chloride. Although the results and conclusions of individual studies can be questioned on scientific grounds, they justify an increased concern about the hazard from increased exposure to vinyl chloride.

(b) Vinylidene Chloride

Ott et al [73] reported the mortality statistics and health examination findings on 138 workers exposed to vinylidene chloride containing small amounts (0.2%) of vinyl chloride. Estimations of exposure were made on the basis of job descriptions and industrial hygiene data. Estimated career doses were then calculated based on duration of exposure and monthly average exposure concentrations. Mortality data for the cohort were compared with those for US white males. Health examination data for the cohort were compared with data from controls paired as closely as possible for age, smoking history, date of employment, and date of participation in the plant health inventory program. Individuals in the control population were exposed to a variety of chemicals other than vinyl chloride used at the plant and therefore represented a background experience for chemical workers.

Comparison of the results of mortality experience of the total cohort, cohort with 15+ years of experience, and cohort with a total calculated dose exceeding 500 ppm-months (1,985 mg/cu m-months) showed no significant increase for any cause of death [73]. Comparison of the results of 17 clinical laboratory parameters showed no significant differences between the matched pairs of exposed and control workers. Regressions of the individual pair differences on estimated cumulative dose and duration of exposure showed no positive correlations at the 0.05 level.

The authors [73] concluded that there were no findings "statistically related or individually attributable to vinylidene chloride exposure" in the cohort studied. The study included few workers, however, and they were all from a single plant. The authors recommended that additional epidemiologic studies be conducted to develop information on chronic exposure to vinylidene chloride. This study does not indicate that there is an occupational hazard from exposure to vinylidene chloride.

(c) Vinyl Bromide, Vinyl Fluoride, and Vinylidene Fluoride

No epidemiologic studies on workers exposed to vinyl bromide, vinyl fluoride, or vinylidene fluoride have been located.

Animal Toxicity

The results of experiments involving exposure of laboratory animals to vinyl halides show that some of these compounds can induce the same toxic effects on rodents as on humans, including the characteristic angiosarcoma of the liver. No lifetime animal experiments have been located that demonstrate a no-observable-adverse-effect concentration for any of the vinyl halides.

(a) Vinyl Chloride

In 1975, Prodan et al [104] published estimates of LC50 values for a 2-hour exposure to vinyl chloride for several animal species. The values were 27,419 ppm for mice, 47,640 ppm for rats, and 236,215 ppm for both guinea pigs and rabbits. However, there was an error in the authors' calculations, and these values should actually have been 117,500 ppm (300.8 g/cu m) for mice, 156,000 ppm (399.4 g/cu m) for rats, and 238,000 ppm (609.3 g/cu m) for guinea pigs and rabbits. The recalculated LC100 values were 150,000 ppm for mice, 210,000 ppm for rats, and 280,000 ppm for guinea pigs and rabbits. The authors reported that death was preceded by excitement, contractions and convulsions, and accelerated respirations. The excitement phase progressed to a state of "tranquility" characterized by Cheyne-Stokes breathing and circulatory disturbances as indicated by cyanosis and conjunctival congestion. This phase then progressed into a state of deep narcosis that was followed by respiratory failure and death.

Abreu and coworkers [105] surveyed the anesthetic effects of 18 halogenated hydrocarbons, including vinyl chloride, on mice. The minimal certain anesthetic concentration (EC100 for anesthesia), highest tolerated concentration (LC0), 50% anesthetic concentration (EC50 for anesthesia) and 50% lethal concentration (LC50) on inhalation were estimated. To establish each point on each substance's effect-concentration of the curve, the authors exposed 10-40 mice to vinyl chloride for 10 minutes by inhalation. The ratio of the highest tolerated concentration to minimal certain anesthetic concentration was calculated as the "certain safety factor." The ratio of the 50% lethal concentration to the 50% anesthetic concentration was calculated as the "50% safety factor." Vinyl chloride had a minimal certain anesthetic concentration of 5.0 millimoles/liter (122,000 ppm; 312.3 g/cu m), a highest tolerated concentration of 9.0 millimoles/liter (220,000 ppm; 563.2 g/cu m), a "certain safety factor" of 1.8, a 50% anesthetic concentration of 4.1 millimoles/liter (100,000 ppm; 256 g/cu m), a 50% lethal concentration of 9.9 millimoles/liter (242,000 ppm; 619.5 g/cu m), and a "50% safety factor" of 2.4.

Aviado and Smith [106] reported the results of studies on sodium phenobarbital anesthetized rhesus monkeys exposed to vinyl chloride at concentrations of 2.5, 5.0, or 10.0% (25,000, 50,000, or 100,000 ppm; 64, 128, or 256 g/cu m). The vinyl chloride was administered to one animal at each concentration through a tracheal cannula for 5 minutes, alternating with 15 minutes of room air. Pulmonary resistance and compliance were estimated from measurements of tracheal airflow and transpulmonary pressure. Although control procedures were not discussed, it is assumed that each animal served as its own control.

Pulmonary resistance increased and pulmonary compliance and respiratory minute volume decreased with increasing vinyl chloride concentrations [106]. The only values of the exposed animals that were significantly different ($P<0.05$) from those of controls were pulmonary resistance (15.35% higher than

controls) and respiratory minute volume (12.30% lower than controls) at vinyl chloride concentrations of 10%. No significant changes in heart rate or aortic blood pressure were observed in these animals.

Oster et al [107], in 1947, investigated the anesthetic effects on dogs of vinyl chloride gas mixed with oxygen. Two dogs were "momentarily" exposed to vinyl chloride at a 50% concentration (500,000 ppm; 1,280 g/cu m) which was then decreased to 7% (70,000 ppm; 179.2 g/cu m) by volume. During exposure, the dogs maintained good abdominal relaxation, but their legs became rigid and muscular movements became uncoordinated. A third dog, anesthetized with 25% vinyl chloride, had signs similar to those in the first two dogs. After exposure the dogs continuously "crowed" and salivated heavily.

Four additional dogs received a local anesthetic, monocaine hydrochloride, and their blood pressures were checked by cannulation of the femoral artery [107]. After control blood pressure measurements were obtained, the animals were anesthetized with 10% vinyl chloride (100,000 ppm; 256 g/cu m) in oxygen. During the vinyl chloride exposure, the dogs had normal blood pressures, but they showed such cardiac irregularities as intermittent tachycardia, ventricular extrasystoles, and vagal beats. Similar irregularities were detected with a stethoscope in noncannulated dogs on the same exposure regimen.

Six other dogs were anesthetized with 10% vinyl chloride (100,000 ppm) in oxygen [107]. Electrocardiographic (ECG) records (lead II) were obtained at exposures to vinyl chloride (not further defined) sufficient to produce light surgical anesthesia, surgical anesthesia, and threatened respiratory collapse. At exposures producing surgical anesthesia, several changes were noted in the cardiac rhythm, especially marked tachycardia followed by bradycardia. In addition, two of the six dogs showed R-wave inversions, and one dog showed incipient ventricular fibrillation. All six dogs showed abnormalities in the ECG record, ranging from sinus arrhythmia and transitory extreme left axis deviation to atrioventricular (A-V) block, ventricular tachycardia, ventricular multifocal extrasystoles, and inversion of the T-wave with elevated ST segments. As the concentration of vinyl chloride was increased toward that necessary for respiratory failure, the ECG abnormalities disappeared except for the greatly reduced R-wave amplitude. The authors concluded that vinyl chloride caused muscular incoordination in the extremities and serious cardiac arrhythmias. They also concluded that vinyl chloride was not safe as an anesthetic and that its use in humans was not warranted.

In 1974, Belej et al [108] reported the changes in cardiac function of rhesus monkeys exposed to vinyl chloride at concentrations of 2.5, 5.0, and 10.0% (25,000, 50,000, and 100,000 ppm; 64, 128, and 256 g/cu m). Three animals were exposed at each concentration. They were anesthetized with sodium phenobarbital, their tracheae were cannulated for artificial respiration, and their chests were opened by midsternal incisions to allow measurement of myocardial contractility, pulmonary arterial, aortic, and left

atrial pressure. The vinyl chloride mixtures were administered for periods of 5 minutes alternating with 10-minute exposures to room air. The number of test periods for each animal was not specified. Control procedures were not reported, but each animal probably served as its own control.

The heart rate and aortic, left atrial, and pulmonary arterial pressures were not significantly different between any of the experimental and control groups [108]. The force of myocardial contraction in animals exposed to 10% vinyl chloride was significantly different ($P<0.05$) from control values, showing a decrease of 28.5%. At 5% vinyl chloride there was a 9.1% decrease, and at 2.5% vinyl chloride there was a 2.3% decrease; however, these latter changes were not significant. Decreases in aortic blood pressure, while not significant, paralleled decreases in contractility.

Carr et al [109] demonstrated that inhalation of certain compounds increased the sensitivity of the canine heart to epinephrine. Lead II of the ECG was recorded from each of seven unanesthetized dogs in the experiment. Epinephrine hydrochloride was injected iv at a dose of 0.01 mg/kg during 25-40 seconds, and the ECG was again recorded. Subsequently, the animals were exposed to vinyl chloride at concentrations of from 15 to 90% with oxygen. After the dogs had inhaled the vinyl chloride for 10-20 minutes, the ECG recording and epinephrine injection were repeated. Three of the seven dogs developed sensitization of the myocardium with multifocal ventricular ectopic tachycardia after vinyl chloride exposure. The authors concluded that vinyl chloride produced sensitization of the myocardium, but less frequently than did its saturated analog, ethyl chloride. The total exposures used were too high to allow a conclusion about whether inhalation of vinyl chloride may pose an acute threat to workers in times of fear-provoking stress or to those who may take medication containing epinephrine for asthma control or other reasons.

Clark and Tinston [110] exposed beagle dogs to vinyl chloride at various concentrations by mask for 5 minutes. During the final 10 seconds of exposure, a 5-μg/kg injection of adrenaline was administered iv. ECG's were recorded and analyzed for serious arrhythmias, such as multifocal ventricular ectopic beats or ventricular fibrillation. At each concentration, four to seven dogs were exposed. Vinyl chloride was calculated, by a moving average interpolation technique, to have induced cardiac sensitization in 50% of the animals at a concentration of 5%.

In 1970, Viola [111] reported on the effects on rats of exposure to vinyl chloride. Twenty-five male albino Wistar rats with an average weight of 150 g were exposed to vinyl chloride at a concentration of 3% (30,000 ppm; 76.8 g/cu m) for 4 hours/day, 5 days/week, for 1 year. Twenty-five similar rats served as controls. Throughout the exposure period, the general physical appearance, behavior, and body weight of the animals were monitored. After the exposures, some of the exposure survivors and some of the controls were killed at 20-day intervals, and gross and microscopic changes in the paws,

brain, liver, kidneys, and thyroid were noted. The number of rats surviving, the number of examinations conducted, and the number of rats killed at each interval were not specified.

Exposure to vinyl chloride did not significantly affect growth but was slightly soporific to the animals during the first 10 months of exposure [111]. During the 11th month, the exposed rats had lower body weights and showed less aggressiveness and less reaction to external stimuli than the controls; they also suffered disturbances in equilibrium. No details about the observations made were presented. Of the rats exposed to vinyl chloride, 13 died from cardiopulmonary complications and 2 died from hematoperitoneum. On microscopic examination, the authors observed that most of the animals showed pathologic changes in the brain, liver, kidneys, and thyroid, and that six rats showed skeletal alterations. He did not report whether these changes were observed in the rats that died during exposure or in those that were killed after the exposure. The paws of the vinyl chloride-exposed rats had areas of hyperkeratosis, superficial thickening of the epidermis, and disappearance of the cutaneous "adnexa." In addition, vacuolization and degeneration of the basal layer, a modest increase in the papillar layer, and edema of the epidermis were noted. The connective tissue of the skin showed fragmentation and decreased elastic reticulum and dissociation of collagen bundles. The small arterial vessels of the paws showed signs of endothelial fibrosis, and some vessels were completely occluded by proliferation of connective tissue.

Extensive metaplastic proliferation of cartilage-like material was found around the small metatarsal bones [111]. The edges of the material were irregular, with differential growth that resulted in outward bending of toes. In areas of mature compact bone, the cartilaginous elements were grouped around a central nucleus of the bone, giving the appearance of chondroid metaplasia. In small bones, this chondroid metaplasia was often extensive, and the bones appeared to be impregnated with a mucoid substance that obliterated the cement lines by altering the characteristic deposition of the bone tissue.

Microscopic examination of the brains of rats exposed to vinyl chloride showed diffuse degenerative lesions of the gray and white matter [111]. Fibrotic processes had often surrounded and invaded the small nerve bundles of the gray matter. There was also evidence of neuronal phagokaryosis with satellitosis and deposition of neuroglial elements around the altered nerve cells of the white matter. In the cerebellum, there were signs of atrophy of the granular layer and profound degenerative changes in some areas of the Purkinje cell layer.

Animals exposed to vinyl chloride had enlarged livers [111]. Some livers appeared yellowish with smooth surfaces and were slightly more brittle than normal. Microscopic examination revealed signs of diffuse interstitial hepatitis, functional degeneration or necrosis of the hepatic cells, and marked cytoplasmic and nuclear polymorphism. The Kupffer cells were often hypertrophic and showed evidence of abnormal proliferation. Numerous areas of

partial necrosis and diffuse fatty degeneration often blocked the portal capillaries, centrilobular veins, and sinusoids. Intense fibrosclerotic reactions were also noted in the areas of degenerative change in the livers of a few exposed rats.

In contrast, the kidneys of the exposed animals were relatively unaltered except for signs of tubulonephrosis and occasional chronic interstitial nephritis [111]. The author also noted colloid goiters and a marked increase in parafollicular cells in the thyroids of several animals. Examination of the controls showed no alterations in any of the organs.

The author [111] concluded that his investigation confirmed that rats were sensitive to vinyl chloride and that the lesions of bone and connective tissue were similar to those described in workers affected by acroosteolysis of the hands and to those in experimental "osteolathyrism." This report is valuable for its characterization of the systemic toxic effects resulting from chronic exposure to vinyl chloride. An assessment of the hazard posed by exposure to vinyl chloride is not possible; however, because the information was not presented in sufficient detail to permit a statistical analysis. Observations on tumors produced in animals that were probably the same as those in this paper were presented in a second paper by Viola et al [112] and are discussed in a subsequent section of this chapter.

In 1961, Torkelson et al [113] reported a three-part investigation on the effect of repeated exposure of rats, rabbits, dogs, and guinea pigs to vinyl chloride at 50, 100, 200, or 500 ppm (128, 256, 512, 1,280 mg/cu m). In the first experiment, groups of 10 male and 10 female rats were placed in a 160-liter inhalation chamber containing vinyl chloride at a nominal concentration of 500 ppm for 7 hours/day, 5 days/week, for 4.5 months. The control group consisted of five male and five female rats.

Male rats exposed repeatedly at 500 ppm for 4.5 months showed a significantly higher ($P<0.001$) liver-to-body weight ratio than the controls [113]. Of the 20 exposed rats, 3 males and 1 female died during the exposure. Microscopic examination showed an increased centrilobular granular degeneration of the liver and interstitial and tubular changes in the kidneys.

In the second experiment, the animals exposed to vinyl chloride at nominal concentrations of 100 or 200 ppm (±15%) for 7 hours/day, 5 days/week, for 6 months, included 20-24 male and 24 female rats, 10 male and 8 female guinea pigs, 3 male and 3 female rabbits, and 1 male and 1 female dog [113]. In addition, eight groups of five male rats each were exposed at nominal concentrations of 100 or 200 ppm (±15%) for 0.5, 1, 2, or 4 hours/day, 5 days/week, for 6 months. For each species and regimen, two control groups carefully matched on the basis of age, condition, and weight were used, one group of colony controls, and the other air-exposed in the chamber on a regimen similar to that of the experimental groups.

Of the 24 rats exposed at 100 ppm for 7 hours/day, 5 males and 1 female died [113]. All rats exposed repeatedly for 7 hours/day showed slight but significant increases ($P<0.05$) in liver weight.

Repeated exposure to vinyl chloride at 200 ppm for 7 hours/day for 6 months resulted in the deaths of 3 of 12 rats [113]. Of the five rats in each "short exposure" group, one, two, one, and one died after exposure for 0.5, 1, 2, and 4 hours/day, respectively. Organ weights were normal in all species, except that liver weights increased in rats exposed repeatedly for 7 hours/day. At 8 weeks after exposure, male rats continued to have increased liver weights, but the weights appeared to be returning to normal. Increases in liver weight of rats exposed for 2 or 4 hours/day were not statistically significant. All the rats exposed for 0.5 or 1.0 hour/day had normal organ weights compared with those of control rats. Microscopic examination showed liver changes characterized by centrilobular granular degeneration and necrosis with some foamy vacuolization in male rabbits and periportal cellular infiltration in female rabbits.

In the third experiment, 24 male and 24 female rats, 12 male and 12 female guinea pigs, 3 male and 3 female rabbits, and 1 male and 1 female dog were exposed to vinyl chloride at a concentration of 50 ppm for 7 hours/day, 5 days/week, for 6 months [113]. Three additional groups of 10 male rats each were exposed for 1, 2, or 4 hours/day, 5 days/week, for 6 months. Matched groups of control animals were again used. Vinyl chloride concentrations in the chamber were determined by micro-Volhard titration. Repeated exposures to vinyl chloride at 50 ppm did not produce toxic signs in any of the animals. A decrease in the kidney weight was observed in the female rats, but it was not attributed to the exposure since it was not observed at higher concentrations.

Animals exposed repeatedly at 100, 200, or 500 ppm were found to have normal biochemical and hematologic values and urinalysis tests [113]. Serum enzyme activities were normal in all dogs, rats, and rabbits. None of the organs examined had macroscopic tissue changes at any exposure concentration. The authors concluded that repeated exposures for 7 hours/day at 100 ppm could cause increased liver weight.

Lester et al [18] conducted four experiments with Sherman strain rats. In the first experiment, an unspecified number of rats were exposed in pairs to vinyl chloride at various concentrations for up to 2 hours in an exposure chamber. No control animals were described. In the rats exposed to vinyl chloride at a concentration of 5% (50,000 ppm; 128 g/cu m), the righting reflex was lost; at 6% (60,000 ppm; 153.6 g/cu m), however, it was said to be still present, but it was absent in rats exposed at 7% (70,000 ppm; 179.2 g/cu m). The corneal reflex disappeared at a vinyl chloride concentration of 10% (100,000 ppm; 256 g/cu m). The animals rapidly returned to normal after removal from the chamber. One rat was killed after exposure to vinyl chloride at 10% but showed no gross signs of adverse effects. Two rats exposed to vinyl chloride at 15% were deeply anesthetized within 5 minutes. One rat had

effusions of fluid from the mouth and died of asphyxia in 42 minutes; autopsy revealed edema and congestion of the lungs. The other rat remained under deep anesthesia for 2 hours but recovered promptly when removed from the exposure.

In their second experiment with rats, Lester et al [18] randomly assigned 18 male and 18 female rats to an experimental group and a control group. The experimental animals were exposed to vinyl chloride at a concentration of 10% (reduced to 8% after 2 days) between 8:30 am and 4:30 pm. Two female rats and an unspecified number of male rats in the experimental group died early in the experiment and were replaced. A total of three female rats died (days 2, 5, and 14) in the course of vinyl chloride exposure, and only two males survived all 15 days of exposure. In addition to mortality, exposure to vinyl chloride at a concentration of 8% was associated with a failure to gain weight until day 9, followed by weight gain at a slower rate than the controls until the cessation of exposure, after which the rates were equal. Neither gross nor microscopic differences were noted between experimental and control livers and kidneys at necropsy. Some rats had parasitic liver cysts and focal necrotizing pneumonia, but kidneys were within normal limits of variability. Microscopic examination of the spleens of experimental rats showed a significantly higher incidence of severe abnormality than was found in controls, although some individual rats in the control group manifested equally severe splenic abnormalities.

Lester et al [18] performed another experiment to ascertain whether vinyl chloride was a lung irritant. Three female and five male rats, matched with controls, were exposed as before for 8 hours daily for 19 consecutive days to vinyl chloride at a concentration of 5% in air. All animals were deprived of food and water while in the exposure chamber. Hemoglobin determinations were made during the exposure period. On the 20th day, all animals were anesthetized with ether, and blood was drawn by cardiac puncture. The animals were killed by an overdose of ether, and necropsies were performed. The terminal blood sample was tested for hemoglobin, prothrombin time, hematocrit, red cell, white cell, and differential white cell counts, and unspecified serum transaminase activities. Prothrombin time, hematocrit, and the serum transaminase activities were normal for both groups. The experimental group had a statistically significant increase ($P<0.05$) in red blood cells and a decrease ($P<0.01$) in white blood cells. The differential white cell counts of control and experimental groups were not significantly different. The ratio of the weight of the liver to the body weight was significantly elevated (males, $P<0.01$; females, $P<0.001$) in the experimental group. The five male experimental animals had thinner coats than normal and scaly tails; all other rats were normal in appearance. One of the experimental males had fibrous pleural adhesions, but these appeared to be old and unrelated to the experimental exposure. Microscopic examination of all organs and tissues failed to disclose abnormalities other than those in the liver in either group. There were no differences in intracellular or total fat in the liver, but livers in the experimental group had widespread swelling and vacuolization of cells with compression of the sinusoids. This difference was significant ($P<0.001$).

In the last experiment of the series, rats were exposed to vinyl chloride at a concentration of 2.0% for 8 hours/day, 5 days/week, for 3 months [18]. Sixty rats weighing about 75 g were separated randomly into two groups, each consisting of 15 males and 15 females. In the week before the rats were exposed to vinyl chloride, they were observed and weighed and blood was taken for hemoglobin determinations. All rats were weighed weekly, and hemoglobin determinations were made monthly. Four control and one experimental animal died in the course of the experiment. After 3 months, all animals were killed for necropsy after blood samples were drawn. The livers and spleens were weighed before being fixed in formalin. No significant differences were noted between the experimental and control body weights, hemoglobin levels, hematocrits, prothrombin times, or white cell monocyte and eosinophil counts. The livers of the experimental animals were significantly heavier ($P<0.001$) and the spleens significantly lighter (males, $P<0.02$; females, $P<0.05$) than in the control group. The experimental rats also had a statistically significant decrease ($P<0.01$) in white blood cell and neutrophil counts and an increase ($P<0.01$) in lymphocyte counts, when compared to the controls. Microscopically, the experimental group had fewer signs of kidney damage but more extensive liver damage, as indicated by swelling of cells and compression of sinusoids, than controls. No microscopic differences between the spleens of the two groups were noted.

In this 1963 paper, Lester and coworkers [18] concluded that the only suggestion of a specific toxic action of vinyl chloride was the increase in liver weight; the increase was not only statistically significant, but also of substantial magnitude (30% heavier than controls). The authors stated that they did not know the significance of the increase in liver weight after exposure to vinyl chloride, nor whether the liver returned to normal after exposure ceased. In addition, the authors pointed out the increasing neurologic deficits with increasing concentrations of vinyl chloride, finally terminating in death from respiratory insufficiency at concentrations of 15% for a single exposure or of 10% for repeated exposures.

(b) Vinylidene Chloride

In 1963, Irish [114], stated that there were at that time essentially no published data on vinylidene chloride toxicity and summarized data from unpublished reports of the Dow Chemical Company. He presented no detailed information on the animals exposed in these studies. "Brief" exposure to vinylidene chloride at concentrations around 4,000 ppm (15.9 g/cu m) was said to have rapidly produced "drunkenness," which progressed to unconsciousness if the exposure was continued. The author stated that vinylidene chloride irritated the eyes, and, in liquid form, irritated the skin. Animals exposed at concentrations of 25, 50, and 100 ppm (99.3, 198.5, and 397 mg/cu m) for 8 hours/day, 5 days/week, for several months exhibited unspecified liver and kidney damage. Irish concluded that vinylidene chloride produced adverse effects at concentrations below those necessary to produce irritation and below the odor threshold of 500-1,000 ppm.

Jaeger et al [115] estimated the LC50's for fed and fasted rats exposed to vinylidene chloride. One group of male Holtzman rats weighing 250-400 g was allowed continuous access to food; another group was fasted for 18 hours before exposure.

Eight groups of fasted rats and six groups of fed rats, each group consisting of five or six animals, were exposed to vinylidene chloride at various concentrations for 4 hours [115]. The 4-hour LC50 at 24 hours for the fasted rats was 600 ppm (2,382 mg/cu m) and the 24-hour minimum lethal concentration was 200 ppm (794 mg/cu m). The estimated 4-hour LC50 at 24 hours for the fed rats was 15,000 ppm (59.6 g/cu m), and the minimum lethal concentration for these animals was 10,000 ppm (39.7 g/cu m).

The authors [115] suggested that decreases in glutathione concentration in the livers of fasted rats was a possible explanation for the differences in lethality. They pointed out that this could be of importance with regard to the occupational risk because of the known circadian pattern of glutathione concentrations in the liver.

Siegel et al [116] published the results of experiments on several dichloroacetylene mixtures. For comparative purposes, they determined LC50's for vinylidene chloride and several other compounds. The 14-day LC50 of vinylidene chloride for male Sprague-Dawley rats was estimated to be 6,350 ppm (25.2 g/cu m) for a 4-hour exposure.

Short et al [117] reported the LC50 at 14 days and the 20-ppm LT50 for CD-1 mice exposed to vinylidene chloride for 22-23 hours. The LC50 for males was 98 ppm (389 mg/cu m) (95% confidence limits, 82-118 ppm), and for females it was 105 ppm (416.9 mg/cu m; 92-121 ppm). When the exposure periods were increased to 2 days at 22-23 hours/day, the LC50 for male mice was 35 ppm (139 mg/cu m). The LT50 for males was 4 days (3.6-4.4) at 20 ppm (79.4 mg/cu m). Concentrations of vinylidene chloride were measured by gas-liquid chromatography; other experimental details for these determinations were not given.

Carpenter et al [118] reported the effects of inhalation of vinylidene chloride on Sherman albino rats weighing 100-150 g. The authors reported that the concentration of vinylidene chloride sufficient to kill two to four of six test animals within 14 days after a 4-hour exposure was 32,000 ppm.

Balmer et al [119] also performed a study to determine the inhalation toxicity of vinylidene chloride in rats. A group of 104 male and 104 female Sprague-Dawley rats was exposed to vinylidene chloride at a concentration of 10 ppm (39.7 mg/cu m) for 6 hours/day, 5 days/week, for up to 18 months. A similar group was exposed at 40 ppm (158.8 mg/cu m) on the same schedule, and another group served as controls. After 30 days, four males and four females from each group were killed and examined. Because examination showed no effects, at the end of week 5 exposure concentrations were increased to 25 and 75 ppm (99.3 and 297.8 mg/cu m). After 6 months, five males and five females

from each group were killed and examined, and after 7 months, four animals of each sex from each group were killed, and bone marrow cells were prepared for cytogenetic examination. After 12 months of exposure, five additional animals of each sex from each group were killed and examined. Exposure ended at 18 months, and any animals remaining alive at 24 months were killed and examined.

Balmer et al [119] stated that "no clinical signs of toxicity were seen at any time." There were no signs of cytogenetic effects, and no hematologic or clinical chemical changes were relatable to the vinylidene chloride exposure. No significant differences in mortality, tumor formation, or gross pathology of the internal organs were observed. On microscopic examination, the livers of the animals exposed at both concentrations showed increased cytoplasmic vacuolation in the hepatocytes.

The authors [119] concluded that inhalation of vinylidene chloride at concentrations of 25 or 75 ppm resulted in "minimal liver changes" and did not cause an increase in tumors in rats.

Gage [120], in 1970, presented the results of an inhalation study of several industrial chemicals, including vinylidene chloride, on Alderley Park specific-pathogen-free rats weighing an average of 200 g. Groups of four male and four female rats were exposed to vinylidene chloride vapor at concentrations of 200 or 500 ppm (794 or 1,985 mg/cu m) for 6 hours/day, 5 days/week, for 4 weeks. Concentrations of vinylidene chloride in the exposure chamber were monitored by gas chromatography. During the exposures, the animals were checked for any change in weight and activity. The rats were killed at the end of the exposures, and blood and organs were collected and tested. Macroscopic and microscopic examinations were performed on organs and fixed tissues. Rats exposed at 500 ppm developed nasal irritation (sneezing), did not gain weight normally, and suffered liver cell degeneration. Rats exposed at 200 ppm suffered only slight nasal irritation, and all organs were normal on necropsy. No further data were presented by the author.

Siletchnik and Carlson [121] published the results of a study to determine the cardiac sensitizing effects of vinylidene chloride. Adult male Charles Rivers rats weighing 250-400 g were lightly sedated with 25 mg of sodium pentobarbital/kg ip, restrained, and then exposed to vinylidene chloride at 25,600 ±700 ppm (101.6 ±2.8 g/cu m) for periods of 10 minutes or longer. The rats were pretreated with 4 µg of epinephrine/kg, and the minimum amount of epinephrine necessary to produce cardiac arrhythmias or demonstrate a difference between pairs of animals was determined. The effect of phenobarbital was determined using weight-matched pairs of animals. One animal of each pair was administered phenobarbital, 50 mg/kg ip, daily for 4 days and exposed to vinylidene chloride 24 hours after the last dose. The pair mate in each case received injections of saline and was similarly exposed to vinylidene chloride.

In rats exposed to air alone, epinephrine at 4 µg/kg did not elicit any cardiac arrhythmias [121]. The authors stated that vinylidene chloride alone

caused sinus bradycardia and such arrhythmias as A-V-block, multiple continuous premature ventricular contractions, and ventricular fibrillation; they presented no further information. Epinephrine at doses as low as 0.5 μg/kg elicited cardiac arrhythmias in 29 animals exposed to vinylidene chloride. Sensitivity to epinephrine increased with increasing length of exposure to vinylidene chloride. The increased sensitivity to epinephrine was reversible, since 5 minutes after removal from exposure the animals were again able to tolerate high doses of epinephrine without showing arrhythmias.

Premature ventricular contractions were seen in animals pretreated with phenobarbital, exposed to vinylidene chloride, and challenged with epinephrine [121]. No arrhythmias were seen in the saline-treated animals challenged with the same amount of epinephrine. Arrythmias were produced in the phenobarbital-treated animals at a lower epinephrine dose and a shorter exposure to vinylidene chloride.

The authors [121] concluded that the phenobarbital probably induced vinylidene chloride metabolizing hepatic enzymes and that a metabolite caused the cardiotoxic effects. They further stated that, since the adrenal gland in humans may release up to 4 μg/kg/minute of adrenalin during stress, workers exposed to high concentrations of vinylidene chloride may be under a risk of these cardiotoxic events.

Prendergast et al [122] conducted a series of inhalation studies to determine the effects of vinylidene chloride and other chlorinated hydrocarbons on Sprague-Dawley or Long-Evans rats, Hartley guinea pigs, New Zealand White rabbits, beagle dogs, or squirrel monkeys. The exposed animals were subjected either to continuous 90-day exposures or to repeated 8-hour exposures, 5 days/week, for 6 weeks. The animals repeatedly exposed to vinylidene chloride included 15 rats, 15 guinea pigs, 3 rabbits, 2 dogs, and 3 monkeys. Continuous exposures involved groups of 15 or 45 rats, 15 or 45 guinea pigs, 3 rabbits, 2 or 6 dogs, and 3, 9, or 21 monkeys. A control group consisted of 304 rats, 314 guinea pigs, 48 rabbits, 34 dogs, and 57 monkeys. The concentrations of vinylidene chloride were monitored by gas-liquid chromatography. The concentrations of vinylidene chloride in the chamber during the continuous exposure were 20 ±2.1, 61 ±5.7, 101 ±4.4, or 189 ±6.2 mg/cu m (5, 15.4, 25.5, or 47.6 ppm), and the concentration during the repeated exposure was 395 ±32 mg/cu m (99.5 ppm).

Immediately after each experiment, the animals were killed and necropsies were performed [122]. To estimate the effects of vinylidene chloride inhalation, the authors measured liver alkaline phosphatase, SGPT, serum urea nitrogen, and liver lipid content, and made hematologic determinations. In the control populations, 7/304 rats, 2/314 guinea pigs, 2/48 rabbits, and 1/57 monkeys died prematurely.

Animals repeatedly exposed to vinylidene chloride at 395 mg/cu m (99.5 ppm) showed no microscopic tissue changes relatable to the exposure when compared with control tissues [122]. Gross examination of tissues showed no

changes in any animals except for one rat that had a gelatinous substance on its kidney and bloody urine in the bladder. The microscopic examination showed only nonspecific respiratory inflammation, which the authors considered not to be the result of vinylidene chloride exposure. They did not give any reasons for this conclusion. None of the repeatedly exposed animals died during the exposure, and only rabbits and monkeys lost weight.

Of the animals exposed continuously at 189 mg/cu m (47.6 ppm), seven guinea pigs died between days 4 and 9, and three monkeys died, one each on days 26, 60, and 64 [122]. Gross examination showed mottled livers in a majority of the experimental animals. Exposed rabbits, dogs, and monkeys lost weight, while rats and guinea pigs gained weight during the exposure. There were increases in liver alkaline phosphatase and SGPT activity in rats and guinea pigs, but there were no significant changes in any other biochemical parameters. Two rats also showed increases of 20% and 34.4% in liver lipid content. Serum urea nitrogen concentrations in exposed rats were comparable with those of control rats. Three of the 15 guinea pigs that were exposed at 101 mg/cu m (25.5 ppm) died between the 3rd and 6th exposure days, and 2 of the 3 monkeys exposed at 101 mg/cu m died, 1 on day 39 and the other on day 47. White or bluish-gray spots and nodules were visible on the lungs of several guinea pigs and rats. Serum urea nitrogen concentrations of exposed guinea pigs were comparable with those of the control group.

Continuous exposure at 61 mg/cu m (15.4 ppm) resulted in the deaths of 3 of the 45 guinea pigs on days 3 and 4 of exposure [122]. The exposed monkeys lost weight, and exposed rats gained less weight than the controls. Several animals of all species had mottled livers and spleens.

Two of the 45 rats, 2 of the 45 guinea pigs, and 1 of the 21 monkeys exposed continuously at 20 mg/cu m (5 ppm) died [122]. The dogs lost weight, and the rats gained less than controls. Gross examination showed mottled livers in about one-third of the exposed animals. Microscopic changes were seen in the kidneys and liver of the exposed monkeys and nonspecific inflammatory changes in the lungs of all animals exposed at each concentration. The authors did not attribute the lung changes to the vinylidene chloride exposure. They did not give any reasons for this conclusion. Liver damage was noted in animals exposed to vinylidene chloride at 189 mg/cu m (47.6 ppm). Although animals exposed at 61 and 20 mg/cu m (15.4 and 5 ppm) also had signs of liver damage, the authors did not consider these attributable to the exposures to vinylidene chloride, since those exposed at 101 mg/cu m (25.5 ppm) did not exhibit these changes. Hematologic test results for exposed animals were similar to those for the controls.

In 1977, Humiston et al [123] and Rampy et al [124] reported the results of a study of the oral toxicity of vinylidene chloride. Groups of 48 male and 48 female Sprague-Dawley rats were given access to vinylidene chloride in their drinking water at nominal concentrations of 50, 100, or 200 ppm (equivalent ranges of daily intake: 5-12, 8-20, 16-40 mg/kg) for 730 days. Eighty male and 80 female rats served as controls. The typical impurities of

the vinylidene chloride used in this study was reported by Rampy et al [124] as: vinyl bromide, 4 ppm; vinyl chloride, 3-5 ppm; trans-1,2-dichloroethene, 138-1700 ppm; cis-1,2-dichloroethene, 24-680 ppm; 1,1,1-trichloroethane, 2-60 ppm; 1,1,2-trichloroethane, 48 ppm; hydroquinone monomethyl ether, 2 ppm.

The appearance and demeanor of the rats ingesting vinylidene chloride were not different from those of controls throughout the study [123]. Body weight gains and food and water consumption were similar for experimental and control animals. No compound-related abnormalities were noted in the results of hematologic studies, urinalyses, or serum chemistry analyses. No significant differences were noted in mean organ weights or organ to body weight ratios.

Gross and microscopic examinations revealed occasional statistical differences between exposed and control animals [123]. The differences considered compound-related were fatty changes or fatty degeneration of the liver in female rats in the 50, 100, and 200 ppm groups and in male rats in the 200-ppm group. Although the incidence of these liver lesions in the males of the 100-ppm group was not significantly increased, it was higher than that in the controls. Centrilobular atrophy and periportal hypertrophy were also seen in the liver of the exposed animals. No target organ was found that showed a tumorigenic effect which was considered compound-related, and the total tumor incidence in male and female rats in the various exposure groups was not considered different from that in the controls.

Humiston et al [123] concluded that the only compound-related deviations in these rats were the fatty changes or fatty degenerations of the liver. All other statistically significant deviations observed were considered to be within the normal variation encountered in lifetime studies with this strain of rat. They also concluded that these results did not indicate an "oncogenic effect for vinylidene chloride ingested by rats." The data indicate that if a carcinogenic potential for rats exists for vinylidene chloride, these cumulative doses must be lower than that necessary to promote the expression of that potential.

Jaeger et al [125] gave single doses of vinylidene chloride dissolved in corn oil by stomach tube to lightly ether-anesthetized male Holtzman rats weighing 250-350 g. The rats were fasted for 4 hours before they received the vinylidene chloride. Anesthetized control rats received corn oil only. Some of the experimental rats were given ip injections of sodium phenobarbital (30 mg/kg) to aid in measuring the time-response relationship for vinylidene chloride. This was indicated by the time between loss and recovery of the righting reflex. Three spontaneous rightings within a minute was considered evidence that the reflex had returned. The liver glucose-6-phosphatase activity, serum alanine-alpha-ketoglutarate transaminase activity, liver triglyceride values, and the phenobarbital sleeping time were determined at different times after the animals received doses of vinylidene chloride and were used as indices of liver damage. The rats were killed at intervals up to 24 hours after they were given the vinylidene chloride or corn oil, and their

blood was collected for preparation of serum. The livers were removed and used to make homogenates for biochemical assays.

The phenobarbital sleeping time increased significantly ($P<0.05$) within 2-4 hours in rats given 400 mg/kg of vinylidene chloride, and the maximum increase was observed at 12-16 hours, although there were no statistical differences between the sleeping times at 4-8, 12-16, and 20-24 hours [125]. Liver damage, as evidenced by either decreased hepatic glucose-6-phosphatase activity or increased serum alanine-alpha-ketoglutarate transaminase activity, was marked at 8 and 16 hours after the vinylidene chloride was given to the rats. Serum alanine-alpha-ketoglutarate transaminase activity increased significantly ($P<0.05$) at 4, 8, 16, and 24 hours and the glucose-6-phosphatase activity decreased significantly ($P<0.05$) at 8 and 16 hours in animals given vinylidene chloride at 400 mg/kg. The serum enzyme activity began to decline after 8 hours. The liver triglyceride concentration increased with increasing doses of vinylidene chloride; at doses of 800 mg/kg, it was almost double the control concentration. None of the control animals showed a change in any of the indices of hepatic toxicity considered.

Jenkins et al [126] studied the effect of vinylidene chloride on adrenalectomized male Holtzman rats weighing 200-470 g. Sham-operated rats were used as controls. Both the adrenalectomized and control rats were fasted for 18 hours before being given single oral doses of 400 mg/kg of vinylidene chloride in corn oil, 2 ml/kg by volume. After 20 hours, more adrenalectomized rats than control rats had died. Biochemical responses to vinylidene chloride could not be measured as a result of the high mortality rate. The LD50 in adrenalectomized rats after 24 or 96 hours was 84 (64-111) and 81 (70-94) mg/kg, respectively. However, with control rats, the LD50 was 1,550 (1,520-1,581) and 1,510 (1,445-1,578) mg/kg after 24 and 96 hours, respectively.

In the same study, Jenkins and associates [126] observed the effects of vinylidene chloride on 9- to 11-week-old male and female rats given single oral doses of 400 mg/kg. They also studied the effects of vinylidene chloride on 21- to 24-week-old male and female rats given single oral doses of 200 mg/kg. Liver and plasma enzyme activities were measured 20 hours after oral administration. The older female rats showed a greater increase in liver glucose-6-phosphatase activity than the older male rats, and both groups of females had increased liver alkaline phosphatase activity in comparison with their respective male counterparts. From these observations, the authors concluded that female rats were more susceptible to the hepatotoxic effects of vinylidene chloride than male rats.

(c) Vinyl Bromide

Abreu et al [105] published the results of a study on the anesthetic properties of 18 halogenated hydrocarbons, including vinyl bromide. Groups of 10-40 mice were exposed to vinyl bromide at several unspecified concentrations to determine the minimal certain anesthetic concentration (EC 100 for

anesthesia), highest tolerated concentration (LCO), and "certain safety factor" (highest tolerated concentration divided by minimal certain anesthetic concentration). Vinyl bromide showed a minimum certain anesthetic concentration of 3.5 millimoles/liter (86,000 ppm; 376.7 g/cu m) and a highest tolerated concentration of 7.0 millimoles/liter (171,000 ppm; 749 g/cu m), for a certain safety factor of 2.0 [105].

Leong and Torkelson [127] conducted two studies of the effects of inhaled vinyl bromide (99.7% pure) on various animal species, including rats, rabbits, and monkeys. The impurities in the vinyl bromide included a polymerization inhibitor (paramethoxy phenol, 0.1%), ethylene oxide (0.12%), vinyl chloride (0.06%), and traces of ethyl bromide, methylene chloride, acetylene, and various aldehydes. The first study involved exposing four groups of five male Wistar rats each to vinyl bromide at 10,000 ppm (43.8 g/cu m) in a 160-liter stainless steel chamber for 7 hours/day, 5 days/week, for either 3 days or, 1, 2, or 4 weeks. Concentrations of vinyl bromide in the chamber were determined by infrared spectroscopy. Two control groups were exposed to room air. Immediately after the exposure, the surviving animals were killed, and macroscopic and microscopic examinations were performed on their organs and on fixed tissue specimens.

The rats exposed to vinyl bromide at 10,000 ppm became hypoactive during the 7-hour exposure period [127]. They seemed drowsy after 1 hour of the first exposure and looked "sluggish" by the 13th exposure. The control animals remained active throughout the exposure period. Exposed animals showed a significantly lower ($P<0.05$) weight gain than controls between the 15th and 20th days of exposure, and the difference was greater ($P<0.01$) after the 20th exposure. Gross examination of killed animals showed multifocal gray areas in the lungs, but the authors stated that no "compound related" tissue changes were observed microscopically.

In the second study, 2 groups of animals each consisting of 60 Charles River rats, 6 New Zealand white rabbits, and 6 cynomologus monkeys, all equally divided according to sex, were exposed to vinyl bromide at 250 or 500 ppm (1,095 or 2,190 mg/cu m) for 6 hours/day, 5 days/week, for 6 months [127]. A third group of 30 male and 30 female rats, 3 male and 3 female rabbits, and 4 female and 2 male monkeys was exposed to filtered room air. The experiments were conducted in the evening during the first 20 weeks of exposure and then were changed to daytime. The authors did not state whether the concurrent control animals were also switched to a daytime schedule. Vinyl bromide concentrations in the chamber were monitored by gas chromatography. Gross and microscopic examinations were performed on organs and fixed tissue of exposed animals at the end of the experiment. The animals exposed for 6 months were observed for changes in activity, body weight, indications of respiratory distress, eye and nasal irritation, and skin condition. Hematologic tests were performed on rats, rabbits, and monkeys in the control and 500-ppm groups prior to exposure and after 2, 10, and 24 weeks of exposure. All monkeys were examined for nonvolatile bromide in whole blood at the end of weeks 1, 2, 4, 8, 16, and 26.

At the end of the 6 months of exposures, all the animals exposed to vinyl bromide at 250 or 500 ppm showed weight increases at rates comparable with controls [127]. Only rats had a decrease in mean weight when the exposure schedule was changed from evening to daytime during the 20th week of exposure. Microscopic examination of major organs of all groups and species showed no changes that resulted from exposure to vinyl bromide. Analysis of blood showed elevated concentrations of bromide in all exposed animals, with monkeys that had been exposed at 250 and 500 ppm having the highest levels. No statistically significant changes were observed in the other measurements.

In the same report, Leong and Torkelson [127] summarized the results from unpublished data on the effects of vinyl bromide on rats. An unspecified number of rats was exposed to vinyl bromide at nominal concentrations of 100,000 (437,600 mg/cu m), 50,000 (218,800 mg/cu m), or 25,000 ppm (109,400 mg/cu m) for 1.5 and 7 hours. Two weeks after exposure to vinyl bromide at 25,000 or 50,000 ppm, the rats that survived were killed and examined for microscopic tissue changes.

Exposure at 100,000 ppm caused deep anesthesia and death within 15 minutes [127]. None of the rats exposed at 50,000 ppm for 1.5 hours died, but an unspecified number of deaths occurred during the 7-hour exposure. Within 25 minutes, the rats became unconscious. Vinyl bromide at 25,000 ppm anesthetized the rats, but they recovered rapidly when removed from exposure. Microscopic examination of tissues showed slight to moderate liver and kidney damage in rats exposed at 50,000 ppm. Examination of tissues from rats exposed at 25,000 ppm showed no abnormalities.

Leong and Torkelson [127] also gave male rats a 50% solution of vinyl bromide in corn oil by oral intubation to determine the LD50. They reported an LD50 of approximately 500 mg/kg but presented no supportive data. They also reported that vinyl bromide was irritating to the eyes but not to the skin of rabbits; data were not presented to support these findings.

(d) Vinyl Fluoride

In a book on the toxicity of anesthetics [128], Clayton reported that a mixture of 800,000 ppm (1,504 g/cu m) of vinyl fluoride in oxygen was not lethal for rats exposed to it for 12.5 hours. He also stated that unpublished data of Limperos (no further identification given) showed that male and female rats exposed to vinyl fluoride at concentrations of 100,000 ppm (188 g/cu m) for 7 hours/day, 5 days/week, for 6 weeks gained weight normally, exhibited no behavioral changes, had no fatalities, and had no tissue changes as evaluated by microscopic examination. Clayton did not supply details of these experiments.

In 1950, Lester and Greenberg [129] reported the effects of single exposures to vinyl fluoride on adult white rats. Rats were exposed for 30 minutes at concentrations ranging from 20 to 80% (200,000 to 800,000 ppm; 376

to 1,504 g/cu m) in an 11-liter glass chamber for 30 minutes. The rats were tested for any abnormalities in the postural, righting, and corneal reflexes after the exposure.

At the 30% (300,000 ppm; 564 g/cu m) concentration, the rats exhibited "hindleg instability" which the authors considered a sign of "slight intoxication" [129]. At 80%, the rats experienced difficulty in breathing but recovered after a 1-minute exposure to room air. Postural and righting reflexes were lost between 50 and 60% and between 60 and 70%, respectively. Loss of the postural reflex was also evident in rats exposed to vinyl fluoride at 80% for 12.5 hours, but these rats also regained the reflexes soon after breathing room air.

Results of an investigation on repeated exposures of rats to vinyl fluoride were summarized in a technical report from E I du Pont de Nemours and Company [130]. An unspecified number of rats were exposed to vinyl fluoride at a concentration of 100,000 ppm for 7 hours/day, 5 days/week, for a total of 30 exposures. There were no differences in the rate of weight gain, in the results of necropsies, of microscopic examinations of fixed tissues, of organ weights, or of clinical observations. It was concluded that vinyl fluoride did not constitute "much of" an inhalation hazard. However, the technical report did not present any data for evaluation.

(e) Vinylidene Fluoride

In a book on the toxicity of anesthetics [128], Clayton reported that a mixture of 800,000 ppm (2,096 g/cu m) of vinylidene fluoride in oxygen was not lethal to rats exposed to it for 19 hours. No experimental details or data were offered to support this statement.

Lester and Greenberg [129], in 1950, reported the effects of inhaled vinylidene fluoride on an unspecified number of adult white rats. The rats were exposed to vinylidene fluoride at concentrations ranging from 10% to 80% (100,000 to 800,000 ppm; 262 to 2,096 g/cu m) in an 11-liter glass chamber for 30 minutes. After the rats were removed from the chamber, their postural, righting, and corneal reflexes were tested.

The rats exposed to vinylidene fluoride at 10%-80% for 30 minutes lost none of the reflexes tested, but those exposed at concentrations of 40% (400,000 ppm; 1,048 g/cu m) or higher showed slight intoxication, which was manifested at 80% by the development of an unsteady gait without loss of the postural reflex [129]. Rats exposed at 80% for 19 hours showed no progressive signs of intoxication, and autopsies showed no evidence of pulmonary irritation.

Carpenter et al [118] reported the effects of short-term static inhalation exposures of Sherman albino rats weighing between 100 and 150 g to vinylidene fluoride. The authors reported that exposure to vinylidene fluoride at a concentration of 128,000 ppm (335.4 g/cu m) for 4 hours was sufficient to kill

two to four of the six test animals. From this observation, they concluded that vinylidene fluoride was slightly hazardous to rats. No further information was presented.

Burgison et al [131] studied the myocardial sensitizing properties of vinylidene fluoride in eight dogs and two cats. The animals were first injected with epinephrine and then exposed by inhalation to vinylidene fluoride at 250,000-500,000 ppm (655-1,310 g/cu m) for 5-15 minute . which time the epinephrine injection was repeated. ECG's, lead II, of h animal were recorded. None of the animals developed myocardial sensitization to epinephrine.

(f) Summary

The results of these studies show that each of the vinyl halides is capable of causing CNS effects. Changes in liver function and structure were also observed in some experiments. The adverse effects noted after exposure were similar to those noted in worker populations exposed to the vinyl halides.

Teratogenicity and Effects on Reproduction

Only two experiments with animals have been located in which the teratogenic, embryotoxic, and fetotoxic potentials of the vinyl halides were evaluated.

(a) Vinyl Chloride

John et al [132] evaluated the effects of the inhalation of vinyl chloride on mouse, rat, and rabbit embryonal and fetal development. Female CF-1 mice (25-30 g) were exposed at nominal concentrations of 50 or 500 ppm (128 or 1,280 mg/cu m), and Sprague-Dawley rats (250 g) and New Zealand white rabbits (3.5-4.5 kg) were exposed at nominal concentrations of 500 or 2,500 ppm (6,400 mg/cu m). Actual concentrations were determined by infrared spectrophotometry. Rats (20-35) and mice (30-40) were exposed on days 6-15 of gestation, and rabbits (15-20) were exposed on days 6-18 of gestation. Controls were matched to each exposure group and exposed in a similar manner to filtered room air. The authors examined the exposed dams for resorption sites in their uteri and the litters for the ratio of the sexes, body weights, and other gross features of the fetuses. The authors also examined the fetuses of each species for soft tissue and skeletal anomalies.

Maternal weight gain, food consumption, and final liver weights were decreased in mice exposed at 500 ppm (1,280 mg/cu m), but these effects were not observed at 50 ppm (128 mg/cu m) [132]. Ethanol (15%) in drinking water was also given to mice, rats, and rabbits exposed at 50 and 500 ppm, at 2,500 ppm (6,400 mg/cu m), and at 2,500 ppm, respectively. The authors stated that

ethanol altered the metabolism of vinyl chloride by blocking the primary and most rapid metabolic pathway. Ethanol with vinyl chloride at concentrations of 50 or 500 ppm decreased food consumption, weight gain, and liver weight in mice. In animals exposed to vinyl chloride at 50 and 500 ppm with 15% ethanol, weight gain and liver weight were lower than in those exposed to vinyl chloride alone. In rats exposed at 500 ppm, there was a significant decrease (P<0.05) in maternal weight gain during days 6-21 of gestation. At 2,500 ppm, the liver weight of the rats was increased significantly (P<0.05) at day 21 of gestation. There was also a significant difference in food consumption but no difference between exposed and control animals in weight gain. Rabbits showed a significantly decreased (P<0.05) food consumption rate at 500 ppm, but no effects were noted at 2,500 ppm. Giving ethanol in addition to exposure to vinyl chloride at 2,500 ppm significantly increased the effects on rabbits and rats. Maternal mortality in mice exposed at 500 ppm was significantly increased (P<0.05) over concurrent control mortality. Also, there was an increase (P<0.05) in the incidence of resorptions, a decrease (P<0.05) in fetal body weight, and a reduction (P<0.05) in litter size. No significant effects other than an increase in crown-rump length (P<0.05) were seen at 50 ppm. Fetuses from mice exposed to vinyl chloride at 500 ppm differed significantly (P<0.05) from concurrent control fetuses in incidences of unfused sternebrae, delays in ossification of the sternebrae, and delays in ossification of bones of the skull. The addition of 15% ethanol to the drinking water of the dams significantly increased (P<0.05) the incidence of skeletal anomalies in the fetuses of these mice, including anomalies in the sternebrae, vertebrae, and skull at 50 and 500 ppm, and in the ribs as well at 500 ppm.

There was only one maternal death in rats exposed to vinyl chloride at 2,500 ppm (6,400 mg/cu m) [132]. The only significant (P<0.05) fetal effects observed after exposure at 500 ppm (1,280 mg/cu m) were a reduction of the body weights, an increase in crown-rump length, and a significant increase (P<0.05) in the number of lumbar spurs. Examination of the fetuses for soft tissue anomalies showed a significant increase (P<.05) in the incidence of dilated ureters at 2,500 ppm. Skeletal anomalies at 2,500 ppm included significant decreases (P<0.05) in the incidence of unfused sternebrae and delayed skull ossifications.

One of the seven rabbits exposed to vinyl chloride at 2,500 ppm died during the experiment [132]. A significant decrease (P<0.05) was seen in the number of live fetuses/litter at 500 ppm, but not at 2,500 ppm. No other gross differences were observed. The incidence of delayed ossification of the fifth sternebra was significantly increased (P<0.05) at 500 ppm. No other skeletal or soft tissue anomalies were seen in rabbits.

John et al [132] concluded that exposure to vinyl chloride at concentrations causing some maternal toxicity did not cause teratogenic effects on or embryonal or fetal toxicity in mice, rats, or rabbits. However, it is apparent that adverse effects did occur on the fetuses of the mice exposed at 500 ppm (1,280 mg/cu m) (sternebrae and skull anomalies) and in the

fetuses of rats exposed at 2,500 ppm (6,400 mg/cu m) (dilated ureters). The authors' determination that these effects did not substantiate an embryotoxic or fetotoxic potential and were secondary to the maternal toxicity were not supported by clinical tests. The authors regarded the changes as minor skeletal and soft tissue variations and concluded that the incidence of "major" skeletal or soft tissue malformations was not significantly greater in exposed animals than in the control groups. In mice exposed at 500 ppm, there were significant increases in the incidence of resorptions and decreases in the fetal body weight and in litter size. In rats exposed at 500 ppm, there was a significant decrease in the fetal body weight and an increase in crown-rump length, and, in rabbits exposed at 500 ppm, there was a significant decrease in the number of live fetuses/litter. In both rats and rabbits, however, there was not a corresponding adverse effect at the 2,500-ppm exposure concentration. Ethanol at 15% in the drinking water enhanced the effects of inhaled vinyl chloride, but the authors concluded that maternal toxicity was more enhanced than was fetotoxicity.

(b) Vinylidene Chloride

In 1977, Murray [133] reported the preliminary results of a study of the effects of inhalation of vinylidene chloride on Sprague-Dawley rats and New Zealand white rabbits. Groups of 40, 28, and 26 pregnant rats were exposed for 7 hours/day to vinylidene chloride at 20, 80, and 160 ppm (79.4, 317.6, and 635.2 mg/cu m), respectively, during days 6-15 of gestation. Eighteen and 15 rabbits were exposed to vinylidene chloride for 7 hours/day at 80 and 160 ppm during days 6-18 of pregnancy. Controls for both species were exposed to filtered chamber air only. The animals were observed for changes in appearance and "demeanor," body weight gain, and food and water consumption. Fetuses were removed by cesarean section, the litters were counted, and the ratio of resorptions to implants, the sex ratio in the litters, and the body weights, crown-rump lengths, external abnormalities, and soft tissue and skeletal alterations of the offspring were noted. The skeletal examinations were performed on the skulls, vertebrae, and ribs.

After exposure at 80 or 160 ppm (317.6 or 635.2 mg/cu m), the rats showed a decrease in their food consumption and body weight gain ($P<0.05$) but an increase in their water consumption ($P<0.05$) [133]. The only change noted in rats exposed at 20 ppm was an increase in water consumption ($P<0.05$). The appearance and demeanor, body weight gain, and food consumption of the exposed animals were comparable with those of controls. Fetuses of rats exposed at 80 or 160 ppm had significantly increased numbers of skeletal abnormalities, but these were not seen in fetuses of rats exposed at 20 ppm (79.4 mg/cu m) Skeletal examinations showed significant increases ($P<0.05$) in the incidences of delayed ossification of the parietal bone, "wavy" ribs, and lumbar spurs in the 292 rats exposed to vinylidene chloride at 80 ppm. Exposures at 160 ppm caused significant increases ($P<0.05$) in delayed ossification of skull bones and cervical vertebrae and "wavy" ribs. No adverse effects were seen on rats exposed at 20 ppm.

For the rabbits exposed to vinylidene chloride at 80 and 160 ppm (317.6 and 635.2 mg/cu m), only those exposed at 160 ppm showed significant decreases (P<0.05) in body weight gain [133]. In addition, exposure at 160 ppm produced a significant increase (P<0.05) in the ratio of resorptions to implants, from 3% (4/115) in the controls to 31% (41/132) in the exposed rabbits. The author stated that high ratios of resorptions to implants in particular females were correlated with high loss of body weight. No increase in resorptions was noted at 80 ppm. Examination of fetuses from dams exposed at 160 ppm found significant (P<0.05) skeletal abnormalities characterized by a decreased incidence of delayed ossification of the fifth sternebrae and by an increased incidence of 13 pairs of ribs. The investigators concluded that only minor and "questionable" adverse effects resulted from exposure of rats and rabbits to vinylidene chloride at concentrations above 20 ppm. They suggested that 20 ppm should be considered as a no-adverse-effect concentration. They further noted that vinylidene chloride was embryotoxic by inhalation in both species, but that the embryotoxic effects were associated with exposure concentrations that were toxic to the mothers.

In another experiment, the investigators [133] gave 26 Sprague-Dawley rats access to vinylidene chloride at a concentration of 200 ppm (0.8 mg/liter) in drinking water during days 6-15 of pregnancy. Twenty-four control rats were given access to water alone. Similar observations to those noted in the inhalation exposures were reported. The only significant difference (P<0.05) reported was an increase in the fetal crown-rump length (controls 46.6 ±2.4 mm; exposed 47.8 ±1.3 mm). The authors concluded that vinylidene chloride in the drinking water was not teratogenic in rats and did not cause adverse effects on rat embryos.

(c) Vinyl Bromide, Vinyl Fluoride, and Vinylidene Fluoride

No reports of studies that examined the teratogenic potential of vinyl bromide, vinyl fluoride, or vinylidene fluoride have been located.

Carcinogenicity

Angiosarcoma of the liver has been shown to be a characteristic tumor in workers exposed to vinyl chloride. Other cancers, of the lungs and brain, have also been linked to vinyl chloride exposure, but these latter types are more common than the former and may have been induced by a variety of other factors. Laboratory animals exposed to the vinyl halides have also shown a wide variety of tumors, especially angiosarcoma of the liver.

(a) Vinyl Chloride

In 1971, Viola et al [112] investigated tumor formation in rats exposed to vinyl chloride by inhalation. Twenty-five 3-month-old male albino Wistar rats were exposed to vinyl chloride at a concentration of 30,000 ppm (76.8 g/cu m)

for 4 hours/day, 5 days/week, for 1 year; 25 similar rats served as controls. Rats were killed at unspecified intervals, and their tissues were examined microscopically. These exposed animals were apparently the same ones used in the study by Viola [111] that was discussed in Animal Toxicity.

Skin tumors, the most frequent types, were found in 65-70% of the animals after 10 months of exposure [112]. These tumors developed in the paraauricular region, and most were epidermoid carcinomas. The authors also found two mucoepidermoid carcinomas and one papilloma of the skin. In addition to the tumors, they found warty subauricular growths and papillary epithelial proliferation with progressive increases in the thickness of the epidermis in a few rats. In four rats, adenocarcinomas of the respiratory tract were found, and in one rat an epidermoid tumor was found. The authors also found osteochondromas in the metacarpal and metatarsal regions of all four limbs in five rats. None of the control rats developed any of the types of tumors observed in the exposed animals.

In addition to the carcinomas, Viola et al [112] observed the same range of nontumorigenic adverse effects that Viola had reported previously [111]. They [112] concluded that the tumorigenic potential of vinyl chloride was greatest for the "cutaneous system." They also suggested that the mucoepidermoid carcinomas could have been caused by ingestion of vinyl chloride from the fur during cleansing, with subsequent concentration of it in the salivary glands. The authors did not analyze their data statistically, and they did not present them in terms of adverse changes/animal. Either of these formats would have allowed a more accurate hazard assessment than is possible from the data as presented by the authors.

In 1974, Caputo et al [134] extended the previous studies of Viola [111] and Viola et al [112] by exposing 3-month-old Wistar rats of both sexes for 4 hours/day, 5 days/week, for 12 months to vinyl chloride at concentrations of 20,000, 10,000, 5,000, 2,000, 500, or 50 ppm [134]. A minimum of 150 animals was exposed at each concentration.

At 50 ppm (128 mg/cu m), no tumors were observed among 200 exposed animals [134]. At 500 ppm (1,280 mg/cu m) and above, a significantly greater ($P<0.03$) percentage of tumors was seen in the exposed animals than was seen in a group of control animals. Tumors most frequently observed included squamous cell carcinoma of the skin, angiosarcoma of the liver and adenocarcinoma of the lung. The authors stated that rabbits exposed to vinyl chloride at concentrations of 10,000 ppm (25.6 g/cu m) also had significantly increased incidences ($P<0.02$) of acanthoma of the skin and adenocarcinoma of the lung appearing during the 9th through the 15th months of exposure. No further information concerning this experiment was reported.

The authors [134] concluded that these results clearly confirmed the carcinogenicity of vinyl chloride and that almost all tissues and organs were sensitive to it. Angiosarcoma of the liver appeared in 4/150 animals (3%) exposed at 500 ppm, 10/200 (5%) at 2,000 ppm (5,120 mg/cu m), 12/200 (6%) at

5,000 ppm (12.8 g/cu m), 16/200 (8%) at 10,000 ppm (25.6 mg/cu m), and 18/150 (12%) at 20,000 ppm (51.2 g/cu m). The prevalences of other tumors showed similar increases with increasing concentrations of vinyl chloride, indicating dose-related responses.

In 1976, Maltoni [135] reported interim and final results of a series of 17 experiments designed to evaluate the carcinogenic properties of vinyl chloride. A detailed plan for the conduct of these experiments [136] and preliminary results [137-139] had previously been published by Maltoni and coworkers. The experiments included inhalation exposure of Sprague-Dawley and Wistar rats, Swiss mice, and golden hamsters to vinyl chloride at concentrations of up to 30,000 ppm (76.8 g/cu m) for 4 hours/day, 5 day/week, for 30-52 weeks. Observation periods in some cases were as short as 46 weeks, because the experiments had not yet been concluded. Other experiments with Sprague-Dawley rats included inhalation exposures at concentrations of 6,000 or 10,000 ppm (15.4 or 25.6 g/cu m) for a total of 100 hours on various schedules; inhalation exposures of pregnant rats at 6,000 or 10,000 ppm for 4 hours/day during days 12-18 of gestation and subsequent examination of the fetuses; and oral administration of vinyl chloride in olive oil at doses of 0.03-50 mg/kg.

The most common malignant tumors seen in Sprague-Dawley rats were Zymbal gland carcinoma (26%), angiosarcoma of the liver (22%), and nephroblastoma (9%), as indicated in Table III-2 [135]. It should be noted that most other species do not have Zymbal glands. With increasing exposure, the percentage of animals with tumors generally increased and the average latent period generally decreased. Angiosarcoma of the liver developed in 1 of 59 rats exposed at 50 ppm (128 mg/cu m) for 52 weeks, with a mean latency of 135 weeks, and in 18 of 60 rats (30%) exposed at 30,000 ppm (76.8 g/cu m) for 52 weeks, with an average latency of 53 weeks. None of the 480 animals exposed at concentrations below 50 ppm had been observed for longer than 46 weeks at the time of publication, and no tumors were seen in them. Preliminary information [139] on tumor formation in the Wistar rats (Table III-3) indicated that these animals might have been less susceptible to vinyl chloride than the Sprague-Dawley rats; however, with the data presented, it was not possible to determine this likelihood mathematically.

Decreased latency with increasing exposure was also observed in the Swiss mice (Table III-4); latency ranged from 51 weeks for lung tumors in animals exposed for 30 weeks at 50 ppm (128 mg/cu m) to 36 weeks for animals exposed at 10,000 ppm (25.6 g/cu m) for the same period [135]. However, the percentage of animals with each tumor reached a maximum concentrations that were different for each tumor. The majority of the tumors in mice were adenomas of the lungs (45%). Angiosarcoma of the liver accounted for 12% of the tumors observed in these animals. The golden hamsters (Table III-5) did not show a dose-related response for tumor induction, and the latent periods seen in this species were not reported.

TABLE III-2

TUMORS OBSERVED IN SPRAGUE-DAWLEY RATS
EXPOSED TO VINYL CHLORIDE BY INHALATION*

Concentration (ppm)	No. Animals Exposed**	Animals with Tumors										
		Zymbal Gland Carcinomas			Nephro-blastomas			Liver Angiosarcomas			All Tumors***	
		No.	%	Latency (wk)	No.	%	Latency (wk)	No.	%	Latency (wk)	No.	%
30,000	60	35	58	43	0	0	-	18	30	53	51	85
10,000	61	16	26	50	5	8	59	9	15	64	38	62
6,000	60	7	12	62	4	7	65	13	22	70	31	52
2,500	59	2	3	33	6	10	74	13	22	78	32	54
500	59	4	7	79	4	7	83	7	12	81	22	37
250	59	0	0	-	6	10	80	4	7	79	16	27
50	59	0	0	0	1	2	135	1	2	135	10	17
Controls	58	0	0	-	0	0	-	0	0	-	6	10

*Results after 135 wk in animals exposed 4 hrs/d, 5 d/wk, for 52 wk
**Number of animals surviving at 24 wk, when first tumor was observed
***Includes angiosarcomas of other sites, angiomas, fibroangiomas, fibroadenomas, adenomas, carcinomas, papillomas, hepatomas, neuroblastomas, neurilemmomas, ependymomas, rhabdomyosarcomas, lymphomas; several animals had two or more tumors

Adapted from Maltoni [135]

TABLE III-3

TUMORS OBSERVED IN WISTAR RATS
EXPOSED TO VINYL CHLORIDE BY INHALATION*

Concentration (ppm)	No. Animals Exposed	No. Animals with Tumors**			
		Zymbal Gland Carcinomas	Nephro-blastomas	Liver Angiosarcomas	All Tumors***
10,000	30	1 (10/69)	1 (3/69)	8 (4/69)	11 (20/69)
6,000	30	0 (3/72)	3 (3/72)	2 (3/72)	8 (13/72)
2,500	30	0 (1/74)	0 (4/74)	3 (5/74)	4 (14/74)
500	30	0 (2/67)	0 (2/67)	3 (0/67)	4 (5/67)
250	30	0 (0/67)	0 (1/67)	1 (1/67)	3 (1/67)
50	30	0 (0/64)	0 (0/64)	0 (0/64)	1 (1/64)
Controls	40	0 (0/68)	0 (0/68)	0 (0/68)	2 (0/68)

*Results after 95 wk in animals exposed 4 hr/d, 5 d/wk, for 52 wk
**Numbers in parentheses indicate proportion of Sprague-Dawley rats at the same exposures with tumors after 95 wk
***Includes angiosarcomas of other sites, adenomas, hepatomas, mesotheliomas, fibroangiomas, carcinomas, lymphomas, pericytosarcomas; several animals had two or more tumors

Adapted from Maltoni [139]

TABLE III-4

TUMORS OBSERVED IN SWISS MICE
EXPOSED TO VINYL CHLORIDE BY INHALATION*

Concentration (ppm)	No. Animals Exposed**	Animals with Tumors									
		Lung Tumors			Mammary Carcinomas			Liver Angiosarcomas		All Tumors***	
		No.	%	Latency (wk)	No.	%	Latency (wk)	No.	%	No.	%
10,000	50	35	70	36	13	47	31	8	16	36	72
6,000	54	38	70	38	8	28	33	5	9	39	72
2,500	53	30	57	43	9	30	35	11	21	31	58
500	58	38	66	41	7	24	37	11	19	43	74
250	58	33	57	45	11	32	39	11	19	40	69
50	57	2	4	51	12	33	43	1	2	18	32
Controls	141	8	6	44	0	0	-	0	0	13	9

*Results after 81 wk in animals exposed 4 hr/d, 5 d/wk, for 30 wk
**Number of animals surviving at 16 wk, when first tumor was observed
***Includes angiosarcomas of other sites, angiomas, fibroangiomas, adenomas, carcinomas, papillomas, acanthomas, adenocarcinomas, basaliomas, leiomyosarcomas, lymphomas; several animals had two or more tumors

Adapted from Maltoni [135]

TABLE III-5

TUMORS OBSERVED IN GOLDEN HAMSTERS
EXPOSED TO VINYL CHLORIDE BY INHALATION*

Concentration (ppm)	No. Animals Exposed	No. Animals with Tumors			
		Liver Angiosarcomas	Skin Tricho-epitheliomas and Basaliomas	Forestomach Epithelial Tumors	All Tumors**
10,000	35	0	6	4	4
6,000	32	1	2	7	10
2,500	33	0	1	10	12
500	33	2	3	7	12
250	32	0	3	2	6
50	33	0	6	4	10
Controls	70	0	2	0	5

*Results after 105 wk in animals exposed 4 hr/d, 5 d/wk, for 30 wk
**Includes angiomas, fibroangiomas, adenocarcinomas, melanomas, lymphomas, hepatomas; several animals had two or more tumors

Adapted from Maltoni [135]

The Sprague-Dawley rats exposed at 6,000 or 10,000 ppm (15.4 or 25.6 g/cu m) had been followed for only 59 weeks at the time of this report [135]. Zymbal gland carcinoma was observed in some exposed animals, but no nephroblastoma or angiosarcoma of the liver was found. The preliminary results shown in Table III-6 suggest that exposure at 6,000 ppm for 100 hours during 25 weeks has a lower carcinogenic potential than exposure at 10,000 ppm on the same schedule or at 6,000 ppm for 100 hours during 5 weeks. These findings indicate that the severity of exposure was more important than the total mass to which the rats were exposed and suggest that metabolic and excretory processes may affect the carcinogenic potential; however, more complete data are needed to substantiate this inference.

The offspring of pregnant rats exposed to vinyl chloride at concentrations of 6,000 or 10,000 ppm (15.4 and 25.6 g/cu m) for 4 hours/day from the 12th to the 18th day of gestation developed tumors [135]. In 54 offspring from dams exposed at 10,000 ppm, the following tumors developed: 3 Zymbal gland carcinomas, 1 nephroblastoma, 1 subcutaneous angiosarcoma, 1 angiosarcoma of the leg, 1 Zymbal gland fibrosarcoma, and 1 ovarian leiomyosarcoma. In the 32 offspring from dams exposed at 6,000 ppm, 1 Zymbal gland carcinoma, 1 subcutaneous angiosarcoma, 1 intraabdominal angiosarcoma, 1 Zymbal gland adenoma, 1 skin carcinoma, 1 subcutaneous fibrosarcoma, and 1 mammary carcinoma were observed following a 143-week observation period. Unfortunately, details of this experiment, including reproduction indices and signs of toxicity to the dams, were not reported. The author's suggestion that the results of this experiment indicate that vinyl chloride has a transplacental effect is reasonable, but it cannot be thoroughly evaluated without more detailed information than was presented.

The experiments outlined by Maltoni and coworkers [135-139] were well designed. The presentation of data in these papers, however, was often confusing, and the information contained in the tables frequently disagreed with that presented in the text. It was often unclear whether they were presenting the number of animals with tumors or simply the number of tumors. Furthermore, preliminary data on tumors when the followup period was less than the probable latent period are of little value. Maltoni's observations and data do show that vinyl chloride induces various tumors in a variety of rodents, and that angiosarcoma of the liver is a characteristic lesion induced by vinyl chloride. The data also indicate that there are strain and species differences in the magnitude of the tumorigenic response elicited by exposure to vinyl chloride.

Lee et al in 1977 [140] and in 1978 [141] reported results of inhalation studies on 2-month-old albino CD-1 mice and CD rats exposed to vinyl chloride (99.8% pure, Matheson Gas Products) at 50, 250, or 1,000 ppm (128, 640, or 2,560 mg/cu m). Groups of 36 females and 36 males of each species were exposed for 6 hours/day, 5 days/week, for 12 months. Two similar control groups were exposed to uncontaminated air. Throughout the exposure period, the animals were observed for changes in weight gain, food consumption, and

TABLE III-6

TUMORS OBSERVED IN SPRAGUE-DAWLEY RATS
EXPOSED TO VINYL CHLORIDE BY INHALATION
FOR 100 HOURS ON VARIOUS SCHEDULES*

Concentration (ppm)	Exposure Schedule	No. Animals Exposed	No. Animals with Tumors		
			Zymbal Gland Carcinomas	Liver Angiosarcomas	All Tumors**
10,000	4 hr/d, 5 d/wk x 5 wk	120	4	0	4
10,000	1 hr/d, 4 d/wk x 25 wk	120	4	0	4
10,000	4 hr/d, 1 d/wk x 25 wk	120	2	0	3
6,000	4 hr/d, 5 d/wk x 5 wk	120	3	0	4
6,000	1 hr/d, 4 d/wk x 25 wk	120	0	0	1
6,000	4 hr/d, 1 d/wk x 25 wk	120	0	0	1
Controls	-	249	0	0	0

*Results after 59 wk
**Includes 1 angiosarcoma, 1 angiopericitosarcoma, 2 lymphomas

Adapted from Maltoni [135]

mortality. Four animals of each species and sex exposed at each concentration were killed at the end of exposure months 1, 2, 3, 6, and 9. Their organs were examined grossly, and tissues were fixed and examined microscopically. Additional laboratory determinations, including hematologic and blood chemistry examinations, cytogenetic analyses of bone marrow cultures, pulmonary macrophage counts, DNA synthesis assays, and urinary analyses were performed at the interim examinations and at the termination of the experiment at 12 months. Roentgenograms of the limbs of those animals exposed for the longest periods were made also.

Of the mice exposed at 1,000 ppm (2,560 mg/cu m), two males and one female died between the 3rd and 9th days of exposure [140]. Between the 6th and 9th months, 13 male and 21 female mice died or were removed from exposure because their health had deteriorated. By the end of the 9th month, all animals had been removed from exposure.

By the 6th month, the most evident effects on exposed mice were rough hair coat, lethargy, loss of appetite, and rapid weight loss [140]. Additionally, abdominal distention and external tumor masses, such as mammary tumors in females, were noticeable between the 7th and 9th months. During the first 8 months of the experiment, exposed and nonexposed mice showed comparable weight gains; however, the exposed group showed a decline in weight by the 9th month. Also, by the 9th month of exposure, one female and most of the male mice had elevated pulmonary macrophage counts (from pulmonary washings) and had developed bronchioloalveolar adenomas.

Microscopic examination of hepatic and renal tissues from one female and two male mice that died after being exposed at 1,000 ppm for 3-9 days revealed a number of lesions characterized by acute toxic hepatitis, focal to marked congestion, diffuse coagulation necrosis of hepatocytes in the centrilobular area, and tubular necrosis in the renal cortex [140]. During the 8th and 9th months of exposure, mitotic figures were observed in mouse livers, but were not seen in livers of mice killed at other times.

Bronchioloalveolar adenomas were observed in 48 of the 72 mice exposed at 1,000 ppm, whereas only 1 of the 72 control mice developed this tumor [140]. These tumors were first noted during the 2nd month of exposure to vinyl chloride and in the 9th month in the control. Mammary tumors, first observed in exposed female mice during the 6th and 7th months, included adenocarcinoma and carcinoma of squamous cells and of anaplastic cells; metastasis was prevalent to the lungs and pleurae. None of the control mice developed mammary tumors. Angiosarcoma of the liver was found in 31 exposed mice, being observed first during the 6th month of exposure. In addition, angiosarcoma was occasionally seen in the mammary glands, heart, gastrointestinal tract, pancreas, kidneys, epididymis, testis, mesenteric lymph nodes, and skeletal muscles. Angiosarcoma of the liver was more prevalent in females than in males. Two male and three female mice developed malignant lymphoma between the 6th and 9th months of exposure, whereas none were seen in controls.

All female mice exposed to vinyl chloride at 250 ppm (640 mg/cu m) died or had to be removed from exposure by the 9th month because of morbidity [140]. Male mice were more resistant to the toxic actions of vinyl chloride than female mice, and some survived the 12-month exposures. No differences in body weight between the exposed and control mice were noted. One female mouse examined after the 9th month of exposure showed an increased pulmonary macrophage count. Bronchioloalveolar adenomas were first detected in exposed mice during the 2nd month; a total of 48, 22, and 12 mice exposed at 1,000, 250, or 50 ppm, respectively, developed this tumor. Only one control male mouse developed bronchioalveolar adenoma during the 9th month. Female mice also developed mammary tumors consisting of adenocarcinomas and squamous and anaplastic cell carcinomas. Of the mice exposed at 250 ppm, 23 (16 females) developed liver angiosarcomas, which were first evident after the 6th month of exposure. During the 9th month of exposure, malignant lymphomas developed in two female mice.

Between the 6th and 12th months of exposure to vinyl chloride at 50 ppm (128 mg/cu m), 6 male and 14 female mice either died or were removed from exposure because of deteriorating health [140]. Throughout the exposure period, the body weights of exposed mice were comparable with those of control mice. Microscopic examination also showed mitotic figures in the liver during the 8th and 9th months of exposure. A significant increase in DNA synthesis, as measured by incorporation of 14C-thymidine, was detected in the livers of male mice exposed at 50 ppm for 11 months. There was no increase in the number of mitotic figures in the livers of these mice. During the 12th month of exposure, one of two surviving male mice had an elevated pulmonary macrophage count.

Bronchioloalveolar adenomas were seen in 12 mice exposed at 50 ppm (128 mg/cu m) [140]. Three male mice developed angiosarcoma of the liver. Mammary tumors were observed in nine of the exposed mice, the first at 7 months. One female mouse developed a malignant lymphoma during the 6th month and another developed a hemangioma in the mediastinum. Control mice developed none of the tumors discussed.

From the effects observed in mice exposed to vinyl chloride at 1,000, 250, and 50 ppm (2,560, 640, and 128 mg/cu m), the authors [140] concluded that weight loss, mortality, and tumor incidence were dependent on the concentration of vinyl chloride and the duration of exposure. Vinyl chloride inhaled at 50-1,000 ppm for 6 hours/day, 5 days/week, was found to be highly carcinogenic in mice. The duration of exposure before tumors were observed varied from 2 months for bronchioloalveolar adenomas to 6 months for mammary gland tumors and for angiosarcoma. The latter tumors occurred first in the liver and then in other organs. Angiosarcoma was more prevalent in female mice than in male mice exposed at 250 or 1,000 ppm. It was found mostly in the livers of mice exposed to vinyl chloride for 7-9 months. Mammary gland tumors were considered to be a complex type and were characterized by anaplastic and squamous cell metaplasia during the early stages of their development. Metastasis of anaplastic and squamous cell carcinomas to the

lungs was common. Bronchioalveolar adenomas occurred in both male and female mice at a very young age and after a short period of exposure. More deaths and tumors were observed at 250 and 1,000 ppm than at 50 ppm. Other types of tumors observed in the exposed mice were hepatic cell carcinomas, renal adenomas, and keratoacanthomas of the skin.

In the second part of the experiment, Lee et al [140] exposed 72 adult male and female rats to vinyl chloride at concentrations of 50, 250, or 1,000 ppm (128, 640, or 2,560 mg/cu m) for up to 12 months, using the same exposure schedule previously reported. During the first 7 months of exposure, no remarkable adverse effects were seen in any rats. However, of the rats exposed at 1,000 ppm, 8 male and 13 female rats either died or were removed from exposure during the 8th-12th months. None of the controls died during the experiment. After the 4th week of exposure, female rats had gained less weight than the controls. During the 9th month, the first cases of angiosarcoma of the liver were observed in four rats. By the end of the study, 22 rats had developed angiosarcoma of the liver, more females than males having the tumors. Of the 22 rats with angiosarcoma of the liver, 13 also developed angiosarcoma of the lungs. Additional angiosarcoma was found in the omentum of one rat, and two rats had hemangiomas in the adrenal glands.

Four male and 10 female rats died or were removed from exposure during months 8-12 of exposure to vinyl chloride at 250 ppm (640 mg/cu m) [140]. None of the control rats died during the exposure period. Body weight gain by exposed rats was comparable with that by the controls. Two rats developed angiosarcoma of the liver during the 9th month of exposure. By the end of the 12th month of exposure, 13 rats, 10 of them females, had developed angiosarcoma of the liver. Of the 10 female rats, 3 also had angiosarcoma of the lungs; angiosarcoma of the omentum or mesentery was also found in two rats. None of the control rats had angiosarcoma.

During months 8-12 of exposure to vinyl chloride at 50 ppm (128 mg/cu m), two female rats died [140]. Exposed and control rats had comparable body weight gain. Subcutaneous angiosarcoma was found in two rats. No angiosarcoma of the liver, lungs, or other organs was observed in any of the rats exposed at 50 ppm, nor did any of the control rats develop angiosarcoma.

None of the laboratory tests performed on rats exposed to vinyl chloride at 1,000, 250, or 50 ppm showed any persistent changes [140]. The authors indicated that female rats were more sensitive to the toxic action of vinyl chloride and that more females than males died between the 8th and 10th months of exposure. In general, rats were considered more resistant to both the carcinogenic and toxic actions of vinyl chloride than mice.

The experiments by Lee et al [140,141] indicate that vinyl chloride is carcinogenic in mice and rats, and that mice are more susceptible to its carcinogenic effects than rats. Since the experiments were conducted at various exposure concentrations, it can be concluded that the different responses probably were a function of species-specific factors and were not

caused by structural differences between the species such as lung surface area. Since there are differences between species, there may be differences between strains within a species also. Such a situation would account for the apparent inconsistencies between the reports of various authors using different strains of the same species. This confounding factor must be considered in any attempt to extrapolate the results from animal experiments to human exposure situations.

Maltoni et al [137], in 1975, gave vinyl chloride by gastric intubation to 13-week-old Sprague-Dawley rats. Groups of 40 male and 40 female rats were given 50.00, 16.65, or 3.33 mg/kg of vinyl chloride dissolved in olive oil 5 times/week for 52 weeks. A fourth group of rats, 40 males and 40 females, was given olive oil without vinyl chloride as the control group. The number of tumors that developed in the rats of each group were recorded.

At 50 weeks, no tumors were apparent in the rats given the lowest dose (3.33 mg/kg), but one of the male rats given 16.65 mg/kg developed angiosarcoma of the liver, which was identified during the 49th week of the experiment, and a female rat given 50 mg/kg had angiosarcoma in the thymus.

In an update of this study [137], Maltoni [135] in 1976 reported that after 84 weeks the rats receiving the 50.00-mg/kg dose had developed eight cases of angiosarcoma of the liver and four other tumors. At 16.65 mg/kg, angiosarcoma of the liver had developed in five rats, a Zymbal gland carcinoma in one, and a nephroblastoma in one. No tumors were observed in animals given 3.33 mg/kg.

(b) Vinylidene Chloride

Lee et al in 1976 [140] and 1978 [141] described the effects of inhaled vinylidene chloride (99% pure, Aldrich Co.) on 36 male and on 36 female 2-month-old albino CD-1 mice and 36 male and 36 female albino CD rats exposed at 55 ppm (218.4 mg/cu m) for 6 hours/day, 5 days/week, for 12 months. Vapor concentrations were monitored by gas-liquid chromatography during the exposures. Control animals were exposed to uncontaminated air. Organs and tissues from rats and mice were examined for microscopic changes at the end of the 1st, 2nd, 3rd, 6th, 9th, and 12th months of exposure.

Two male mice died on the 13th day of exposure and were replaced in the study with healthy males [140]. Microscopic examination showed a number of lesions in the two dead male mice. They were characterized by acute toxic hepatitis, focal to marked congestion, marked diffuse coagulation necrosis of hepatocytes, and marked tubular necrosis in the renal cortex. No additional deaths occurred, nor did any control animals die. Six mice were observed to have small nodules of bronchioalveolar adenoma by the 12th month of exposure; only one control male mouse developed a bronchioalveolar adenoma. The mice removed from exposure after the 9th month (two males) and 10th month (one

female) had developed angiosarcoma in the liver. Neither mammary tumors nor malignant lymphomas were found.

After exposures at 55 ppm (218.4 mg/cu m), fatty changes were seen in the livers of rats [140]. Two rats had also developed extrahepatic angiosarcoma (mesenteric lymph node, subcutaneous) by the end of the exposure period. None of the control rats developed liver tumors of any type.

In 1977, Maltoni et al [142] reported the research plan and preliminary results for a series of experiments with vinylidene chloride. Groups of 60-120 Sprague-Dawley rats were exposed by inhalation to vinylidene chloride at concentrations of 10, 25, 50, 100, or 150 ppm (39.7, 99.3, 198.5, 397, or 595.5 mg/cu m) for 4 hours/day, 4-5 days/week, for 12 months. An increased incidence of mammary tumors was noted in the exposed animals; however, there was no apparent dose-related effect. One Zymbal gland carcinoma was found in an animal exposed at 100 ppm.

Swiss mice were exposed to vinylidene chloride at 10 and 25 ppm (39.7 and 99.3 mg/cu m) on the same schedule [142]. Exposure of these mice at concentrations of 50 ppm or higher produced an unacceptably high mortality in the study population within 4 days. Adenocarcinoma of the kidneys was observed in two groups exposed at 25 ppm with an incidence of 8% and 4%. None of these tumors were observed in animals exposed at 10 ppm or in control animals.

Sprague-Dawley rats were given vinylidene chloride in olive oil by gavage at dose rates of 0.5, 5, 10, and 20 mg/kg/day, 4-5 days/week for 52 weeks [142]. No increase in mammary tumors was observed; one rat developed a Zymbal gland carcinoma at the 10-mg/kg dose.

Although Lee et al [140,141] had reported that their mice were exposed at 55 ppm of vinylidene chloride, Maltoni et al [142] found that exposure of his mice at concentrations of 50 ppm or more produced unacceptable mortalities within 4 days. Maltoni's mice exposed at 25 ppm of vinylidene chloride for 12 months did not develop angiosarcoma of the liver, whereas Lee et al stated that 3 of 72 mice exposed at 55 ppm for 12 months did develop this tumor. The discrepancies in the results of these studies may be caused by differences in the strains of animals used, and has been discussed previously. Maltoni et al [142] stated that neither inhalation exposures nor ingestion experiments with rats demonstrated a specific carcinogenic effect from vinylidene chloride such as had been demonstrated for vinyl chloride (Zymbal gland carcinoma and angiosarcoma of the liver). It should be remembered that these data are preliminary for rats, and that a longer followup period may reveal that these tumor types will be formed. The authors also stated that mice were susceptible to a specific carcinogenic effect, adenocarcinoma of the kidney, and that this species difference probably resulted from a more "favorable metabolic condition for expressing the oncogenic potentiality." The authors pointed out that further research using different species was necessary to evaluate this hypothesis.

(c) Vinyl Bromide

In 1977, BL Van Duuren, in a written communication to NIOSH, reported unpublished results of bioassays with vinyl bromide. Thirty female ICR/Ha Swiss mice were injected subcutaneously (sc) with 25 mg of vinyl bromide in 0.05 ml of trioctanoin once a week for 420 days, and none of the mice developed tumors at the site of injection.

Van Duuren also tested the effect of vinyl bromide on mouse skin. Thirty female ICR/Ha Swiss mice had vinyl bromide in acetone (15.0 mg/0.1 ml) applied to their skin three times/week for 420 days. Control mice received no exposures. After 412 days of applications of vinyl bromide, one mouse developed a papilloma and another developed a subcutaneous fibrosarcoma. None of the control mice developed skin tumors. These data indicate that vinyl bromide is not carcinogenic by these routes of exposure.

Dorato et al [143], in 1978, submitted to NIOSH the 1-year interim results of a 2-year study on the inhalation toxicity of vinyl bromide. Rats were exposed to vinyl bromide at nominal concentrations of 1,250, 250, 50, and 10 ppm (5,475, 2,095, 219, and 43.8 mg/cu m) for 6 hours/day, 5 days/week. Hematologic values did not show dose-related effects that were attributed by the authors to the vinyl bromide exposure, and the only clinical chemistry abnormalities considered significant were elevations in serum LDH and bromide in the rats exposed at 1,250 ppm. These rats also showed decreases in body weight beginning at week 16 for the females and week 45 for the males.

Increased liver weights were noted in the rats exposed to vinyl bromide at 250 and 1,250 ppm, and increased spleen weights were noted in rats exposed at 50, 250, and 1,250 ppm [143]. Angiosarcoma of the liver seen in 9/48 rats exposed at 1,250 ppm and in 2/30 exposed at 250 ppm. Zymbal gland carcinoma was observed in 8/47 exposed at 1,250 ppm and in 2/30 exposed at 250 ppm. Lung, mammary, and brain tumors were also observed in these animals. Metastatic angiosarcoma was present in the lungs of four animals. The authors concluded that vinyl bromide after exposure for up to 52 weeks at 250 and 1,250 ppm had a carcinogenic effect on rats.

A comparison of the frequencies of angiosarcomas at these exposure concentrations in this experiment [143] with the frequencies of angiosarcoma observed in rats exposed to vinyl chloride after a similar followup period [139] suggests that vinyl bromide may be more effective in inducing this tumor than vinyl chloride. Further comparison with the vinyl chloride data indicates that after a longer observation period, angiosarcoma will be seen in those rats exposed to vinyl bromide at lower concentrations.

(d) Vinyl Fluoride and Vinylidene Fluoride

No reports examining the carcinogenic potential of vinyl fluoride or vinylidene fluoride have been located.

Mutagenicity

Each of the vinyl halides has been shown to be mutagenic in one or another test system. The mutagenic activity of these compounds increases with metabolic activation by mammalian microsomal systems, showing that metabolites as well as the parent compounds have mutagenic potential.

(a) Vinyl Chloride

Because the reported carcinogenic action of vinyl chloride in workers attracted considerable attention, several investigators became interested in evaluating its mutagenicity and that of its known or presumed metabolites. Ames et al [144] showed that many known carcinogens activated by mammalian liver enzymes produced back mutations in auxotrophs of the bacterium *Salmonella typhimurium*. Studies of the genetic activities of vinyl chloride and some of its metabolites have been performed in several bacterial species, yeasts, *Neurospora*, *Drosophila*, mammalian cell cultures, and mice.

In 1974, Rannug et al [145] used the system described by Ames and his coworkers [144] to investigate the mutagenicity of vinyl chloride. Four histidine-requiring strains of *Salmonella typhimurium* were exposed to vinyl chloride under various experimental conditions and then cultured on histidine-deficient media to determine the frequency of back mutations to histidine independence. Strain TA1535 reverts to histidine independence by a base-pair substitution, while in the other strains used, TA1536, TA1537, and TA1538, reversion results from addition or deletion of a base pair (frameshift mutation). In addition to their inability to synthesize histidine, these test strains include a mutation that increases the permeability of the cell and another that decreases the repair of damaged DNA; these deletions enhance the sensitivity of the bacteria to certain mutagenic agents.

Because bacteria may not duplicate mammalian metabolic processes in activating potentially mutagenic substances, the substances under test were incubated with a 9,000 x gravity (G) microsomal extract from the livers of Sprague-Dawley rats [145]. For some experiments, a microsomal system was produced by mixing the microsomal supernatant with a dihydronicotinamide adenine dinucleotide phosphate (NADPH) generating system.

In preliminary tests with strain TA1535, Rannug and coworkers [145] bubbled vinyl chloride gas through either water or a suspension containing the microsomal system, or they exposed the bacteria for 75 minutes to an atmosphere containing 11% (281.6 g/cu m) vinyl chloride. The vinyl chloride was analyzed by gas chromatography and mass spectroscopy and was reported to be of "very high" purity, containing only trace amounts of isopropanol. In a subsequent experiment, strain TA1535 was exposed to an atmosphere of 20% vinyl chloride with the microsomal system or with the microsomal supernatant with no NADPH-generating system, and to vinyl chloride alone, for intervals of 30, 60, or 90 minutes. Controls were incubated with the microsomal system without vinyl chloride. Finally, all four strains were exposed for 90 minutes under

similar conditions. To evaluate the lethality of the vinyl chloride preparations, identically treated bacteria were cultured on a medium containing histidine, and the number of surviving cells was determined. To evaluate the comparative mutagenicity of the vinyl chloride preparations, histidine-independent mutant colonies were counted on five plates of minimal media for each test, and the number of mutants/100 million surviving cells was compared to the spontaneous mutation rate using the Student's t-test.

In the initial tests, Rannug et al [145] found that vinyl chloride dissolved in water or directly in the microsomal suspension had no effect on the rate of back mutation to histidine independence in strain TA1535. However, exposure to an atmosphere of 11% vinyl chloride gas for 75 minutes resulted in 17.6 ±2.6 (SE) mutants/100 million surviving cells, compared with 5.3 ±0.2 in controls, significant at the 1% level (P<0.01). A mutation rate two to three times that of controls was observed in strain TA1535 exposed to 20% vinyl chloride in the presence of a microsomal system for intervals of 30 minutes (P<0.01) and 60 or 90 minutes (P<0.001). Bacteria exposed to vinyl chloride without the microsomal system showed no increase in mutagenic response. Exposure of strain TA1535 to vinyl chloride and the microsomal supernatant in the absence of the NADPH-generating system also caused no significant change in the mutation rate. In the final experiment, involving all four test strains, only TA1535 showed a mutagenic response to vinyl chloride. At the concentrations tested, vinyl chloride with the microsomal system did not affect bacterial survival on a complete medium, although vinyl chloride alone reduced the survival rate slightly.

Rannug et al [145] concluded that vinyl chloride was mutagenic in Salmonella only after metabolic activation by mammalian microsomal enzymes. They suggested that the most plausible primary metabolite from vinyl chloride would be chloroethylene oxide, which could be formed in the NADPH-dependent oxidation by microsomal enzymes. Since only strain TA1535 was affected, the mutagenically active metabolites of vinyl chloride appeared to be capable of causing base-pair substitutions but not frameshift mutations. The ineffectiveness of water solutions in inducing mutations was attributed to the low solubility and high volatility of vinyl chloride.

In 1975, Bartsch et al [146] reported tests of vinyl chloride on Salmonella, in which they evaluated the mutagenic response as a function of dose and exposure duration and compared the effectiveness of several mammalian tissue fractions in manifesting the mutagenic potential of vinyl chloride. The Salmonella typhimurium strains used in this series of experiments were TA1535, TA1530, and G-46, which are reverted to histidine-independence by base-pair substitutions, and TA1538, which is reverted by a frameshift mutation. Bacteria were exposed to vinyl chloride (99.9% pure) at nominal concentrations of 0.2, 2.0, and 20% (5.1, 51.2, and 512 g/cu m) in air for up to 48 hours. Gas chromatography showed that these nominal concentrations of vinyl chloride in air produced vinyl chloride concentrations in the media of 0.04, 0.4, and 4 millimoles, respectively, after 6 hours, with no further increase in up to 48 hours.

Fractions of liver, kidney, and lung tissue were prepared from BD-IV rats and OF-1 mice, some of which had been pretreated with sodium phenobarbital to increase hepatic microsomal enzyme activity [146]. Microsomal supernatants were prepared by centrifuging homogenized tissue at 9,000 x G. For some experiments, the microsomal supernatant was then centrifuged at 100,000 x G to produce a purified microsomal fraction and a supernatant containing microsomal protein. The 9,000 x G microsomal supernatants from four human liver biopsy specimens were also tested.

In the presence of a microsomal fraction derived from mice treated with sodium phenobarbital, with an NADPH-generating system, the mutation rate in TA1530, the most sensitive of the strains tested, increased 6, 12, and 28 times over the spontaneous rate after 48 hours of exposure to vinyl chloride in air at 0.2, 2.0, and 20% [146]. Strains TA1535 and G-46 showed similar mutagenic response, while TA1538 showed no significant increase over control rates after exposure to 20% vinyl chloride for 48 hours. Exposure of strain TA1530 to 20% vinyl chloride without the microsomal system caused a linear increase in the mutation rate as a function of length of exposure, reaching 20 times the spontaneous rate after 48 hours. The prevalence of mutations in the presence of the microsomal system was seven times as high as with vinyl chloride alone after 1.5 hours, but only twice as high after 48 hours; the difference in the mutation rate induced by vinyl chloride alone and in the presence of a microsomal system reached a plateau after about 9 hours of exposure.

Bartsch et al [146] attempted to characterize the enzymes involved in vinyl chloride mutagenicity by testing the activity of various mouse liver fractions on strain TA1530. In the absence of the NADPH-generating system, the 9,000 x G microsomal supernatant with 2% vinyl chloride produced no increase above the mutation rates produced by exposure to vinyl chloride alone for periods of up to 48 hours. Purified microsomes with an NADPH-generating system produced an increase in the vinyl chloride-induced mutation rate of about half of that obtained with the 9,000 x G microsomal system after 48 hours. The 100,000 x G liver protein supernatant (cytosol) did not increase the mutagenic response compared to that induced by vinyl chloride alone. Addition of alcohol dehydrogenase and NAD+ to the microsomal systems did not affect the mutation rate, although alcohol dehydrogenase would be expected to convert any chloroethanol produced to chloroacetaldehyde. This compound is known to be mutagenic to TA1530 [147].

Comparison of tissues from various sources showed that rat liver microsomal supernatant was comparable to that from mouse liver in activating the mutagenic response to vinyl chloride [146]. Pretreating the animals with phenobarbital increased the activity of the liver microsomal supernatant 15-40%. Kidney and lung fractions from either pretreated or untreated animals increased the mutagenic activity of vinyl chloride only marginally over control values. One of the four human liver specimens tested was nearly twice as active as those of rat or mouse liver, while the remaining three were somewhat less active than liver tissue from phenobarbital-treated rats and

mice. Based on data from pretreated animals, the relative mutagenic activities of tissue fractions for strain TA1530 were: mouse liver 100%; rat liver, 80%; mouse kidney, 20%; rat kidney, 16%; mouse and rat lung, 7%; and human liver, 170, 70, 64, and 46%.

Bartsch et al [146] concluded that vinyl chloride was mutagenic to Salmonella in the absence of mammalian microsomal enzymes, probably through the action of breakdown products produced either by bacterial enzymes or nonenzymatically. They pointed out that their findings strongly supported the enzymatic formation of mutagenic vinyl chloride metabolites. Since the 9,000 x G microsomal extract gave a stronger response than purified microsomes, the authors concluded that soluble liver proteins (cytosol) played a role in the metabolic activation of vinyl chloride, either by involvement in a two-step activation mechanism or by prolonging the viability of the microsomal enzymes.

In a subsequent review paper, Bartsch [148] noted that the wide variation in human liver enzymatic activity, which was confirmed in mutagenicity testing of N-nitrosomorpholine, indicated that some individuals are genetically liable to a higher risk from exposure to carcinogens. Bartsch suggested that those estimating acceptable environmental levels of such substances should consider the possible risk for the most susceptible individuals.

In 1976, Garro et al [149] reported studies of the modification of the mutagenic activity of vinyl chloride on Salmonella typhimurium TA1530 by suspensions of untreated and Aroclor 1254-pretreated rat or mouse hepatic microsomes in the presence of an NADPH-generating system and by a system generating free radicals from riboflavin and N,N,N',N'-tetramethylethylenediamine (TMED) under irradiation from fluorescent lamps. They described TMED as an accelerator of vinyl chloride photopolymerization. Although incubation of vinyl chloride with native microsomal suspensions increased its mutagenic activity by about 65%, incubation of this chemical with similar suspensions that had been heated to destroy their enzymatic activity also increased, but at a somewhat reduced level, its mutagenic activity. Mutagenesis in the presence of liver extracts was not NADPH dependent. Incubation of vinyl chloride with the free-radical generating system apparently increased its mutagenic activity by nearly tenfold. The authors took these findings to indicate "that the stimulatory effect of liver extracts on the mutagenic activity of vinyl chloride in the Salmonella auxotroph reversion test cannot be ascribed to enzymatic activation by a microsomal mixed function oxidase and...that the mutagenic effect of vinyl chloride may involve a free radical mechanism."

Although Garro et al [149] found that riboflavin and the tertiary amine had an effect on the mutagenic activity of vinyl chloride for Salmonella typhimurium TA1530 only in the presence of light, they made no measurements that would confirm the relationship of the presence of free radicals in their photoactivated test system to increased mutagenic activity of vinyl chloride. Also, they did not rule out the possibility that the light itself altered the apparent mutagenic activity of vinyl chloride. These two uncertainties about

the photochemical activation experiments makes the conclusion that free radicals may have a special importance in increasing the mutagenic activity of vinyl chloride questionable.

In 1976, Andrews et al [150] confirmed the earlier reports that exposure to vinyl chloride increased the mutagenic frequency in Salmonella TA 1535 in the absence of a microsomal enzyme system.

Greim et al [151], Henschler [152], and Henschler et al [153] reported a study of the mutagenic effects of vinyl chloride and several related compounds on Escherichia coli K12. This bacterium, they suggested, might be a more useful test organism than Salmonella because it was more resistant to the nongenetic toxic effects of some of the test compounds [152]. E. coli K12 can be evaluated for mutagenic response at four loci by plating on selective media; back mutations at the gal+, arg+, and nad+ loci cause reversions to prototrophy, while forward mutations are measured at the gal+ and MTR loci [154]. Vinyl chloride (99.9% pure) was bubbled through a medium containing the bacteria, a microsomal supernatant from mice pretreated with phenobarbital, and an NADPH-generating system [152]. This produced a concentration of vinyl chloride in the medium of 10.6 millimoles, as determined by gas chromatography. The mutagenic response at the four test loci, as a percentage of the spontaneous mutation frequency, was 231 ±20 (SD) for gal+, 663 ±141 for arg+, 148 ±24 for nad+, and 172 ±35 for MTR. Vinyl chloride was by far the most mutagenically active of the compounds tested.

In 1976, Loprieno et al [155] reported on the genetic effects of vinyl chloride activated by mammalian enzymes both in vitro and in vivo (host-mediated assays). Two species of yeasts were used as the test organisms. A haploid strain of Schizosaccharomyces pombe containing a mis-sense mutation in the ade6 locus and a mutation in the rad10 locus was used to study forward mutations at the five loci involved in the biosynthesis of adenine. A diploid strain of Saccharomyces cerevisiae with double heterozygotic alleles in the ade2 and trp5 loci was used to study the induction of gene conversion at these loci. For the in vitro experiments, vinyl chloride (about 99.9% pure) at concentrations of 5 and 50% (128 and 1,280 g/cu m) in air was bubbled through a suspension containing the yeast cells for up to 4 hours, resulting in vinyl chloride concentrations in the treatment flasks of 16 and 48 millimoles, respectively. The vinyl chloride was activated with a purified microsomal fraction (105,000 x G) from the livers of Swiss albino mice combined with an NADPH-generating system. For the host-mediated assays, Swiss albino mice weighing 25 g each were administered orally 1 ml of olive oil containing 1.85% vinyl chloride, ie, at 740 mg/kg. Controls were given olive oil. Cells of Schizosaccharomyces were injected into the peritoneum of four to six mice and permitted to incubate for 3-12 hours before being plated to study mutation frequency.

Mutagenic activity was observed in the in vitro studies with Schizosaccharomyces only when a purified microsomal system was included in the preparation [155]. Maximum mutagenic activity was reached after 30-60 minutes

of treatment, and the activity increased with the vinyl chloride concentration in the medium. At a vinyl chloride concentration of 48 millimolar for 60 minutes, the mutation frequency at the five loci was 62.43/10,000 surviving cells, compared with a spontaneous mutation rate of 2.00/10,000. In studies with Saccharomyces, treatment with 48 millimoles vinyl chloride for 300 minutes in the presence of purified microsomes produced a gene conversion frequency of 8.47/100,000/locus in the ade2 system and 4.36/100,000/locus in the trp5 system; spontaneous frequencies for these systems were 0.49/100,000/locus and 0.85/100,000/locus.

In the host-mediated assay, Loprieno et al [155] found that Schizosaccharomyces showed a mutagenic response after incubation for 12 hours in the peritoneum of mice given vinyl chloride orally at 740 mg/kg. The observed mutation frequency was 6.89 (±0.60)/10,000 cells compared with a control rate of 1.33 (±0.19)/10,000 in yeast incubated in rats not given vinyl chloride; regression analysis showed the effect to be significant at the 1% level. Comparing the mutagenic effectiveness of the 16 millimoles of vinyl chloride used in vitro with that of 11.2 millimoles of vinyl chloride administered to the mice for in vivo studies, the authors concluded that in vitro treatment was more effective. They attributed this to the fact that, in the host-mediated assay, the presence of an active concentration of the purported mutagenic metabolite was minimal as a consequence of its short half-life.

In a later study with Saccharomyces cerevisiae, Shahin [156] reported that vinyl chloride added directly to the medium in concentrations of 0.275-0.55% caused neither mutagenesis nor recombination (crossing-over) in the absence of a microsomal activating system. He did not describe any experiments in which a microsomal system was used.

Drozdowicz and Huang [157] tested the mutagenic activity of vinyl chloride in the fungus Neurospora crassa. The test organisms were a wild-type strain, which was scored for forward mutation to acriflavin resistance, and a nicotinic acid-deficient auxotrophic strain. Amounts of a 1.78 M ethanol solution of vinyl chloride (apparently up to 10%) were added directly to the medium containing the fungal conidia for 3- to 4-hour exposures, or cultures were exposed to gaseous vinyl chloride at concentrations of up to 50% in oxygen for 3.5 or 24 hours.

Neurospora conidia were then plated on nonselective and selective media and incubated for 5-7 days for scoring of survival and mutation frequencies. Activating systems used were a 9,000 x G hepatic microsomal supernatant from phenobarbital-induced rats for the 3.5-hour exposures and a purified microsomal fraction from uninduced Buffalo rats for the 24-hour exposures, both mixed with an NADPH-generating system. Ultraviolet light and methyl methanesulfonate were used as positive mutagenic controls. Both of the latter treatments induced dose-dependent increases in mutation rates, but vinyl chloride showed no mutagenic activity under any of the test conditions.

Two possible explanations for this lack of vinyl chloride-induced mutagenicity in Neurospora were suggested by the authors [157]. Vinyl chloride or its mutagenic metabolites might have been unable to penetrate into the conidia; however, the authors also noted that the Salmonella [146,150] and yeast tester strains [155] used carried mutations affecting genetic repair, while the Neurospora crassa strains used in this study [157] were not repair deficient and thus might not have been sensitive enough to indicate mutagenicity for vinyl chloride. Drozdowicz and Huang also mentioned unpublished data by Huang et al showing that vinyl chloride was not mutagenic in Haemophilus influenzae when DNA from this bacterium was subjected to vinyl chloride treatment and used in transformation assays. No additional details were provided.

Bartsch and Montesano [158], in a 1975 review of the mutagenic and carcinogenic effects of vinyl chloride, mentioned a personal communication from F Verburgt reporting unpublished observations of positive results in a recessive lethal test on Drosophila. Verburgt exposed male Drosophila to vinyl chloride at 800 ppm for 4 or 17 days. He observed a significant increase in the frequency of recessive lethals and noted that peak mutagenic activity was found in metabolically active germ cells. No further information was provided. Magnusson and Ramel [159], in 1976, also obtained positive results in recessive lethal tests with Drosophila. Male Drosophila were exposed to vinyl chloride at unspecified concentrations in air for 3 hours and then mated to Muller 5 females. The authors reported a significant increase of recessive lethals in both the first and second generations after treatment, indicating that vinyl chloride induced recessive lethal mosaics, but no quantitative data were given.

In 1977, Verburgt and Vogel [160] reported the results of recessive lethal tests on Drosophila males exposed to vinyl chloride at concentrations of 200, 850, 10,000, 30,000, or 50,000 ppm for 2 days and to 30 or 850 ppm for 17 days. At each concentration, chromosomes were tested and the number of lethals was scored during days 0 to 12 after exposure.

Vinyl chloride exposure for 2 days at concentrations of 0, 30, and 200 ppm produced 0.10, 0.18, and 0.09% lethals, respectively [160]. Concentrations of 850 ppm for 2 or 4 days produced 0.39 and 0.59% lethals, respectively, and concentrations of 10,000, 30,000, and 50,000 ppm for 2 days induced lethals in 2.19, 2.22, and 2.30%, respectively. After 17 days of exposure and examination for 10 subsequent days, lethals were found in 0.19% of those exposed at 0 ppm, 0.35% of those exposed at 30 ppm, and 1.02% of those exposed at 850 ppm.

Verburgt and Vogel [160] stated that the data, which demonstrated an increasing frequency of lethal effects between concentrations of 0 and 10,000 ppm, demonstrated that the mutagenic activity of vinyl chloride was concentration dependent. Since exposure at 30,000 and 50,000 ppm did not significantly increase the mutation frequency above that seen at 10,000 ppm,

they inferred that above a certain concentration (between 850 and 10,000 ppm) Drosophila was incapable of metabolically "activating" (for mutagenesis) further vinyl chloride, and that the enzymatic mechanisms were saturated.

Anderson et al [161] conducted a dominant lethality study with mice to determine whether vinyl chloride can induce genetic effects. Male CD-1 mice were exposed in groups of 20 to vinyl chloride (purity not described) at concentrations of 3,000, 10,000, and 30,000 ppm (7.68, 25.6, and 76.8 g/cu m) 6 hours/day for 5 days. A concentration of 30,000 ppm was the highest exposure level chosen because it had been shown in preliminary tests to be in the "toxic range" and it was desired that the maximum tolerated dose or higher be included in the study protocol. Control mice were exposed to air alone. Two positive control groups of 15 and 25 mice were given 200 mg/kg of ethyl methanesulphonate orally for 5 days or one ip dose of 200 mg/kg of cyclophosphamide on the 5th day. After the exposure period, each of the surviving males, then 10-12 weeks old, was mated with two 8- to 10-week-old females each week for 8 consecutive weeks. The females were killed 13 days after the assumed date of mating, and their uteri were examined for live implantations, early fetal deaths, and late fetal deaths.

Of the male mice, only those exposed to vinyl chloride at 30,000 ppm showed significant mortality, with only 9 of 20 mice surviving 5-day exposures [161]. In females mated to vinyl chloride-exposed males, the frequency of pregnancy and the number of early and late fetal deaths did not differ significantly from untreated control values. The number of implantations/pregnant female was not affected by vinyl chloride, except that it was significantly below the negative control value in females mated during the 4th week to mice that had been exposed at 30,000 ppm (10.00 vs 12.38, $P<0.05$); however, implantation frequency in this group was slightly above control values for 3 of the 8 weeks of mating. Both positive control groups were significantly higher than negative (air) controls in all the indicators of dominant lethality examined, attesting to the sensitivity of the system. The authors [161] concluded that vinyl chloride at the stated exposure concentrations was not mutagenic in mice as measured by the dominant lethal test. This suggests that the active mutagenic compound in the metabolism of vinyl chloride did not affect the germinal cells of these mice.

Several investigators have attempted to obtain information on the mechanism of mutagenic and carcinogenic activity of vinyl chloride by testing its known or suspected metabolites for their ability to cause mutations in microorganisms. Malaveille et al [147] used Salmonella strain TA1530 to evaluate the mutagenic activity of three presumed metabolites, chloroacetaldehyde, chloroethanol, and chloroethylene oxide (chlorooxirane), and a known urinary metabolite, chloroacetic acid. The substances were tested, with and without a liver microsomal system from phenobarbital-pretreated mice, at concentrations of 40, 4.0, and 0.4 µmol/ml of medium; chloroethylene oxide was also tested at 0.04 µmol/ml.

Chloroacetaldehyde was highly toxic to bacteria at each test concentration used, reducing survival to less than 0.004% of control levels [147]. Chloroacetic acid was toxic at concentrations of 4 and 40 µmol/ml and was the only substance tested that showed no mutagenic activity. Chloroacetaldehyde caused a sixfold increase over the spontaneous rate at a concentration of 4 µmol/ml in combination with the microsomal system; this substance also showed direct mutagenic activity in the absence of mammalian microsomes. At a concentration of 40 µmol/ml, chloroethanol, which did not affect bacterial survival, increased the mutation frequency more than 10 times in the presence of the microsomal system and about 6 times in its absence; at 4 µmol/ml, chloroethanol approximately doubled the mutation frequency of the bacteria, and its mutagenic activity was apparently unaffected by the microsomal system. Chloroethylene oxide was tested without microsomal activation only.. At a concentration of 0.4 µmol/ml, it reduced bacterial survival to 11% and produced a sixfold increase in the mutation frequency; at 0.04 µmol/ml, it caused no increase over spontaneous mutation frequency. These findings indicate that chloroethylene oxide is a far more effective mutagen than chloroacetaldehyde, supporting the suggestion of Henschler and his colleagues [151-153] that the unstable oxirane was directly involved in vinyl chloride mutagenesis.

McCann et al [162] compared the mutagenicity of vinyl chloride with that of its probable metabolites chloroacetaldehyde and chloroethanol, both with and without activation by a rat liver microsomal system. They used Salmonella strains TA1535 and TA100, the latter being identical with TA1535 except that it contains a factor that interferes with DNA repair, thus increasing its sensitivity to many mutagens. Bacteria were exposed to 20% vinyl chloride in air for 3-9 hours, while chloroacetaldehyde and chloroethanol were added directly to the media in concentrations of up to 30 µg/plate and 1-5 mg/plate, respectively.

The mutagenic responses of strains TA1535 and TA100 to vinyl chloride were similar, and McCann et al [162] observed very little activation by a microsomal system from phenobarbital-pretreated rats with exposure periods of up to 9 hours. From the authors' graphs, the direct action of vinyl chloride on strain TA100 produced about 25 revertants/plate above spontaneous levels (which averaged 150 revertants/plate) with 3 hours of exposure and 200 at 9 hours; the corresponding levels with the microsomal system added were 65 and 225 revertants/plate above control levels. However, the authors added in a footnote that increasing the concentration of microsomes in the system had produced a twofold increase over the direct activity of vinyl chloride in both TA100 and TA1535.

At the concentrations tested, chloroacetaldehyde, with no microsomal system, effectively reverted strain TA100 to histidine independence but did not affect strain TA1535 [162]. The mutagenic activity in strain TA100 increased with the concentration of chloroacetaldehyde, reaching 285 revertants/plate above the spontaneous rate at a concentration of 30 µg/plate. Chloroethanol increased the mutagenic reponse of strain TA100 to over twice

the spontaneous levels and showed a trace of activity with strain TA1535. However, addition of the microsomal supernatant caused a small increase in the mutagenic response of strain TA1535 and a much greater increase in that of strain TA100; quantitative data were not given. The authors noted that an NADPH-generating system was not necessary for this activation. Comparing the mutagenic activity of the three substances in strain TA100 on an equimolar basis, the authors found that the number of revertants/μmol, with control levels subtracted, was 1.0 for vinyl chloride, 0.6 for chloroethanol, and 746 for chloroacetaldehyde.

Despite the high mutagenic activity of chloroacetaldehyde in strain TA100, McCann and coworkers [162] concluded that this substance was probably not the active metabolite involved in vinyl chloride mutagenicity. Vinyl chloride, with or without microsomes, was about equally active in the two bacterial strains tested, while chloroacetaldehyde affected only strain TA100. By contrast, activation of chloroethanol by a microsomal system produced a relatively large increase in the mutation rate of TA100, indicating that chloroacetaldehyde might be the mutagenically active metabolite of chloroethanol. The authors suggested that chloroethylene oxide might be the metabolic intermediate responsible for vinyl chloride mutagenicity. This conclusion is supported by the findings of Malaveille et al [147] and of Rannug et al [163] that chloroethylene oxide was several times more active than chloroacetaldehyde in the nonrepair-deficient strains TA1530 and TA1535.

In 1976, Rannug et al [163] compared the mutagenicity of chloroethlyene oxide, chloroacetaldehyde, 2-chloroethanol, and chloroacetic acid in Salmonella typhimurium strain TA1535. The test substances were added to the bacteria before plating, in concentrations ranging from 0.1 to 1.5 millimolar; 2-chloroethanol and chloroacetic acid were also studied at concentrations of up to 1 M. Ethylene oxide, described as a "well known mutagen," was used as a positive control. The authors also tested vinyl chloride at a concentration of 2% (51.2 g/cu m) in air for 3 hours, using the procedure described in their earlier study [145].

Chloroethylene oxide showed both a definite toxic effect and strong mutagenic activity [163]. At a concentration of 0.75 millimolar, it produced 180 revertants/100 million surviving cells, 60 times the spontaneous mutation rate. Chloroacetaldehyde also showed a mutagenic effect in this concentration range but was only about 5% as effective as chloroethylene oxide on a molar basis. 2-Chloroethanol and chloroacetic acid showed no mutagenic effect up to 1.5 millimolar and were therefore retested at higher concentrations. 2-Chloroethanol produced a weak mutagenic response only at 1 molar. Chloroacetic acid was highly toxic to bacteria at concentrations up to 0.5 molar, and no increase in mutagenic response could be detected. Ethylene oxide, the positive control substance, did not produce an increase in mutagenic response at concentrations below 5 millimolar, and ethylene oxide at 95.5 millimolar produced an increase in the mutation rate equivalent to that produced by chloroethylene oxide at a concentration of 0.15 millimolar.

In their experiments with 2% (51.2 g/cu m) vinyl chloride, Rannug et al [163] found that vinyl chloride at this concentration in the presence of a microsomal system produced 10.0 ±0.9 (SE) revertants/100 million cells, significantly more (P<0.001) than the control rate of 3.8/100 million cells; neither vinyl chloride alone nor the microsomal system alone produced a significant increase. Noting the difficulty of comparing vinyl chloride mutagenicity to that of the other compounds because of the difference in experimental conditions, the authors estimated that only if all the vinyl chloride was converted to 2-chloroethanol would the concentration of this compound be great enough to account for the observed mutagenic activity of activated vinyl chloride.

Calculating that chloroethylene oxide was 10,000-15,000 times as mutagenic as ethylene oxide, Rannug et al [163] concluded that this was in reasonable agreement with the ratio of the preliminary rate constants of the two compounds for reaction with the appropriate nucleophiles. They considered this to be an indication that chloroethylene oxide acts in the same way as ethylene oxide, as a monofunctional alkylating agent. The authors concluded on the basis of interpretations by Hussain and Osterman-Golkar [164] that chloroacetaldehyde was far more active as a mutagen than would be expected from its reactivity as an alkylating agent.

In an addendum to the paper by Rannug et al [163], Hussain and Osterman-Golkar [164] analyzed the data of Rannug et al on a kinetic basis. They noted that the higher-than-expected mutagenic activity of chloroethylene oxide, based on comparison of its rate constant for alkylation with that of ethylene oxide, indicated "a certain role of the aldehyde groups." Chloroacetaldehyde, however, was several orders of magnitude more effective than expected, indicating "a reaction mechanism different from simple alkylation."

In 1977, Loprieno et al [165] tested vinyl chloride metabolites for mutagenic activity in yeasts. Chloroethylene oxide, 2-chloroacetaldehyde, and 2-chloroethanol, added to the media in various concentrations, were tested in vitro; 2-chloroacetaldehyde was also tested in the host-mediated assay. Test organisms and experimental procedures were the same as those used in their previous study [155].

Chloroethylene oxide showed the highest mutagenic activity in all systems examined [165]. At a concentration of 0.1 millimole, the forward mutation frequency in S. pombe was 340 times the control rate, and at 1 millimole the gene conversion rate in S. cerevisiae was 40-50 times that in controls. 2-Chloroethanol in concentrations up to 50 millimoles showed no mutagenic activity in yeast cells, with or without microsomes. 2-Chloroacetaldehyde showed a weak mutagenic effect in vitro, increasing mutation rates 2-7 fold at concentrations up to 12.5 millimoles. When administered to male Swiss albino mice (25 g) in oral doses of 250 mg/kg, 2-chloroacetaldehyde produced no increase in the mutation rate of S. pombe incubated in the peritoneum for 3-6 hours.

Elmore et al [166] evaluated vinyl chloride and its metabolites in a study designed to permit accurate comparisons of their mutagenic activity and to provide additional insight as to the mechanism of this activity. Since previous investigators had not ascertained the purity of the metabolites used, Elmore and coworkers tested pure forms of chloroethanol, chloroacetic acid, chloroethylene oxide, and chloroacetaldehyde. The last compound can exist in combinations of four forms, depending on its preparation. The authors therefore tested pure preparations of the monomer, dimer hydrate, and trimer, plus the 50:50 mixture of monomer and monomer hydrate formed when the monomer is dissolved in water or physiologic systems. They also tested a solution of 0.0106 M vinyl chloride in nutrient broth.

Salmonella typhimurium strain TA100 was used for quantitative mutagenicity testing [166]. The authors also tested DNA repair-deficient strains of Bacillus subtilis in repair assays, as an indirect test for mutagenicity. 4-Nitroquinoline-N-oxide was used as a positive mutagenic control. The number of replicate plates used in these studies was not mentioned and the authors did not indicate the statistical significance of their results.

Incubation of Salmonella for 48 hours in a medium containing initially 0.0106 M vinyl chloride produced no increase over the spontaneous mutation rate [166]. Chloroethanol and chloroacetic acid at concentrations of 1 millimole also showed no mutagenic activity. All forms of chloroacetaldehyde were mutagenic to Salmonella strain TA100. Chloroacetaldehyde monomer, the most active form, caused a maximum mutagenic response of 404 revertants/plate above spontaneous levels at a concentration of 0.84 µmol. The mixture of monomer and monomer hydrate caused 512 revertants/plate at 14 µmol. The dimer hydrate at 120 µmol and the trimer at 240 µmol produced 193 and 159 revertants/plate, respectively. Mutagenic response was linear up to these concentrations but became nonlinear at high concentrations for all four forms of chloroacetaldehyde.

Chloroethylene oxide, which decomposed rapidly to chloroacetaldehyde, with a half-life of 1.6 minutes under the 37 C incubation conditions, was preincubated with the bacteria at 3 C for up to 6 hours before plating to ensure that the undecomposed compound penetrated the cells [166]. With 4 hours of preincubation, chloroethylene oxide at a concentration of 0.26 millimole produced 114 revertants/plate above spontaneous levels.

The repair assay used by Elmore et al [166] involves incubating Bacillus subtilis strains on 6-mm filter paper discs saturated with the test substances, as described by Kada et al [167]. Lethality and mutagenic potential were evaluated by comparing inhibition zones in the wild-type strain 168 with those in strains deficient in their ability to repair DNA lesions due to lack of either excision repair (hcr- or uvr-) or recombination repair (rec-) capability.

Vinyl chloride, chloroethanol, and chloroacetic acid did not inhibit bacterial growth in the repair assay [166]. The various forms of

chloroacetaldehyde affected growth of the wild type and excision repair-deficient strains only slightly but had a much greater effect on the recombination-deficient strain, with the monomer and monomer hydrate again being the most active forms. Chloroethylene oxide also selectively inhibited growth of the recombination-deficient strain.

Since the wild-type and excision repair-deficient strains of B. subtilis (both capable of recombination repair) were essentially unaffected by these substances, Elmore and colleagues [166] concluded that recombination repair is induced to correct DNA lesions caused by vinyl chloride metabolites. They suggested that the post-replication repair of DNA in mammalian cells, which, like recombination repair in bacteria, is an error-prone process, might be responsible for the production of human cancer by vinyl chloride. The ultimate carcinogenic metabolites of vinyl chloride, the authors considered, are chloroacetaldehyde monomer hydrates, known to react with DNA, and chloroethylene oxide, which rearranges to chloroacetaldehyde or to a stabilized diradical intermediate also known to react with DNA. They suggested that the lower mutagenicity of chloroethylene oxide might result from its being detoxified faster than chloroacetaldehyde.

Laumbach et al [168] extended the investigation of Elmore et al [166] by studying the effect of chloroacetaldehyde on transforming DNA extracted from Bacillus subtilis. Previous studies [169,170] had shown that chloroacetaldehyde could bind to DNA in vitro, modifying bases and causing mismatched base pairs; however, Laumbach et al [168] found that transforming DNA isolated from wild-type B. subtilis and treated with chloroacetaldehyde did not affect the number of transformants in any of four auxotrophic B. subtilis strains used as recipients. When the wild-type strain was treated with chloroacetaldehyde for 15 minutes before the DNA was extracted, there was a major depression of biologic activity in the transforming DNA, as evidenced by a decrease of 50% or more in the number of transformants produced. This depression showed genetic-marker specificity, reducing transformation at some loci by over 90%. The authors noted that DNA segments that have previously been shown to be associated with macromolecular structures such as the cell wall or cell membrane appeared to be selectively protected from attack by chloroacetaldehyde. The addition of a mammalian microsomal system did not significantly alter the effect of chloroacetaldehyde on transformation efficiency.

Huberman et al [171] evaluated the mutagenicity of chloroethylene oxide and 2-chloroacetaldehyde directly on mammalian cells, using cultures of V79 cells derived from the kidneys of Chinese hamsters; this cell system had been successfully used in detecting mutagenic activity of other carcinogenic substances. Cells were seeded on media containing 8-azaguanine or ouabain and exposed to the test substances at concentrations of up to 25 µmol for 3 hours. After an additional period of incubation, colonies resistant to 8-azaguanine were scored for 10-12 plates at each concentration and ouabain-resistant colonies were scored for 32-40 plates at each concentration.

The mutagenic response at both loci increased as a function of the concentrations of chloroethylene oxide and 2-chloroacetaldehyde [171]. At a concentration of 6 μmol, chloroethylene oxide produced mutation frequencies 4 times that of controls for 8-azaguanine resistance and 10 times the control frequency for ouabain resistance. The corresponding values for 2-chloroacetaldehyde at these concentrations were 13 and 2 times control frequencies. Chloroethylene oxide at 13 μmol produced mutation frequencies exceeding control frequencies by factors of 8 at the 8-azaguanine locus and 23 for ouabain. 2-Chloroacetaldehyde at this concentration was strongly cytotoxic, and no mutagenic effect could be detected.

(b) Vinylidene Chloride

In a 1975 study of vinyl chloride mutagenicity, Bartsch et al [146] stated that they had unpublished data showing that vinylidene chloride metabolically activated by rat or mouse liver microsomal enzymes was a more active mutagen than vinyl chloride. A subsequent 1975 paper by Bartsch et al [172] presented data on the mutagenicity of vinylidene chloride in *Salmonella typhimurium*, using histidine-deficient tester strains TA1530 and TA100. Plated bacteria, in a medium containing a microsomal system from the livers of phenobarbital-pretreated OF-1 mice supplemented with an NADPH-generating system, were exposed to vinylidene chloride, containing 0.3% 4-methoxyphenol as an antioxidant, at concentrations of 0.2, 2.0, or 20% (7.94, 79.4, or 794 g/cu m) in air [172]. This produced respective vinylidene chloride concentrations of 0.33, 3.3, and 33 millimoles in the media after 2 hours of exposure, as determined by gas chromatography. After exposure, the bacteria were cultured for up to 48 hours, and histidine-revertant colonies were counted on each plate. From one to four experiments were conducted at each concentration, each using a pool of four mouse livers; bacteria were plated in triplicate for each experiment. The authors also compared the efficiency of liver, kidney, and lung microsomal systems from male OF-1 mice and female BD-VI rats in inducing mutagenic response to vinylidene chloride in strain TA100.

Both *Salmonella* strains showed a positive mutagenic response to vinylidene chloride in the presence of a mammalian microsomal system [172]. No mutagenic activity was observed in the absence of the NADPH-generating system. In strain TA100, exposure to vinylidene chloride for 4 hours at 0.2% produced an average of about 300 revertants/plate, compared with control levels of less than 50. At 2% vinylidene chloride, the number of revertants/plate in strain TA100 reached 500 ±23 (SE) above control levels, while at 20% vinylidene chloride, the number was only 330 ±29. Strain TA1530 followed the same pattern but was somewhat less sensitive. The authors suggested that the reduction in mutagenic response at the highest exposure concentration might have resulted from inhibition of the microsomal enzymes responsible for the metabolic activation of vinylidene chloride.

The mutagenic response of the bacteria to 2% vinylidene chloride increased linearly with time up to 4 hours, and this test period was therefore used in experiments comparing various tissue fractions [172]. All of the mouse tissue

fractions tested induced some mutagenic response to vinylidene chloride, although the liver fraction was the most active. Rat tissue fractions were much less active than those of mice, and rat lung tissue showed minimal activity. Phenobarbital pretreatment, used only in mice, approximately doubled the mutagenic activity of vinylidene chloride. Exposure to 2% vinylidene chloride in the presence of microsomal systems from untreated mouse liver caused 330 ±49 revertants/plate above control levels. Using this value as a standard (100%), relative activities of the other tissue fractions from untreated animals were: mouse kidney, 20%; mouse lung, 6%; rat liver, 30%; rat kidney, 5%; rat lung, less than 3%. In a 1976 review paper, Bartsch [148] also noted that the relative activities of four human liver biopsy specimens, under similar experimental conditions, were 38, 17, 16, and 11% of the corresponding mouse liver values.

In another set of experiments, using Salmonella strain TA1530, Bartsch et al [172] attempted to explicate the mechanism whereby vinylidene chloride exerted mutagenicity by adding sulfur-containing compounds to the media. With exposure to 2% (79.4 g/cu m) vinylidene chloride, the addition of 12 µmol/ml of N-acetyl cysteine and the same amount of N-acetyl-methionine to the medium, in the presence of a mouse liver microsomal system, caused an 80% reduction in the number of revertants. The authors concluded that the mutagenic metabolites of vinylidene chloride are trapped by nucleophilic sulfur groups, thus competing for binding to bacterial DNA.

In a review published in 1976, Bartsch et al [173] again noted that vinylidene chloride was more mutagenically active than vinyl chloride, based on calculations from linear dose- and time-dependent response curves from separate experiments. They indicated that vinyl chloride produced 3.7 revertants/µmol of substrate/hour of exposure/plate in strain TA1530 and 5.6 in strain TA100, while vinylidene chloride produced 9.5 and 14.6 revertants/µmol/hour/plate, respectively, in these test strains.

Greim et al [151], Henschler [152], and Henschler et al [153] reported on the mutagenic activity of vinylidene chloride, as well as that of vinyl chloride and related compounds, in Escherichia coli strain K12. Analytical grade vinylidene chloride was injected into the incubation medium to produce a concentration of 2.5 millimoles, as determined by gas chromatography.

Like vinyl chloride, vinylidene chloride was most active in causing mutations at the arg+ locus, producing revertants at 229 ±26% of the spontaneous mutation rate [151-153]. Revertants at the gal+ locus increased to 120 ±14% of the spontaneous rate in bacteria exposed to vinylidene chloride, while the MTR and nad+ loci were not observably affected. No mutagenic activity was observed in the absence of a microsomal system.

The authors [151-153] stated that the mutagenic activity of vinyl chloride, which caused a 563% increase in mutations at the arg+ locus, was "several times higher" than that of vinylidene chloride. It should be noted, however, that the measured concentration of vinyl chloride in the medium (10.6

millimoles) was over four times that of vinylidene chloride. In addition, the difference in exposure technique required for the highly insoluble gaseous vinyl chloride necessitates that such comparisons be made with caution. Since Bartsch et al [172] exposed bacteria to both vinyl chloride and vinylidene chloride in air, and thus determined the concentration in the medium under similar conditions, their comparisons of relative mutagenic activity are probably more accurate. Furthermore, Henschler et al [153] pointed out in their discussion that vinylidene chloride is the most polar of the chlorinated ethylenes they tested, and its oxirane, which has not been successfully synthesized, would be expected to be the least stable. Thus, it should be expected to have a higher mutagenic activity than vinyl chloride.

(c) Vinyl Bromide

VF Simmon and R Mangham, in a written communication to NIOSH in August 1977, reported the results of a study on the mutagenic effects of vinyl bromide on Salmonella typhimurium strains TA100 and TA1535. Plated bacteria, with and without a liver microsomal system from male rats pretreated with Aroclor 1254, were exposed for 12 hours to atmospheres containing 0-20% vinyl bromide (source and purity not described). After an additional 48-hour incubation period, histidine revertants were counted on three replicate plates for each concentration.

Vinyl bromide at concentrations of 2-20% (87.6-876 g/cu m), in the absence of a microsomal activation system, increased the mutagenic response in both Salmonella test strains as compared with control activity according to Simon and Mangham. Addition of a microsomal system enhanced the mutagenic activity of vinyl bromide. At 20% vinyl bromide in the presence of a microsomal system, there was an average of 1,129 revertants/plate in strain TA100 and 959/plate in TA1535; without the activating system, the mutation rates were 620/plate for TA100 and 721/plate for TA1535. Control rates of about 130/plate for TA100 and less than 20/plate for TA1535 were not significantly affected by the addition of the microsomal system alone.

Although Simmon and Mangham did not conduct simultaneous studies of vinyl chloride or measure the concentration of vinyl bromide produced in the medium, they postulated that vinyl bromide was slightly more mutagenic by these procedures than vinyl chloride. No data were offered in support of this comparison. The authors' findings do support a conclusion that vinyl bromide induces mutations in Salmonella strain TA100 and TA1535 without microsomal activation, although microsomes enhance its mutagenic activity. The mutagenic responses of both strains were concentration-dependent in a nearly straight-line relationship, which showed a tendency to level out at the highest concentration tested (20%). Since Simmon and Mangham did not provide data on bacterial survival, it is not possible to determine whether this saturation resulted from a toxic effect of vinyl bromide at high concentrations.

In a 1976 review, Bartsch [148] mentioned unpublished data on vinyl bromide mutagenicity and noted that microsomal systems from three human liver

samples were 23-36% as effective as a mouse liver microsomal system in inducing the mutagenic effects of vinyl bromide on Salmonella. No experimental data were presented, nor did Bartsch make any comparison of vinyl bromide mutagenicity with that of other vinyl compounds.

(d) Vinyl Fluoride and Vinylidene Fluoride

Putative mutants of Escherichia coli B and E. coli Sd-4 were isolated by the penicillin method of Lederberg and Zinder and by plating in the absence of streptomycin, respectively [174]. The authors stated that the E. coli B cultures exposed to vinyl fluoride and vinylidene fluoride mutated at about 100 times the rate at which the control cultures mutated. They also noted a similar influence of vinylidene fluoride on the Sd-4 strain of E. coli.

Despite this apparent observation of extremely high mutation rates, the authors were unable to isolate any auxotrophic strains from the treated cultures [174]. They presented some evidence for the induction of heritable changes in the fermentation patterns of certain carbohydrates, although it was not unequivocal.

While it is possible that qualitative changes in the mutation frequencies may have occurred, the failure to isolate auxotrophic strains from the treated cultures suggests that any such effects were minimal [174]. Also, the selection method used by these authors does not allow the direct measurement of either the spontaneous or induced mutation rates or frequencies of a culture, although it may greatly enrich the ratio (frequency) of mutated cells to normal cells in the culture.

Vinylidene fluoride has been tested for mutagenic potential in the "Ames" Salmonella auxotroph reversion assay procedure, and the "in vitro transformation of BALB/3T3 cell assay (J Watson, written communication, April 7, 1978). The "Ames" analysis was conducted with Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-100, and TA-98, with and without activation by rat liver microsomes. Solvent and positive control plates were run concurrently with test plates. Test plates were exposed to vinylidene chloride gas for 1, 24, 48, or 72 hours and incubated at 37 C for 48 hours, after the 1-hour exposure, and 24 hours after the 24, 48, and 72-hour exposures.

Without activation, strain TA-1535 exposed for either 24 or 48 hours showed a threefold increase in the number of revertants/plate and those exposed for 72 hours showed a sevenfold increase when compared with solvent controls according to Watson. Ten micrograms of methylnitrosoguanidine/plate (positive control) increased the number of revertants 43-fold. With activation, the increases in revertants/plate were reported as sevenfold for one 24-hour exposure, eightfold for one 48-hour exposure, and tenfold for a 72-hour exposure. A positive control of 2-anthramine at 100 μg/plate gave eightfold increases. Other Salmonella strains did not show substantial increases in revertants/plate when compared with solvent controls in either the activated or nonactivated systems.

These results show that vinylidene fluoride induces a base-pair substitution, but no frameshift mutations according to Watson. Although strain TA-100 should also identify any potential mutagen capable of inducing base-pair substitution, the results were negative for this strain in these tests. The most plausible explanation for this apparent discrepancy is that the presence of the resistance transfer factor on the TA-100 was in this case protective. The results also indicate that some product of the metabolism of vinylidene fluoride is more mutagenic than the parent compound.

Watson also tested the ability of vinylidene fluoride to transform BALB/3T3 cells. Cells were exposed for various periods ranging from 0 to 48 hours, with and without tissue culture media. Only the cells exposed with culture media showed an elevated number of transformations above background; however, these elevations were not significant.

Metabolism

Metabolic pathways have not been completely and convincingly delineated for any of the vinyl halides. That for vinyl chloride apparently is nearest to completion, but, even here, several key steps in the initial reactions are only postulated and have not been conclusively proven by experimentation designed specifically to elucidate intermediate metabolic products in vivo. The proposed pathways for vinylidene chloride are sketchy at best, while the determinations of pathways for vinyl bromide, vinyl fluoride, and vinylidene fluoride have only just begun.

(a) Vinyl Chloride

Vinyl chloride metabolism has been studied extensively since the discovery of vinyl chloride-induced angiosarcoma in humans in early 1974. The major urinary excretion products of vinyl chloride have been characterized following both inhalation and oral exposures, and the compound has been shown to be readily absorbed and widely distributed in body tissues and to be metabolized into several major and minor metabolites.

(1) Distribution and Elimination

Hefner et al [4], in 1975, found that the metabolism of vinyl chloride during the first 15 hours after exposure of three male rats to 14C-(1,2-)vinyl chloride at 49 ppm (125.4 mg/cu m; a total estimated intake of 0.49 mg/kg) for 65 minutes resulted in the formation of polar metabolites that were excreted predominantly in the urine (58% of the 14C activity). Lesser amounts of radioactivity were excreted in the feces (2.7%) and in the expired air as carbon dioxide (9.8%). At 75 hours after administration, 67.1% of the radioactivity had been excreted in the urine, 3.8% in the feces, and 14.0% as expired carbon dioxide. Trace amounts of radioactivity (0.02% of that administered) were eliminated in expired air as unchanged vinyl chloride. A small but significant amount of radioactivity (1.6%) was retained in the liver