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

for as long as 75 hours after exposure. The skin retained 3.6% of the radioactivity, 0.2% was found in the kidneys, and 7.6% was found in the remaining carcass.

Hefner et al [4] also reported that the in vivo kinetics of uptake (metabolism) of inhaled vinyl chloride, determined for four male rats exposed together in a chamber at initial concentrations ranging from 50.5 to 1,167.0 ppm (129.3 to 2,987.5 mg/cu m) for 52.5-356.3 minutes, differed at different concentrations of vinyl chloride. For concentrations of 50-105 ppm, the apparent first-order rate constant derived after seven separate exposures was 0.00804 ±0.0034/minute, corresponding to a half-life of 86 minutes. After five separate exposures to vinyl chloride at concentrations ranging from 220 to 1,167 ppm, the first-order rate constant was determined to be 0.00265 ±0.00135/minute, a half-life of 261 minutes. Based on these results, Hefner et al concluded that there were different pathways for vinyl chloride metabolism and that at least one of them was readily saturated, so that the amounts of substrate degraded by the individual pathways were dependent on the concentration of vinyl chloride presented to the organism. However, these variations in apparent first-order constants are equally consistent with the existance of a single saturable process and do not provide any conclusive experimental evidence for the existence of multiple biochemical pathways.

Watanabe et al [175] reported that the largest amounts of radioactivity in rats exposed to 14C-(1,2-)vinyl chloride at 10 or 1,000 ppm (25.6 or 2,560 mg/cu m) for 6 hours remained, 72 hours after exposure, in the liver and skin. For the liver, skin, carcass, muscle, lungs, and kidneys, μg equivalents of radioactivity per g of tissue were higher at 1,000 ppm than at 10 ppm. When the data were normalized for metabolized vinyl chloride, an apparent increase, although not a statistically significant one, was observed in the 14C activity of the liver and skin at 1,000 ppm.

In the rats exposed at 10 ppm, a greater percentage of the total recovered radioactivity was excreted in the urine and a smaller percentage in the expired air than in rats exposed at 1,000 ppm [175]. The apparent first-order rate constants for pulmonary excretion at 10 and 1,000 ppm were 0.034 ±0.002 and 0.031 ±0.01/hour, respectively, equivalent to half-lives of 20.4 and 22.4 minutes, respectively.

The urinary excretion of radioactivity as a function of time for both concentrations of airborne vinyl chloride was nonlinear [175]. This could indicate that elimination was occurring from at least two compartments [176]. From the initial linear portions of these data, apparent first-order rate constants were 0.151 ±0.009 and 0.168 ±0.001/hour, corresponding to half-lives of 4.6 and 4.1 hours for 10 and 1,000 ppm, respectively. No kinetic analysis was performed on the slow phase, or second part, of the curve, since it represented less than 3% of the total urinary radioactivity.

In 1976, Watanabe et al [177] reported that, in male rats given 14C-(1,2-)vinyl chloride in single oral doses of 0.05, 1.0, or 100 mg/kg, the

percentage of radioactivity in the expired air varied in a dose-dependent manner. At 100 mg/kg, 67% of the administered radioactivity was recovered as unmetabolized 14C-vinyl chloride, while at the lower doses, only 1-2% was recovered as 14C-vinyl chloride. The percentage of 14C activity detected as carbon dioxide was greater (13%) at 1.0 mg/kg than at 0.05 (9%) or at 100 mg/kg (2%). Urinary and fecal excretion of 14C activity was higher at 0.05 and 1.0 mg/kg than at 100 mg/kg. The same trend was observed for the carcass and tissues. Analysis of tissues for 14C activity at 72 hours indicated that the liver contained three to six times as much radioactivity/g of tissue as the other tissues. The proportion of the dose remaining in the tissues after 72 hours in the 0.05- and 1.0-mg/kg dose groups was greater than that in the 100-mg/kg group.

Watanabe et al [177] indicated that the kinetics of the pulmonary elimination of oral doses of vinyl chloride at 0.05 and 1 mg/kg were essentially similar and monophasic. The apparent first-order rate constants for the lower doses were 0.013 ±0.001 and 0.012 ±0.001/minute, corresponding to half-lives of 53.3 and 57.8 minutes, respectively. The characteristics of excretion of a dose of 100 mg/kg, however, indicated a biphasic response during the first 4 hours after administration. Separation of the response into a rapid and a slow component gave apparent first-order rate constants of 0.048 ±0.005 and 0.017 ±0.008/minute, equivalent to half-lives of 14.4 and 40.8 minutes.

Urinary excretion curves after single oral doses of vinyl chloride indicated a multiphasic mode of elimination [177]. The linear portion of each curve extended from 12 to 36 hours after administration. The first-order rate constants were estimated to be 0.155 ±0.006, 0.15 ±0.002, and 0.152 ±0.011/hour, equivalent to half-lives of 4.5, 4.6, and 4.6 hours for doses of 0.05, 1.0, and 100 mg/kg, respectively. Ninety-seven percent was excreted within 36 hours. After 36 hours, the excretion curves were quite variable and represented less than 3% of the total urinary activity; hence, no estimates of rate constants were made.

Hefner et al [178], in 1975, reported that when the whole body (excluding the head) of a male rhesus monkey was exposed to 14C-(1,2-)vinyl chloride at 7,000 ppm (17.9 g/cu m) for 2 hours, and a second monkey was similarly exposed at 800 ppm (2.1 g/cu m) for 2.5 hours, radioactivity could be detected only in the liver, bile, and kidneys. They also reported that very little gaseous vinyl chloride was absorbed percutaneously, 0.023 and 0.031% of the total available radioactivity at 7,000 and 800 ppm, respectively.

Gehring et al [179] reported that metabolism of vinyl chloride by rats did not increase proportionately as the concentrations increased from 1.4 to 4,600 ppm (3.58 to 11,776 mg/cu m). The nonlinearity of the amount of vinyl chloride metabolized during the 6 hours of exposure was reported to be in accordance with Michaelis-Menten kinetics, rather than with apparent first-order kinetics. From the Michaelis-Menten kinetic model, the authors

estimated that the theoretical maximum amount (Vm) of vinyl chloride that could be metabolized in 6 hours was 8,558 ±1,147 μg and the apparent Michaelis constant (Km) was 860 ±159 μg/liter of air.

Gehring et al [6] analyzed the results obtained by Watanabe et al [177] and concluded that vinyl chloride elimination tended to be dose dependent and that the primary pathways of vinyl chloride metabolism became saturated as the oral dose of vinyl chloride increased, permitting elimination by another pathway(s). From the urinary excretion curves of Watanabe et al [177], Gehring et al [6] concluded that, since the slopes, and therefore the rate of elimination (half-lives of 4.5 hours), did not change, urinary excretion was not affected by dose. They also concluded that elimination kinetics of vinyl chloride in expired air after a dose of 0.05 or 1.0 mg/kg indicated a monophasic process, whereas at a higher dose (100 mg/kg), biphasic elimination kinetics were indicated. The half-life for elimination for the 100-mg/kg dose was found to be 14 minutes, which corresponded with the half-life for vinyl chloride in blood reported by Withey [180]. Gehring et al [6] interpreted these results to mean that "vinyl chloride is bound reversibly to some site in the body having a finite capacity. As the dose increases, the availability of the binding sites decreases and the chemical is free to find its way to other sites as well or to be eliminated." The authors also concluded that the fate of vinyl chloride within the body changed with dose, and that pulmonary excretion of vinyl chloride was not a rate-limiting step in metabolism.

In 1975, Green and Hathway [5] reported on a whole-body autoradiographic study in which rats were given single oral doses of 30 microcuries of 14C-(1,2-)vinyl chloride, which showed that, at 4 hours after administration, the gastrointestinal tract was free of radioactivity except for the large intestine, which contained trace amounts. After 2 hours, a distinct localization of 14C was noted within sectioned tubules of the paraauricular region, which the authors thought might be localized in the Zymbal glands.

Green and Hathway [5] also found that excretion of radioactivity by four rats given single iv or ip doses of 250 or 450 mg/kg of 14C-(1,2-)vinyl chloride, containing 2 microcuries of 14C, in a beta-hydroxyethyllactamide solution was completed within 72 hours. Rats given similar oral doses of 14C-vinyl chloride were still excreting small amounts of 14C after 72 hours. The greatest percentage of the radioactive label in the lower oral dose was excreted in the urine; only small amounts of radioactivity were eliminated in expired air as unchanged vinyl chloride and [4,6,175,177,186,187] the high oral dose, approximately 92% of the label, was eliminated in expired air as unchanged vinyl chloride and less than 1% as carbon dioxide. The authors stated that, nevertheless, about 100 times more vinyl chloride was metabolized at the higher oral dose than at the lower one.

At an iv dose of 250 μg/kg, 99% of the vinyl chloride was excreted unchanged in expired air within an hour after injection, including 80% within 2 minutes [5]. The excretion profile of vinyl chloride after a single ip injection at the low dose was intermediate between that occurring with oral or

iv administration. The authors suggested that some of the vinyl chloride in blood was excreted unchanged through the lungs and some was absorbed into the hepatic-portal system and metabolized by the liver.

From these data, the authors [5] concluded that the change in excretion pattern between high and low doses was due to a "saturable drug metabolism and to a highly efficient arterial-alveolar transfer of unchanged vinyl chloride from systemic blood that leaves a relatively low concentration of material available for biotransformation in successive passes through the liver."

In another experiment by Green and Hathway [5], three rats that received 3, 30, or 300 mg/kg/day of nonradioactive vinyl chloride by oral intubation for 60 days were given single oral doses of 14C-(1,2-)vinyl chloride (0.6 mg/kg, containing 2 microcuries) on days 1 and 60. For the first 24 hours after administration of the radiolabeled vinyl chloride, urine and expired air were monitored for radioactivity. The authors concluded that chronic exposure for 60 days did not affect the excretion rate for a single oral dose of 14C-vinyl chloride. The authors also concluded that vinyl chloride did not induce its own metabolism and that excretion data for a single dose also applied to the chronic situation.

(2) Identification of Metabolites

Gothe et al [3], in 1974, analyzed the metabolites produced from vinyl chloride in vitro by a microsomal supernatant from rat liver in the presence of an NADPH-generating system. Vinyl chloride was bubbled through the microsomal system, to which 3,4-dichlorobenzenethiol had been added as a trapping agent for reactive metabolites. After 2 hours of exposure, the products identified were consistent with the formation of chloroethylene oxide, chloroacetaldehyde, or both.

A similar study by Barbin et al [2], using a mouse liver microsomal system and 4-(4-nitrobenzyl)pyridine, showed that a volatile metabolic adduct of vinyl chloride was formed that had an absorption spectrum identical to that of the adduct of chloroethylene oxide but different from the adduct of chloroacetaldehyde. They concluded that chloroethylene oxide was the primary metabolite of vinyl chloride in their system.

Chloroethylene oxide was chemically synthesized by Zief and Schramm [181] and Gross and Freiberg [182]. Upon standing, chloroethylene oxide was observed to readily rearrange to chloroacetaldehyde. In an aqueous solution at pH 7.4 and 37 C, chloroethylene oxide had a half-life of 1.6 minutes and its rearrangement followed first-order kinetics [183].

Radwan and Henschler [184] reported that small quantities of monochloroacetic acid could be detected when vinyl chloride at 100-2,000 ppm (256-5,120 mg/cu m) was perfused through isolated rat livers. They reported a slight increase in the concentration of methemoglobin in the system, which they considered indicative of the formation of peroxide intermediates.

Watanabe et al [175] reported that during the first 24 hours after exposure of rats to 14C-(1,2-)vinyl chloride 10 or 1,000 ppm (25.6 or 2,560 mg/cu m) the nonvolatile urinary metabolites were N-acetyl-S-(2-hydroxyethyl)cysteine, thiodiglycolic acid, and a third metabolite that was not identified. They concluded that the proportion of 14C eliminated by various routes was concentration dependent. Moreover, the dominant route of excretion at both concentrations was in the urine and the metabolites were predominantly nonvolatile or polar. This finding supports the earlier conclusions of Hefner et al [4] and Watanabe et al [177] that the elimination of vinyl chloride metabolites was dose dependent. The authors also suggested that metabolism occurred at a reduced rate because body burden in terms of equivalents of radioactivity increased by only 27-fold as the concentration of vinyl chloride was increased from 10 to 1,000 ppm. They concluded that this also indicated that the primary metabolic pathway for vinyl chloride was saturable at high concentrations, specifically at 1,000 ppm. This work, in addition to the work of Hefner et al [4], tends to support a hypothesis that, at concentrations of vinyl chloride above 220 ppm, alternate metabolic pathways exist.

Hefner et al [4] reported that the urinary metabolites from rats exposed for 4, 5, or 7 weeks to vinyl chloride at 5,000 ppm (12.8 g/cu m) were similar. The polar metabolites in the urine appeared to be conjugated with glutathione or cysteine through covalent linkages to the sulfhydryl groups. Chromatographic analysis suggested the presence of S-(2-hydroxyethyl)cysteine. Two theoretically possible metabolites, S-(2-chloroethyl)cysteine and S-(2-carboxymethyl)cysteine, were not detected, but Hefner et al postulated that S-(2-carboxymethyl)cysteine might not have been adequately resolved from the urine background. When rats were exposed to vinyl chloride at 5,000 ppm for 9 weeks, chromatographic analysis of their urine showed the additional presence of monochloroacetic acid. Muller et al [185] exposed male rats continuously to vinyl chloride at 1,000 ppm for 48 hours, and they found thiodiacetic acid as well as S-(carboxymethyl)cysteine in the urine.

To identify the probable urinary metabolites of vinyl chloride after its administration by oral intubation, Green and Hathway [5] gave each of four rats three doses of 50 mg of 14C-(1,2-)vinyl chloride/kg at 3-hour intervals, for a total dose of about 10 microcuries of 14C. Major metabolites of vinyl chloride were identified by mass spectral analysis as thiodiglycolic acid, S-(2-chloroethyl)cysteine, and N-acetyl-S-(2-chloroethyl)cysteine. Thiodiglycolic acid contained 47% of the excreted label, whereas S-(2-chloroethyl)cysteine and N-acetyl-S-(2-chloroethyl)cysteine each accounted for 23% of the urinary label. Urea, glutamic acid, and monochloroacetic acid were responsible for 6, 0.5, and 0.5% of the urinary 14C, respectively. Radiolabeled methionine and serine were present in trace amounts. The authors also stated that thiodiglycolic acid is the major metabolite (61%) of monochloroacetic acid in the rat.

Watanabe et al [177] also found three major and several minor high-pressure liquid chromatographic peaks that contained about 95% of the total

14C activity in urine samples from rats after oral doses of 0.05, 1.0, or 100 mg of 14C-(1,2-)vinyl chloride/kg. Two of the major peaks were identified as N-acetyl-S-(2-hydroxyethyl)cysteine and thiodiglycolic acid; the third major peak could not be identified.

(3) Proposed Pathways

Investigators have postulated that there are at least two pathways for the metabolism of vinyl chloride, both leading to similar end products [4,6,175,177,186,187]. Some have stated that one pathway, suggested to be active at low levels of absorption, begins with a hydration reaction whose product is chloroethanol. A proposed second pathway, suggested to be predominant at high absorption levels, involves the oxidation of vinyl chloride to chloroethylene oxide by microsomal enzymes. These two pathways converge with the formation of chloroacetaldehyde as the second step in each. Investigators have also postulated that the metabolism of vinyl chloride involves the formation of free radicals [5,157].

Reynolds et al [188], in 1976, proposed a scheme in which an epoxide (chloroethylene oxide) was produced as a primary reactive metabolite of vinyl chloride. This would involve the hepatic mixed-function oxidase system [189,190], particularly the cytochrome P-450 component [191,192]. A wide variety of oxidative reactions, including epoxidation, can be mediated by these enzymes, located in the membranes of the endoplasmic reticulum of liver cells, with NADPH serving as an electron donor. Rearrangement of the epoxide could then occur, producing a beta-chlorinated acetaldehyde, a diol, or a glutathione conjugate as a secondary metabolite. These products could be formed by interaction of the epoxide with epoxide hydrase or with glutathione epoxide transferase [188].

Watanabe et al [177] reported that, at oral doses of 0.05 or 1 mg of 14C-(1,2-)vinyl chloride/kg, 14C was consistently eliminated in the urine as nonvolatile, polar metabolites and in the expired air as carbon dioxide. At an oral dose of 100 mg/kg, however, the primary route of excretion was by expiration of unchanged vinyl chloride. This indicated to the authors not only that metabolic pathways for vinyl chloride are dose dependent, but also that the process contains a saturable component. Comparing these results with their previous findings [175], the authors also concluded that the metabolic fate of vinyl chloride is independent of the route of administration.

In both oral and inhalation exposures to vinyl chloride, N-acetyl-S-(2-hydroxyethyl)cysteine was a major urinary metabolite [175,177]. One study [193] has indicated that this compound could be formed from S-formylmethyl cysteine and S-formylmethyl glutathione, which at one time were considered either not to be formed or to be metabolized to S-carboxymethyl cysteine [193]. Although Green and Hathway [5] identified (2-chloroethyl)cysteine and N-acetyl-S-(2-chloroethyl)cysteine as urinary metabolites of vinyl chloride in rats, the formation of these products may be an artifact [177,185] of the

method of separation (methanol derivatization produces the chloro-compound, whereas diazomethane derivatization produces the hydroxy-compound) [194,195].

From data presented by other authors [5,175,177], showing the formation of 14C-carbon dioxide after administration of 14C-vinyl chloride, Plugge and Safe [196] proposed two additional alternatives for its pulmonary metabolism. The first assumed that the metabolism of vinyl chloride proceeded by the addition and transfer of a chloroacetyl group to coenzyme A and by subsequent metabolism in the Krebs cycle. The second scheme assumed formation of glycolate followed by oxidation to glyoxylate, which entered the C-2 and C-1 pools. By analogy with the metabolic pathway for chloroethylene oxide, the glycolate alternative seems to be the more feasible one [163].

Chloroethanol has been reported to be transformed in vivo and in vitro via rat liver enzymes to S-carboxymethyl glutathione, which can also be derived from the two compounds, S-formylmethyl glutathione and chloroacetate [193]. Chloroacetate has been detected as a metabolite of chloroethanol [197]. Metabolism of either chloroethanol or chloroacetate yields S-carboxymethyl cysteine, thiodiacetate (thiodiglycolate), and small amounts of glycolate [197,198]. In another investigation, thiodiacetate was detected as a product of the metabolism of S-carboxymethyl cysteine [199]; this conversion has been confirmed by Yllner [198].

Bonse and Henschler [1], in a 1976 review of the metabolism of chlorinated ethylenes, concluded that their oxidation via monooxygenases to corresponding oxiranes (epoxides) consituted the initial metabolic reaction. The authors suggested that electrophilic reactions or alkylation of cellular components were essentially responsible for the toxicity of the chlorinated ethylenes and that the other pathways were generally part of a detoxification mechanism. After rearrangement, additional metabolic steps, including oxidation of either aldehydic or alcoholic derivatives to carboxylic acids were suggested. The authors also suggested hydrolysis of acyl chlorides to acids as an alternate pathway.

Green and Hathway [5,195] speculated that several routes might exist for vinyl chloride metabolism in rats. Their first suggestion was oxidative biotransformation involving molecular oxygen and the formation of chloroethylene oxide, which would then spontaneously rearrange to chloroacetaldehyde. Both chloroethylene oxide and chloroacetaldehyde can react with glutathione to form S-(formylmethyl)glutathione, which in turn can be converted to S-(2-hydroxyethyl)cysteine. Chloroacetic acid is also formed from chloroacetaldehyde and subsequently metabolized via the Krebs cycle to glutamic acid. Formaldehyde would also be formed and metabolized to carbon dioxide and urea. Since serine and methionine are synthesized in part from formaldehyde, they also could be formed. The second suggested reaction was the formation of the previously identified metabolites, S-(2-chloroethyl)cysteine and N-acetyl-S-(2-chloroethyl)cysteine. These reaction products were thought to be the result of equilibrium interactions of S-(2-

hydroxyethyl)cysteine and N-acetyl-S-(2-hydroxyethyl)cysteine through cyclic intermediates [195]. The third suggested reaction was conversion of S-(carboxymethyl)cysteine, by oxidation and transamination, to thiodiglycol, and its subsequent oxidation to thiodiglycolic acid. The authors [195] concluded that chloroacetic acid was not a part of the major degradative pathway for vinyl chloride, but simply a byproduct of chloroacetaldehyde metabolism, unless however, the glutathione conjugation mechanism were inhibited, whereupon the conversion of chloroacetaldehyde to chloroacetic acid would become an important detoxification alternative. They further supported this hypothesis by administering several vinyl chloride metabolites to rats and showing that chloroacetaldehyde and S-(carboxymethyl)cysteine, but not chloroacetic acid, were in the direct pathway for the formation of thiodiglycolic acid. These authors [195] also identified small quantities of N-acetyl-S-vinylcysteine as a urinary metabolite of vinyl chloride in rats, thus lending credence to the hypothesized pathway of an equilibrium between the chloro- and the hydroxy-ethyl glutathione derivatives, possibly through an episulphonium ion intermediate.

Plugge and Safe [196], in a 1977 review postulated that the metabolism of vinyl chloride occurs both in vivo and in vitro via the mixed-function oxidase system, primarily through the cytochrome P-450 system, to an oxirane [153], in this case chloroethylene oxide. The oxirane (epoxide) formed from vinyl chloride would be a strong electrophilic molecule, and it may be unstable because of the presence of its asymmetric chlorine. This instability could result in intramolecular rearrangement of the chloroethylene oxide to chloroacetaldehyde. The authors considered that both chloroethylene oxide and chloroacetaldehyde would bind either directly or enzymatically to glutathione, thereby forming S-formylmethyl glutathione. Via an NAD+-dependent aldehyde dehydrogenase, chloroacetaldehyde could also be oxidized to chloroacetate. This particular compound, if it was not excreted, could bind with glutathione to form S-carboxymethyl glutathione, which in turn could be hydrolyzed to S-carboxymethyl cysteine. S-carboxymethyl cysteine could be either deaminated and decarboxylated to form thiodiglycolate, N-acetylated, or excreted.

Van Duuren [200], in 1975, hypothesized that when rats were given large amounts of vinyl chloride, an epoxidation reaction would occur in which vinyl chloride was converted to chloroethylene oxide by the microsomal mixed-function oxidase system. Chloroethylene oxide would subsequently be reacted or would spontaneously rearrange to chloracetaldehyde [201].

Hefner et al [4] conjectured that, with exposures below 100 ppm of vinyl chloride, its metabolism would occur by hydration to 2-chloroethanol followed by oxidation to 2-chloroacetaldehyde by the very rapid alcohol dehydrogenase pathway. Since chloroacetaldehyde reacts rapidly with the sulfhydryl of glutathione, only a trace amount of monochloroacetic acid would be formed.

At higher concentrations of vinyl chloride, Hefner et al [4] suggested that the alcohol dehydrogenase pathway could easily be saturated. Compensatory mechanisms, in terms of alternate pathways, were suggested to

these authors by the fact that two different rate constants were obtained from the results of their kinetic analyses. The rate constant was reduced, from 0.00804 ±0.0034/minute (half-life of 86 minutes) for concentrations ranging from 50 to 105 ppm to 0.00265 ±0.00135 (half-life of 261 minutes) for concentrations ranging from 220 to 1,167 ppm, suggesting that additional pathways were mobilized in conjunction with the alcohol dehydrogenase pathway. Hefner et al speculated that one of the mobilized metabolic paths may involve the oxidation of accumulated 2-chloroethanol, and a second plausible alternative appeared to involve direct epoxidation of vinyl chloride. The authors stated that their results were preliminary and inconclusive for support of their hypotheses. Gehring et al [6] concluded that the reactive metabolites formed from the first pathway should possess a lower carcinogenic potential.

In 1977, Bolt et al [202] stated that vinyl chloride in the atmosphere of a closed system equilibrated with that in the tissues of rats within 15 minutes at various concentrations below 250 ppm (the concentration at which the authors stated that saturation of the vinyl chloride-metabolizing enzymes is achieved) when metabolism of vinyl chloride was blocked by 6-nitro-1,2,3-benzothiadiazole (an inhibitor of some cytochrome P-450-dependent oxidations). When rats not given the metabolic blocking agent were tested, the concentration of vinyl chloride in the atmosphere declined exponentially with a half-life of about 1.1 hours.

Bolt et al [202] also reported that exposure of rats to vinyl chloride at concentrations below 250 ppm (640 mg/cu m) produced a straight first-order decline of vinyl chloride in the exposure system indicating concentration dependence, while exposures at concentrations above 250 ppm produced decline curves best described by zero-order kinetics indicating no concentration dependence. They stated that vinyl chloride leaves the body rapidly due to urinary excretion. They observed that approximately 50% of the dose from a 1-hour exposure at an initial concentration of 50 ppm appeared in the urine after 8 hours; approximately 70% appeared after 22 hours. Excretion via feces and via expiration of carbon dioxide was not taken into account. The authors also noted that the calculated rate constant for urinary excretion of 0.19/hour is valid only in their system. They concluded that on continuous or repeated exposure below 220 ppm, no appreciable accumulation of either unchanged vinyl chloride or its major metabolites should be expected. This is consistent with the conclusions of other authors [146,200,203-205], who support the theory that a reactive, short-lived metabolite occurring in low concentrations is responsible for the adverse effects of vinyl chloride, primarily liver damage and carcinogenesis.

Barrio et al [170], Secrist et al [206], and Barbin et al [2] reported that chloroacetaldehyde reacts directly with adenosine or cytidine at pH 3.5-4.5 and 37 C to produce either 3-beta-D-ribofuranosyl-imidazo-(2,1-i) purine or 5,6-dihydro-5-oxo-6-beta-D-ribofuranosyl-imidazo-(I,2-c) pyrimidine. Bartsch and Montesano [158] postulated that covalent binding of

chloroacetaldehyde directly to DNA in the cell could explain the induction of mutagenesis by base-pair substitutions in Salmonella typhimurium TA1530.

An integrative scheme of putative metabolic pathways for vinyl chloride is presented in Figure XVII-3.

(4) Mechanisms

Hefner et al [4] evaluated the effects of potential inhibitors of vinyl chloride metabolism. Pyrazole, an inhibitor of alcohol dehydrogenase, xanthine oxidase, and other enzymes, administered to rats 1 hour before exposure to vinyl chloride at 65 or 1,234 ppm (166.4 or 3,159 mg/cu m) inhibited the metabolism of vinyl chloride by 71.2 and 86.9%, respectively. Other rats were pretreated with ethanol 1.5 hours before exposure to vinyl chloride at 56 or 97 ppm (143.4 or 248.3 mg/cu m) to competitively inhibit alcohol dehydrogenase activity. This resulted in 96.0 and 82.9% inhibition of vinyl chloride metabolism, respectively. Exposure to vinyl chloride at 1,025 or 1,035 ppm (2,624 or 2,649.6 mg/cu m) after pretreatment with ethanol produced inhibition of vinyl chloride metabolism by 46.5 and 35.7%, respectively. Rats pretreated with SKF-525A (a mixed function oxidase inhibitor) before being exposed to vinyl chloride at 65 ppm (166.4 mg/cu m) exhibited no metabolic inhibition. However, when SKF-525A pretreated rats were exposed to vinyl chloride at 1,038 ppm (2,657.3 mg/cu m), inhibition of vinyl chloride metabolism was 18.8%.

Jaeger et al [207,208], Conolly et al [209], and Conolly and Jaeger [210] also evaluated the effects of various inhibitors and promoters of vinyl chloride metabolism in male and female rats. Female rats were not susceptible to hepatotoxic effects of vinyl chloride in any of the experiments. In polychlorinated biphenyl (Aroclor 1254) or phenobarbital-pretreated male rats, pyrazole and SKF-525A protected against acute hepatotoxicity, while disulfiram (an inhibitor of acetaldehyde dehydrogenase) and ethanol potentiated the toxic effects of vinyl chloride. The authors speculated that the protection afforded by SKF-525A was due to mixed-function oxidase inhibition, indicating a disruption in the metabolism of vinyl chloride to an active metabolite. Protection by pyrazole was probably due partially to inhibition of mixed-function oxidases and partially to inhibition of acetaldehyde dehydrogenase. The lack of competitive effects by ethanol indicated to the authors that the conversion of chloroethylene oxide to chloroethanol was decreased, possibly because of increased spontaneous rearrangement of chloroethylene oxide to chloroacetaldehyde (bypassing the acetaldehyde dehydrogenase conversion step) or because of catalase conversion of chloroethanol to a peroxide and subsequent rearrangement to chloroacetaldehyde (again bypassing acetaldehyde dehydrogenase).

Hefner et al [4] determined the effects of vinyl chloride on liver sulfhydryl levels. Male rats were exposed for 7 hours/day to vinyl chloride at concentrations of 15,000 ppm (38.4 g/cu m) for 5 days, 5,000 or 500 ppm (12.8 or 1.3 g/cu m) for 5 days/week for either 1, 3, or 7 weeks, or 50 ppm

(128 mg/cu m) for either 1 hour, 7 hours, or 5 days. No overt signs of toxicity were observed in rats exposed at any concentration. Significant reductions in nonprotein sulfhydryl levels were found in rats exposed to vinyl chloride at 50 ppm for 7 hours, at 500 and 5,000 ppm for 1 and 3 weeks, and 15,000 ppm for 1 week. Although the reduction in nonprotein sulfhydryl levels could not be definitively correlated with exposure concentration, the authors concluded that there was a tendency for such reduction to become less obvious with repeated exposures. Four rats treated with ethanol before exposure to vinyl chloride at 1,070 ppm (2.7 g/cu m) for 105 minutes had significant decreases in hepatic nonprotein sulfhydryl levels (77.0 ±12.8%) as compared with the controls (95.0 ±3.4%). The authors indicated that ethanol alone did not affect the liver nonprotein sulfhydryl levels.

Reynolds et al [211], in 1975, showed that male rats given drinking water containing 0.1% pentobarbital 7 days before either one or five consecutive 6-hour exposures to airborne vinyl chloride at a concentration of 5% (128 g/cu m) exhibited a diffuse vacuolization of the cytoplasm of cells of the centrilobular liver parenchyma and focal areas of necrosis of midzonal parenchyma after the exposure. In the livers of pretreated rats exposed to vinyl chloride for 5 consecutive days, the authors found broad tracts of stroma depleted of parenchymal cells that corresponded in distribution and extent to the areas of necrosis found 24 hours after a single exposure. Since pentobarbital, an inducer of mixed-function oxidase activity, appeared to increase the liver toxicity of vinyl chloride, the authors concluded that the endoplasmic reticulum was the primary site for generation of toxic vinyl chloride metabolites. Moreover, Reynolds and colleagues suggested that these metabolites, possioly epoxides, were presumably responsible for the observed cellular injury as well as the potential for tumorigenesis.

Several reports [202,204,205,212,213] have detailed the in vivo and in vitro requirements for covalent binding of vinyl chloride (or its metabolites) with cellular macromolecular constituents, including DNA, RNA, and protein. In addition, these reports have identified some effects of various chemical inducers or inhibitors of cellular metabolic processes on the metabolism of vinyl chloride.

In 1975, Bolt et al [204] reported that rat liver microsomes metabolized vinyl chloride to more polar metabolites during a 90-minute incubation and that these metabolites became covalently bound to the microsomal proteins. In addition, vinyl chloride metabolites became covalently bound to other sulfhydryl-containing proteins or to RNA when added to the incubation mixture. NADPH was reported to be essential to the binding process, hence essential to this metabolic route for vinyl chloride. Similar results were reported with microsomes from human liver, but the authors did not give experimental data.

Kappus et al [205], using the same incubation procedure as that used by Bolt et al [204], confirmed the essentiality of NADPH in the covalent binding process. In an additional paper, Kappus et al [212] reported that continued uptake of 14C-vinyl chloride by liver microsomes depended on NADPH. Without

NADPH, uptake of 14C-(1,2-)vinyl chloride increased rapidly during the first 2 minutes and reached saturation after 5 minutes. With NADPH, the uptake of vinyl chloride continued beyond the incubation time of 60 minutes and a tenfold increase in the uptake of 14C-vinyl chloride by the microsomal preparation was noted. The authors also showed that vinyl chloride could be taken up by both protein and lipid components of microsomal membranes; the single difference noted was that the time course differed from that for uptake by microsomes. Their data also suggested a greater ability on the part of liposomes to bind vinyl chloride reversibly. From these studies, the authors inferred that the NADPH-independent part of microsomal vinyl chloride uptake was at least partially due to the reversible binding of vinyl chloride to the lipids and proteins of the microsomal membranes.

Kappus et al [212] also found that, of the total 14C-(1,2-)vinyl chloride taken up by the microsomes, about 1% was bound irreversibly to the microsomal protein. Moreover, irreversible binding of the vinyl chloride metabolites to the microsomal proteins appeared to depend on the presence of NADPH, the incubation time, and the concentration of the metabolites. In addition, the authors demonstrated that when atmospheric air was replaced by nitrogen in the presence of NADPH, vinyl chloride uptake was reduced. The amount of vinyl chloride metabolites irreversibly bound to protein also was lowered.

Kappus et al [205] reported that vinyl chloride metabolites were also bound to added albumin, but not to concanavalin A, which contains no sulfhydryl groups. They found that the addition of glutathione or glutathione-containing cytosol to the incubation medium caused a 30% depression in covalent binding to cellular proteins [204,212], thus indirectly supporting the concept that free sulfhydryl groups must be present for binding to proteins to occur or for detoxification to occur. In further support of this concept, they reported that inhibition of the microsomal cytochrome P-450-dependent mixed-function oxidases by 4-(1-naphthyl)imidazole inhibited covalent binding by about 85%. Microsomal uptake of vinyl chloride was completely blocked by carbon monoxide, an inhibitor of cytochrome P-450 oxidation reactions [212]. Irreversible binding of vinyl chloride metabolites to proteins was also blocked by carbon monoxide, whether NADPH was present or not. Boiling of the microsomes prior to incubation reduced vinyl chloride uptake in the presence of NADPH, and no irreversibly protein-bound vinyl chloride metabolites were detected. Addition of reduced glutathione to the microsomal incubation mixture with NADPH resulted in very little change in vinyl chloride uptake by the microsomes, but did result in a 25% inhibition of irreversible protein binding.

Microsomal uptake of vinyl chloride was not affected by trichloropropene oxide, an inhibitor of epoxide hydrase [212]. However, irreversibly protein-bound vinyl chloride metabolites were increased twofold. No induction effect on vinyl chloride uptake could be demonstrated in microsomes from phenobarbital-pretreated rats. There also were no changes in irreversible protein binding. Cytochrome P-450, however, was increased in the liver

microsomes from rats pretreated with phenobarbital. Vinyl chloride uptake was similar in liver microsomal preparations containing added glutathione and cytosol obtained from control and pretreated rats. From the data presented, the authors suggested that the initial step in vinyl chloride metabolism involved an oxygenation reaction catalyzed by an enzyme system containing cytochrome P-450. In addition, the authors concluded that chloroethylene oxide was probably the initial reactive metabolite.

Kappus et al [205] presented additional evidence for involvement of the epoxide in the covalent binding reaction by using the xanthine oxidase model system, which generates hydrogen peroxide and an oxygen-radical. They concluded that demonstration of the binding of vinyl chloride metabolites to albumin in the presence of a complete xanthine oxidase system strongly suggested that oxygen radicals, known to be involved in epoxidation by the microsomal enzyme system, convert vinyl chloride to a metabolite that then binds covalently to a protein, such as albumin.

Watanabe et al [214], in 1977, attempted to characterize the binding of vinyl chloride to hepatic macromolecules and nucleic acids by exposing male rats to 14C-(1,2-)vinyl chloride at nominal concentrations of 1, 10, 25, 50, 100, 250, 500, 1,000, or 5,000 ppm (range 2.96-12,800 mg/cu m). The results suggested that increases in exposure concentration did not proportionately increase the total amounts of radioactivity bound to hepatic macromolecules. The percentage of total 14C activity bound to hepatic macromolecules ranged between 20 and 22%, except in rats pretreated with phenobarbital where it reached 39%. Both the metabolized and bound vinyl chloride increased with increasing nominal concentration, but the ratio of bound to metabolized vinyl chloride declined with increasing nominal concentration.

In rats exposed at nominal concentrations of 1, 10, 25, or 50 ppm (range 2.56-128 mg/cu m), Watanabe et al [214] found that the nonprotein sulfhydryl content of the liver was depleted, but not significantly so. However, at 100 ppm (256 mg/cu m) and above, a significant depletion ($P<0.05$) was noted. Since it seemed a reasonable assumption that the major detoxification of the reactive metabolites of vinyl chloride occurs by reaction with nonprotein sulfhydryl groups [186], Watanabe et al [214] considered it important that at nominal concentrations of 100 ppm and higher, a signficant dose-related depletion of that pool could be demonstrated. They also suggested that the carcinogenicity of vinyl chloride was related to the decreased ability of exposed organisms to detoxify the reactive metabolites of vinyl chloride.

Watanabe et al [214] reported that no evidence could be found that would suggest covalent binding of 14C from vinyl chloride to the isolated nucleic acids in their experiments. The authors concluded that vinyl chloride did not preferentially react with intracellular nucleic acids. They mentioned, however, that this finding conflicted with the findings of a previous study by Bolt et al [215] which demonstrated that covalent binding to RNA and DNA occurred after a static exposure to 14C-vinyl chloride at 145 ppm (371.2 mg/cu m). Both Watanabe et al [214] and Bolt et al [215] concluded that vinyl

chloride does not preferentially react with hepatic nucleic acids. However, Watanabe et al [214] reported that five rats pretreated with phenobarbital (80 mg/kg/day) by ip injection 3 days before exposure to 14C-vinyl chloride at a nominal concentration of 100 ppm showed a markedly increased binding of 14C to hepatic macromolecules when compared with nonpretreated animals, even though there was no observable increase in vinyl chloride metabolism.

Watanabe et al [214] concluded that their findings did not associate the carcinogenicity of vinyl chloride with a disproportionate increase in binding of its electrophilic metabolites to hepatic macromolecules as the exposure concentration was increased. Because there was no demonstrable evidence to support preferential binding of the electrophilic metabolites to nucleic acids of the hepatocytes, they raised the possibility that the carcinogenic potential of vinyl chloride could not be associated with this commonly accepted mechanism for carcinogenesis. The authors suggested, however, that before alkylation of nucleic acids was excluded as the carcinogenic mechanism, more studies to determine the absence of such alkylation activity in the target tissue rather than the hepatocytes are required. They suggested that the hepatocyte itself, since it is susceptible to the toxicity of vinyl chloride, may function as a detoxifier. This would imply that tissues having a lesser ability to detoxify the reactive metabolites of vinyl chloride may themselves become the victims of toxicity.

Gehring et al [6] stated that since "Toxicity, including carcinogenesis, is a dynamic process involving absorption of a chemical into the body, distribution to various tissues, reversible or irreversible reactions with cellular components, and ultimately clearance from the tissues and the body via metabolism and/or excretion," it follows that "the predictability of animal toxicological data for assessing the hazard of a chemical to man is enhanced if the fate of the chemical per se and/or its degradation products in animals is equated to the fate in man." They stated that under ordinary conditions and over a selected range of doses, chemical kinetics fit a linear differential equation, the implication being that a known increase in dose will result in a linear increase in tissue levels. They added, however, that many metabolic and excretory processes were easily overwhelmed and saturated, thus leading to a situation in which nonlinear Michaelis-Menten pharmacokinetics would prevail. The authors suggested that the results of Hefner et al [4], in which the rate of metabolism of vinyl chloride at low concentrations was rapid and the primary metabolic pathways were overwhelmed at high concentrations, supported this theoretical model.

The data presented above suggest that alkylating metabolites of vinyl chloride, such as chloroacetaldehyde and chloroethylene oxide, are formed in vivo. Both chloroacetaldehyde and chloroethylene oxide can conjugate with glutathione and cysteine and subsequently form the vinyl chloride metabolites that have been identified in urine, such as N-acetyl-S-(2-chloroethyl)cysteine [195], which may itself be a potent alkylating agent by virtue of its half-mustard forming ability. In terms of assessing the hazard of exposure to vinyl chloride, experimental data support the conclusion that the

carcinogenicity of vinyl chloride is a function of the metabolic formation of alkylating metabolites. The urinary metabolites identified so far indicate that the primary deactivation mechanism is conjugation with glutathione, a nonprotein, free-sulfhydryl containing compounds. Experimental data have indicated that as the nonprotein, free-sulfhydryl groups are depleted as a sequel of the absorption and metabolism of vinyl chloride reaction of the alkylating metabolites with tissue macromolecules, such as DNA or RNA, may be more likely to occur. As a result, toxicity of a different order of magnitude may be elicited at higher exposure concentrations.

(b) Vinylidene Chloride

McKenna et al [216] showed that in rats exposed to airborne 14C(1,2-) vinylidene chloride at concentrations of 10 and 200 ppm (39.7 and 794 mg/cu m) for 6 hours covalent bonding occurred in the liver. At 10 ppm, 1-2% of the body burden was expired as unchanged vinylidene chloride, and at 200 ppm, between 4 and 8% was expired unchanged, ie, a twentyfold increase in the exposure concentration increased the total body burdens of vinylidene chloride of both fasted (fasting depletes liver glutathione) and fed rats only fifteenfold.

The pulmonary elimination of vinylidene chloride at both 10 and 200 ppm (39.7 and 794 mg/cu m) was found to be biphasic [216]. First-order rate constants for the rapid and slow portions of the biphasic curve were 0.0345 ±0.0095 and 0.0032 ±0.0013/minute (half-lives of 20 and 217 minutes) at 10 ppm and 0.0324 ±0.002 and 0.0052 ±0.0042/minute (half-lives of 19 and 133 minutes) at 200 ppm. The largest amounts of radioactivity at both concentrations were found in the liver and kidneys. The fasted rats exposed at 10 ppm (39.7 mg/cu m) had more radioactivity in the liver and plasma than the fed animals exposed at 10 ppm. The amount of covalently bound radioactivity in the liver, as compared with body burden of 14C and total metabolism of vinylidene chloride, increased about 26 times with a twentyfold increase in exposure concentrations for the fed rats. The percentage of covalently bound radioactivity in the livers of fasted rats exposed at 200 ppm also was greater than that in the fed animals, although the fasted rats metabolized less vinylidene chloride than the fed animals.

The elimination of radioactivity in rat urine at both 10 and 200 ppm was also biphasic [216]. First-order rate constants for the rapid and slow portions of the biphasic curve were 0.226 ±0.041 and 0.036 ±0.005/hour, (half-lives of 3.1 and 19.3 hours) at 10 ppm and 0.155 ±0.015 and 0.043 ±0.007/hour, (half-lives of 4.5 and 16 hours) at 200 ppm. The urinary metabolites, at 24 hours after exposure, were identified as N-acetyl-S-(2-hydroxyethyl)cysteine and thiodiglycolic acid, with two additional unidentified 14C-labeled fractions.

McKenna et al [217] reported that rats given single oral doses of 50 mg/kg of 14C-(1,2-)vinylidene chloride exhaled 19-29% of the radioactivity as unchanged vinylidene chloride while rats receiving 1 mg/kg exhaled only 1-3%

of the radioactivity as unchanged vinylidene chloride. The increase in pulmonary elimination of vinylidene chloride at the higher dose was accompanied by decreases in 14C carbon dioxide production and in urinary and fecal excretion of 14C. Fasted rats excreted less radioactivity in the urine (35%) than fed animals (47%) at 50 mg/kg. The pulmonary elimination of radioactivity was inconsistent after administration of vinylidene chloride at a dose of 1 mg/kg, although these elimination curves also were biphasic. Rate constants were not calculated, but half-lives for the rapid and slow portions of the curves were estimated to be 25 and 117 minutes, respectively, for both fed and fasted rats. The pulmonary elimination curve at 50 mg/kg was biphasic, with rate constants for the rapid and slow portions of the curve being 0.0332 ±0.0036 and 0.0105 ±0.001/minute (half-lives of 21 and 66 minutes). Elimination reached a peak at 30 minutes in fed rats and at 60 minutes in fasted rats.

The greatest tissue concentrations of 14C after 72 hours were found in the liver [217]. A fiftyfold increase in dose resulted in only a 35-fold increase in nonvolatile radioactive compounds. There was a fiftyfold increase in covalently bound radioactivity in the liver, however, which was greater than the observed increase in metabolism. The urinary excretion profiles were biphasic, with apparent first-order rate constants for the rapid and slow phases of 0.116 and 0.042/hour (half-lives of 6 and 16.8 hours) at both 1 and 50 mg/kg. The major urinary metabolites were the same as those identified after inhalational exposure [216], ie, N-acetyl-S-(2-hydroxyethyl)cysteine and thiodiglycolic acid. McKenna et al [217] also reported that unchanged vinylidene chloride was eliminated only in expired air and was not retained in the tissues for longer than a few hours.

Madrid et al [218] identified two of the four major urinary metabolites isolated from rats exposed to 14C-vinylidene chloride by inhalation at 10 or 200 ppm (39.7 or 794 mg/cu m) for 6 hours and by oral administration (1 or 50 mg/kg) as N-acetyl-S-(2-hydroxyethyl)cysteine and thiodiglycolic acid. The former compound represented about 45% of the total urinary radioactivity, whereas the latter accounted for 25%. The authors concluded that the identification of these two compounds as urinary metabolites supported the hypothesis that glutathione conjugation was a major step in the biotransformation of vinylidene chloride.

Jaeger et al [219], in 1977, reported that fed and fasted male rats exposed to 14C-vinylidene chloride at 2,000 ppm (7.9 g/cu m) for 2 hours did not differ significantly in the rate of vinylidene chloride uptake or of urinary excretion of 14C during the first 24 hours. Of the calculated dose, 36.7 and 36.5% were recovered within 24 hours in the urine of fed and fasted animals, respectively. Thirty minutes after a 2-hour exposure, the kidneys of fasted rats contained both the greater amount of total radioactivity and the larger amount of metabolites that were soluble in trichloroacetic acid (hence, not bound). Fasted rats also had significantly greater amounts of total radioactivity in the spleen, heart, and serum than did fed rats. There was no

significant difference between the 14C contents of the brains of fed and fasted rats.

The livers of fasted rats contained substantial amounts of radioactivity that was trichloroacetic acid-insoluble [219]. This component represented either 14C that was tightly bound to microsomal or mitochondrial macromolecules or that had entered the metabolic pool. The rates of disappearance of the trichloroacetic acid-insoluble 14C from the microsomal and mitochondrial fractions of the liver in fed and fasted rats were similar, although the amounts differed significantly; both had estimated half-lives of less than 3 hours. Significantly more radioactivity was found in the hepatic cytoplasmic fractions of fasted rats than in those of fed rats. The authors suggested that on the basis of their data metabolism of vinylidene chloride was quite rapid, with trichloroacetic acid-soluble components being excreted by the kidney and trichloroacetic acid-insoluble components entering the metabolic pool. They concluded that since a rapid turnover of bound 14C material occurred (half-life less than 3 hours), covalent binding to protein or tissue constituents must have been minimal.

Jaeger et al [219] concluded that fasting had no effect on the rate or on the amount of in vivo metabolism, but that the in vivo metabolic pathway appeared to be significantly different in fasted rats than in fed rats. They also reported that pretreatment with trichloropropane epoxide (an epoxide hydrase inhibitor) significantly increased the toxicity of vinylidene chloride in rats, and on this basis, suggested the possible formation of an epoxide intermediate as a result of the hepatic metabolism of vinylidene chloride.

Several studies [115,220-224] have shown the hepatotoxic effects of inhaled vinylidene chloride on rats to be associated with decreased glutathione concentrations in the liver and liver mitochondria. Fasting of rats before exposure to vinylidene chloride at concentrations of 2,000 ppm (7.9 g/cu m) for 4 hours [115,221], 1,980 ppm (7.9 g/cu m) for 4 hours [220], 250 ppm (992.5 mg/cu m) for 1-24 hours [223], or 200 ppm (794 mg/cu m) for 4 hours [222] resulted in markedly enhanced hepatotoxicity as compared with that seen in fed control rats. Hepatic necrosis was reported in fasted but not in fed rats and was associated with glutathione depletion Elevation of serum alanine alpha-ketoglutarate transaminase activity levels (indicative of liver injury) occurred in fasted rats at 150-200 ppm but only at or above 2,000 ppm in fed rats [115,220]; this difference was also evident in an isolated perfused rat liver system [115].

Diethylmaleate, a material that depletes glutathione, potentiated the hepatotoxic effects of vinylidene chloride on fed rats and on perfused livers from fed rats [115]. Surgical or chemical thyroidectomy (resulting in increased liver glutathione) reduced the severity of hepatotoxic injury in fasted rats exposed to vinylidene chloride at a concentration of 2,000 ppm for 4 hours, whereas thyroxine (which increases the general metabolic rate and decreases glutathione) enhanced the hepatotoxicity of vinylidene chloride [224]. SKF-525A (an inhibitor of mixed-function oxidases) did not affect the

response of fed rats exposed to vinylidene chloride nor did it protect fasted rats from the hepatotoxic effects of such exposure [222]. However, Reichert and Bashti [225] reported that SKF-525A in perfused rat liver preparations diminished the rate of metabolism of vinylidene chloride. These data support the hypothesis that a major pathway for the detoxification of vinylidene chloride involves conjugation with glutathione and that blockage of its conjugation with glutathione greatly enhances its hepatotoxicity.

Jaeger [223] noted that a significant elevation ($P<0.05$) of hepatic citric acid occurred after exposure of rats to vinylidene chloride at a concentration of 250 ppm. He concluded that vinylidene chloride probably affected the mitochondria, leading to inhibition of the Krebs cycle and subsequent mitochondrial damage. Jaeger et al [219], on the basis of prior work [5,152], hypothesized that chloroacetyl chloride, a metabolite of vinylidene chloride, was converted to monochloroacetic acid, which in turn was converted into chlorocitric acid, an inhibitor of the enzyme aconitase. Inhibition of this enzyme would result in an accumulation of citric acid. This hypothesis was predicated by analogy on the hypothesis of lethal synthesis, which suggests that fluoracetic acid is converted to fluorocitric acid, which inhibits aconitase [226].

Short et al [117] reported that the lethality of vinylidene chloride in male and female mice exposed at various concentrations for 22-23 hours was reduced substantially by pretreatment with disulfiram. When exposures were increased to 22-23 hours/day for 2 days, pretreatment with disulfiram, diethyldithiocarbamate, or thiram reduced the lethality of vinylidene chloride in male mice. Pretreatment with methionine or cysteine also reduced the lethality of vinylidene chloride, but instead of protecting in terms of increasing the acute LC50 (as did the other three compounds), these compounds protected by delaying the onset of mortality after exposure.

Short et al [117] also reported that disulfiram pretreatment protected male mice from the hepatotoxic effects of exposure to vinylidene chloride at 60 ppm (238.2 mg/cu m) for 22-23 hours. However, after two consecutive 22- to 23-hour exposures at 60 ppm, there was no protection from hepatotoxicity.

Short et al [117] showed that covalently bound radioactivity could be detected in the macromolecules of the liver and kidneys of mice 4 and 24 hours after an ip injection of 3 mg of 14C-vinylidene chloride/kg. The kidneys contained more bound 14C/mg of protein than did the liver at 4 and 24 hours after administration of vinylidene chloride. Pretreatment with disulfiram greatly reduced the bound 14C in both tissues at both time periods.

Bonse and Henschler [1] proposed in a 1976 review of polychlorinated aliphatic compound metabolism that an oxirane, a postulated metabolic intermediate of vinylidene chloride, spontaneously rearranged to chloroacetyl chloride and was then hydrolyzed to monochloroacetic acid. Since monochloroacetic acid is also a product of vinyl chloride metabolism, they speculated that its formation and fate in vinylidene chloride metabolism followed a similar scheme.

An integrative scheme for vinylidene chloride metabolism is presented in Figure XVII-3.

(c) Vinyl Bromide

In 1940, Abreu and Emerson [227] reported that a slight increase (approximately 2.7 times control value, 0.016-0.043 mg/g wet liver) occurred in the amount of total inorganic bromide in the livers of mice exposed to vinyl bromide vapor at a concentration of 2.5 millimoles/liter (267,400 mg/cu m) for 60 minutes. The authors concluded that this increase in liver bromide content was caused by the in vivo hydrolysis of vinyl bromide.

Barbin et al [2], in 1975, showed that a volatile metabolite was formed when a 50% vinyl chloride-oxygen mixture was passed through an incubation medium containing phenobarbital-pretreated mouse liver microsomes and an NADPH-generating system. The volatile metabolite was identified as chlorethylene oxide. A 1:1 mixture of vinyl bromide and air, when passed through the same microsomal system, produced a reaction product having an absorption spectrum identical to that of the vinyl chloride/oxygen mixture. From these data, the authors concluded that vinyl chloride and vinyl bromide were converted in vitro by microsomal enzymes to the corresponding epoxides.

Conolly et al [209] and Conolly and Jaeger [210] reported that polychlorinated biphenyl (Aroclor 1254)-treated male rats that were then exposed to vinyl bromide at concentrations of 10,000-51,000 ppm (43.8-223.4 g/cu m) for 4 hours developed acute hepatic injury as indicated by increases in serum sorbitol dehydrogenase activity. The authors [209,210] concluded that these data, along with observations of increased toxicity in fasted animals and those treated with an inhibitor of epoxide hydrase, suggested that the acute hepatotoxicity of vinyl bromide was mediated through epoxide intermediates. They felt that the similarity between the acute effects of vinyl bromide and of other vinyl halides indicated that hepatic mixed-function oxidase induction had occurred to such an extent that the rate of epoxidation exceeded the rate of detoxification, so that hepatic damage occurred. Conolly and Jaeger [210] concluded also that both glutathione and epoxide hydrase were involved in the detoxification of vinyl bromide metabolites in polychlorinated biphenyl-treated rats.

(d) Vinyl Fluoride and Vinylidene Fluoride

Dilley et al [228] found that vinyl fluoride and vinylidene fluoride could undergo biotransformation and that the carbon-fluorine bond could be broken. They reported that male rats exposed by inhalation to vinyl fluoride or vinylidene fluoride for 30 minutes at concentrations of 3,000 and 2,200 ppm (6.64 and 5.76 g/cu m), respectively, excreted significantly increased ($P<0.01$) amounts of fluoride ion daily when compared with the nonexposed control animals. The value for excreted fluoride ion 6 days after exposure to vinyl fluoride was 3.77 ±0.23 μmol. There was a significant increase ($P<0.05$) in urinary output for vinyl fluoride-exposed animals that persisted for the 14

days of measurement. The values for excreted fluoride 1, 5, 6, and 7 days after exposure to vinylidene fluoride ranged from 3.02 ±0.14 to 4.30 ±0.49 μmol. No diuresis was observed in vinylidene fluoride-exposed animals.

The authors also noticed a cyclic excretion pattern for vinylidene fluoride in which there was major excretion directly after exposure and again 5 days after exposure [228]. The authors proposed that either the fluoride ion, the parent compound, or the fluorine-containing metabolites were stored in some biologic compartment and then released with a turnover rate of about 5 days.

Conolly and Jaeger [210] reported that fasting had no effect on hepatotoxicity in polychlorinated biphenyl-treated rats exposed to vinyl fluoride at 10,000 ppm (18.8 g/cu m) for 4 hours. Three consecutive daily doses of trichloropropane epoxide (an inhibitor of epoxide hydrase) before exposure to vinyl fluoride produced increased mortality in rats; however, fasting in addition to administration of trichloropropane epoxide did not exert a synergistic effect. This indicated to the authors that glutathione may not be important in the detoxification of vinyl fluoride metabolites in polychlorinated biphenyl-pretreated rats, but that epoxide hydrase may be involved in detoxifying fluoroethylene oxide.

Structure-Activity Considerations

Vinyl chloride has been shown to be a human and animal carcinogen, inducing characteristic angiosarcoma of the liver [79,84,87,134,135,140]. Vinylidene chloride [140,141] and vinyl bromide [143] have also been shown to induce this characteristic tumor in animals. No information is available concerning the induction of tumors by vinyl fluoride or vinylidene fluoride. An assessment of the carcinogenic potential of the vinyl halides as a group can be attempted by applying the available carcinogenicity and mutagenicity data to the structure-activity interrelationships of the five compounds.

Such an assessment of the carcinogenic potential of the vinyl halides requires an understanding of their mechanisms of action. A review of the available metabolic data on vinyl chloride indicates that several reactive intermediates are produced (Figure XVII-3). These intermediates are electrophiles, which have been shown to form covalent bonds with cellular macromolecules. A similar metabolic route may be operative for the other vinyl halides, producing similar intermediates that also may bind covalently to cellular constituents. The carcinogenic potential of each of the vinyl halides would therefore be the resultant potentials of these possible metabolites and of any unmetabolized compound.

One hypothesis for the mechanism by which vinyl chloride expresses its carcinogenic potential involves its metabolism, via the microsomal cytochrome P-450 mixed-function oxidase system, to the oxirane (epoxide), chloroethylene oxide [1,196,200,212]. One basis for predicting the carcinogenicity of

inadequately tested vinyl compounds is the assumption that metabolic epoxidation is involved in their metabolism as well, and that the epoxide is the major reactive intermediate. The relative carcinogenic potential would then be influenced by the relative ease with which the epoxides are formed from the corresponding halo-olefins and the relative reactivity of a particular epoxide toward cellular nucleophiles.

The propensities of vinyl halides to undergo chemical transformation to epoxides are dependent on the electronegativities of the substituents on the olefinic nucleus [229]. Increasing electronegativity results in rarefaction of the electron density of the double bond, and thereby in a decrease in the susceptibility of the olefin to epoxidation. Thus, for the vinyl halides under consideration, the expected order of non-enzymatic epoxidation would appear to be vinyl bromide > vinyl chloride > vinylidene chloride > vinyl fluoride > vinylidene fluoride. Bonse and Henschler [1] supported this scheme of epoxidation by showing that the order of the rate of ozonization of a series of olefins was ethylene > vinyl chloride > trichloroethylene > tetrachloroethylene. Also, a study of the reaction rates of ozone, a strong oxidizing agent, with chlorinated and conjugated olefins showed that the rate of ozone attack decreased strongly (1,000-fold from vinyl chloride to tetrachloroethylene) as the number of chlorine atoms in the olefin molecule increased [230].

Hanzlik et al [231] found that a series of para-substituted styrenes (vinyl benzenes) underwent metabolism via the cytochrome P-450 system of rat-liver microsomes at rates that were essentially substituent independent. From the kinetic data of these authors, RL Schowen (written communication, August 1977) calculated a Hammett rho value (a measure of the sensitivity of the reaction series to ring substitution) of around -0.2. Using this value and the Hammett equation, Schowen suggested that substituted vinyl compounds for which the substituents have effective Hammett sigma values (measures of the substituents' abilities to attract or repel electrons by inductive and resonance effects) which differ by as much as 2 units, should differ in rates of oxidation by only a factor of 2.5. Since the sigma* values (measures of inductive effects alone) for the halogens are fluoride, 1.10; chloride, 1.05; and bromide, 1.02 [232], Schowen concluded that these vinyl compounds would be expected to generate epoxide metabolites at similar rates.

Schowen also addressed the problem of estimating the reactivity of the epoxide intermediates that are assumed to be formed. He made two analyses, each dependent on an experimentally derived measure of reactivity for halide compounds. One analysis used Swain-Scott substrate factors, S, which reflect electrophilicity [232]. Based on S values determined for other halide alkylating agents, ag, S = 1.00 for ClCH2CO2-, 1.10 for BrCH2CO2-, 1.33 for ICH2CO2-, and for nonhalide agents, Schowen suggested that alkylating potentials of the vinyls relative to vinyl chloride would vary over a range of no more than 16. In a second analysis using a leaving group parameter, L, from the Swain-Lohmann equation [233] for the halides (bromine, 0.0; chlorine, -1.61; fluorine, -3.60) and reaction constants determined from these L values

for halide compounds, he estimated that the relative reaction rates of chloro to fluoro compounds would range from 63 to 1,585 and that those of bromo to chloro would range from 25 to 398. Thus, the fluoro derivatives could be roughly 1,500-fold less reactive than the chloro derivatives, while the bromo derivatives could be roughly 400 times more reactive. Although the second method predicts less alkylating potency for the fluoro compounds than the first method, both methods indicate that at least the chloro- and bromo-vinyls would be expected to form active alkylating intermediates.

The available experimental evidence lends some support to the theoretical considerations regarding epoxidation, but the information is spotty, and comparative studies on the relative rates of epoxidation of the five vinyl halides under consideration are not available. Besides the evidence for formation of the epoxide of vinyl chloride, the production of epoxides in the metabolism of vinylidene chloride [1,173] and vinyl bromide [2] has only been postulated. No evidence has been located for the metabolic epoxidation of vinyl fluoride or vinylidene fluoride. Other chlorinated olefins whose metabolism via an epoxide has been postulated are trichloroethylene and tetrachloroethylene. Evidence in support of the formation of the corresponding epoxides has been reviewed [1].

Bonse and Henschler [1] also attempted to estimate the relative reactivities of vinyl intermediates. They noted that a common feature of those vinyl halides found to be mutagenic is their potential for the formation of asymmetric oxirane intermediates. Asymmetrically substituted oxiranes have been found to be less stable and, hence, presumably more reactive than the symmetrical ones such as those that would be expected to be formed from some of the vinyl halides that have been tested and found inactive as mutagens, eg, cis- and trans-1,2-dichloroethylene [151-153]. The authors suggested that the formation of an asymmetrical intermediate might be a useful criterion for predicting mutagenic and carcinogenic potentials of the vinyl halides.

The solubilities of the vinyl halides in water increase in the following order: fluoride, difluoride, chloride, dichloride, and bromide. Their molar solubilities are essentially zero, 0.003, 0.018, 0.026, and 0.053, respectively (see Table XVII-1). Their solubilities in blood might well follow this same order although lipid solubility and various types of macromolecular binding cannot be ignored. That is to say, the relative amount absorbed through the lungs may be about 20 times greater for vinyl bromide than for vinylidene fluoride.

The experimental evidence and theoretical considerations are compatible with the hypothesis that the five vinyl halides are capable of undergoing biochemical epoxidation but that the two fluoro olefins may be somewhat less susceptible than the other vinyl halides to this reaction. Extrapolation of the estimates of Schoven suggests that vinylidene chloride, and vinyl bromide, may have a similar or greater carcinogenic potential than vinyl chloride, but that vinyl fluoride and vinylidene fluoride, although possibly carcinogenic,

on the basis of limited mutagenicity data, are likely to be less reactive (possibly up to 1,500 times less) than the other three.

The suggestion that the other vinyl halides resemble vinyl chloride in their effects and mechanism of action is debatable on the basis that normal mammalian biochemical constituents such as steroids and unsaturated fatty acids contain the vinyl moiety and are not suspected of carcinogenicity. It should be noted that variation either above or below a physiologically critical concentration range for many of these compounds may lead to toxic effects. NIOSH is unable to suggest any systematic relationship between the toxicities of all compounds containing the vinyl group, although vinyl halides are treated here as a group because of the similarities in chemical and physical properties. It may well be that it is the presence of the halide moiety which imparts a particular toxicity range to these agents by allowing them to be activated, epoxidated, or hydrated at sufficiently high rates, and, at the same time imparting to the metabolites a characteristic stability and intrinsic activity that allows them to reach and interact with vital cellular constituents.

In the absence of additional toxicologic data on the vinyl halides under consideration, the relevance of the theoretical considerations discussed above to estimation of the potential toxicity of these compounds might be tested by developing mathematical relationships between biologic activities and physicochemical parameters. These parameters could also consider any variations in steric interactions. Sufficient consistent biologic data are not yet available, however, to permit the development of such equations.

Correlation of Exposure and Effect

The vinyl halides have been shown to induce effects on the nervous, circulatory, respiratory, integumentary, skeletal, and digestive systems in humans and laboratory animals. These effects are summarized in Tables III-2 to III-10. The modes of action of the vinyl halides are not clearly understood, and there is little published information on any of them other than vinyl chloride. It has been proposed that expression of the tumorigenic and mutagenic potential of vinyls depends on metabolic intermediates; this possibility is discussed in the section on Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction.

Lethal concentrations of the vinyl halides have been determined. For vinyl chloride, the LC50 for mice exposed for 10 minutes and observed for an unspecified period was 239,580 ppm [105]. In another study, the LC50's for 2-hour exposures were calculated as 117,500 ppm for mice, 156,000 ppm for rats, and 238,000 ppm for guinea pigs and rabbits [104]. The LC100's were also

determined in this experiment; they were 150,000 ppm for mice, 210,000 ppm for rats, and 280,000 ppm for guinea pigs and rabbits. The duration of the observation period was not reported.

For vinylidene chloride, the 4-hour LC50 in rats has been reported as 6,350 ppm with a 14-day observation period [116]. Other authors [118] reported that from two to four of six rats died within 14 days after one 4-hour exposure at a concentration of 32,000 ppm. A single 22- to 23-hour exposure of rats produced LC50 values of 98 ppm for males and 105 ppm for females [117]. Two exposures for 22-23 hours gave LC50 values of 35 ppm for male rats, and the LT50 for exposure at 20 ppm was reported as 4 days. This experiment showed that mortality in rats exposed to vinylidene chloride is in part a function of the total accumulated dose. The 24-hour LC50 value in rats after one 4-hour exposure was determined as 15,000 ppm for fed animals and 600 ppm for animals fasted for 18 hours [115]. The oral LD50 has also been calculated for adrenalectomized rats as 84 mg/kg for 24 hours and 81 mg/kg for 96 hours; in sham-operated controls, the LD50 was 1,550 mg/kg for 24 hours and 1,510 mg/kg for 96 hours [126]. This indicated that adrenal hormones provide some protection against the acute effects of vinylidene chloride in rats.

Exposure to vinyl bromide at concentrations of 171,000 ppm has been tolerated by rats for 10 minutes [105]. In another experiment [127], concentrations of 100,000 ppm were shown to be 100% lethal in rats after 15 minutes, and some rats died (percentage unspecified) at concentrations of 50,000 ppm for 7 hours. An oral LD50 of 500 mg/kg (given in corn oil) for vinyl bromide was also determined for rats [127].

Vinyl fluoride has been reported to be not lethal to rats exposed at a concentration of 80%, with 20% oxygen, for 12.5 hours [128]. Vinyl fluoride at 100,000 ppm, 7 hours/day, 5 days/week, for 6 weeks caused no observed adverse effects on rats according to Lorieno, quoted by Carpenter [128]. However, Connoly et al [209] reported that four of seven rats pretreated with Aroclor 1254 and exposed to vinyl fluoride at concentrations of 102,000 ppm died after a 4-hour exposure.

Vinylidene fluoride has been reported not to be lethal to rats exposed at a concentration of 80%, with 20% oxygen, for 19 hours [128]. Another author [118], however, stated that a static exposure to vinylidene fluoride at 128,000 ppm for 4 hours was sufficient to kill from two to four of six rats within 14 days. Not enough information was presented to evaluate whether or not the static exposure conditions, eg, decreased partial pressure of oxygen, were directly responsible for this difference in lethality.

These studies indicate that vinyl chloride, vinylidene chloride, and vinyl bromide present a low degree of acute toxic hazard in animals, and that, as the duration of exposure increases, the concentration necessary to produce lethal effects decreases. One study [117] showed that exposure of male mice to vinylidene chloride at concentrations as low as 20 ppm was lethal after daily exposures of 22-23 hours for 4 days. The information available suggests

that the acute toxicity of these compounds is dependent in part on the rates of metabolism and excretion and the subsequent total accumulated dose. The variability of the estimates of LC50 values, resulting in part from variability in the experimental protocols used, does not allow a ranking of the compounds according to acute toxicity, except that vinyl fluoride and vinylidene fluoride are less toxic than the other vinyls and have not been found to have acute lethal actions on dynamic inhalation exposure in normal rats.

Cardiovascular effects have been reported for vinyl chloride, vinylidene chloride, and vinylidene fluoride. Twenty percent of 51 workers examined in 1965, who were exposed to vinyl chloride at a TWA concentration of 49 ppm, and 42% of 60 workers examined in 1969, exposed at a TWA concentrations of 43 ppm, showed elevated pulmonary arterial pressure [19]. Durations of exposure for these workers were not presented. In another report [78], workers who were currently exposed to vinyl chloride or who had been exposed in the past had a 39.4-41% frequency of diastolic hypertension, while workers from the same plant with no known exposure to vinyl chloride had a frequency of diastolic hypertension of 24.3% ($P<0.05$). Symptoms of circulatory disturbances in workers exposed to vinyl chloride have been reported in several studies. The symptoms included cold hands and feet (13%) [33], cold fingers and hands (25.7%), numbness or tingling in fingers or toes (31.4%), and other indications of Raynaud's syndrome (8.6%) [20]. Raynaud's syndrome (155) and unspecified circulatory disturbances (12%) appeared in another population also [32]. Cardiac sensitization to epinephrine has also been demonstrated in dogs exposed to vinyl chloride at 50,000 ppm for 5 minutes [110]. Changes, such as tachycardia, sinus arrhythmia, and ventricular multifocal extrasystoles, have been observed in the ECG records of dogs exposed to vinyl chloride at 100,000 ppm without epinephrine stimulation [107]. A significant decrease ($P<0.05$) of 28.5% in myocardial force of contraction has been observed in monkeys exposed to vinyl chloride at the same concentration for 5 minutes [108]. Monkeys exposed at 50,000 ppm showed a decrease of 9.1% in myocardial force of contraction, and those exposed at 25,000 ppm showed a 2.3% decrease in force of contraction. Decreases in aortic blood pressure followed a similar pattern in the monkeys. This experiment indicates that the cardiovascular effects of vinyl chloride are dose dependent.

Vinylidene chloride, inhaled at concentrations of 25,000 ppm for 10 minutes, caused sinus bradycardia and such cardiac arrhythmias as AV-block and ventricular fibrillation in rats [121]. Vinylidene chloride also enhanced the cardiac sensitivity to epinephrine, and this sensitivity increased with increasing duration of vinylidene chloride exposure.

No cardiac sensitization to epinephrine was noted in cats or dogs exposed to vinylidene fluoride at concentrations of 250,000-500,000 ppm for 5-15 minutes [131]. This suggests that vinylidene fluoride does not have the same mode of action on the cardiovascular system as vinyl and vinylidene chlorides. Similar information is not available for vinyl bromide or vinyl fluoride.

CNS effects have been observed on animals after exposure to each of the vinyl halides. In some instances, these effects might have been secondary to systemic effects caused by cardiovascular changes. The authors of the reports did not address this possibility. Vinyl chloride caused "certain anesthesia" in mice after a 10-minute exposure at 122,000 ppm, and 50% anesthesia after exposure a 10-minute exposure at 100,000 ppm [105]. Exposures at LC50's caused excitement, convulsions, and contractions in mice, rats, and rabbits [104]. Exposure of dogs to vinyl chloride at 500,000 ppm momentarily, then at 70,000 ppm for an unspecified period, caused rigidity of the legs and uncoordinated muscular movements [107].

An experiment with human subjects showed that 5-minute exposures to vinyl chloride at 12,000 ppm caused dizziness in two, dizziness, nausea, lightheadedness, and dulling of vision and hearing at 16,000 ppm in five, and the same symptoms with more intensity in all six, and a headache in one of them at 20,000 ppm [18]. Among 168 workers exposed to vinyl chloride at a TWA concentration of 899 ppm for up to a few months, 47% complained of dizziness, 45% of somnolence, 36.6% of headache, 13% of loss of memory, 11% of euphoria, and 9% of nervousness [19]. Of 168 workers exposed to vinyl chloride at a TWA concentration of 98 ppm (prior exposure information not available), 10.2% reported dizziness, 16.6% somnolence, 6.9% headache, 8% loss of memory, 1.2% euphoria, and 0.6% nervousness. Frequent dizziness and weakness in the legs were also reported by other workers exposed to vinyl chloride [20,33], as was fatigue in 38.6% and headache in 12.9% [20] and in 13.7% of another group of workers [78]. These data indicate that a decrease in the TWA concentration of vinyl chloride led to a decrease in the frequency of CNS symptoms, and that exposure at TWA concentrations as low as 38 ppm produced adverse effects.

Other findings that might be considered manifestations of CNS effects have included a "large proportion of accidents" occurring in a worker population [32] and an excess of suicides as the actual causes of death in another worker population (10 observed vs 5.3 expected) [84]. Effects on brain cells have also been observed, ie, a localized atrophy of cerebellar Purkinje cells and necrosis of the frontoparietal cerebral cortex in one worker [41]. Also, fibrotic processes surrounding and invading the small nerve bundles of the gray matter, neuronal phagokaryosis with satellitosis and deposition of neurologic elements around altered nerve cells of the white matter, and atrophy of the granular layer and degenerative changes in the Purkinje cell layer were observed in rats exposed at 30,000 ppm, 4 hours/day, 5 days/week, for 1 year [111].

Exposure to vinylidene chloride at about 4,000 ppm for a brief period caused "drunkenness" that progressed to unconsciousness in animals [114]. Workers exposed to a vinylidene chloride copolymer while cleaning tanks developed pains in the lips, nose, and eyes, headache, somnolence, facial anesthesia, corneal anesthesia, hypoesthesia, difficulty in speaking and eating [72], and fatigue, weakness, nausea, and dizziness [70,71]. Because of the composition of the copolymers to which these workers were exposed in

aqueous suspension, it is not reasonable to assume that vinylidene chloride was the only causative agent of these CNS effects.

Vinyl bromide caused a "certain anesthesia" at a concentration of 86,000 ppm for a 10-minute exposure in rats [105]. In another experiment, a 1.5-hour exposure at 25,000 ppm anesthetized all rats, and a 7-hour exposure at 10,000 ppm caused drowsiness in rats [127].

Rats exposed for 30 minutes to vinyl fluoride at 300,000 ppm showed instability of the hindlegs; at 500,000 ppm there was loss of postural reflexes, and at 600,000 ppm there was loss of the righting reflex [129].

Rats exposed to vinylidene fluoride for 30 minutes at 400,000 ppm showed slight intoxication, and at 800,000 ppm showed an unsteady gait without loss of the postural reflexes [129].

The available data indicate that vinyl chloride and vinylidene chloride can produce similar CNS manifestations that may be secondary to the cardiovascular effects of these compounds. Human exposure information is not available concerning CNS symptoms for vinyl bromide, vinyl fluoride, or vinylidene fluoride, and anesthetic effects from vinyl fluoride and vinylidene fluoride have only been observed at very high concentrations.

Workers exposed to vinyl chloride have also experienced adverse respiratory effects ranging from coughing and sneezing to bronchial rales, pulmonary emphysema, decreased respiratory volume, decreased vital capacity, decreased maximum expiratory volume/second, and linear type pulmonary fibrosis [19]. Pulmonary function changes were also noted in 12 of 180 workers with "vinyl chloride disease" [32]. Monkeys exposed to vinyl chloride at 100,000 ppm for 5 minutes have shown a significantly increased ($P<0.05$) pulmonary resistance (15.35%) and significantly lowered ($P<0.05$) respiratory minute volume (12.3%) [106]. In addition, bronchioalveolar adenomas have been observed in mice exposed to vinyl chloride at 50 ppm, 6 hours/day, 5 days/week, for up to 12 months [140].

Bronchioalveolar adenomas have been found in mice exposed to vinylidene chloride at 55 ppm for 6 hours/day, 5 days/week, for up to 12 months [140]. Respiratory irritation was seen in rats, guinea pigs, rabbits, dogs, and monkeys when exposed to vinylidene chloride at 87.6 ppm continuously for 90 days or 1,730 ppm for 8 hours/day, 5 days/week, for 6 weeks [122]. Nasal irritation was seen in rats exposed to vinylidene chloride at 200 ppm for 6 hours/day, 5 days/week, for 4 weeks [120]. Inflammation of the epipharynx was also noted in two workers exposed to the emanations from an aqueous polyvinylidene chloride copolymer suspension [70,71]. Because of the mixed composition of this suspension, the authors did not attribute the effect to vinylidene chloride alone. No data were located on respiratory effects of vinyl bromide, vinyl fluoride, or vinylidene fluoride.

Scleroderma of the hands and forearms was observed in 8 of 70 workers exposed to vinyl chloride at unspecified concentrations [20]. Fourteen of 36 workers from whom samples of skin for biopsy were taken had fragmentation of the elastic fibers of the skin of the fingers. Dermatologic complaints were also elicited from 80% of 168 workers during the 1st month of exposure to vinyl chloride at a TWA concentration of 899 ppm [19]. These changes progressed over a period of time from pruritus to contact dermatitis with papules to scleroderma. In the 1st year, 4.4% of the workers developed contact dermatitis, and this frequency increased to 7.4% during 7 years, even though the TWA concentration decreased from 899 to 43 ppm. Other possible routes of exposure of the skin were not evaluated by the authors. Scleroderma was diagnosed in 10% of 180 workers with "vinyl chloride disease" [32]. Another four workers found to have scleroderma showed thickening of the skin of the fingers, adhesions on the deep layers, palmar erythema, hyperhydrosis, and hard projecting nodules in the areas of the flexor tendons [21]. The paws of rats exposed to vinyl chloride at 30,000 ppm, 4 hours/day, 5 days/week, for 1 year showed hyperkeratosis, superficial thickening of the epidermis, disappearance of the cutaneous adnexa, vacuolization and degeneration of the basal layer, a thickening of the papillar layer, with edema, and a decrease in the elastic reticulum and dissociation of the collagen bundles [111]. Arterial vessels of the paws also showed endothelial fibrosis. The same rats also showed skin papillomas and warty subauricular growths [112]. No reports were located of integumentary effects with exposure to vinylidene chloride, vinyl bromide, vinyl fluoride, or vinylidene fluoride.

Skeletal changes have also been observed in humans and animals exposed to vinyl chloride. Acroosteolysis has been observed in workers [20,21,32,35,74,75,78]. In one study [78], exposures to vinyl chloride were estimated at 50-100 ppm in air and 600-1,000 ppm close to the workers' hands during reactor cleaning operations. No information was available in any of these reports on the duration of employment or exposure for the workers with acroosteolysis. It was noted that all workers with acroosteolysis had been employed in reactor cleaning, whereas only 21% of the entire vinyl chloride-exposed population had had experience as reactor cleaners [74,75]. In rats exposed to vinyl chloride at 30,000 ppm for 4 hours/day, 5 days/week, for 1 year, observed effects included alteration in the characteristic bone deposition in the small bones with a mucoid impregnation [111] and osteochondromas in the metacarpal and metatarsal regions of all of the limbs of of 25 rats [112]. No information concerning skeletal abnormalities was located for vinylidene chloride, vinyl bromide, vinyl fluoride, or vinylidene fluoride.

Liver and spleen abnormalities have been observed in humans and animals exposed to vinyl chloride. Hepatomegaly was observed in 65% of 48 workers [30], in one worker exposed 3-4 hours/day, 2-3 days/week, for 3 years and 5 months [41], in 1 of 17 workers [33], in 64% of 11 workers [37], and in 30.2% of 168 workers exposed at a TWA concentration of 899 ppm, and in 11.4% of 168 workers exposed at a TWA concentration of 38 ppm [19] (durations of exposure not reported). Hepatomegaly was also reported in 13.2% of 126 workers

estimated as having "moderate" exposures to vinyl chloride, while only 7.3% of 80 workers estimated to have "light" exposures and 7.1% of 134 workers with "no" exposure showed hepatomegaly [78]. Degenerative changes found in the livers through biopsy included single cell necrosis, hyperplasia, hydropic swelling of the cells, periportal and centrilobular fibrosis and fatty degeneration [31]; dilatation of sinusoids, formation of connective tissue septa, fibroblastic activation, and enlarged hepatocytes with hyperchromatic nuclei [39]; nonalcoholic-type cirrhosis [36]; and fibrosis [30,34,37,40,41].

Several authors have reported clinical test results indicative of liver damage. Workers exposed to vinyl chloride showed abnormal SGOT values in 31% of 277 [78], in 73% of 11 [37], in 50% of 36 [19], in 28% of 68 [34], and in 46.9% of 59 [35]. Results of BSP retention tests were found to be abnormal in 47% of 15 workers [33], in 85% of 26 workers [19], in 9% of 11 workers [37], and in 67.2% of 70 workers [20] and to increase significantly ($P<0.001$) with increasing exposure to vinyl chloride [76]. LDH values were elevated in 36% of 11 workers [37], in 11.1% of 72 workers [34], and in 9.4% of 59 workers [35]. Alkaline phosphatase values were also found to be abnormal in 13% of 277 workers [78], in 55% of 11 workers [37], in 49.4% of 72 workers [34], and in 59.4% of 59 workers [35]. Other clinical values found to be abnormal in vinyl chloride-exposed workers in some of these reports included thrombocyte counts, leukocyte counts, reticulocyte counts, erythrocyte sedimentation rates, total bilirubin, albumin, cholesterol, icterus index, SGPT, isocitrate dehydrogenase, and gamma-glutamic transpeptidase activities, serum beta-globulin, and 17-ketosteroids [19,20,32-35,37,76-78]. Unfortunately, the authors of these studies did not supply information concerning exposure concentrations or durations that might facilitate a correlation of the exposures with the effects. It should also be noted that the frequencies of these abnormal test results were often not significantly different from those in other cohorts of workers such as rubber workers.

Splenic abnormalities, including enlargement, were observed in 57.4% of 70 workers [20], 45% of 11 workers [39], 10% of 1,180 workers [38], 26% of 180 workers with "vinyl chloride disease" [32], and 77% of 48 workers [30] exposed to vinyl chloride. No information is available on exposure concentrations or durations for these workers.

In 30 rats exposed to vinyl chloride at 20,000 ppm, 8 hours/day, 5 days/week, for 3 months, the livers were significantly heavier ($P<0.001$) than those of controls, there was widespread swelling and vacuolization of the liver cells and compression of the sinusoids; the spleens of the rats were significantly lighter ($P<0.05$) [18]. Rats and guinea pigs exposed to vinyl chloride at 100 ppm for 7 hours/day, 5 days/week, for 6 months also showed significantly increased liver weights ($P<0.05$) [113].

No abnormal clinical chemical values were noted either in mice or rats exposed to vinyl chloride at 50, 250, or 1,000 ppm for 6 hours/day, 5 days/week, for up to 12 months [140] or in rats, guinea pigs, rabbits, or dogs exposed at 100 or 200 ppm for 7 hours/day, 5 days/week, for 6 months [113].

In a group of 46 affected workers exposed to vinylidene chloride at TWA concentrations of 0-5 ppm with occasional peaks of 300 ppm, 11% showed hepatomegaly, 13% showed abnormal serum alkaline phosphatase values, and 21-39% showed abnormalities in serum LDH, GGTP, GOT, and GPT values [69]. Of 256 additional workers tested, 29% had abnormal clinical values, and these workers averaged 5.11 years of exposure, whereas the employees who showed normal values averaged 3.64 years of exposure. Serum alkaline phosphatase and GPT values were increased in rats and guinea pigs exposed to vinylidene chloride at 47.6 ppm continuously for 90 days [122]. These animals also had mottled livers and spleens. In rats exposed to vinylidene chloride at 25 or 75 ppm 6 hours/day, 5 days/week, for 17 months, there was increased hepatocytic cytoplasmic vacuolization [119]. Liver cell degeneration was also noted in rats exposed to vinylidene chloride at 500 ppm for 6 hours/day, 5 days/week, for 4 weeks, but not in animals exposed at 200 ppm for the same period [120]. Necrosis and fatty focal vacuolization was also seen in mice and rats exposed at 55 ppm for 6 hours/day, 5 days/week, for up to 12 months [140]. Liver damage has also been demonstrated in rats given vinylidene chloride orally in corn oil in a single dose of 200 mg/kg [125,126], and in drinking water at normal concentrations of 50, 100, or 200 ppm for 730 days [123].

In a 1-year interim report [143], vinyl bromide was stated to have caused increased liver weights in rats exposed at 250 and 1,250 ppm and increased spleen weights in rats exposed at 50, 250, and 1,250 ppm. Gross examinations indicated mottled livers in a few of the animals exposed at these concentrations. Significant elevations in serum LDH and bromide values were also reported in those rats exposed at 1,250 ppm.

No information was located concerning the hepatotoxicity of vinyl fluoride or vinylidene fluoride.

The systemic effects that reportedly resulted from exposure to vinyl halides suggest that the vinyls or their metabolites interfere with cellular processes primarily in the liver and the cardiovascular system. Exposures to large quantities may also involve the skeletal, integumentary, and central nervous systems; however, these systems may also be affected secondarily by changes in the cardiovascular system, and the relative magnitudes of the effects resulting from primary action of the vinyl halides or their metabolites and from the suggested secondary ones are uncertain.

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

The vinyl halides produce reactive metabolic intermediates, possibly including asymmetric oxiranes. Several proposed intermediates in the metabolic pathways of the vinyl halides are capable of covalent binding with cellular macromolecules, and may therefore induce carcinogenesis and mutagenesis. Although few experiments have been reported in which the carcinogenic or mutagenic potentials of the vinyl halides other than vinyl

chloride are evaluated, the vinyl halides considered in this document, as suggested earlier in this chapter, may be qualitatively if not quantitatively similar in their induction potentials.

One report has suggested that exposure of male workers to vinyl chloride can induce excess fetal deaths among their offspring [101]. However, as has been previously discussed, the methods of data collection used in this report are considered inappropriate.

The fetuses of mice, rats, and rabbits from dams exposed to vinyl chloride during pregnancy showed significant abnormalities, including increased crown-rump length, in mice exposed at 50 ppm, and increased incidence of resorptions, decreased fetal body weight, reduction in litter size, increased numbers of unfused sternebrae, and delayed ossification of the bones of the skull in mice exposed at 500 ppm [132]. Fetuses from rats exposed at 500 ppm showed a significant reduction in the fetal body weights and a significant increase in the number of lumbar spurs and in crown-rump length. Fetuses from rats exposed at 2,500 ppm showed a significant increase in the incidence of dilated ureters and significant decreases in the incidence of unfused sternebrae and delayed skull ossifications. The only other significant difference noted was an increase in the incidence of delayed ossification of the fifth sternebra in rabbits exposed at 500 ppm. The authors of this study regarded these effects as due to maternal toxicity and not to direct toxicity to embryos and fetuses. However, the observation of characteristic cancers in progeny of dams exposed to vinyl chloride during their pregnancies [135] suggests that vinyl chloride or its metabolites cross the placental barrier and may have induced the abnormalities reported.

Pregnant rats exposed to vinylidene chloride at 80 ppm had significantly increased numbers of resorptions/implants and fetuses with significant increases in skeletal abnormalities such as delayed ossification of parietal bones, wavy ribs, and lumbar spurs [133]. Delayed ossification of skull bones and cervical vertebrae, along with wavy ribs, were also observed in fetuses of rats exposed at 160 ppm. No adverse effects were observed at 20 ppm. In the rabbits exposed at 160 ppm, there was a significant increase in the number of resorptions/implants and a significantly decreased incidence of delayed ossification of the fifth sternebra, and an increased incidence of a 13th pair of ribs. No adverse effects were noted on the fetuses from dams exposed at 80 ppm. In the fetuses of rats given access to drinking water containing vinylidene chloride at 200 ppm, the only significant difference from controls was an increase in the fetal crown-rump length.

No reports have been located of studies in which the reproductive or teratogenic effects of vinyl bromide, vinyl fluoride, or vinylidene fluoride were investigated.

Covalent binding of the vinyl halides to cellular macromolecules such as DNA, RNA, and proteins may be a critical step in a genotoxic effect [1]. Vinyl chloride, after metabolic activation by microsomal enzymes, has been

shown to enhance mutagenesis in Salmonella [151,152,155,156]. Similar metabolic enhancement has been shown for vinylidene fluoride (J Watson, written communication, April 1978), vinyl bromide (VF Simmon and R Mangham, written communication, August 1977) and vinylidene chloride [151,152,172]. The common molecular features of these compounds, the formation of the asymmetrical oxirane and the subsequent spontaneous rearrangement to the aldehyde or acyl halide, have been suggested as producing the most reactive intermediates [1-3,210], although the oxirane of vinylidene chloride has not yet been synthesized in the laboratory [1]. Direct binding with proteins in vitro has been reported for chloroethylene oxide (the oxirane of vinyl chloride) [196,212] and for chloroacetaldehyde [170,206]. In vitro and in vivo binding of vinyl chloride or its metabolites to microsomal proteins has also been reported [204,205,212,214,215] as has macromolecular binding of vinylidene chloride or its metabolites [117,219]. In addition to direct binding to proteins, metabolites of vinyl chloride have been reported to bind to RNA in vitro [170,204,205] and in vivo [215].

Even when the route of administration of the vinyl halides differ, the metabolic end products, and, presumably, the intermediates, with each route are the same [4,5,175,177]. Only unchanged vinyl chloride or vinyl chloride-derived carbon dioxide were exhaled after oral, ip, iv, or inhalation exposures [4,5,175,177]. Urinary metabolites from all routes of exposure include cysteine-conjugated products and small amounts of monochloroacetate [5,175,177].

Absorption of vinyl chloride through the intact skin was reportedly minimal, amounting to less than 0.031% of the total available gaseous vinyl chloride in 2.5 hours [178]. Vinyl chloride concentrations in the air rapidly equilibrated (within 15 minutes) with vinyl chloride in the tissue when metabolism was blocked [202]. Under these conditions, the half-life of vinyl chloride was 4.3 minutes. Absorption and subsequent metabolism of vinyl chloride has been reported to be concentration dependent, with a saturable enzyme system responsible for metabolism at low concentrations and a secondary oxidative system predominant at higher concentrations [4-6]. It has been postulated that the oxirane is formed only at the higher concentrations through the secondary oxidative pathway, but that halogenated acetalydehyde is common to both pathways. Thus, even without the formation of the oxirane at low concentrations, the possibility of macromolecular alkylation exists through the action of the aldehyde intermediate.

Vinyl chloride has been shown to produce a characteristic tumor, angiosarcoma of the liver, in both humans [31,32,34,36,37,40,41] and animals [134,135,140]. The cases of angiosarcoma of the liver in workers exposed to vinyl chloride are summarized in Table XVII-4 [61]. This summary shows that the average exposure of the affected workers was 17.3 ±6.3 years (range 4-30 years) and the average latency for diagnosis of the tumor was 20.5 ±6 years (range 9-38 years). Accurate exposure information, to allow calculation of total accumulated dose, is not available for these workers. Kuzmack and McGaughy [62], using data derived from epidemiologic studies, calculated that

the incidence rates of angiosarcoma in workers exposed at concentrations of 350 ppm, 7 hours/day, for 5 days/week should be 0.0031/person/year of exposure. An incidence rate of 0.0052/person/year of exposure was also calculated from the incidence rate in rats.

Angiosarcoma of the liver was also observed in 1 of 59 rats exposed to vinyl chloride at 50 ppm, 4 hours/day, 5 days/week, for 52 weeks [135]. The percentage of animals with angiosarcoma of the liver increased from 2% at 50 ppm, to 7% at 250 ppm, 12% at 500 ppm, 22% at 2,500 ppm, 22% at 6,000 ppm, 15% at 10,000 ppm, and 30% at 30,000 ppm. Average latency increased with decreasing exposure concentration, from 53 weeks at 30,000 ppm to 135 weeks at 50 ppm. In another experiment [134], rats exposed at 50 ppm for 4 hours/day, 5 days/week, for 12 months showed no tumors over an unspecified observation period. At 500 ppm, however, there was a significant increase ($P<0.03$) in the number of tumors seen, with 3% of the animals having angiosarcomas of the liver. Increasing exposure concentrations to 2,000, 5,000, 10,000, and 20,000 ppm increased the frequency of all tumors and of angiosarcoma of the liver to 5, 6, 8, and 12%, respectively. Other tumors in rats, such as Zymbal gland carcinoma and nephroblastoma, have been reported to have similar latencies [135]. Statistical estimates were made by CR Norwood and FW Dresch (written communication, December 1977) using the data in these reports [134,135,140] and based on the assumptions that the background incidence of this tumor is zero and that the no-observable-effect concentration is that at which the probability of one tumor developing in 100 replications of the same experiment is less than 0.01. They found that conservative, no-observable-effect concentrations for angiosarcoma of the liver could be calculated as any of 0.2 ppm [140], 0.01 ppm [134], or 0.000004 ppm [135].

A variety of other tumors have been seen in workers exposed to vinyl chloride, such as cancer of the respiratory system [80,81,84], buccal cavity, pharynx, and peritoneum [64,81], brain [84,89], gall bladder [84], and glioblastoma, reticulum cell sarcoma, and lymphosarcoma [87]. Similar tumors have been seen in animals exposed to vinyl chloride [112,135,136,138,140]. These tumors are much more common than angiosarcoma of the liver, however, and can be induced by a wide variety of other carcinogens; they are therefore not considered pathognomonic of vinyl chloride exposure. The relatively long latent period for induction of angiosarcoma of the liver in both humans and animals, along with the lack of adequate followup information in most cases, makes a purely objective assessment of risk on the basis of the available data unwarranted.

No cases of cancer in humans have been reported to be related to occupational exposure to vinylidene chloride, and only three animal experiments relative to its carcinogenicity have been located. In one experiment [140], 3 of 72 CD-1 mice exposed to vinylidene chloride at a concentration of 55 ppm, 6 hours/day, 5 days/week, for up to 12 months developed angiosarcoma of the liver. Two of 72 rats similarly exposed

developed extrahepatic angiosarcoma. In another experiment [119], 208 rats exposed at 40 ppm for 6 hours/day, 5 days/week, for 1 month and then exposed at 75 ppm on the same schedule for 17 more months developed no angiosarcoma. In addition, these rats did not show increased incidences of other tumors compared with those in controls. A further experiment [142] showed an increased incidence of adenocarcinoma of the kidney in Swiss mice exposed to vinylidene chloride at 25 ppm for 4 hours/day, 4-5 days/week, for 12 months, but no increase in other tumors. The authors [142] stated that exposures at 50 ppm and higher were lethal to these animals. Sprague-Dawley rats exposed on the same schedule at concentrations of from 10 to 150 ppm showed an increased incidence of mammary tumors; however, this increase was not dose dependent.

No cases of cancer in humans have been attributed to vinyl bromide exposure. The preliminary results of one animal experiment [143] indicated that vinyl bromide at 1,250 or 250 ppm induced a significant increase in angiosarcoma of the liver and other tumors in rats after 1 year of exposure. At 1,250 ppm, 9/48 rats developed angiosarcoma, and, at 250 ppm, 2/30 rats developed angiosarcoma. No increase in tumor formation was observed in rats exposed at 10 and 50 ppm for 1 year.

No reports concerning the carcinogenicity of vinyl fluoride or vinylidene fluoride in humans or animals have been located.

Comparison of the limited animal data available on induction of angiosarcoma by the vinyl halides supports the postulations outlined in the Structure-Activity Considerations section of this chapter. The expected order of susceptibility of the olefins toward epoxidation, calculated from the sum of the electronegativities of the substituents, is vinyl bromide > vinyl chloride > vinylidene chloride > vinyl fluoride > vinylidene fluoride. The carcinogenicity data show that vinyl bromide at a concentration of 250 ppm induced angiosarcoma in 7% of the animals after 1 year of exposure [143], and that vinyl chloride at a concentration of 250 ppm also induced angiosarcoma in 7% of the animals, but after 2 years of exposure [135]. This indicates that, for this characteristic tumor, vinyl bromide has a stronger induction potential, since it is reasonable to assume that, within another year, more of the vinyl bromide exposed animals would develop the tumors. A comparison of the induction potential of vinylidene chloride with that of vinyl chloride or vinyl bromide is more difficult. One experiment with vinylidene chloride showed 4% angiosarcoma of the liver in CD-1 mice exposed at a concentration of 55 ppm for 85,800 ppm-hours [140], another experiment showed no angiosarcoma in Swiss mice exposed to vinylidene chloride at 25 ppm for 23,000 ppm-hours [142], while an experiment with Swiss mice exposed to vinyl chloride at a concentration of 50 ppm for 30,000 ppm-hours showed a 2% incidence of angiosarcoma of the liver [135]. These data are not so easily compared as the data from the vinyl bromide and vinyl chloride experiments, but they do suggest that vinylidene chloride may be less active in inducing angiosarcoma of the liver than vinyl chloride. Because the behaviors of these compounds appear to fit the expected structure-activity relationship, it is reasonable

to suppose that vinyl fluoride and vinylidene fluoride might also fit this relationship. Because of the lack of information concerning the vinyl fluorides, it is not possible to substantiate this hypothesis at the present time.

No mutagenic effects on humans have been related to exposure to any of the vinyl halides. Studies of chromosomal aberrations in the lymphocytes of workers exposed to vinyl chloride [94-99] have indicated a significant increase in abnormalities; however, the combined frequency of abberations in these studies was only a fraction of the background frequency [100].

Each of the vinyl halides has been found to be mutagenic in some test system. Vinyl chloride has been shown to produce mutations in Salmonella, in the Ames test procedure [145,146,149,150,162,163], in E. coli [151-153], in yeasts, both in vitro and by host-mediated assay [155], and in Drosphila by the recessive lethal test [158,159]. Negative results have been obtained in Neurospora [157].

Several investigators have shown that vinyl chloride has a direct mutagenic effect on Salmonella [149,150,162,163]. One study [146] reported a twentyfold increase over the spontaneous mutation rate after 48 hours of exposure to a medium containing 4 millimoles of vinyl chloride. Activation by a 9,000 x G mammalian microsomal system increased the mutation rate to 28 times the spontaneous rate [146], and exposures of as short as 30 minutes at vinyl chloride concentrations of 200,000 ppm induced twice the spontaneous rate of mutation [145]. Purified microsomal fractions (100,000 x G) have been shown to be less active than the 9,000 x G fraction [146], while the supernatant cytosol produced no increase in mutagenic activity above that found for vinyl chloride alone. Vinyl chloride has been shown to affect only loci at which mutation occurs by substitution [145,146]. One dominant lethal study [161] with male mice exposed to vinyl chloride at 30,000 ppm for 6 hours/day for 5 days showed no mutations, which suggests that mammalian gametes may not be affected by exposure.

Vinylidene chloride has been shown to be similarly mutagenic in bacterial systems [172,173]. Exposure of Salmonella for 2 hours, in media containing 0.33-33 millimoles of vinylidene chloride and a mammalian microsomal system, resulted in 6-10 times the spontaneous number of revertants [172]. Bartsch et al [173] reported that vinylidene chloride was about three times as active as a mutagen as vinyl chloride on an equimolar basis. In a study using E. coli, Greim et al [151] concluded that vinyl chloride was "several times" more mutagenically active than vinylidene chloride; their data showed that vinylidene chloride produced a onefold increase over the spontaneous number of mutations at a concentration of 2.5 millimolar, while vinyl chloride produced six times the spontaneous number of mutations at the same locus at a concentration of 10.6 millimolar.

Vinyl bromide at concentrations of 20% in air was shown to be mutagenic in Salmonella TA100 after a 12-hour exposure (VF Simmon and R Mangham, written

communication, August 1977). This exposure produced revertants at 10 times the spontaneous rate with microsomal activation and at about 5 times the spontaneous rate without activation.

Both vinyl fluoride and vinylidene fluoride have been shown to produce direct mutagenic responses in E. coli at up to 100 times the spontaneous rate [174]. However, no data were given on the exposure concentrations for these experiments and no auxotrophic mutants were isolated from the putative mutant colonies. Vinylidene fluoride has also been reported to increase the frequency of mutations in Salmonella both with and without microsomal activation (J Watson, written communication, April 1973).

Several investigators have tested known or presumed metabolites of vinyl chloride in an attempt to identify the mutagenically active agents. The most active compounds tested were chloroacetaldehyde and chloroethylene oxide [166,147,163,165]. Chloroethanol showed lower mutagenic activity [147,162, 163], and chloroacetic acid was not mutagenic in Salmonella [147,163,166]. The direct activity of chloroethanol resembled that of vinyl chloride in that it affected Salmonella strain TA1535 and strain TA100 (identical to TA1535 except that it is DNA repair-deficient) about equally [162]. Chloroethanol, like vinyl chloride, was also further activated by the addition of mammalian microsomes [147,162].

McCann et al [162] suggested that, although chloroacetaldehyde was the most active of the metabolites of vinyl chloride that they tested, it was probably not the major mutagenic metabolite of vinyl chloride, since chloroacetaldehyde affected only the repair-deficient Salmonella strain TA100. These authors suggested that chloroethylene oxide was the most likely mutagenic metabolite, although they did not test its activity. However, the compound has been tested by other investigators on both repair-deficient [166] and nonrepair-deficient [147,163] strains, and it appears, like vinyl chloride, to be similarly active in each.

These studies show that each of the vinyl halides is mutagenic in various test systems and that the putative metabolites are more strongly mutagenic than the parent compounds. The studies support the conclusions drawn from the limited carcinogenic data available and, further, raise a suspicion that the fluorides also may be carcinogens. The failure of vinyl chloride to be mutagenic in the mouse dominant-lethal test may indicate that the mutagenic factor may not be distributed to mammalian gametes in sufficient concentrations to produce significant changes; however, further research is necessary to determine fully the potential of these compounds for mammalian mutagenicity.

The available evidence concerning the vinyl halides indicates that each produces similar biologic effects, albeit possibly at different concentrations. Indications from the experiments on mutagenicity suggest that these compounds are able to exert their action directly but that metabolism of the parent compounds produces intermediates that are much more reactive.

TABLE III-7

EFFECTS ON HUMANS FROM EXPOSURE TO VINYL CHLORIDE OR VINYLIDENE CHLORIDE*

Subjects	Concentration (ppm)	Duration	Signs and Symptoms	Reference
6 Volunteers	20,000	5 min	Dizziness, nausea, lightheadedness, dulling of vision and hearing in 6; headache in 1	18
"	16,000	"	Dizziness, nausea, lightheadedness, dulling of vision and hearing in 5	18
"	12,000	"	Dizziness in 2	18
"	4,000-8,000	"	No effects	18
168 Workers	899	a few mo	Dizziness (37%), somnolence (69%), headache (36.6%), anorexia (23%), epigastric pain (16%), loss of memory (13%), euphoria (11%), nervousness (9%); hepatomegaly (30.2%); dermatologic complaints (80%); Raynaud's syndrome (6%)	19
51 Workers	69	-	Elevated pulmonary arterial pressure in 20%	19
60 Workers	63	-	Elevated pulmonary arterial pressure in 42%	19
157 Workers	"	-	Decrease in respiratory volume, FVC, FEV 1	19
168 Workers	38	-	Dizziness (10.2%), somnolence (16.6%), headache (6.9%), anorexia (10.3%), epigastric pain (9%), loss of memory (8%), euphoria (1.2%), nervousness (0.6%); hepatomegaly (11.4%); dermatitis (7.4%); Raynaud's syndrome (2.9%)	19
1,673 Workers	20-39	-	Acroosteolysis in 1%	74, 75
46 Workers*	0-5 (peaks to 300)	-	Hepatomegaly (11%), abnormal alkaline phosphatase (13%); abnormal LDH (39%), GGTP (30%), SGOT (23%), SGPT (21%)	69
73 Workers*	"	avg 5.11 yr	Abnormal clinical blood values	69
181 Workers*	"	avg 3.64 yr	Normal clinical blood values	69

TABLE III-8

NONTUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYL CHLORIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Rat	210,000	2 hr	LC100	104
"	136,000	"	LC50; contractions, convulsions	104
"	30,000	4 hr/d, 5 d/wk x 1 yr	Nerve cell changes, thickening of skin, papillomas, warty growths	111
"	20,000	8 hr/d, 5 d/wk x 3 mo	Liver weight increase, cellular changes; spleen weight decrease	18
"	2,500	d 6-15 of gestation	Dilated ureters in offspring	132
"	500	"	Decreased fetal weight, increased lumbar spurs in offspring	132
"	100	7 hr/d, 5 d/wk x 6 mo	Increased liver weight	113
Mouse	242,000	10 min	LC50	105
"	150,000	2 hr	LC100	104
"	122,900	10 min	Anesthesia in 100%	105
"	117,500	2 hr	LC50; contractions, convulsions	104
"	100,000	10 min	Anesthesia in 50%	105
"	500	d 6-15 of gestation	Decreased litter size, fetal weight; increased resorptions;	132
"	50	"	Increased crown-rump length in offspring	132
Guinea pig	280,000	2 hr	LC100	104
"	238,000	"	LC50	104
"	100-200	7 hr/d, 5 d/wk x 6 mo	Liver weight increase; no blood changes	113
Rabbit	280,000	2 hr	LC100	104
"	238,000	"	LC50; contractions, convulsions	104
"	2,500	d 6-18 of gestation	Death of 1/7; no reproductive effects	132
"	500	"	Delayed ossification, wavy ribs	132
"	100-200	7 hr/d, 5 d/wk x 6 mo	Liver weight increase; no blood changes	113
Dog	100,000	-	Cardiac irregularities	107
"	50,000	5 min	Cardiac sensitization to epinephrine	110
"	100-200	7 hr/d, 5 d/wk x 6 mo	Liver weight increase; no blood changes	113
Monkey	100,000	5 min	Increased pulmonary resistance,	106
"	"	"	Myocardial force decreased 28.5%	108
"	50,000	"	Myocardial force decreased 9.1%	108
"	25,000	"	Myocardial force decreased 2.3%	108

TABLE III-9

NONTUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYLIDENE CHLORIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Rat	32,000	4 hr	Death of 2-4 of 6 in 14 d	118
"	25,000	10 min	Cardiac irregularities, sensitization to epinephrine	121
"	15,000	4 hr	LC50 (24 hr) for fed animals	115
"	6,350	"	LC50 (14 d)	116
"	600	"	LC50 (24 hr) for fasted animals	115
"	500	6 hr/d, 5 d/wk x 4 wk	Liver cell degeneration	120
"	200	"	Nose irritation; no liver effects	120
"	160	d 6-15 of gestation	Delayed ossification, wavy ribs in offspring	133
"	105	22-23 hr	LC50--14 d (females)	
"	100	8 hr/d, 5 d/wk x 6 wk	Respiratory irritation	122
"	98	22-23 hr	LC50--14 d (males)	
"	80	d 6-15 of gestation	Increased fetal resorptions; skeletal abnormalities	133
"	25-75	6 hr/d, 5 d/wk x 17 mo	Liver cell degeneration	119
"	48	90 d	Mottled livers; elevated SGPT, alkaline phosphatase	122
"	55	6 hr/d, 5 d/wk x 12 mo	Fatty infiltration of liver	140
"	35	22-23 hr x 2	LC50 (males)	117
"	20	4 d	LT50	117
"	5	90 d	Respiratory irritation	122

TABLE III-9 (CONTINUED)

NONTUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYLIDENE CHLORIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Mouse	55	6 hr/d, 5 d/wk	Necrosis of liver	140
Guinea pig	100	8 hr/d, 5 d/wk x 6 wk	Respiratory irritation	122
"	48	90 d	Mottled livers, elevated	122
"	5	90 d	Respiratory irritation	122
Rabbit	160	d 6-18 of gestation	Increased fetal resorptions, extra ribs in offspring	133
"	100	8 hr/d, 5 d/wk x 6 wk	Respiratory irritation	122
"	5	90 d	Respiratory irritation	122
Dog	100	8 hr/d, 5 d/wk x 6 wk	"	122
"	5	90 d	"	122
Monkey	100	8 hr/d, 5 d/wk x 6 wk	"	122
"	5	90 d	"	122

TABLE III-10

NONTUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYL BROMIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Rat	171,000	10 min	Not lethal	105
"	100,000	15 min	Lethal to 100%	127
"	86,000	10 min	Anesthesia in 100%	105
"	50,000	7 hr	Death of unspecified number	127
"	25,000	1.5 hr	Anesthesia in 100%	127
"	10,000	7 hr	Drowsiness, sluggishness	127

TABLE III-11

NONTUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYL FLUORIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Rat	800,000 in oxygen	12.5 hr	Not lethal	128
"	800,000 in oxygen	30 min	Breathing difficulty, loss of postural reflex	129
"	600,000	"	Loss of righting reflex	129
"	500,000	"	Loss of postural reflex	129
"	300,000	"	Instability of hindlegs	129
"	100,000	7 hr/d, 5 d/wk x 6 wk	No effects	128

TABLE III-12

NONTUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYLIDENE FLUORIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Rat	800,000 in oxygen	19 hr	Not lethal	128
"	800,000	30 min	Unsteady gait	129
"	400,000	"	Slight intoxication	129
"	128,000 static exposure	4 hr	Death of 2-4 of 6 in 14 d	113

TABLE III-13

TUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYL CHLORIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Rat	30,000	4 hr/d, 5 d/wk x 12 mo	Tumors in 85%; liver angiosarcomas in 30%, average latency 53 wk	135
"	20,000	"	Liver angiosarcomas in 12%	134
"	10,000	"	Tumors in 62%; liver angiosarcomas in 15%, average latency 64 wk	135
"	"	"	Liver angiosarcomas in 8%	134
"	6,000-10,000	4 hr/d d 12-18 of gestation	Tumor induction in offspring	135
"	6,000	4 hr/d, 5 d/wk x 12 mo	Tumors in 52%; liver angiosarcomas in 22%, average latency 70 wk	135
"	5,000	"	Liver angiosarcomas in 6%	134
"	2,500	"	Tumors in 54%; liver angiosarcomas in 22%, average latency 78 wk	135
"	2,000	"	Liver angiosarcomas in 5%	134
"	500	"	Liver angiosarcomas in 3%	134
"	"	"	Tumors in 37%; liver angiosarcomas in 12%, average latency 81 wk	135
"	250	"	Tumors in 27%; liver angiosarcomas in 7%, average latency 73 wk	135
"	50	"	Tumors in 17%; liver angiosarcomas in 2%, average latency 135 wk	135
"	"	"	No tumors	134

TABLE III-13 (CONTINUED)

TUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYL CHLORIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Mouse	10,000	4 hr/d, 5 d/wk x 30 wk	Tumors in 72%; liver angiosarcomas in 16%	135
"	2,500	"	Tumors in 58%; liver angiosarcomas in 21%	135
"	250	"	Tumors in 69%; liver angiosarcomas in 19%	135
"	50	"	Tumors in 32%; liver angiosarcomas in 2%	135
"	"	6 hr/d, 5 d/wk x 12 mo	Bronchiolar adenomas	140
Hamster	50-10,000	4 hr/d, 5 d/wk x 30 wk	Tumor induction; liver angiosarcomas; not dose related	135
Rabbit	10,000	-	Increase in skin acanthomas and lung adenocarcinomas	134

TABLE III-14

TUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYLIDENE CHLORIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Rats	10-150	4 hr/d, 4-5 d/wk x 12 mo	Increase in mammary tumors	142
"	55	6 hr/d, 5 d/wk x 12 mo	Extrahepatic angiosarcomas in 3%	140
"	40-75	6 hr/d, 5 d/wk x 18 mo	No angiosarcomas; no increase in tumors	119
Mice	55	6 hr/d, 5 d/wk x 18 mo	Liver angiosarcomas in 4%; bronchiolar adenomas	140
"	25	4 hr/d, 4-5 d/wk x 12 mo	Increase in kidney adenosarcomas and lung adenocarcinomas	142

TABLE III-15

TUMORIGENIC EFFECTS ON ANIMALS FROM EXPOSURE TO VINYL BROMIDE BY INHALATION

Species	Concentration (ppm)	Duration	Effects	Reference
Rat	1,250	12 mo	Liver cancer in 19%; significant increase	327
"	250	"	Liver cancer in 7%; significant increase in other tumors	327
"	10-50	"	No tumors	327