). In the third school, 105 students exposed to chloroprene at 0.04-0.05 ppm excreted 4.11 ± 0.23 μg of coproporphyrin. Whether these values were related to daily excretion or excretion for each liter of urine was not stated. The authors stated that the increase in coproporphyrin excretion was statistically significant and attributed it to the increasing air concentration of chloroprene, but no quantitative statistical reliance was assigned. The normal range of urinary coproporphyrin in children is 0-80 μg/24 hours [36], and all the above data are within this range.

Mnatsakanian [37] measured urinary 17-ketosteroids as an evaluation of adrenal function in the same groups of children during the same time period as in the study by Mnatsakanian and Mushegian [33]. Five hundred urine specimens were collected over a 5- to 6-hour period and the volumes recorded. The concentration of 17-ketosteroids in each sample was determined spectrophotometrically by the method of Uvarovskaia [38]. Steroid content was reported as total milligrams/urine sample. The control value was 0.73 ± 0.045 mg. At chloroprene concentrations in the air of 0.07-0.12 and 0.08-0.13 ppm, urinary 17-ketosteroid excretions were observed to be 0.919 ± 0.086 and 1.021 ± 0.086 mg, respectively. Total urine volume for each child was not reported. From these studies, the author claimed a statistically significant increase in urine volume and in 17-ketosteroid excretion as a function of the chloroprene concentration. He concluded that there was an effect on adrenal function in children at chloroprene concentrations of about 0.11 ppm. The normal excretion of 17-ketosteroids for children under 12 years old is less than 5 mg in a 24-hour period [36]. All the observed values are within the normal range.

Vanuni [39,40] investigated the effect of chloroprene in the air on human milk production near the Kirov chloroprene works in Erevan, Armenia. Four groups of 30 pregnant women each were examined: group I worked in the plant (25.94 ± 0.34 years old, 4.07 ± 0.47 years of working exposure), group II lived 500 meters distant, group III lived in a village 1,500 meters distant, and group IV lived 3,000 meters from the plant. Data on average age and exposure of groups II, III, and IV were not specified. On the 8th day after parturition, a milk sample was taken, and total protein and individual amino acid composition were measured by paper chromatography

(method not given in detail). Two control groups of 25 women each, one from a village 25 km distant and the other from the northern portion of the city of Erevan (distance not specified), were selected and examined in a similar manner.

A significant increase was noted in total milk protein concentration as a function of distance from the plant [39,40]. Milk protein concentrations from mothers working in the plant were 964 ± 9 mg/100 ml ($P<0.001$). Total protein concentrations were 1,049 ± 15 mg/100 ml ($P<0.001$), 1,074 ± 24 mg/100 ml ($P<0.01$), and 1,140 ± 24 mg/100 ml ($P<0.001$) for groups at 500, 1,500, and 3,000 meters, respectively. Control concentrations of milk protein averaged 1,321 ± 22 mg/100 ml in women from the northern section of the city and 1,289 ± 17 mg/100 ml for mothers 25 km away. Mothers from the plant had milk protein concentrations that were 73% of those of the Erevan controls.

Concentrations of some individual amino acids in milk were significantly decreased when samples from mothers who worked in the plant were compared with those from the two control groups [39,40]. Cysteine concentrations were 90% of the controls ($P<0.05$), lysine was 66.3% ($P<0.001$), arginine was 58.8% ($P<0.001$), valine plus methionine was 75.7% ($P<0.001$), and leucine plus isoleucine was 93.0% of the controls ($P<0.05$). The decrease in lysine and valine plus methionine appeared to be distance dependent, whereas decreases in cysteine, arginine, and leucine plus isoleucine were not.

Since the mothers had similar diets and lived under the same climatic conditions, Vanuni [39,40] concluded that depression of the quantity of amino acids in the milk, and hence of its nutritional value, apparently

depends on the depressed lactation function of the mammary glands due to chloroprene intoxication. This argument appears to be spurious, since depressed lactation reduces milk volume (quantity) but does not necessarily affect the nutritional value (quality). Chloroprene was not reported to have been found in the mothers' milk, and the concentration of chloroprene in the plant air was not reported. The chloroprene concentrations at the various distances from the plant were also not indicated. Therefore, a precise dose-reponse relationship cannot be determined.

In 1976, Volkova et al [41] surveyed conditions in a Soviet plant manufacturing rubber gloves from polychloroprene latex. A total of 65 workers were examined: 43 had less than 5 years of exposure to chloroprene latex, 15 had worked for from 10 to 20 years, and the exposures of the other 7 are not clear. Most (no number specified) of the 65 persons worked as dippers. This job was not described in any detail. The concentration of chloroprene in the air in the dipping area varied between 0.8 and 1.95 ppm. The authors stated that they observed an increase in the frequency of complaints of fatigue, headache, and chest pain with increasing time of service. No quantitative data were presented. Nineteen percent of the workers had chronic tonsillitis (the control frequency was not presented). Of the women in the study group, 47% (the total number of women was not given) had menstrual disorders versus 10% in a control population. The major menstrual disorder observed was decreased blood flow, and the frequency of the disorder increased with length of service.

Volkova et al [41] concluded that concentrations of chloroprene near the maximum allowed in air in the USSR at that time (0.56 ppm) adversely affected the workers' health. However, in an operation using

polychloroprene latex, there is concomitant exposure to many substances besides chloroprene [42]. The significance of the symptoms described is difficult to assign. Fatigue and chest pain have usually been reported only at much higher concentrations [20].

Recently, cytogenetic analysis of lymphocytes from the blood of persons working in chloroprene manufacture was carried out by Katosova [43] in the USSR. Of three groups of employees with chloroprene exposure studied, subjects in group A were also exposed to chlorine, acetylene, ammonia, mercaptans, and monovinyl acetylene; those in group B were exposed to essentially pure chloroprene (mercaptan and ammonia below the MPC); and those in group C were exposed to chloroprene only. Since no significant differences between the percentages of chromosomal aberrations in the exposed groups were observed, the data from the three groups were combined. Blood samples were taken from 18 healthy workers (13 men and 5 women), and lymphocytes were cultured. The lymphocyte cultures were coded, and all available metaphase cells that met certain unstated requirements were analyzed for aberrations and gaps. Blood samples from nine workers not exposed to chloroprene were used to obtain control lymphocytes. The average air concentration of chloroprene was 5 ppm (nine times the 1975 USSR standard). Data presented for each examined worker included age, sex, number of cells in metaphase studied, and the frequencies of aberrations and gaps. The author stated that the maximum frequencies of cells in metaphase with aberrations were observed in the blood cells of cleaners, manual laborers, and loaders, but no breakdown by occupation was shown in the tabular data presented. Chromosomal aberrations were mainly paired

fragments, while chromatid-type aberrations (68% of total aberrations) were individual fragments.

There was no relationship between the frequency of aberrations and the number of years of service, but the average frequency of aberrations in the occupationally exposed group was 4.77 ± 0.57%, versus 0.65 ± 0.56% in the controls ($P<0.001$) [43]. The frequency of cells in metaphase with gaps was also significantly ($P<0.01$) higher in the exposed group, 3.71 ± 0.59% versus 1.14 ± 0.43%. The author concluded that, since the number of chromosomal aberrations in the workers exposed to chloroprene was significantly increased in comparison with both that for the control group and that reported for spontaneous change (1.6%), the cytogenetic effect was probably related to the influence of chloroprene. However, the author suggested that further experimental studies were necessary because this study did not demonstrate directly that chloroprene was mutagenic.

In 1975, further study of chromosomal aberrations in lymphocytes from workers exposed to chloroprene was reported by Bochkov (written communication, March 1976) at an International Symposium on New Developments in Mutagenicity Testing. Control subjects (437) had a mean of 1.19% of chromosomal aberrations in lymphocytes, whereas 57 workers exposed to chloroprene had a mean of 2.90%. No measures of variability in these two populations were given, so that the significance of the differences between these two percentages cannot be judged.

In 1976, Volkova et al [41], as part of a survey of working conditions in a Soviet polychloroprene rubber glove manufacturing plant, studied 20 women, 19-23 years old, with 2-4 years of service. These women worked in the dipping area, where the chloroprene air concentration varied

between 0.8 and 1.95 ppm. They had blood pressures and olfactory sensitivities below normal and short attention spans. In 16 of 20 subjects, the frequency of chromosomal aberrations in lymphocytes was greater than normal (1.5-9%). The average frequency of occurrence of aberrant cells in the blood of exposed women, 3.49 ± 0.51%, was significantly higher than that reported by Bochkov et al [44] in 1972, 1.19 ± 0.06%. The authors concluded that these cytogenetic changes indicated that chloroprene at concentrations of 0.8-1.95 ppm had mutagenic properties. Since there are other compounds emitted by Russian polychloroprene latex besides chloroprene, eg, ammonia, dodecylmercaptan, and methacrylate [42], the study did not demonstrate conclusively that chloroprene was mutagenic. Data on the control group were gathered from previously reported studies [44] that were not conducted under similar conditions.

Fomenko et al [45] stated that, although cytogenetic analysis of blood cultures of workers is a promising technique for detecting occupationally induced chromosomal aberrations, the interpretation of results from such studies is difficult because of the many other environmental factors in and around the plant.

In 1965, Gasparian and Arutiunian [46] reported on chloroprene-induced changes in the electroencephalographs (EEG's) of 70 workers involved in the production of chloroprene. Twenty persons with no chloroprene exposure were studied as controls. The age, sex, work history, location, and chloroprene exposure of each worker were not presented. The authors did state, however, that young and middle-aged men with 5-15 years of experience predominated in the occupationally exposed group. The

authors stated that chloroprene caused a sense of drunkenness, sleepiness, suppression of memory, dizziness, and increased reflex excitability. Early in a series of repeated occupational exposures, toxic neurasthenia was evident. In later stages, encephalopathy with epileptiform seizures may have occurred.

The authors [46] stated that chloroprene exposure for 5-15 years led to five different types of abnormal EEG's. The three most common of these types were (1) deflections of low voltage and frequency, (2) deflections of low frequency but long duration (delta-type), and (3) inconsistent wave patterns with alternating alpha-, beta-, and delta-activities and occasional spikes. In workers with comparatively short durations of exposure to chloroprene, the predominant EEG changes were disruption of the alpha-rhythm or predominance of beta- or delta-activity. When the exposed workers were subjected to a flashing light during recording of the EEG, 82.8% (11.4% high, 14.3% moderate, 57.1% low) reacted, while 17.2% were completely unreactive. In the control group, 100% of the subjects reacted (50% high, 40% moderate, 10% low) to the flashing light, and none were completely unreactive. Of the workers, 78.6% did not synchronize with the frequency of the visual stimulus or only synchronized at comparatively low frequencies, whereas only 25% in the control group failed to synchronize. The authors concluded that the EEG changes induced by chloroprene exposure may have been either functional or partially organic in nature, and that the balance between functional and organic abnormalities of the brain was related to the length of contact of the workers with chloroprene.

The significance of these data [46] is difficult to assess. Chloroprene can cause CNS effects, but it was not convincingly demonstrated

in this paper. Comparison of the EEG's taken on the workers with similar recordings made prior to exposure would have been much more meaningful. The methods of recording and analysis were not described in sufficient detail in the text.

Animal Toxicity

The general toxicity of chloroprene in various species of animals has been evaluated by several routes of exposure. Effects of chloroprene exposure on reproduction have been studied extensively in rats and mice. A smaller number of studies has been found on the carcinogenic and mutagenic effects of chloroprene exposure in these animals. Bacteria and Drosophila have also been used to evaluate the mutagenicity of chloroprene.

In 1936, Von Oettingen et al [18] first examined chloroprene toxicity and the resulting adverse effects. Their report described a large number of studies, but involved a small number of animals, thus making statistical evaluation difficult. The minimum fatal oral dose, resulting in (by the authors' definition) 70-100% deaths, was determined for chloroprene using a total of 39 rats of unspecified strain, 3-15 rats at each dose. At chloroprene doses of 0.4 ml/rat or greater (body weight not given), 75% or more of the rats died. Lung edema, internal bleeding, liver necrosis, and gastric inflammation were found upon autopsy. The investigators did not report the relationship between toxic signs and specific dose. Doses/unit of body weight cannot be calculated, because the latter values were not given.

The minimum fatal concentration (defined as the concentration killing at least 70% of a group of exposed animals) for mice via inhalation for 1

hour was 278-834 ppm [18]. In another experiment, mice, rats, cats, and rabbits were exposed to chloroprene at varying concentrations in air from 40 to 43,800 mg/cu m for 8 hours. At each individual chloroprene concentration, three to nine mice, two to four rats, one cat, and one rabbit were exposed. The minimum fatal concentrations for these animals were 167 ppm for mice, 4,170-5,860 ppm for rats, 695 ppm for cats, and 2,085 ppm for rabbits.

Von Oettingen et al [18] also investigated the effect of nutritional states on susceptibility to chloroprene and skin toxicity. Three fed rats were exposed to chloroprene at an air concentration of 3,030 ppm for 8 hours. This exposure was not lethal. A second group of three rats was starved for 120 hours prior to exposure at 3,030 ppm for 8 hours. Two of these rats died on the day of exposure and the third on the following day. Similar results were observed when two rats, starved for 24 hours, were exposed to concentrations of chloroprene in the air of 4,114 ppm. Both fed and starved rats were equally susceptible to chloroprene at concentrations of 5,778 ppm (20,800 mg/cu m).

Skin-painting studies were described in which 0.5 ml of chloroprene was applied to the unshaved backs of seven rats for 1 week [18]. The rats then had the hair removed from their backs with barium sulfide and were rested for 2 weeks. After that, a dose of 1.5 ml of chloroprene was applied daily to the bare skin for up to 55 days. Administration of chloroprene to one of the seven experimental rats was discontinued on day 49. After each daily application of chloroprene, the rats showed some signs of local irritation and later developed a state of depression that continued for about 2 hours.

After the higher dose of chloroprene had been applied to their skin for about 2 weeks, the exposed rats gained weight less rapidly than the controls [18]. The rat that had the chloroprene application terminated on the 49th day regained practically all its deficit of gain of weight by the 71st day. Four of the six rats that had applications of chloroprene up to the 55th day regained some of their deficit of gain of weight by the 71st day, one continued to lose weight, and the remaining rat maintained an approximately constant body weight from day 55 to day 71.

Three rats were killed for examination on day 74. The skin of all the experimental rats was normal in structure, although the hair shafts appeared to have been dissolved partially by the chloroprene. The internal organs were normal in gross appearance, but there were mild nephroses, hyperemic spleens, and slight degeneration and calcification of the testes. The livers of two rats showed signs of scattered hydropic degeneration and lysis of the nuclei of the hepatocytes.

The authors [18] concluded that chloroprene was a toxic material that should be handled with great caution, that contamination of the skin should be avoided, and that inhalation of the vapor at concentrations as low as 83 ppm could cause toxic symptoms. However, adverse reproductive effects in male mice were observed below this level and are discussed further in part (c) of this section, Teratogenicity and Effects on Reproduction. These studies are difficult to evaluate quantitatively because the number of animals in each experiment was small and the authors did not report the purity of the chloroprene.

Roubal [19], in 1942, conducted experiments on five cats, five rabbits, and one dog that were administered chloroprene by inhalation,

injection, or skin application. In one experiment, a single cat (2.6 kg) was given a 5-cc dose of chloroprene subcutaneously. Respiratory changes were noted within a few minutes; after about 0.5 hour, breathing stopped. Blood pressure, after an initial increase, dropped gradually until the heart stopped. The total elapsed time between injection and heart stoppage was 41 minutes. A second cat (2.6 kg) was anesthetized and made to inhale chloroprene vapor for 7.5 minutes through a mask saturated with 10 cc of the liquid. Irregularity of breathing was observed at first, but, after 7 minutes, respiration returned to normal. Blood pressure initially rose and then fell rapidly after an additional 5 cc of chloroprene were applied to the mask. The mask was left in place until death occurred. The total time elapsing until death was not given.

A rabbit of unspecified strain (2.3 kg) was injected subcutaneously with distilled chloroprene at a dose of 1 cc. Although it died after 20 hours, Roubal [19] found no physical changes in the rabbit after the injection. Another rabbit (2.7 kg) received four separate subcutaneous injections of chloroprene at a dose of 1 cc on days 1, 5, 13, and 27. The rabbit died 26 hours after the last injection.

The unshaven skin of a dog was painted daily with chloroprene on an area 10 x 10 cm for 12 days [19]. Hair fell out of the painted area for 3 days after the application, leaving the area bare. Twenty days after the start of hair loss and 8 days after the last application of chloroprene, hair was observed to be growing back.

An experiment was also conducted by Roubal [19] with a cat exposed to chloroprene in an inhalation chamber. Twice-distilled chloroprene was administered at an unspecified concentration and duration of time. Loss of

muscle coordination developed first, followed by difficulty in breathing. The cat died 6 weeks later. On post-mortem examination, Roubal [19] observed the following in one cat and in all rabbits: edema, degenerative changes in the liver, some kidney and adrenal tissue degeneration, and hair loss.

Nystrom [20] presented a comprehensive study of chloroprene toxicity carried out between 1944 and 1948. The author examined the differences in toxicity between pure, freshly distilled chloroprene and oxidized material stabilized against polymerization with pyrocatechol but exposed to the air for several days. Using 280 rats, strain and sex not specified, Nystrom calculated the LD50's after subcutaneous administration to be 0.002 ml/g of body weight (1,916 mg/kg) for pure chloroprene and 0.0005 ml/g of body weight for the oxidized material, a fourfold difference. Twenty rats were exposed at each of seven doses. The mean survival time was stated to be distinctly shorter for animals exposed to oxidized chloroprene than for those exposed at similar doses of pure material. It was stated that the lungs showed a greater extent of hyperemia and edema when rats were administered the oxidized chloroprene above the LD50. Administration of similar doses of pure chloroprene resulted in less extensive changes. This was the first report to describe and examine the two types of chloroprene. The author made no statements on the chemical nature of the oxidized chloroprene.

Exposure of 10 rats to chloroprene at air concentrations of 334 ppm for 8 hours each day for 5 months resulted in the deaths of half the rats by the end of the 13th week of exposure [20]. This exposure led to significant decreases in body weight, red blood cell count, and hemoglobin

concentrations, but blood leukocyte levels increased. The rats ate and drank little during the first 10-14 days of the exposure, but thereafter ate and drank about as much as the controls. Despite this increased appetite and thirst, a continuous loss of weight occurred throughout the exposure. Statistical analysis of these changes is not possible, since only mean values were given. Exposure of a second group of 10 rats at a chloroprene concentration of 56 ppm for 8 hours each day for 5 months resulted in no deaths. No changes were observed in body weight and red blood cell, leukocyte, or hemoglobin levels in these rats. Changes observed at autopsy were described by the author as "inconsiderable."

Nystrom [20], in an attempt to delineate physiologic changes at unspecified "high" concentrations of oxidized chloroprene, measured the volume of plasma in the blood, the oxygen content and oxygen capacity of the blood, and blood coagulation time; both rats and rabbits were used. After 20 minutes of inhalation of air bubbled through chloroprene, Nystrom observed decreases in blood plasma content in 10 rats, 55.8% ± 0.77 before exposure versus 49.3% ± 0.42 after exposure. After 30 minutes of exposure, the mean coagulation time of the blood in 40 rats was reduced, 2.36 minutes before exposure versus 1.67 minutes after exposure. The mean value of the ratios of the coagulation times after exposure to those before exposure was 0.738 ± 0.018. After unspecified exposures to chloroprene, arterial blood of 15 rats showed a mean decrease of 17% in oxygen content, and that of 6 rabbits showed mean decreases of 8.3% in oxygen content and 10.1% in oxygen capacity.

A striking finding in this series of experiments with rats is that from the changes in the percent of plasma in whole blood, the blood of a

rat can be calculated to lose about 1 g of plasma, and the weights of the lungs of exposed and unexposed rats indicated that exposed lungs could gain up to 1.25 g of fluid. Nystrom suggested, therefore, that transudation of plasma from pulmonary capillaries explains both the increased concentration of the blood and the pulmonary edema found after heavy exposures to oxidized chloroprene. The increase in weight of the lungs after exposure of the rat to oxidized chloroprene was not reproduced by exposure to unoxidized chloroprene. No comparison of the effects of oxidized and unoxidized chloroprene on the percent of plasma in whole blood was made.

In 1969, Paulet and Malassis [24] investigated chloroprene toxicity in Wistar rats. Rats of unspecified sex (10 at each dose) were injected ip with chloroprene in an unspecified type of polyethylene glycol at 300, 500, 800, 1,000, 1,200, or 1,500 mg/kg. The LD50 was reported to be 520 mg/kg.

In 1971, Asmangulian and Badalian [47] studied the oral toxicity of chloroprene to rats and mice. Sixty white mice (20-24 g) of unspecified strain and sex were given chloroprene in sunflower oil at single oral doses (10 mice/dose) of 50, 100, 200, 300, 400, or 500 mg/kg. The LD50 was reported to be 260 mg/kg. A total of 54 white rats of unspecified sex and strain (180-200 g) were also given single oral doses of chloroprene in sunflower oil; the actual amounts were not specified. The LD50 was reported to be 251 mg/kg. The authors [47] stated that acute (single-dose) poisoning was characterized by signs of CNS depression; the animals were listless and sluggish. After 2 weeks of recovery, no abnormal behavior was observed in surviving animals. The criteria for this were not discussed. Autopsy of dead animals showed vascular congestion and edema in the lungs, liver, brain, spleen, and epicardial region. The stomach showed signs of

inflammation, and myocardial degeneration was evident.

A second group of rats (number and sex unknown) was exposed to chloroprene for 5 months at a daily oral dose of 15 mg/kg [47]. Although this is cumulatively over four times the LD100 (400 mg/kg for rats and 500 mg/kg for mice), none of these animals died. No information was presented on possible sublethal toxic effects. The authors concluded that chloroprene was a strongly toxic substance but not a cumulative poison by the oral route.

Jaeger et al [48] have reported that single 4-hour exposures to concentrations of 500, 1,000, or 2,000 ppm of airborne chloroprene did not cause an elevation of the activity of alanine alpha-ketoglutarate transaminase in the serum of fed rats, but did have this action in rats fasted for about 18 hours before exposure and caused death in the fasted rats exposed at each of these concentrations. The difference between the responses of fed and fasted rats exposed to chloroprene vapor disappeared at a concentration of 10,000 ppm. The hepatotoxic effect of chloroprene, evidenced by an increase in the activity of alanine alpha-ketoglutarate transaminase in the serum, increased progressively from 12 to 24 hours after exposure. The difference between fasted and fed rats was thought to be related to the lower concentrations of glutathione found in the liver of the fasted rats.

(a) Carcinogenicity

Khachatrian [49] subjected 10 groups of mice and rats (a total of 210 mice and 180 rats) to repeated subcutaneous injections, cutaneous applications, or intratracheal administrations of preparations of chloroprene latex and nonpolymerized chloroprene, alone or mixed with other

materials. Presumably, the latex was primarily polymerized chloroprene. The number of doses was stated in only 5 of the experiments and was 1, 16, or 40. Although it is unclear what form of the chemical was given, Khachatrian observed that high percentages of the animals that survived the series of administrations developed toxic hepatitis with extensive necrosis, hemorrhages in the liver, lungs, gastrointestinal tract, and kidneys, leukosis, and tumors in various sites.

Khachatrian [49] stated that malignant tumors were found in the internal organs following skin application or subcutaneous injection, but not at sites of either injection or application to the skin, but she gave no quantitative information about the types of tumors and their sites of occurrence. The development of more than one tumor in a single animal was said to be a common result of exposure to chloroprene; one example of this response included a pulmonary adenoma, sarcomatosis of the skin, and intestinal tumors.

Leukosis appeared rapidly in animals whose skins were painted with a solution of chloroprene in acetone [49]. In some experiments, mixtures of chloroprene latex and shellac or of chloroprene latex, shellac, and oil paint were applied to the skins of the experimental animals; myeloid leukosis occurred. All 11 surviving animals (from an unspecified number) exposed to the two-part mixture and 7 of 11 surviving animals (from an unspecified number) exposed to the three-part mixture had tumors of unspecified origin and type. The author concluded that both nonpolymerized chloroprene and chloroprene latex were carcinogens. Evaluation of these data are particularly difficult because the descriptions of the methods used to generate the data are incomplete. Moreover, the absence of control

studies prevents NIOSH from making dependable conclusions from these data.

In 1972, Zilfian and Fichidjian [50] published the results of a study investigating the effect of chloroprene on the growth of implanted Crocker's murine sarcoma. Thirty mixed-breed mice, 18-20 g, were injected subcutaneously with chloroprene in peach oil (0.1 g/kg of body weight) five times prior to inoculation with Crocker murine sarcoma suspension. Six additional subcutaneous injections of chloroprene were given after the tumor inoculation. Thirty mixed-breed mice, 18-20 g, were injected with peach oil and the Crocker's tumor suspension in the same manner and kept as controls. The frequency and spacing of chloroprene and peach oil injections were not specified, but the doses are presumed to have been given at intervals of 1-2 days because the experiment was said to have been terminated on the 12th day after the sarcoma cell suspension was injected into the mice.

The authors [50] observed that the mice injected with chloroprene exhibited palpable tumors 1-2 days earlier than mice in the control group. The number of mice in each group developing tumors and the latent period were unspecified. Autopsies were carried out 12 days after inoculation of the tumor suspension. Tumors in the mice injected with chloroprene were almost twice as large and 4.5 times as heavy as tumors in the control mice. The authors suggested that chloroprene depressed the immune system of the mice, allowing the tumors to grow more rapidly.

A brief report describing a 2-year carcinogenicity study was published by Zilfian et al [51] in 1975. A total of 290 mice were exposed to chloroprene, 9,10-dimethyl-1,2-benzanthracene (DMBA), or both by skin painting. Benzene was used as the solvent for all skin-painting studies.

Fifty percent chloroprene (100 mice), 0.1% DMBA (80 mice), or 50% chloroprene plus 0.01% DMBA (number not stated) was painted on shaved shoulder regions of each mouse. Chloroprene or DMBA was applied (the amount/application was not stated) twice weekly for 25 weeks, while the chloroprene-DMBA mixture was applied five times in all. The exposure duration and frequency for chloroprene-DMBA administration were not stated.

After skin painting, 42 of 100 mice painted with chloroprene died within 6 months [51]. In addition, 20 of 80 exposed to DMBA alone died, while 38 died after application of the mixture (total number not stated). Examination of an unspecified number of mice surviving to the end of the experiment found no tumors in animals painted with chloroprene alone or with the mixture of chloroprene and DMBA. However, 92% of the mice painted with 0.1% DMBA developed skin tumors. No data on the overall frequency of tumors or the causes of death in the mice expiring before the end of the study were given. It is difficult to assess the significance of the consequences of painting the skin with chloroprene when the exact dose for each animal is unknown.

In a second section of the study [51], 390 rats were given subcutaneous injections of chloroprene or DMBA in sunflower oil. Four groups of rats were used: the first group (110 rats) was given 10 injections of chloroprene at 400 mg/kg, the second group (number not specified) received 50 injections of chloroprene at 200 mg/kg, the third group (60 rats) received DMBA at a single dose of 0.5 mg, and the fourth group (number unspecified) was given 50 injections of chloroprene at 200 mg/kg plus a single dose of 0.5 mg of DMBA. The timing and frequency of the injections were not given. The rats were observed for a 2-year period.

Sarcomas of the subcutaneous cellular tissue developed in rats exposed to DMBA, a known carcinogen. With DMBA alone, the first tumor appeared after 3.5 months. Of 60 rats, 50 survived the injections, but only 32 (64%) developed sarcomas. With the mixture of chloroprene and DMBA, the first tumor appeared at 4 months. Surviving the 6-month injection period were 42 rats (the total number exposed was not mentioned), 24 (57%) of which developed sarcomas. No malignant tumors were observed in any of the chloroprene-treated rats over the entire 2-year observation period. Deaths were noted after 6 months (ie, during the period of injections). Twenty percent of the rats died at the 400 mg/kg chloroprene dosage, and 46 rats (unknown percentage) died at the 200 mg/kg dosage. The authors concluded that chloroprene was not a carcinogen. When chloroprene was given in combination with DMBA, the formation and growth of tumors were decreased and the tumor growth appeared to be somewhat delayed. Therefore, the authors concluded that chloroprene may suppress the growth of DMBA-induced tumor cells.

(b) Mutagenicity

In 1975, Bartsch et al [52] described a mutagenic study of chloroprene in the Salmonella typhimurium strain TA-100 histidine auxotroph. Bacteria were exposed to chloroprene at 0.5, 2, or 8% concentrations in air (v/v) for 4 hours at 37 C in the presence of an NADPH-generating system and a 9,000 G liver supernatant from a homogenate of liver from male mice. The chloroprene, manufactured from acetylene, was 98.94% pure. Contaminants were alpha-chloroprene (0.98%), butadiene (370 ppm), vinyl acetylene (280 ppm), and tertiary-butyl-catechol (250 ppm added as a stabilizer). The number of plates incubated was not given.

Chloroprene in air caused back mutations as a linear function of the chloroprene concentration to which the bacteria had been exposed. At 8% chloroprene, mutation rates were approximately three times the spontaneous rate. An additional two- or threefold increase in mutagenic response was observed when postmitochondria supernatant from phenobarbital-treated or control male mice was added. The results are summarized in Table III-3. Bartsch et al concluded that chloroprene was mutagenic with or without microsomal activation.

In 1976, Bartsch et al [15] published an abstract of data presented at the 67th annual meeting of The American Association for Cancer Research. The authors investigated the mutagenicity of several airborne compounds using the Salmonella typhimurium TA-100 tester strain in the presence or absence of a microsomal enzyme activation system. Mutation rates were calculated from linear regions of dose and time-dependent plots and were expressed as revertant colonies/μmol/hour/plate. The plots were not presented; as a consequence dose ranges could not be ascertained.

Vinyl chloride exposure resulted in 6 reversions/μmol/hour/plate (2 without mouse microsomal activation), 2-chloroprene exposure resulted in 51 reversions (9 without activation), 1-chloroprene exposure resulted in 157 reversions (81 without activation), and 3,4-dichlorobutene exposure resulted in 490 reversions (345 without activation). The authors [15] also stated that human liver fractions activated vinyl chloride and 2-chloroprene to compounds with mutagenicities comparable to those induced by activation by the mouse liver microsomal system.

TABLE III-3

SUMMARY OF MUTAGENIC TESTS WITH CHLOROPRENE VAPOR

Air Exposure (% v/v)	Microsomal Activation	Salmonella typhimurium Strain				Reference
		TA-1535*	TA-1537*	TA-1538*	TA-100**	
8	None	-	-	-	117 ± 6	52
8	Liver	-	-	-	306 ± 25	52
2	None	-	-	-	70 ± 4	52
2	Liver	-	-	-	154 ± 15	52
1.26	None	2.25	8.56	10.20	-	***
0.63	"	3.15	8.65	12.69	-	***
0.63	Lung	3.92	2.09	8.45	-	***
0.63	Liver	1.85	3.83	10.69	-	***
0.5	None	-	-	-	49 ± 3	52
0.5	Liver	-	-	-	101 ± 4	52
0.30	None	2.56	14.09	5.54	-	***
0.30	Lung	2.22	1.91	9.84	-	***
0.30	Liver	7.70	2.02	8.77	-	***
0	None	2.34	11.84	8.48	-	***
0	"	1.36	9.68	8.57	-	***
0	"	-	-	-	35 ± 2	52
0	Liver	-	-	-	35 ± 3	52

*Revertants/100 million survivors, unspecified exposure duration
**Revertants/plate, 4-hr exposures
***Adapted from RS Barrows (personal communication, August 1976)

Bartsch et al [15] reported that 2-chloroprene was activated to an alkylating agent as measured by trapping with 4-nitro-(4-benzyl-pyridine). No details were provided on this aspect of the study. Although the authors stated that this work demonstrated the conversion of 2-chloroprene to potentially carcinogenic metabolites, extrapolation from mutagenicity to potential carcinogenicity may or may not be warranted.

Recently, RS Barrows (written communication, August 1976) transmitted to NIOSH results of Litton Bionetics' testing of the mutagenicity of chloroprene in Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 using the Ames procedure [53,54]. These mutagenicity tests were carried out in 1974 and 1975. Mutagenicity was measured with and without microsomal activation, apparently by using single plates, ie, no replication of data. Known mutagens including dimethylnitrosoamine, 2-acetylaminofluorene, 7,12-dimethylbenzanthracene, ethyl methanesulfonate, 2-nitrofluorene, and quinacrine mustard were also tested. Saline was used as the control. Cells were exposed to liquid chloroprene at 0.1, 1, 10, or 100 μl/plate and to chloroprene vapor at 0.30, 0.63, or 1.26% (v/v). In the first series of tests, microsomally activated and nonactivated plates showed no reversions above the spontaneous levels with atmospheric chloroprene exposure in TA-1535, TA-1537, or TA-1538. In activated and nonactivated suspension tests, Barrows (written communication, August 1976) reported that no mutagenic response was observed. The data can also be interpreted as showing a weak mutagenic response in only strain TA-1535. The results are presented in Table III-3.

When activated and nonactivated plate tests were repeated in 1975, using liquid chloroprene and strains TA-98 and TA-100 in addition to TA-

1535, TA-1537, and TA-1538, moderately positive results were obtained in strain TA-1535. In contrast to the results of Bartsch et al [15,52], mutagenicity was not observed in TA-100 (RS Barrows, written communication, August 1976). The results are presented in Table III-4. Bartsch et al [52]

TABLE III-4

SUMMARY OF MUTAGENIC TESTS WITH LIQUID CHLOROPRENE

Exposure Concentration (μl)	Microsomal Activation	Salmonella typhimurium Strain*				
		TA-1535	TA-1537	TA-1538	TA-98	TA-100
100	None	11	12	22	19	14
100	Liver	115	12	23	44	102
100	"	175	-	-	-	-
10	None	14	11	23	21	45
10	Liver	53	15	25	24	105
10	"	21	-	-	-	-
1	None	13	9	20	22	24
1	Liver	23	12	19	18	102
1	"	23	-	-	-	-
0	None	10	11	23	21	42
0	Liver	34	15	16	30	85
0	"	18	-	-	-	-

*Revertants/plate, unspecified exposure duration

Adapted from RS Barrows (personal communication, August 1976)

used only TA-100, but reported a response that increased linearly with increasing concentrations of chloroprene, whereas the study by Barrows (written communication, August 1976) found variable responses to increasing concentrations of chloroprene.

Using the Berlin K strain of Drosophila melanogaster, a strain that has been found to be especially susceptible to mutagenic activity by ethyl methanesulfonate and 1-(2,4,6-trichlorophenyl)-3, 3-dimethyltriazine, E Vogel (written communication, July 1976) found that chloroprene fed to adult males for 3 days at a concentration of either 5.7 or 11.4 millimolar resulted in increases in the percent of X-linked recessive lethal mutations from 0.18% ± 0.04 to 0.58% ± 0.3 or 1.0% ± 0.4, respectively. The last value is clearly significant evidence of potential mutagenic activity in other species. It is noteworthy that the recessive lethal mutations produced in Drosophila include the small deletions that are the most important type of genetic damage indicating a potential hazard to man.

A brief study of chromosomal aberrations in bone marrow cells from male rats of unspecified strain exposed over a period of 4 months to chloroprene vapor and other substances present in latex was presented by Bagramian and Babaian [42]. Six rats were used in each exposure group and six were kept as controls. The frequency of chromosomal aberrations was determined using histochemical methods (no method specified) and was reported to be 5.5% in the controls. Inhalation of chloroprene at 0.54 ± 0.29 ppm in conjunction with dodecyl mercaptan at 5.02 ± 1.96 mg/cu m and ammonia at 19.8 mg/cu m for 1 day produced 8.8% chromosomal aberrations in rat bone marrow cells. When methylmethacrylate at 4.0 ± 0.25 mg/cu m was combined with chloroprene at 2.8 ± 2.0 mg/cu m, 10.7% of the chromosomes

were altered after 1 day. Controls exposed to only dodecyl mercaptan, chloroprene, or methyl methacrylate were not run in this study. The authors concluded that the mixture tested caused a mutagenic effect in rats.

In 1976, Volkova et al [41] presented a brief study of metaphase configurations in bone marrow cells of mice exposed to chloroprene. Separate groups of 6-10 mice were exposed for 2 months to airborne chloroprene at each of the following six concentrations: 0.054, 0.064, 0.13, 0.32, 1.85, and 3.5 mg/cu m. Two groups of six and eight unexposed mice served as controls. Chromosomal disorders of an undefined nature were measured and appeared with increasing frequency as the chloroprene concentration was increased (units are percent of occurrence): 3.05 ± 0.46 and 2.0 ± 0.58 (in the two control groups), 3.4 ± 0.7, 2.8 ± 0.33, 4.65 ± 0.89 ($P<0.05$), 6.07 ± 0.4 ($P<0.001$), 10.9 ± 1.34 ($P< 0.001$), and 10 ± 0.68 ($P< 0.001$). Volkova and coworkers stated that this was an increased frequency of cell aberration. The types of marrow cells examined were not indicated, making evaluation of the data difficult. The authors also stated that chloroprene induced what were called dominant lethal mutations in mouse reproductive cells at the two highest air concentrations, 1.85 and 3.5 mg/cu m.

Davtian et al [55] measured mutagenicity in bone marrow cells of 36 male rats exposed to airborne chloroprene at 1.1 and 11 ppm (3.8 and 39 mg/cu m) using a cytogenetic anaphase-telophase analysis. The authors stated that the number of chromosomal aberrations was increased above the control level at both 1.1 and 11 ppm; no data were given to support this conclusion.

(c) Teratogenicity and Effects on Reproduction

In 1936, Von Oettingen et al [18] investigated the action of chloroprene on male reproductive processes in the rat. A total of 18 male rats of unknown strain were exposed to chloroprene in air for 8 hours at concentrations of 121-6,227 ppm. Five control males were unexposed. After 0-30 days of isolation, the males were mated with normal female rats, and the frequency of successful matings was determined. Only 6 of 19 matings produced pregnancies, with an average of 6.8 offspring/liter. Unproductive matings occurred at all exposure concentrations of chloroprene. The five control rats mated successfully, producing an average of 6.4 offspring/liter.

The chloroprene inhalation experiment was repeated with a total of 14 male mice exposed to chloroprene at air concentrations of 12, 75, 115, 119, 121, 150, or 152 ppm for 8 hours [18]. The exposed males were mated with females 1 to 9 days after exposure. Only 6 of the 14 matings produced pregnancies, with an average of 6 offspring/litter. Five of six control mice reproduced normally, producing seven offspring/litter.

Five female mice were also exposed for 8 hours to chloroprene at air concentrations of 151 ppm [18]. All these animals reproduced normally when mated 1 day after the exposure, producing an average of 4.8 offspring/litter. No degenerative changes in the female sexual organs were observed microscopically.

The authors [18] concluded that chloroprene interfered with reproductive processes in male rats and mice; testicular atrophy was demonstrated in the rat. On the other hand, female mice exposed at similar concentrations reproduced normally when mated.

There have been several recent publications on the effect of chloroprene on the reproductive capacities of male and female rats. Davtian et al [55] exposed a total of 36 male rats to airborne chloroprene for 4 hours/day over a 48-day period at concentrations of approximately 1.1 or 11 ppm. The animals were subsequently observed for overall toxic effects and for specific effects on reproductive function. Weight gain, oxygen requirement, detoxification by the liver (sodium benzoate loading followed by measurement of hippuric acid excretion), total serum albumin and sulfhydryl content, diuresis, total urinary albumin and chloride, and the relative weights of the internal organs were measured. Male reproductive function was determined by measurement of fertilizing ability, spermatozoic motility, testicular atrophy, and preimplantation and postimplantation mortality. Each male was mated with two females (strain unspecified). The males were killed after mating; some females were killed on day 21 and the fetuses were examined. The development of the offspring of some females was also followed after birth.

The only reported indices of toxicity to the males were increased concentrations of chloride in the urine and decreased concentrations of what were called stable intermediate products [55]. The nature of this latter index was not given. It was, however, observed to decrease significantly ($P<0.1$), from 5.8 to approximately 4.5 units (units not defined) at both 1.1 and 11 ppm. Urinary chlorides increased only at 11 ppm from 1.14 ± 0.21 to 1.74 ± 0.16 mg/ml ($P<0.05$). No other significant changes were observed in the other parameters measured, except that both exposure concentrations were said to have increased the number of chromosomal aberrations in the cells of the bone marrow; no data to support

this statement were included.

Davtian et al [55] observed no effects of inhaled chloroprene on spermatozoal motility or fertilizing ability. The effect on postimplantation embryonic death increased at chloroprene concentrations of 1.1 and 11 ppm, from 2.2 ± 1.1 in the offspring of controls to 4.7 ± 1.5 in offspring of animals exposed to the lower concentration, and to 8.4 ± 3.4 in offspring of animals exposed to the higher concentration (no units specified), but these changes were not significant ($P>0.05$) in any case. Preimplantation losses were increased significantly ($P<0.02$) when compared with those of the control dams, at both exposures to chloroprene. Total embryonic mortality was demonstrated to increase significantly ($P<0.05$), from 12.9 ± 2.7 in the offspring of control rats to 30.7 ± 5.7 and 32.0 ± 7.4 in those of rats exposed at 1.1 and 11 ppm, respectively.

Davtian et al [55] suggested that sex and somatic cells had identical sensitivities to chloroprene and concluded that embryonic death was apparently linked to the mutagenic activity of chloroprene. Although the two exposure concentrations of chloroprene apparently had no graded effects on either preimplantation or total embryonic mortality, they seemed to have graded effects on postimplantation mortality. The authors noted the need to study the effects of still lower concentrations of chloroprene.

Davtian [56] described the effects of chloroprene on the reproductive function of male rats. A total of 100 rats of unspecified strain was exposed to chloroprene at concentrations of 0.47, 0.042, or 0.014 ppm by inhalation. The animals were exposed 4 hours/day for periods up to 5.5 months. Unspecified numbers of animals were killed after 1.5, 2.5, 3.5, and 4.5 months of exposure, and spermatozoa were examined microscopically

with respect to duration of motility, vitality (percentage of live sperm), and resistance to hypertonic and acid solutions. Testicular weight coefficients (percentage of total body weight) were also determined. Liver function was determined after 5.5 months of exposure by sodium benzoate loading experiments; results were reported as milligrams of hippuric acid excreted. Oxygen uptake was also measured. No details were given on how these indices were determined. After 2.5 months of exposure, eight males at each exposure concentration were mated with virgin female rats of unspecified strain to determine fertilization efficiency. The females were killed after the 20th day, and the preimplantation and postimplantation losses, overall embryonic mortality, and fetal size were determined.

Oxygen uptake decreased significantly, as did hippuric acid excretion, after chloroprene exposure at the concentration of 0.47 ppm for 5.5 months [56]. Neither the number of rats nor the collection interval was reported. Atrophy of the testicles and decreased vitality, motility, and acid resistance of spermatozoa were found in some of the males exposed to chloroprene at 0.47 ppm; similar changes also occurred in some male rats exposed at 0.042 ppm. No effects were reported at 0.014 ppm; however, no data were presented. Davtian [56] found that untreated females mated to males exposed at 0.042 ppm showed significant increases in total embryonic mortality and preimplantation deaths.

Davtian [56] concluded from this study that the threshold for general toxicity (oxygen consumption and liver function, both poorly defined) was approximately 0.56 ppm, but that the thresholds for interferences with reproductive function were one order of magnitude lower. He also stated that the increases in embryonic death were the result of increased

preimplantation deaths; however, preimplantation death may not be a good indication of genetic damage [57,58]. Postimplantation losses were not reported.

Volkova et al [41] described an inhalation study in which chloroprene-induced reproductive effects were examined. Randomly bred male rats and C57BL/6 mice were exposed to chloroprene in air at concentrations of 0.14-0.47 ppm for 4.5 months. The numbers of rats and mice exposed at each concentration were not given. The number of hours of exposure each day was not given. The authors stated that the highest concentration of chloroprene caused a decrease in rat spermatozoal motility and acid resistance, atrophy of the testicles in five of eight rats, and an increase in the number of dead spermatozoa. Tabular data were presented for "animals"; the tabular material did not distinguish between rats and mice, but the discussion implied that clearly it was limited to rats. Without more specific information, the significance of this study is difficult to assess.

In 1968, Salnikova [59] first discussed the combined embryotoxic effects of volatile chloroprene and ammonia, derived from polychloroprene latex, on pregnant rats and mice of randomly bred strains. Neither the method of generating the vapors nor the identities of volatile dimers and contaminants were described, but the concentrations of chloroprene and ammonia were 4 ppm (14.4 mg/cu m) and 4.8 ± 0.3 mg/cu m, respectively. The analytical methods were not described. Thirteen mice and 11 rats were exposed to chloroprene and ammonia for 4 hours/day for the first 18 or 19 days of pregnancy, respectively. Two control groups were used in the study, one exposed to ammonia alone at 58 ± 6 mg/cu m (10 mice and 7 rats)

and the other exposed to air alone (11 mice and 9 rats). The following measurements were made on day 17 of treatment: body weight, hemoglobin content, and red and white blood cell counts. No actual values were given, but the author reported that there were no significant changes from normal physiologic limits.

On the last day of exposure, day 18 or 19, the females were killed and autopsied [59]. The following variables were measured: liver and kidney weights, urinary albumin and chloride concentrations, numbers of corpora lutea, sites of implantation, postimplantation deaths, and living fetuses, and the weights of the fetuses. Preimplantation deaths were defined as the difference between the number of corpora lutea and the number of implantation sites.

The only physiologic changes observed that could be attributed to chloroprene alone were slight, but significant ($P=0.01$), increases of liver and kidney weights (no units were given) of the female mice compared with those of the controls, 6.18 ± 0.03 versus 5.64 ± 0.18 and 1.65 ± 0.06 versus 1.16 ± 0.03, respectively [59]. The kidney weights of female rats were also increased, 0.65 ± 0.02 versus 0.57 ± 0.02 ($P=0.05$). In the mice exposed to both chloroprene and ammonia, the average number of postimplantation deaths was significantly ($P<0.001$) increased, 8.1 ± 1.1 versus 1.47 ± 0.43 for the air controls and 1.9 ± 0.79 for the ammonia-inhalation controls. There was a complete loss of all litters in the pregnant mice exposed to vapors derived from latex. Female rats exposed to both chloroprene and ammonia vapors under the same conditions showed no significant ($P>0.05$) change in the number of postimplantation embryonic deaths when compared with rats exposed to ammonia and with rats exposed

only to air. After exposure of the dams to latex fumes, the number of rat fetuses with hematomas or cyanoses was elevated, 2.50 ± 1.04 versus 0.40 ± 0.29; however, this was not significant (P between 0.05 and 0.1). The mean number of normal rat fetuses/litter was 52% below that of the controls ($P<0.01$, 4.70 ± 1.24 versus 9.80 ± 1.76). No criteria for distinguishing abnormal fetuses from normal ones were presented.

Salnikova [59] concluded that the polychloroprene latex studied liberated volatile substances that possessed considerable embryotoxic action. The effect was not attributed to ammonia, since a tenfold higher exposure to ammonia alone did not have comparable embryotoxicity. However, the possibility of ammonia and chloroprene acting together to cause embryotoxicity cannot be entirely ruled out, as a chloroprene control was not included in the study. The amounts of oxidized chloroprene and other contaminants released from the latex were also not determined, making the assignment of toxic activity to chloroprene somewhat difficult. The composition of the latex and the method for generation of fume from it were not described. Complete reliance on the data presented is impossible without this information.

In 1973, Salnikova and Fomenko [60] published the results of an investigation of chloroprene's influence on embryogenesis in pregnant rats. In these studies, 205 rats in groups of 22 to 30 were exposed to chloroprene, via inhalation, at 1 of 5 concentrations for 4 hours/day during the entire period of pregnancy, and the results were compared with those from control groups. The concentrations were 1.11, 0.83, 0.17, 0.036, and 0.016 ppm of chloroprene. The purity of the chloroprene was not indicated. The experimental protocol was not outlined.

The embryotoxicity experiments were done at three different times; consequently, three sets of control animals were examined [60]. Variables considered for embryos and fetuses were total mortality (no breakdown into preimplantation and postimplantation embryonic losses), liver weight, femoral and fibular diaphysis lengths, and disturbances in vascular permeability. Variables considered in the study of 2-month-old weanlings included urinary proteins, cholinesterase (no tissue specified), oxygen requirement, serum sulfhydryl content (no method described), urinary hippuric acid after benzoate loading, weight gain, and weight ratios of brain, lung, liver, and kidney. No data were supplied on organ weights. The additional gain in liver weight after further hepatotoxic stress with alcohol was measured, but the results were not indicated.

In dams exposed at 0.83 and 1.11 ppm, total embryonic mortality was significantly increased by 273% ($P<0.01$) and by 193% ($P<0.05$), respectively. Exposure of dams to chloroprene at 0.17, 0.036, or 0.016 ppm led to nonsignificant increases in embryonic and fetal mortalities of 76, 71, and 14%, respectively, over those of the controls. The weight of fetuses was stated to be significantly ($P<0.001$) below that of those from controls, 1.8 ± 0.18 versus 2.3 ± 0.2 g, when dams were exposed at 1 ppm. Disturbances in the vascular permeability and decreases in the lengths of long bones (femur and fibula) also occurred at around 1 ppm (no data were presented).

The mortalities during the 3 weeks after birth of progeny from dams exposed at 0.17 and 0.036 ppm were increased more markedly than embryonic mortality, 34.1% ± 12.0 versus 2.2% ± 1.5 ($P=0.05$) and 26.0% ± 3.4 versus 11.2% ± 4.2 ($P<0.02$), respectively [60]. The documenting of physiologic

changes observed during the study of first-generation progeny was incomplete and variable. Some effects seen with low-exposure concentrations were not observed at higher concentrations, so no dose-response relation was derived. Because of the varying statistical results and incomplete details of the study, interpretation of the physiologic effects is not possible. In addition, the lack of information on the purity of the chloroprene affects the overall value of this study.

A rather novel approach to studies of the effects of industrially produced chloroprene vapor on pregnant rats was described by Apoian [61]. Four groups of pregnant rats were housed in, or at various distances from, the Kirov chloroprene complex. The lengths of exposures and numbers of rats were not indicated for the teratogenicity portion of the study. Chloroprene concentrations (detection method not specified) were determined to be as high as 61 ppm within the plant (average not given) and means of 0.2 (range 0.056-0.44), 0.14 (range 0.039-0.52), and 0.05 (range 0.038-0.11) ppm were observed at distances of 500, 1,500, and 7,000 meters from the plant, respectively. Rats housed at these distances were identified as groups 1-4, respectively. Group 4 was used as a control. Data were reported on 15 rats (highest number only listed in the tables) exposed in the plant (group 1), 23 exposed at 500 meters (group 2), 9 at 1,500 meters (group 3), and 14 controls at 7,000 meters (group 4) for 20 days. The author stated that increased fetal mortality was noted particularly in the preimplantation period (no specific chloroprene concentration or location was mentioned), and that there were reductions in placental weight. For rats housed at the highest exposure concentration (group 1), the weights of livers of 20-day fetuses were lower, and the period of pregnancy in dams

was lengthened, when compared with those of groups 2-4. This elongation led to an increased number of prenatal (23.2%) and neonatal (38.2%) deaths.

Apoian [61] measured placental weight in all four groups and fetal liver weight in groups 1 and 3. He also measured vitamin C content in the brain, liver, adrenals, and placenta of all the dams and in the brain and liver of 20-day-old fetuses. A significant (no P value) decrease in placental weights in all exposed groups was reported when compared with those of control dams housed at 7,000 meters, but the decreases were not dose dependent: group 1, 625.6 ± 22.3 mg; group 2, 563.8 ± 12.9 mg; group 3, 521.5 ± 14.1 mg; and control group 4, 690.8 ± 13.3 mg. Decreases described as reliable were also observed in embryonic liver weights of groups 1 and 3: 251.3 ± 7.9 mg and 231.6 ± 9.4 mg, respectively, versus 273.8 ± 7.8 mg in control group 4; the response again was not dose dependent. There was no significant change in the concentration of vitamin C in any tissue of either the dams or the fetuses in which it was measured.

The author [61] also examined the effect of chloroprene on DNA and RNA concentrations in tissues of pregnant rats handled in the manner described in the preceding paragraphs. In the Kirov plant, chloroprene daily mean air concentrations ranged from 4.1 to 14.8 ppm. When RNA and DNA concentrations were determined in the brain, liver, and placenta of dams and in the brain and liver of 20-day-old fetuses, the only significant change in group 1 was a decrease in the mean concentration of RNA in the liver of the fetuses [61]. In group 2, the mean concentrations of RNA in the placenta and liver of the dams and of DNA in the brain and liver of the fetuses were decreased with at least 95% reliability. In group 3, the only change stated to be reliable at the 95% level was a decrease in the

concentration of RNA in the liver of the dams. Apoian explained the predominance of significant alterations in the nucleic acid concentrations in various organs of the dams and fetuses of group 2 by supposing that comparatively large concentrations of chloroprene have general toxic effects that are more apparent than the biochemical ones, and that an intermediate concentration has more apparent biochemical effects because it has less general toxic effect. He asserts that Gofmekler, Pushkina, and Klevtsova have reported a similar situation in pregnant rats exposed to formaldehyde vapor. With the dearth of experimental detail and data and the possibility of mixed exposure, interpretation of this paper is not possible.

In 1971, Mnatsakanian et al [62] published a brief, preliminary report of a study similar to that of Apoian [61] and in the following year published a more detailed paper [63] on this research. In the preliminary report [62], four groups of pregnant rats were caged in apparently the same locations used by Apoian [61]. The animals in these two studies [61,62] may have been the same. In the later paper of Mnatsakanian et al [63], the results of caging at locations 1 and 2 were compared with those of caging at location 4. The rats caged at location 4 were used as control animals; the group caged at location 3 was not discussed. The three groups of pregnant white rats were exposed to chloroprene at three concentration ranges: 4.1-14.8 ppm in the plant, 0.056-0.44 ppm at 500 meters away, and 0.033-0.11 ppm at 7,000 meters away from the plant (considered as controls). The total number of females exposed at each concentration was not stated. Prenatal deaths were stated to occur in 20.93% of the embryos in 9 females of the first group (4.1-14.8 ppm), in 6.38% in 5 females of

the second group (0.056-0.44 ppm), and in 10.88% in 15 females in the control group (0.033-0.11 ppm). Prenatal deaths were determined by comparing the number of points of uterine-placental attachment to the number of fetuses within a few days after giving birth.

Neonatal deaths were also considered [63]. At the highest concentration of chloroprene in air, 38.2% of the offspring from nine rats were stillborn or died immediately after birth. No deaths were observed at the lower exposure concentration, and 2.3% of the offspring from 30 females studied in the control group died. The only postnatal deaths (two) were observed in the group exposed at 0.056-0.44 ppm. A study of weight gain during the first 6 months of offspring growth (the number was unstated) showed some deviation from the controls at various times but no general trends.

Mnatsakanian et al [63] concluded that, on exposure to vapors freed during production of polychloroprene, the course of pregnancy in the rat was disrupted, labor was prolonged, and neonatal deaths were increased. They stated that the embryotoxic effect of chloroprene was characterized by early embryonic death in both exposed groups; however, the data to demonstrate this were not presented. No distinction was made between early and late embryonic deaths (preimplant and postimplant), and the methodology described would not have allowed such a distinction. In addition, the group exposed to chloroprene at 0.056-0.44 ppm had a smaller proportion of prenatal deaths than the control group, 6.4% versus 10.9%. It also had a smaller proportion of unviable offspring than the control group, 0 versus 2.3%.

In 1975, Salnikova and Fomenko [64] studied the embryotoxic and teratogenic effect of chloroprene administered orally and by inhalation. Each group of pregnant white rats (no particular strain specified) contained 8-15 rats. Six groups were given daily oral doses of 0.5 mg/kg for 2-day periods through the 14th day of pregnancy, ie, days 3 and 4, 5 and 6, etc. One group was given the same dose every day for the entire 14-day period, and a control group was left unexposed. Paralleling the oral regimen, eight other groups of pregnant rats were exposed to airborne chloroprene at 1.1 ppm for staggered 2-day periods to the 18th day of pregnancy, and one group was exposed on days 1 through 20. The number of hours of exposure each day was not stated. The fetuses of all rats were examined on the 20th day of pregnancy. This procedure included examination for teratogenic effects as well as quantification of total embryonic and fetal toxicity. Preimplantation and postimplantation embryonic deaths were determined separately but reported only as total embryonic deaths.

Embryonic deaths in rats receiving oral chloroprene doses were significantly ($P<0.001$) increased for rats exposed for 14 days, 9.4 (about 92%) versus 0.4 (about 5%) for the controls [64]. The authors stated that deaths were primarily preimplantation. The total number of embryonic and fetal deaths was elevated in those rats given chloroprene on days 3 and 4 (7.7%) and on days 11 and 12 (5%). All fetuses from rats given chloroprene orally for 14 days showed hydrocephalus and internal bleeding.

Total deaths of concepta in dams inhaling 1.1 ppm of airborne chloroprene were approximately 20% for those exposed on days 1 and 2, 3 and 4, 9 and 10, 11 and 12, or 1 through 20, versus 8% for the controls [64]. Dams exposed at periods other than those listed above had lower embryonal

mortalities, that for dams exposed on days 7 and 8 actually being below that for unexposed dams. No teratogenic effects were observed in offspring of controls or of those animals exposed by inhalation throughout pregnancy. Internal bleeding was found in 70% of the fetuses of the dams exposed throughout pregnancy versus 16% in those of the controls. Internal bleeding, hydrocephalus, and cerebral herniations were also observed in fetuses from dams exposed for the 2-day periods after day 5, the frequencies being 34-47%, 6-34%, and 1.6-23.5%, respectively. The only effect classified by the authors as teratogenic was hydrocephalus with cerebral herniation. The largest number of cerebral hernias was seen in fetuses from dams exposed to chloroprene on days 5 and 6 of pregnancy.

In 1976, Melik-Alaverdian et al [65] presented the results of a three-generation study of reproductive function and sexual maturation in female rats. Ninety female rats (150-180 g, no strain identification given) were exposed at concentrations of airborne chloroprene of 8.34 ppm for 5 hours each day, 6 days each week, during 6 months. Thirty-six females were not exposed to chloroprene and served as controls. At the end of the exposure period, the rats were mated with nonexposed males. The percentage of females giving birth to progeny, number of progeny/litter, intrauterine development of fetuses, and fetal weights were all determined.

The first generation of exposed animals had the same percentage of fertility (62.2 versus 63.8%) and average fetal weight (4.76 ± 0.09 versus 4.61 ± 0.05 g) as the control group [65]. Intrauterine development was normal; no stillbirths or deformities were observed in either group. The average number of fetuses/litter was decreased in the exposed dams to 3, versus 5.2 in the control group. The authors also stated that the estrus

cycle was altered in the 3rd month of exposure at 30 mg/cu m. The length of the heat period was significantly ($P<0.05$) increased in the exposed rats, 1.3 versus 1.1 days in the control group. A significant ($P<0.001$) decrease in the length of anestrus was also observed, 3.4 days in exposed rats versus 5.1 in the controls.

Female offspring were chosen from the second generation, 60 from exposed dams and 65 from control dams [65]. These females were mated with nonexposed males. None of the second-generation animals were exposed to chloroprene. Fertility in the female rats derived from exposed dams was decreased, 56.6% versus 66% in the second-generation control animals. Intrauterine development and pregnancy duration were normal in both groups, and average fetal weight was unchanged, 4.69 ± 0.05 in experimental progeny versus 4.50 ± 0.06 in control progeny.

In the 3rd month of a 6-month observation period, experimental dams showed significant ($P<0.001$) decreases in the duration of anestrus, 3.5 versus 5.1 days, and in the number of estrus cycles, 6.13 versus 7.9 in the control group [65].

Sixty-one female offspring of the third generation and 65 females of the same generation of controls were chosen and mated with unexposed males [65]. Nearly all the reproductive indices were normal. Fertility was unchanged, 60.6 versus 63% in controls, but the average litter size was decreased, 3.3 versus 4.1. No stillbirths or deformities were observed, but fetal weights were decreased significantly: 4.48 ± 0.07 versus 5.03 ± 0.08 in the control progeny. The duration of estrus was significantly ($P<0.05$) increased, 4.56 days in the experimental group versus 3.5 days in the controls.

The authors [65] concluded that 8.34 ppm of chloroprene caused decreased litter size in the first and third generations and a decrease in the frequency of conception in the second generation. No substantial changes were observed in estrus or other indices of development.

Culik et al [66] described the exposure of pregnant Charles River-CD rats to freshly distilled chloroprene at nominal concentrations of 25, 10, and 1 ppm in inhalation chambers for 4 hours/day. In the embryotoxic study, 50 pregnant rats at each concentration and 50 control rats were observed after exposure from day 1 through day 12 of pregnancy; the rats were killed on day 17. For determination of teratogenicity, 25 rats were exposed at each concentration (along with 25 control rats) from day 3 through day 20 before being killed on day 21. Resorptions and preimplantation and postimplantation losses were measured in addition to the examination of surviving fetuses for viability and teratogenic effects. Chloroprene concentrations in the chambers were analyzed every 30 minutes by gas chromatography using methods similar to those described in Appendix II. The nominal concentrations of 1, 10, and 25 ppm were determined to average 0.8, 8.6, and 22.7 ppm, respectively. The chloroprene was freshly distilled from antioxidant-stabilized solutions and was protected from exposure to air until injection into the airstream of the chamber.

Culik et al [66] stated that there were no embryotoxic or teratogenic effects at any of the concentrations of chloroprene tested. They also stated that chloroprene did not affect the body weight or gravid uterus weights of the dams. No gross abnormal changes were noted in the uterine horns, ovaries, or other organ systems at any of the test concentrations. The only effect seen in the teratogenic study was a tendency toward

increased size and weight of the fetuses from dams exposed at a nominal concentration of 25 ppm; however, the increase was not significant and the number of fetuses was not decreased. Although 21.1% of the litters of the dams exposed at 25 ppm had bipartite thoracic centra, compared with 9.5% of those of the control dams, the authors stated that this difference was not significant by Fisher's exact probability test. Pregnancy outcome, as measured by preimplantation and postimplantation losses of fertilized ova and number of live fetuses in each litter, was not significantly different from that of the controls. Median preimplantation loss in control dams was 20%; at 25 ppm, this loss was 16%. Fifty-one percent of the control litters showed early resorptions, compared with fifty percent of those exposed at 25 ppm. In terms of absolute preimplantation losses, there were 168/653 (25.7%) in controls and 144/637 (22.6%) in dams exposed at 25 ppm. Control dams had an absolute number of 39/485 (8.0%) early resorptions versus 34/493 (6.9%) in animals exposed at 25 ppm. Total postimplantation fetal loss was 39/485 (8%) in control litters and 34/493 (6.9%) in litters from dams exposed at 25 ppm. No changes were observed at 1 or 10 ppm.

A second portion of the study [66] addressed the effects of pure chloroprene on fertility of male rats. Five male Charles River-CD rats (150-200 g) were exposed for 4 hours daily for 22 consecutive days to chloroprene vapor at 25 ppm. Five males were kept as controls. On day 23, eight sequential mating trials were initiated with five test and five control rats. In each trial, a male was housed with three unexposed virgin females for a total of 7 days. After mating, the females were housed separately and allowed to deliver and raise their pups to weaning. The number of pups in each litter and their average body weight at weaning were

calculated along with the percentage of successful matings, the percentage of pups surviving 4 days or longer after birth, and the percentage of pups surviving through weaning. After the eighth mating trial, the males were killed and their reproductive organs examined microscopically after staining. No clinical signs of toxicity were observed during the test. Gross and microscopic examination showed no changes attributable to chloroprene. There were no significant differences between test and control animals for any of the reproductive variables measured.

This study [66] shares with others [55,56,60,64] one principal shortcoming limiting its applicability to occupational exposures to chloroprene: daily 4-hour exposures for 7 days/week are not representative of actual industrial situations. Further, many of the findings are stated as means or medians without any indication of the variability of the quantity measured within the various groups of rats. Although the authors concluded that no embryotoxic or teratogenic effects were seen at the exposure concentrations used, NIOSH believes that the data may justify a conclusion that the highest concentration to which the pregnant females were exposed may have increased significantly the incidence of abnormal centra in the thoracic vertabrae of the pups, which could be considered a teratogenic action. This abnormality appeared in the fetuses of the various groups with the following frequencies: control, 2/126; 0.8 ppm, 3/122; 8.6 ppm, 2/112; and 22.7 ppm 8/122. None of the exposed groups, when compared individually with the control group, had a significantly increased incidence of abnormal vertebral centra in the pups. It is apparent, however, that the group of dams exposed to the highest concentration of chloroprene produced pups that had a different incidence

of abnormal centra than did pups of the other three groups of dams. If the control groups and the groups of pregnant females exposed to the two lowest concentrations of chloroprene are lumped together, the incidence of abnormal vertebral centra in the pups of the group of pregnant female rats exposed to the highest concentration of chloroprene is statistically significant. NIOSH believes, therefore, that the highest concentration of chloroprene used by Culik et al may have had a teratogenic effect on the offspring of the female rats exposed to it.

Correlation of Exposure and Effect

No well-documented epidemiologic studies correlating occupational environmental concentrations of chloroprene with observed toxic effects have been found in the literature. The few epidemiologic studies of long-term, low-level occupational exposure have been reviewed, but these give no indication of the chloroprene air concentrations in the occupational environment [28-30].

Occupational exposure to chloroprene occurs chiefly by inhalation and skin contact. Chemical burns resulting from contact with chloroprene have been reported [11,24,67]. Few details are available, but the severity of the burns was dependent on the duration of contact. Dermal application of chloroprene has been found by several investigators [18,20,51] to cause damage to the skin and induce systemic poisoning in rats or mice as well.

There are few reports concerning the toxic effects of chloroprene inhalation on humans where the airborne concentrations were known. Observed signs and symptoms include CNS effects [1,20,46], chest pains [19,20,25,41], loss of scalp hair [23,24,68-70], hypotension [19,20],

conjunctivitis [19,24], extreme fatigue [19,20,41], slow pulse rate [25,41], fast pulse rate [25], and irritability [20]. These reports are summarized in Table III-5. Since nearly all these human effects involve mixed exposure, it is difficult to assign every one of these signs and symptoms to chloroprene alone. From Table III-5, the lowest occupational concentration of chloroprene reported to produce definite symptoms is given as a range of 1.95-0.8 ppm. Although these symptoms are nonspecific, they are in part the same as those produced by much larger concentrations of chloroprene.

Inhalation of chloroprene by animals has been reported to lead to CNS depression [19,20], primary irritation of the respiratory tract [19], and hypotension [19]. The results of the animal studies are summarized in Table III-6.

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

Khachatrian [28,29] reported that working where exposure to chloroprene was likely increased the risk of developing lung and skin cancer. Such information as work history, dietary and hygiene practices, exposure concentrations, smoking habits, and other compounds in the air are lacking in particular instances. The very high probability of mixed exposure at the Kirov Synthetic Rubber Complex renders interpretation of these data difficult. Volek et al [71], using gas-liquid chromotography, detected more than 25 compounds, mostly chlorinated hydrocarbons, in technical grade chloroprene manufactured from acetylene.

The study reported by Pell [30] suggests an excess of lung cancer in maintenance mechanics in a chloroprene manufacturing facility. Since the

task of the maintenance mechanics is to replace leaking pipefittings, to install equipment, and to do general maintenance in reactor areas, this group of workers would be expected to have relatively high exposures to chloroprene. Because the mean age of the lung cancer patients among maintenance mechanics is not compared with that of lung cancer patients among other types of employees at the plant, the lung cancer data for the maintenance mechanics are difficult to interpret.

Zilfian et al [51] reported that chloroprene did not induce tumors in mice when administered alone or in conjunction with dimethyl-1,2-benzanthracene by skin painting or by subcutaneous injection. This demonstrated that chloroprene was neither carcinogenic nor cocarcinogenic in an unspecified number of surviving mice or rats.

Results of mutagenicity testing of chloroprene by Litton Bionetics (RS Barrows, written communication, August 1976) were negative in Saccharomyces cerevisiae and in some Salmonella tester strains, but positive in TA 1535. In two studies [15,52], investigators demonstrated a dose-dependent mutagenic response to chloroprene in TA-100, both with and without metabolic activation. Bartsch [72] has reported that chloroprene is mutagenic in S. typhimurium TA-1530 also. The chloroprene used by Bartsch et al [15,52] was manufactured from acetylene, whereas that used by Litton Bionetics was made from butadiene. Different contaminants or testing methods and procedures may explain the differences between experimental results with different samples of chloroprene.

Further, in regard to mutagenicity, E Vogel (written communication, July 1976) has demonstrated sex-linked recessive lethal mutations in Drosophila. Several investigators [22,41,43] have demonstrated a

significant excess of chromosomal aberrations in blood cells of workers exposed to chloroprene in comparison with those of controls. Although one could speculate that the excess of chromosomal aberrations in chloroprene-exposed workers may be the result of air contamination with other agents, the study by Katosova [43] demonstrated no significant differences in the percentages of chromosomal aberrations in the blood cells of workers exposed to essentially pure chloroprene or chloroprene only and in those of workers exposed to chloroprene mixed with several other starting materials and byproducts. Sanotskii [22] has reported morphologic disturbances in sperm of workers exposed to chloroprene, as well as a threefold excess of miscarriages in the wives of chloroprene workers. No reports clearly attributing mutagenic effects on mammalian cells to chloroprene have been found.

Reports of experimental attempts to induce the formation of birth defects by exposing pregnant female animals to chloroprene have been described [64,66]. Oral administration resulted in teratogenic effects in rats [64]. Inhalation of chloroprene during the full period of pregnancy at nominal air concentrations of 1.0, 1.1, 10, or 25 ppm led to no clearly teratogenic effects [64,66], although there is a possibility that the highest concentration used by Culik et al [66] may have increased the incidence of abnormal vertebral centra in pups of the exposed pregnant rats. Inhalation at 1.1 ppm for 2 days between the 5th and 14th days of pregnancy did lead to greater incidence of hydrocephalus and cerebral herniation in fetuses [64]. The greatest incidence of cerebral herniation was found in the offspring of rats that inhaled chloroprene on days 5 and 6 of pregnancy. The greatest incidence of hydrocephalus occurred in

offspring of dams that inhaled chloroprene on days 11 and 12. Inhalation of chloroprene throughout pregnancy did not produce these effects, leading the authors to suggest that under this condition, the fetus adapts to chloroprene in some way and remains unaffected.

Experimental attempts to induce postimplantation embryonic deaths by chloroprene exposure in pregnant rats were not successful [56,59,64]. Some increases of preimplantation embryonic deaths by exposure of dams at concentrations of chloroprene below 2 ppm (as low as 0.04 ppm) have been reported [55,56,61] and have been contradicted [66] by results showing no effect at higher chloroprene concentrations of 1, 10, or 25 ppm. Many of the papers claiming to demonstrate chloroprene-induced preimplantation deaths lacked controls or reported exposures to other compounds in addition to chloroprene. The implication presented was that preimplantation death is a strong indication of a dominant-lethal genetic change. However, only postimplantation embryonic death is a sound indicator of a dominant-lethal effect [57,58]. Preimplantation deaths are quite variable, even in control populations, due in part to the imprecise basis on which they are calculated. For this reason, apparent changes in the incidence of preimplantation deaths are not reliable indications of mutational activity.

Effects on the male reproductive process in rats and mice, including testicular atrophy and decreased reproductive functions, have been found by Von Oettingen et al [18] to occur between 75 and 6,232 ppm. Davtian [56] observed a significant excess of total embryonic mortality following exposure of male rats to concentrations of airborne chloroprene as low as 0.042 ppm. With exposures to 1 and 11 ppm, Davtian [56] found testicular atrophy but no effect on male reproductive function. At 0.04 and 0.5 ppm,

Volkova et al [41] reported testicular atrophy and decreased spermatozoal motility. In contrast, Culik et al [66] could not demonstrate changes in male reproductive success at 25 ppm and found no histologic changes in the reproductive organs.

Recently, a confirmed case of angiosarcoma of the liver in a worker who had extensive exposure to finished polychloroprene (Neoprene) has been identified (PF Infante, written communication, March 1977). The worker had been employed as a roll builder during the period 1952-1962 when he applied neoprene to metal cylinders, which were then vulcanized. After this procedure, the material often would be cut to the desired size with a metal saw. The worker did not wear a mask, but an attempt to control dust by water sprays was made. Data for atmospheric levels of chloroprene were not available. A history of exposure indicated that this worker had never had occupational exposure to vinyl chloride, nor had he ever received Thorotrast, a diagnostic preparation also associated with the induction of angiosarcoma of the liver. Because of the chemical similarity between vinyl chloride and chloroprene, this observation may be important. On the other hand, this case of angiosarcoma of the liver could be one of the rare spontaneous tumors of this type and location.

In summary, the presently available data appear to be insufficient to formulate firm conclusions on the carcinogenicity of chloroprene. However, chloroprene is mutagenic in Salmonella [15,52]. Likewise, sex-linked recessive lethal mutations have been induced in Drosophila (E Vogel, written communication, July 1976). Infertility has been reported following exposure of male mice and rats to chloroprene [18]. Administration of chloroprene to male rats has also been associated with embryonic mortality

[55,56], testicular atrophy [41], and reduced numbers and motility of live spermatozoa in animals with nonatrophied testicles [41,56]. Although exposure of humans to chloroprene has not produced all the effects summarized above, male workers have had decreased numbers and motility of viable spermatozoa after exposure to chloroprene [22]. A threefold excess of miscarriages in wives of chloroprene workers has been reported [22]. Most investigators have found no apparent teratogenic risk from inhalation of chloroprene by rats and mice, although one study [64] reported hydrocephalus and cerebral herniation, and another [66] reported some increased, but statistically nonsignificant, skeletal abnormalities in offspring of exposed dams. The transplacental effects of chloroprene on embryos are somewhat less clear cut. There have been several studies on this subject [55,56,61,63,64], and some have indicated increased preimplantation deaths in rats [55,56,61]. Chloroprene has also been associated with increased chromosomal aberrations in bone marrow cells of rats [42,55] and mice [41]. Likewise, two studies have reported a significant excess of chromosomal aberrations in blood cells of chloroprene-exposed workers in comparison with those of controls [41,43]. NIOSH believes that, although any single study cited in this document may not allow definite conclusion that chloroprene is mutagenic, the consistency of positive mutagenic responses in various test systems and the number of systems yielding them, as well as additional observations indicating that chloroprene may affect the spermatozoa, testicles, and male reproductive function, establish a clear need to control chloroprene as a mutagenic agent.

TABLE III-5

EFFECTS OF CHLOROPRENE ON HUMANS

Routes of Exposure	Subjects	Exposure Concentration and Duration	Effects	Reference
Respiratory	-	973 ppm 5 - 15 min	Nausea and giddiness	20
"	30 persons	334 - 56 ppm -	Fatigue, chest pains, heart palpitations, giddiness, irritability, dermatitis, hair loss	20
"	6 women	80.6 - 16.7 ppm -	Hair loss in 4	23
"	5 women and 13 men	5 ppm 1 - 15 yr	Increased chromosomal aberrations in blood lymphocytes	43
"	65 men and women	1.95 - 0.8 ppm Up to 20 yr	Fatigue, headache, chest pains, chronic tonsilitis, menstrual disorders	41
"	246 boys and girls	0.13 - 0.04 ppm 9 mon	Increased steroid hormones in urine, diuresis	37
"	148 boys and girls	0.13 - 0.04 ppm 9 mon	Increased coproporphyrin in urine	33
"	155 persons	Unknown 1 - 15 yr	Hypoglycemia, hypocholesterolemia, decreased carbonic anhydrase and reserve alkalinity in blood, decreased clotting time; increased total proteins, albumin, calcium, oxidized glutathione, fibrinogen, and chlorides in blood; hypotension	31, 32

TABLE III-5 (CONTINUED)

EFFECTS OF CHLOROPRENE ON HUMANS

Routes of Exposure	Subjects	Exposure Concentration and Duration	Effects	Reference
Respiratory	273 men and women	Unknown 7 - 13 yr	Chest pains, variable pulse rate, hypotension, increased capillary permeability, myocardial dystrophy	25
"	2,934 men and women	Unknown 9.1 yr	59 cases of skin cancer	28
"	"	Unknown 8.7 yr	34 cases of lung cancer (2 expected)	29
"	120 women	Unknown	Decreased protein, cystine, lysine, arginine, valine plus methionine, leucine, and isoleucine in milk	39 40
"	208 men and women	"	Increased titer of "OH" agglutins and phagocytic index, decreased immune response	26
"	39 persons	"	Increased gamma globulins, decreased beta globulins	27
"	130 men and women	"	Chemical burns, hair loss, conjunctivitis, sexual impotency	24

TABLE III-6

EFFECTS OF CHLOROPRENE ON ANIMALS

Routes of Exposure	Species	No.	Exposure Concentration and Duration	Effects	Reference
Respiratory	Rats	13 M	6,227-121 ppm 8 hr	Reproductive failure	18
"	"	10	470 ppm 8 hr/d x 13 wk	Decreased body weight, red blood cells, and hemoglobin value	20
"	"	-	Up to 60 ppm -	Increased preimplantation deaths, reduction in placental weight	61
"	Mice	14 M	152 - 12 ppm 8 hr	Reproductive failure	18
"	Rats	-	14.8- 4 ppm -	Increased prenatal and neonatal death	63
"	"	36 M	10 ppm 4 hr/d x 48 d	Increased chlorides in urine, increased embryonic mortality	55
"	"	73 F	8.6- 0.14 ppm Up to 21 d	Increased embryonic deaths	62
"	"	11 F	4.1 ppm with 1.3 ppm ammonia 4 hr/d 18 - 19 d	Increased liver weights	59
"	"	-	1.1 ppm 2 - 14 d	Internal bleeding, hydrocephalus, cerebral herniations, and death of fetuses	64
"	"	205 F	1.11- 0.16 ppm 4 hr/d on d 1 - 21 of gestation	Increased embryonic mortality with increased concentration, decreased fetal weights and long bone lengths	60

TABLE III-6 (CONTINUED)

EFFECTS OF CHLOROPRENE ON ANIMALS

Routes of Exposure	Species	No.	Exposure Concentration and Duration	Effects	Reference
Respiratory	Rats	36 M	1 ppm 4 hr/d x 48 d	Decreased chlorides in urine	55
"	Rats Mice	- -	0.47- 0.14 ppm 4.5 mon	Testicular atrophy, decreased spermatozoal motility and resistance to acid, chromosomal disorders, increased dead spermatozoa	41
"	Rats	6 M	0.78 ±0.56 ppm 4 mon	Chromosomal aberrations in bone marrow cells	42
"	"	100 M	0.47 ±0.02 - 0.04 ±0.002 ppm 5 hr/d up to 5.5 mon	Increased embryonic mortality and preimplantation deaths in mated females, decreased spermatozoal vitality, motility, and acid resistance	56
"	"	100 M	0.014±0.0008 ppm 5 hr/d up to 5.5 mon	No effects	56
"	Mice	13 F	4.1 ppm with 1.3 ppm ammonia 4 hr/d 18 - 19 d	Increased liver and kidney weights and increased embryonic mortality	59
"	Cats	1	10 cc* 7.5 min	Irregular breathing, returning to normal after 7 min, lung edema, liver and kidney degeneration, hair loss	19

TABLE III-6 (CONTINUED)

EFFECTS OF CHLOROPRENE ON ANIMALS

Routes of Exposure	Species	No.	Exposure Concentration and Duration	Effects	Reference
Respiratory	Cats	1	Unknown	Difficult breathing, loss of muscular coordination, lung edema, liver and kidney degeneration, hair loss, death in 6 wk	19
Dermal	Mice	100	50% in benzene twice/wk x 25 wk	No skin tumors, 42 deaths	51
Oral	Rats Mice	54 60	500 - 50 mg/kg 1 dose	CNS depression, listlessness, sluggishness, vascular congestion; lung, liver, brain, spleen, and epicardial edema; inflammation of stomach, myocardial degeneration	47
"	Rats	100	200 mg/kg twice/wk x 25 wk	No tumors, 60 deaths	51
"	"	-	0.5 mg/kg x 14 d	Increased preimplantation and embryonic deaths	64
Subcutaneous	"	280	1,916 mg/kg -	LD50, pulmonary edema, hyperemia	20
"	"	110	400 mg/kg x 10 doses**	No connective tissue tumors, 22 deaths after 6 mon	51
"	"	-	200 mg/kg x 50 doses**	No connective tissue tumors	51
"	Rabbits	1	684 mg/kg on d 1, 5, 13, and 27	Lung edema, liver and kidney degeneration, hair loss, death on d 28	19

TABLE III-6 (CONTINUED)

EFFECTS OF CHLOROPRENE ON ANIMALS

Routes of Exposure	Species	No.	Exposure Concentration and Duration	Effects	Reference
Subcutaneous	Rabbits	1	417 mg/kg	No physical changes initially, death after 20 hr, lung edema, liver and kidney degeneration, hair loss	19
"	Cats	1	1,843 mg/kg	Initial increase in blood pressure, then gradual decrease until death in 41 min	19
ip	Rats	50	520 mg/kg 1 dose	LD50	24

*Liquid poured onto a mask
**Interval between doses not specified