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# Vinyl Chloride Mechanistic Data and Risk Assessment: DNA Reactivity and Cross-Species Quantitative Risk Extrapolation

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**ABSTRACT.** Vinyl chloride produced several tumor types among species. Angiosarcoma of the liver is found in all tested species, including humans with occupational exposures. Vinyl chloride is biotransformed by CYP2E1 to DNA-reactive chloroethylene oxide producing cyclic etheno adducts, which are mutagenic. The dose-response for angiosarcoma of the liver formation in rodents is supralinear, which is consistent with saturation of metabolic activation, and the tumor rate in humans at occupational exposure levels is similar to that for equivalent exposures in rodents. PHARMACOL. THER. 71(1/2): 7–28, 1996.

KEY WORDS. Angiosarcoma, DNA adducts, genotoxicity, risk assessment, vinyl chloride.

CONTENTS



ABBREVIATIONS. $\epsilon$A, 1,N$^6$-ethenoadenine; $\epsilon$Ado, 1,N$^6$-ethenoadenosine; ASL, angiosarcoma of the liver; CAA, chloroacetaldehyde; CEO, chloroethylene oxide; CI, confidence interval; $\epsilon$C, 3,N$^4$-ethenocytosine; $\epsilon$Cyo, 3,N$^4$-ethenocytidine; $\epsilon$dA, 1,N$^6$-ethenodeoxyadenosine; $\epsilon$dC, 3,N$^4$-ethenodeoxycytidine; $\epsilon$dG, N$^2$,3-ethenodeoxyguanosine; $\epsilon$G, N$^2$,3-ethenoguanine; 7-OEdG, 7-(2-oxoethyl)-deoxyguanosine; PVC, polyvinyl chloride; SCE, sister chromatid exchange; SMR, standardized mortality rate; VC, vinyl chloride.

## 1. INTRODUCTION

Vinyl chloride (VC) is a colorless gas with a boiling point of −13.7°C. It polymerizes in light or in the presence of a catalyst. It is soluble in ethanol, diethyl ether, carbon tetrachloride and benzene, and is slightly soluble in water. The structure of VC is shown in Fig. 1. VC is used to produce polyvinyl chloride (PVC), which is formed into many products as well as copolymer products, such as films and resins. Human exposure to VC occurs mainly in occupational settings, but environmental exposures may have occurred since VC is released into the air and waste water from the plastics industry. Tobacco smoke has also been found to contain 1.3–27.3 ng of VC per cigarette (Hoffmann *et al.*, 1976). Also, due to VC impurities in PVC, small amounts of VC may enter the drinking water from PVC pipes or may dissolve in foods packaged in PVC plastic food wraps.

Occupational exposure to VC has been associated with angiosarcoma of the liver (ASL), which is a rare tumor among the unexposed population. In some studies, exposure to VC has also been associated with increases in cancer of the CNS, lung and lymphatic/hematopoietic system. Due to these effects, the International Agency for Research on Cancer has determined that VC is a human carcinogen

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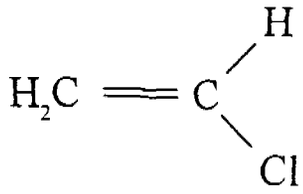

FIGURE 1. Chemical structure of VC.

(IARC, 1979). VC has been chosen for review by the Panel because it is an example of a DNA-reactive chemical for which the same tumor type has been reported in humans as in several other animal species. Extensive dose-response bioassays and epidemiological studies are available, which include quantitative estimates of exposure, thereby presenting a unique opportunity to compare the relative potency of this genotoxic carcinogen in humans and animals.

This review is one of a series, which documents bioassay, epidemiological and mechanistic studies for 10 chemicals that are carcinogenic in rodents. The purpose of these documents is to illustrate the use of mechanistic information that assists in the determination of cancer risk at human exposure levels. The data for each topic are first presented with a minimum of analysis. The data are summarized and analyzed in the last sections, which the reader may wish to consult first for an overview.

## 2. RESULTS OF ANIMAL BIOASSAYS

Maltoni *et al.* (1984) investigated the carcinogenicity of VC using male and female Sprague-Dawley rats, Wistar rats, Swiss mice and Syrian golden hamsters. In the initial experiment, 15 different VC doses, between 0 and 30,000 ppm, were administered by inhalation for a 52-week period to male and female Sprague-Dawley rats. Other VC inhalation studies were performed over 5-, 17- and 25-week periods. Studies were also conducted on pregnant rats and embryos. In addition, Wistar rats were tested so that strain variability could be evaluated; Swiss mice and Syrian golden hamsters were also tested so that species differences could be determined. VC was administered by stomach tube (gavage) and by i.p. injection to determine route-specific effects. Although the study was the most extensive analysis of carcinogenicity due to VC conducted to date, other investigators have provided significant additional information. All of these studies are discussed in more detail in the following sections.

### 2.1. *Inhalation Studies*

**2.1.1. Sprague-Dawley dose-response studies.** In the initial series of experiments, groups of male and female Sprague-Dawley rats, 11–17 weeks of age, were exposed via inhalation to 15 different concentrations of VC ranging from 0 to 30,000 ppm 4 hr/day, 5 days/week for 52 weeks, followed by lifetime observation (Maltoni *et al.*, 1984). Six types of malignancies occurred in statistically significant excess.

*Angiosarcoma of the liver:* The frequency of ASL in Sprague-Dawley male and female rats exposed to VC for 52 weeks is presented in Fig. 2. The display of incidence vs. the logarithm of air concentration is presented based upon the relationship of log (dose) to hepatic macromolecular binding (Watanabe *et al.*, 1978). Dose-related increases in the proportion of rats developing ASL were observed in the range of exposures from 10 to 30,000 ppm. The incidence became statistically significant at doses of 200 ppm and greater for males and 50 ppm and greater for females.

*Mammary gland tumors:* Significant increases in the incidence of malignant mammary gland tumors in female rats occurred at airborne concentrations of 5 ppm and above. The dose-response relationship was unusual in that at concentrations above 150 ppm, the incidence of malignant mammary gland tumors was not significantly greater than the incidence observed in control rats. However, the decreased incidence may be at least partially explained by the decreased survival in these groups due to other tumor types in comparison with the latency for development of malignant mammary gland tumors.

*Zymbal gland carcinoma:* The proportions of animals developing carcinomas of the Zymbal gland, which is the sebaceous gland of the ear canal, were significantly increased at 10,000 ppm and 30,000 ppm.

*Nephroblastoma:* Statistically significant increases in numbers of nephroblastomas occurred in males in the dosage range of 100–2500 ppm and in females at 250 ppm and 500 ppm. The decreased incidence of this tumor type at higher doses may have been due to increased mortality of the animals from other tumor types, precluding development of a neoplasm with long latency.

*Neuroblastoma:* Various types of encephalic tumors of neuronal cells, usually without metastasis, were observed in the higher-dosage groups. Only in females of the 10,000 ppm dose group was the incidence statistically significant in this

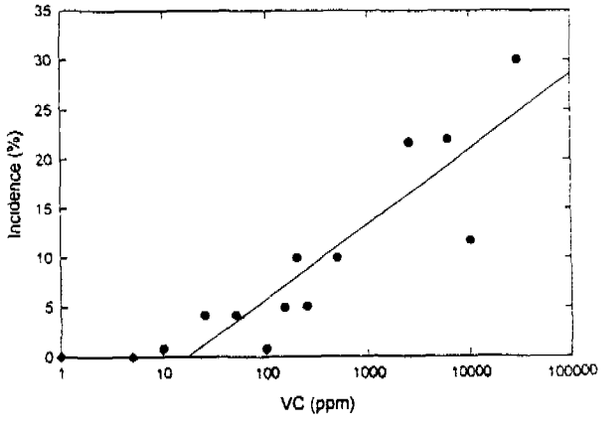


FIGURE 2. Dose-response of VC-induced angiosarcoma of the liver in rats. (◆) indicates data not used in regression analysis. Data from Maltoni *et al.* (1984).

dose-response study, although an elevated incidence appeared beginning at 2500 ppm.

*Forestomach tumors:* Papillomas and squamous cell carcinomas of the forestomach occurred in 18.3% of rats exposed to 30,000 ppm VC and in several of the lower-dose groups in nonsignificant proportions.

*Other tumors:* The incidences of the following tumors associated with VC exposure were increased, although the increases reported were not statistically significant using the Fisher exact probability test ($P < 0.05$): angiomas and fibroangiomas of the liver; hepatocellular carcinomas; angiosarcomas, angiomas and fibroangiomas at sites other than the liver; lung adenomas; and cutaneous epithelial tumors (squamous cell carcinomas).

**2.1.2. Studies in other strains and species.** *Studies of Wistar Rats:* In an early experiment, 26 male Ar/IRE Wistar rats, 3 months old, were exposed to 30,000 ppm VC 4 hr/day, 5 days/week, for a period of 12 months (Viola *et al.*, 1971). In all 17 rats surviving 54 weeks, skin tumors in the submaxillary parotid region were reported. Lung tumors were reported in 7 rats and osteochondroma in 5 rats. No tumors were reported in 25 control rats. Maltoni *et al.* (1984) later concluded that the skin tumors were carcinomas originating from the Zymbal gland and that the lung tumors were probably metastases from Zymbal gland carcinomas. Inhalation studies on Wistar rats were also conducted by Feron *et al.* (1979), who exposed groups of males and females to 0 or 5000 ppm VC for 52 weeks; after 4, 13, 26 and 52 weeks, 10 rats/group were killed. In rats killed after 26 weeks, only foci of cellular alteration, consisting of clear cells (14/20) or basophilic cells (5/20), were observed in the livers, but angiosarcoma (9/19) and hepatocellular carcinoma (2/19) were observed after 52 weeks. Primary tumors in the brain, lung, Zymbal gland and nasal cavity were also reported in these rats (Feron and Kroes, 1979). Controls had few liver changes and no liver tumors or other tumors.

Male Wistar and Sprague-Dawley rats were utilized in life-span inhalation studies to investigate the effect of strain differences on tumor incidence (Maltoni *et al.*, 1984). Both strains of rats were exposed 4 hr/day, 5 days/week for 52 weeks to VC concentrations of 1, 50, 250, 500, 2500, 6000 and 10,000 ppm. The incidence of liver angiosarcomas was comparable between the two strains at doses up to 10,000 ppm. Fewer Zymbal gland tumors were recorded in male Wistar rats than in male Sprague-Dawley rats. For example, at 10,000 ppm the Zymbal gland tumor incidence was 2/18 (11.1%) for Wistar rats compared with 10/30 (33.3%) for Sprague-Dawley rats. The incidence of many other tumor types that occurred in Wistar rats was comparable with the tumor incidence in Sprague-Dawley rats. Forestomach papillomas and squamous cell carcinomas, however, were not recorded in the Wistar rats (Maltoni *et al.*, 1984). In another investigation, Bi *et al.* (1985) reported that 36.8% (7/19) adult male Wistar rats developed liver angiosarcoma and 10.5% (2/19) developed lung angiosarcoma after exposure to 100 ppm VC 6 hr/day, 6 days/week for 12 months; killing was at 18 months.

*Studies Comparing Rats, Mice and Hamsters:* When CD rats and CD-1 mice, approximately 2 months of age, were exposed to 0, 50, 250 and 1000 ppm VC 6 hr/day, 5 days/week for up to 12 months by Lee *et al.* (1978), ASL was the most prevalent neoplasm induced in the rats occurring in 17% and 30% at 250 and 1000 ppm VC, respectively. Hemangiosarcoma of the lung also occurred in rats at 250 ppm (3/70) and 1000 ppm (13/70). A few hemangiosarcomas were also found at other sites. The mice also developed bronchioalveolar adenomas and mammary gland tumors in high incidences. A subsequent study, in which both CD rats and CD-1 mice were exposed to 0, 50, 250 and 1000 ppm VC in an identical regimen for up to 10 months, but followed by a 12-month observation period (Hong *et al.*, 1981), resulted in comparable values for ASL in rats and mice. The incidence of mammary gland tumors in female mice, however, was somewhat reduced compared with the earlier study. In another study, groups of 5-week-old male CD1 Swiss/ChR mice were exposed to 2500 or 6000 ppm VC 5 hr/day, 5 days/week for 5 or 6 months by Suzuki (1978). Almost all of the dosed mice (26/27) killed 2–37 days after termination of exposures had developed lung neoplasia diagnosed as "alveogenic cancer"; none of 16 controls developed lung cancer. Light and electron microscopic examination of the tissues suggested that the neoplastic cells had arisen from Type II alveolar epithelium (Suzuki, 1978). A small proportion of the mice had developed ASL.

F344 rats and ICR mice were exposed by inhalation to 50–50,000 ppm VC for 1 hr (Hehir *et al.*, 1981). The age range of these animals was 15–18 weeks, and the animals were observed for their lifetime, which was 18–24 months. VC produced no significant increases in pulmonary neoplasia in ICR mice until the chamber level attained 5000 ppm (1/143 carcinoma and 24/143 adenomas) or 50,000 ppm (3/137 carcinomas and 45/137 adenomas) compared with controls (0/120 carcinomas and 12/120 adenomas). Pneumonitis occurred in all groups of mice exposed to 500 ppm or more for 1 hr. None of the F344 rats developed tumors even at the highest dose. No dose-related increases in other types of tumors were found in this study.

Studies by Drew *et al.* (1983) indicated that B6C3F$_1$ and Swiss female mice had the highest incidence of ASL related to the lowest concentration of exposure for any of the studies. Mice exposed to 50 ppm VC for 12 months and observed for an additional 12 months showed ASL incidence of 77% for B6C3F$_1$ and 64% for Swiss mice. No other doses were tested in this experiment. Syrian golden hamsters dosed at 200 ppm showed much lower ASL incidences, whereas female F344 rats dosed at 100 ppm showed a 20% incidence of these tumors.

Swiss mice and Syrian golden hamsters were also utilized by Maltoni *et al.* (1984) in order to investigate species differences in response to VC exposure. In Swiss mice, ASL appeared in greater proportions at lower-dose levels after

30-week exposures than in Sprague-Dawley rats exposed for 52 weeks; at higher doses, however, the incidences were comparable. The incidence of ASL in male Syrian golden hamsters was considerably less after 30-week exposures than the incidence in male Sprague-Dawley rats or in male Swiss mice at corresponding daily exposure levels, indicating a definite species difference for hamsters. At high exposure levels, the incidence of mammary carcinomas in female Swiss mice was greater, but at lower levels, it was similar to the incidence in female Sprague-Dawley rats (43.3% vs. 10.0% at 2000 ppm, 40.0% vs. 35.0% at 50 ppm) exposed for 52 weeks. Other tumor types that were common in rats, e.g., Zymbal gland carcinomas, neuroblastomas and nephroblastomas, did not occur in Swiss mice or Syrian golden hamsters. Melanomas, lymphomas and leukemias occurred only in Syrian golden hamsters; corresponding neoplasms did not occur in rats or mice.

**2.1.3. Studies of exposure parameters and exposure age.** In one protocol, 12-week-old Sprague-Dawley rats were exposed to several concentrations of VC ranging from 50 to 10,000 ppm 4 hr/day, 5 days/week for 17 weeks. Animals were observed for their lifetime (156 weeks) to determine if a reduction in the number of total hours of exposure influenced the tumor yield. Compared with incidences in groups exposed for 52 weeks, dramatic decreases in the proportions of rats with ASL (1.7% vs. 22.0% for 6000 ppm and 0.0% vs. 11.7% for 10,000 ppm) were observed, but the effect of reducing the exposure duration by a factor of three on the incidence of Zymbal gland carcinomas in the 6000 and 10,000 ppm groups was equivocal. Some reductions in nephroblastomas were also recorded in the rats exposed for 17 weeks (Maltoni *et al.*, 1984). In another study, variations in the exposure regimen were tested, keeping the total exposure time constant. Sprague-Dawley rats, 11 weeks old, were exposed to 6000 and 10,000 ppm VC 4 hr/day, 5 days/week for 5 weeks; 4 hr/day, 1 day/week for 25 weeks; or 1 hr/day, 4 days/week for 25 weeks (100 hr total in each instance), followed by lifetime observation. The incidence of ASL was very low; however, the incidences of mammary and Zymbal gland carcinomas in these rats did not differ significantly among the various dosage groups, suggesting that the total exposure time rather than dosage regimen is the most important determinant of tumorigenicity (Maltoni *et al.*, 1984).

In investigations on the effect of age, embryo and newborn Sprague-Dawley rats appeared to be much more sensitive to VC exposures than 11- to 13-week-old rats in developing brain neuroblastomas, ASL and hepatocellular carcinomas after exposure (Maltoni *et al.*, 1984; Maltoni and Cotti, 1988). Newborn Sprague-Dawley animals were exposed to 6000 and 10,000 ppm VC for 5 weeks according to the same protocol as adults with lifetime observation. The resulting ASL incidences for newborns were 17/42 (40%) and 15/44 (34%) for 6000 and 10,000 ppm, respectively. Large numbers of adenomatous tumors of the lung were also associated with perinatal exposure to VC. Eleven-week-old rats exposed according to the same doses and regimen had ASL incidences of 0/120 (0%) and 1/118 (1%), respectively. The incidences of hepatomas showed similar differences. Another experiment compared tumor incidence between breeder animals and embryos for 2500 ppm VC exposure beginning at 13 weeks of age for breeders and 12 days of gestation for embryos and continuing for 76 weeks (20-35 hr/week). In this experiment, the number of neuroblastomas and angiosarcomas were similar between breeders and offspring, but hepatocellular carcinoma incidences were 9.2% and 51.2% for breeders and offspring, respectively.

Further investigations on the role of exposure intensity compared with number of exposures, resulting in the same total VC dose, were conducted by Hehir *et al* (1981) in mice. When A/J mice had 10 1-hr exposures to 500 ppm VC, the incidence of pulmonary adenoma (124/166) and lung carcinoma (22/166) was significantly greater than matched controls (31/90 pulmonary adenomas and 3/90 lung carcinomas, $P < 0.001$) and also considerably greater than when they received 100 1-hr exposures to 50 ppm VC (65/158 pulmonary adenomas and 7/158 lung carcinomas). Consequently, it appeared that these mice were somewhat more sensitive to less frequent, more intense exposures; however, the differences were only 2- to 3-fold.

An extensive study by Drew *et al* (1983) further demonstrated that age of female Fisher 344 rats, C57BL/6N mice, CD-1 Swiss mice and Syrian golden hamsters influenced the number and type of tumors observed after VC administration. For example, 4/76 (5.3%) of 5- to 6-week-old female Fisher 344 rats exposed to 100 ppm VC 7 hr/day, 5 days/week for 6 months developed a statistically significant number of hemangiosarcomas. (Only total hemangiosarcomas were reported in this study.) In contrast, 7-month-old female Fischer rats exposed to VC by an identical experimental protocol developed 2/52 (3.8%) hemangiosarcomas, and rats held for 12 months or 18 months prior to the daily exposures for 6 months failed to develop any hemangiosarcomas. When VC exposure was for a total of 12 months, deferring the period of exposure for 12 months reduced the ASL incidence from 11/55 (20%) to 2/49 (4%). In Swiss mice, 6 months' exposure to VC (50 ppm) produced significant incidences of hemangiosarcomas, mammary gland carcinomas and lung carcinomas. After 12 months of exposure, the incidence of ASL was 30/47 (64%). If initiation of exposures was deferred until the mice were 12 months of age, the incidence for 12 months of exposure was decreased to 3/50 (6%). The incidence of hemangiosarcomas and mammary gland carcinomas was also reduced in $B6C3F_1$ mice that were dosed at ages older than 5–6 weeks; the reduction of total hemangiosarcomas after a 12-month deferral of dosing was from 69/90 (77%) to 29/48 (60%). Female Syrian golden hamsters developed hemangiosarcomas, mammary gland carcinomas, stomach adenomas and skin carcinomas after exposure to 200 ppm VC beginning at 2 months of age for as little as 6 months' duration. When the exposures were started at 8 months of age, the incidence of

hemangiosarcomas and mammary gland carcinomas was again markedly reduced.

### 2.2. *Dietary and Gavage Studies*

Male and female Sprague-Dawley rats were dosed 5 times weekly by gavage with 0, 0.03, 0.3, 1.0, 3.33, 16.6 and 50.0 mg/kg/day VC in olive oil for 52 weeks and observed for their life span by Maltoni *et al.* (1984). ASL were induced in males at dose levels as low as 0.3 mg/kg/day; the incidence was 12% at 16.6 mg/kg/day and 21% at 50.0 mg/kg/day. Dose-related increases in liver nodular hyperplasia and diffuse hyperplasia were also observed; however, the levels of neoplastic nodules did not achieve statistical significance. Also, nephroblastomas (2% and 3.7% in respective dose groups) and forestomach papillomas and squamous cell carcinomas (2.5% and 1.2% in respective dose groups) occurred in the 50.0 mg/kg/day and 16.6 mg/kg/day groups. Zymbal gland carcinomas occurred at 1.0 mg/kg/day (3.3%), 16.6 mg/kg/day (2.5%) and 50.0 mg/kg/day (1.2%). Extrahepatic angiosarcomas and angiomas were recorded in the 50.0 mg/kg/day (2.5% incidence, each type) and 3.33 mg/kg/day gavage dose groups (2.5% extrahepatic angiosarcomas and 1.2% angiomas).

A lifetime oral toxicity study of VC monomer was performed by incorporating PVC containing 0, 1.8, 5.6 and 17.0 mg VC/kg/day into the diet of Wistar rats for 135 weeks in males or 144 weeks in females. VC was also administered at 300 mg/kg/day, 5 days/week for 83 weeks by gastric intubation in soy bean oil (Feron *et al.*, 1981). Statistically significant numbers of ASL were observed at 5.6 mg/kg/day in males and at 17.0 mg/kg/day in females. The tumor response demonstrated a shift from 0/116 ASL and 5/116 hepatocellular carcinomas at the 1.8 mg/kg/day dietary dose to a mixture of 8/114 ASL and 21/114 hepatocellular carcinomas in the 5.6 mg/kg/day dose group, 36/116 ASL and 37/116 hepatocellular carcinomas in the 17.0 mg/kg/day diet group, and finally to a preponderance of 56/109 ASL and 1/109 hepatocellular carcinomas in the 300 mg/kg gavage group. Angiosarcomas of the lung, extrahepatic abdominal angiosarcomas and tumors of the Zymbal gland appeared in rats at doses of 5.6 mg/kg/day or greater. A second study in the same laboratory was conducted using male and female Wistar rats, which were fed VC in powdered PVC in diet for 149 weeks. The doses used were 0, 0.014, 0.13 and 1.3 mg VC/kg/day (Til *et al.*, 1991). On the basis of the results, it was concluded that induction of tumors, neoplastic nodules and hyperplastic foci in the liver were the most sensitive responses to dietary VC and that 0.13 mg/kg/day was the "no-observed-adverse-effect-level" for the induction of liver tumors in Wistar rats.

### 2.3. *Effects of Intraperitoneal and Subcutaneous Injection*

Intraperitoneal or subcutaneous injections of VC were not effective in causing various types of tumors (Maltoni *et al.*, 1984); however, the total doses administered were small. The incidence of ASL was 0 in 4 groups of 60 rats administered 4.25 mg/kg VC i.p. either 4, 3, 2 or 1 time at 2-month intervals; 2/188 angiosarcomas occurred at other sites in the 4 dosage groups. In another study, a single nephroblastoma was diagnosed in 32 Sprague-Dawley rats surviving to 85 weeks after a single i.p. dose of 4.25 mg VC/kg; no nephroblastomas occurred in 49 control rats (Maltoni *et al.*, 1984).

## 3. EPIDEMIOLOGICAL STUDIES

In a number of studies, VC has been found to cause ASL, a rare tumor in humans. Some quantitative information is also available regarding the historical VC levels in the workplace. Barnes (1976) provided estimates that exposure concentrations for various years were the following: 1945–1955, 1000 ppm; 1955–1960, 400–500 ppm; 1960–1970, 300–400 ppm; mid-1973, 150 ppm; 1975, 5 ppm. Based upon measurements of VC and interviews with workers concerning VC odor detection, Heldaas *et al.* (1984) estimated exposure levels of 2000, 1000, 500 and 100 ppm for the years 1950–1954, 1955–1959, 1960–1967 and 1968–1974, respectively. Thus, the estimates of Barnes (1976) and Heldaas *et al.* (1984) are within a factor of two.

### 3.1. *Studies in the United States and Canada*

Monson *et al.* (1975) reported an epidemiological study among workers at two plants in Kentucky, USA involved in the production of VC and PVC. Death certificates were obtained for all 161 deceased workers who had been actively employed or pensioned. There were eight deaths due to cancer of the liver and biliary tract, and five of these were diagnosed as ASL. A proportional analysis revealed that the ratios of observed vs. expected mortality were 11.0 for liver and biliary tract, 1.6 for lung, 1.6 for digestive tract and 4.2 for brain. Heath *et al.* (1975) reported that seven workers at a plant in Louisville, KY, USA developed ASL after having been exposed for between 12–28 (average 17) years. This was among a work force of approximately 270 workers exposed to VC beginning in 1942.

Wu *et al.* (1989) reported the most recent update of a cohort analysis utilizing death certificates and a nested case-control study of 3635 male workers exposed to VC and PVC. Workers were exposed between 1942 and 1974, with follow-up extending through 1986. Prior published reports of these workers have included Waxweiler *et al.* (1976) and Waxweiler *et al.* (1981). Only liver cancer incidence was elevated [standardized mortality rate (SMR)=333; confidence interval (CI)* = 202–521]. Estimates of cumulative dose were made, and a statistically significant association with VC and liver cancer was determined. Analyses for lung and brain cancer were also reported, and no association with VC exposure was found. After review of medical records and death certificates, there were 12 ASL cases and

*CI is the 95% CI unless otherwise stated.

7 hepatocellular carcinoma cases. Only the ASL cases exhibited a positive dose-response with VC.

Theriault and Allard (1981) reported that 451 Canadian workers who were heavily exposed to VC for more than 5 years, employed from 1943 through 1972 and followed through 1977, had an excess of digestive system tumors. Histopathological confirmation of all cancer cases was determined, and there were 10 total ASL cases found, resulting in an SMR of 5714. The exposures of these workers were thought to be quite heavy because several episodes of unconsciousness were reported. In this study, the exposure durations for both the exposed population and the ASL cases were reported. They were found to be comparable, although there were no ASL cases among the 105 men with the longest exposures. This study will be analyzed in Section 9.4.

Rinsky *et al.* (1988) and Teta *et al.* (1990) reported results of exposure to VC at Union Carbide Corporation facilities in the Kanawha Valley of West Virginia, USA, between 1940 and 1978. The Rinsky *et al.* (1988) study reported on a particular set of facilities within the Teta *et al.* (1990) study. In the Rinsky *et al.* (1988) study, the results were specifically linked to VC exposure. Increased incidences of liver cancer (SMR = 174; CI = 102–280) and lympho- and reticulosarcomas (SMR = 140; CI = 104–187) were reported. The highest SMRs occurred among persons who worked at least 25 years (SMR = 239) and who died 30 years or more after first employment (SMR = 301). The results for lympho- and reticulosarcomas did not show effects of latency or years of employment.

A study of a cohort in which there were 1536 deaths out of 10,173 men who had at least 1 year of exposure to VC due to employment at 37 plants in the United States was completed by Wong *et al.* (1991). A previous report of this cohort was reported by Tabershaw and Gaffey (1974). The updated report included observed mortality from 1942 through 1982. Fifteen ASL cases were reported among the 37 deaths due to cancers of the liver and biliary tract (SMR = 641; CI = 450–884). The excess mortality due to cancers of the liver and biliary tract (SMR = 386) was still present even after the removal of ASL cases. Also reported were increased cancers of the brain and central nervous system (SMR = 180; CI = 114–271). No excess mortality was found for cancers of the respiratory system, lymphatic and hematopoietic system or digestive system other than the liver. Analysis of mortality by latency for liver and biliary tract tumors (including ASL) showed a clear positive trend; however, for brain and CNS, this trend was not obvious. There was the expected inverse relationship of age of first exposure for liver and biliary tract tumors, but for brain and CNS tumors, there was an opposite relationship. Consequently, the increased mortality due to cancers of the brain and CNS appears to be equivocal.

### 3.2. *Studies in Europe*

Byren *et al.* (1976) reported three cases of angiosarcoma among 58 cancer cases in 771 workers who had worked in various chemical operations with exposure to VC in Sweden. These workers were compared with standardized mortality statistics from the Swedish Central Bureau of Statistics. Four cases of cancer of the liver/pancreas, which included two of the ASL cases, resulted in an SMR of 413 (P = 0.017). One of the cases of ASL was added to the paper as a note in proof. Also, these authors found two cases of brain tumors, yielding an SMR of 612 (P = 0.043). However, one of these cases had been employed for less than 1 year at the time of diagnosis. Fox and Collier (1977) reported the results of a study of 393 deaths among 7000 men exposed to VC from 1940 to 1974 in the United Kingdom. There were four deaths due to liver cancer, and two of these deaths were ASL. The two cases of ASL had 8 and 25 years of high constant exposure, which was estimated to average 200–500 ppm. No other cancers were found to be at elevated incidence. A follow-up of this study through 1984 was reported by Jones *et al.* (1988). The number of cases was restricted to the 5498 workers having exposure to VC for at least 1 year and during at least 25% of the working period. At this point, 11 cases of primary liver tumor had been identified (SMR = 567; CI = 283–1015) from the 780 deaths from all causes. Among autoclave workers, there were seven deaths due to liver tumors, and they were all identified as ASL. The mean latency period from time of first exposure was 25 years. One of the cases had an exposure of 2–4 years, 2 had between 5–9 years and 4 had greater than 10 years. There were no increases in neoplasms of the brain, lung, colon, lymph system or in malignant melanoma. An increase in urogenital cancers was not correlated with degree of VC exposure. Weber *et al.* (1981) reported on German workers exposed to VC or PVC who died before 1974. Death certificates of these workers were compared with mortality rates of the West German population and also to a group of chemical workers not exposed to VC. The SMR for workers exposed to VC was 1523, compared with 401 for the reference group. VC exposure was also associated with statistically significant increased incidences of malignancies of the lymphatic and hematopoietic tissues (SMR = 214) and of the gastrointestinal tract and peritoneum (SMR = 149). The SMRs for 13–60, 61–120 and >121 months were 874, 1525 and 2528, respectively. Heldaas *et al.* (1984) reported one ASL case among 23 cancer cases in 454 VC and PVC workers in Finland. The one case was in a person exposed for 22 years. During most of this person's employment history, VC levels were between 500 and 2000 ppm. There were also four cases of malignant melanoma reported, resulting in an SMR of 510. Pirastu *et al.* (1990) investigated 253 deaths among persons who had been involved with using VC or PVC at plants in Italy. Death certificates and clinical and pathological data were reviewed, and seven cases each of ASL and hepatocellular carcinoma were verified. The mean duration of exposure was 16 and 18 years, respectively. It was not possible to compare these results with expected values. The mean latencies were 19 and 20 years, respectively. A large multicenter cohort mortality study of European VC workers

found a 3-fold increase in cancer of the liver (Simonato *et al.*, 1991). This study included 14,351 workers from Italy, Norway, Sweden and the United Kingdom. Some of this data was included in other reported studies (Byren *et al.*, 1976; Jones *et al.*, 1988; Heldaas *et al.*, 1984; Pirastu *et al.*, 1990); however, this study included additional workers, and follow-up was extended. Only cancers of the liver and intrahepatic bile ducts were statistically significantly elevated (SMR = 286; CI = 183–425). Fourteen cases of brain cancer vs. 13 expected were reported.

## 4. BIOTRANSFORMATION

VC can be biotransformed to chloroethylene oxide (CEO), which readily reacts with DNA or chloroacetaldehyde (CAA), which primarily reacts with protein (Fig. 3). The main enzymatic pathway for metabolism of VC requires microsomal mixed-function oxidase. Microsomal activation of VC has been confirmed both *in vivo* (Reynolds *et al.*, 1975; Guengerich and Watanabe, 1979; Bartsch *et al.*, 1979; Guengerich *et al.*, 1981) and *in vitro* (Barbin *et al.*, 1975; Guengerich *et al.*, 1979; Guengerich, 1982); oxygen and NADPH are required as cofactors. When a mixture of VC and pure oxygen was passed through a mouse liver microsomal fraction fortified with an NADPH-generating system, volatile alkylating metabolites were trapped by reaction with excess 4-(4-nitrobenzyl)pyridine. The absorption spectra of the adducts formed were identical to those obtained by reaction of CEO with 4-(4-nitrobenzyl)pyridine, indicating that CEO, the highly reactive epoxide of VC, was formed (Barbin *et al.*, 1975; Bartsch *et al.*, 1979). Microsomes prepared from human surgical specimens of liver exhibited a 9-fold variation in the capacity to convert VC into electrophiles mutagenic to *Salmonella typhimurium* strain TA 1530 (Sabadie *et al.*, 1980). The specific mixed-function oxidase required for bioactivation of VC to its epoxide CEO was identified as CYP2E1 by selective inhibition of the enzyme with diethyldithiocarbamate and by studies with anti-CYP2E1 in liver microsomes (Guengerich *et al.*, 1991). Additional evidence for the role of CEO will be described in Section 5.

CEO (Fig. 4) has a half-life of 1.6 min in aqueous solution and can spontaneously rearrange to CAA (Barbin *et al.*, 1975). In addition, CEO can be hydrolyzed by the enzyme epoxide hydrolase. When epoxide hydrolase was added to an aqueous solution of CEO, the $t_{1/2}$ for disappearance of CEO was decreased. When epoxide hydrolase was added to a rat liver microsomal preparation, NADPH-dependent covalent binding of $^{14}C$ (from $^{14}C$-VC) to proteins was decreased by 50% (Guengerich *et al.*, 1979). However, subsequent studies have shown almost a complete inhibition of DNA-adduct formation from CEO following the addition of epoxide hydrolase to the incubation (Guengerich, 1992).

VC metabolism was also reported to be partially inhibited by pyrazole and ethanol, both of which can inhibit alcohol dehydrogenase; this suggests that VC may be oxidized to 2-chloroethanol and then via alcohol dehydrogenase to CAA (Hefner *et al.*, 1975; Guengerich *et al.*, 1979). However, it is also possible that decreased VC metabolism may

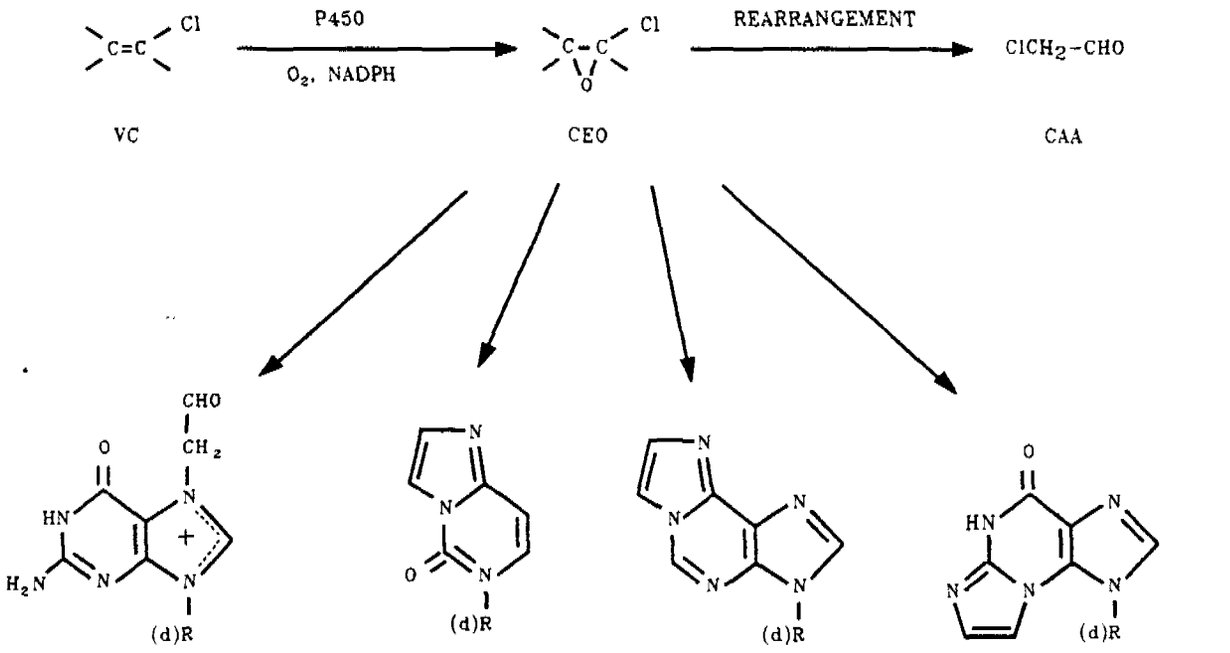


FIGURE 3. VC metabolism and adducts. Data from Ciroussel *et al.* (1990).

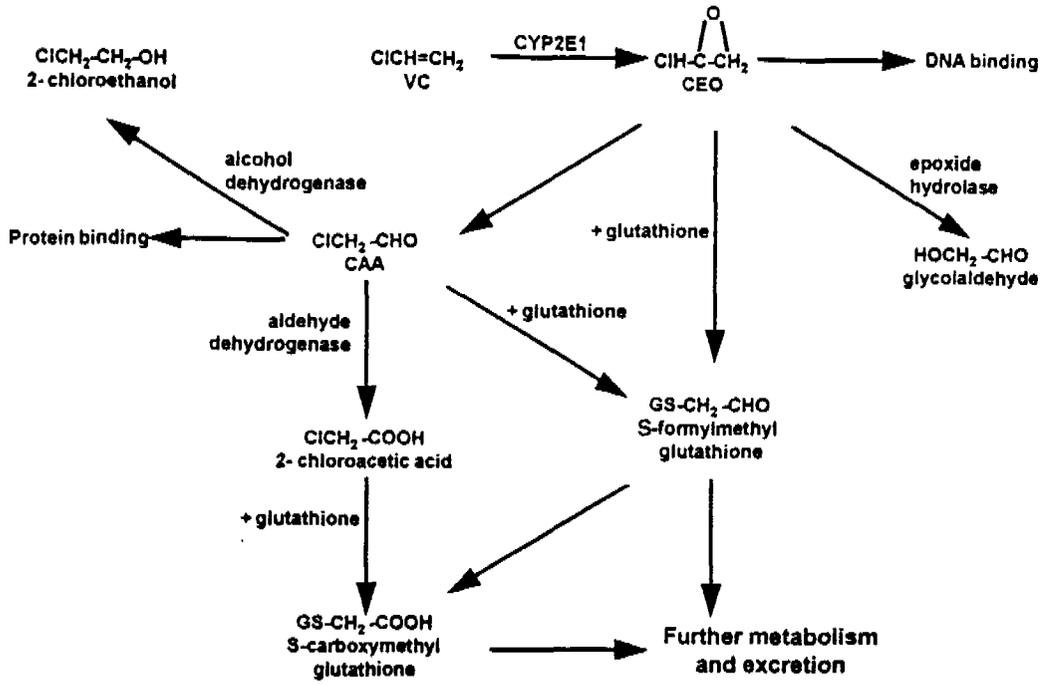


FIGURE 4. Proposed metabolic pathways for VC.

have been due to competitive inhibition of CYP2E1 by ethanol. Oxidation of 2-chloroethanol in the presence of hydrogen peroxide and catalase has also been proposed (Hefner *et al.*, 1975), but evidence for this is not available. CAA can be oxidized by aldehyde dehydrogenase to 2-chloroacetic acid (Fig. 4), which has been found in the urine of VC-dosed rats. 2-Chloroacetic acid can also bind to glutathione to form S-carboxymethylglutathione, which can also be formed from S-formylmethylglutathione (Plugge and Safe, 1977). CEO and CAA can bind covalently to sulfhydryl-containing groups in protein (Kappus *et al.*, 1975) or react with glutathione via glutathione epoxide transferase (or other glutathione transferases) to form S-formylmethylglutathione. Further metabolism (not shown in Fig. 4) occurs with conversion of S-formylmethylglutathione to S-carboxymethylcysteine and S-(2-hydroxyethyl)-cysteine; these products have been identified in the urine of VC-dosed rats. N-acetylation of S-(2-hydroxyethyl)-cysteine then yields N-acetyl-S-(2-hydroxyethyl)-cysteine, thiodiglycolate is formed from S-carboxymethyl-cysteine by deamination, and subsequent decarboxylation products have been identified as urinary metabolites of VC (Watanabe *et al.*, 1976a,b; Plugge and Safe, 1977).

Several studies have indicated that the epoxide metabolite of VC, CEO, is the ultimate carcinogen that reacts with DNA. Using *in vitro* studies, Guengerich *et al.* (1981) found that epoxide hydrolase (which reacts with CEO), but not alcohol dehydrogenase (which reacts with 2-chloroethanol, a CAA precursor), inhibited binding of VC metabolites to calf thymus DNA (Fig. 4), proving that CEO was the DNA-reactive species. Gwinner *et al.* (1983) determined *in vivo* that the likely proximal carcinogen was CEO rather than CAA. After exposing young rats to VC and to the precursors 2,2'-dichlorodiethyl ether and chloroethanol, which are metabolized only to CAA and not to CEO, only the VC-exposed rats developed preneoplastic hepatocellular ATPase-deficient foci, indicating that VC was DNA reactive (presumably through a CEO intermediate) and that CAA was not DNA reactive *in vivo*. A single 1 mg (12.7 μmol) s.c. dose of CEO to 8- to 10-week-old XVIInc/Z mice was administered followed by 2 μg 12-O-n-tetradecanoylphorbol-13-acetate 3 times per week for 42 weeks (Zajdela *et al.*, 1980). After 590 days, 18/22 (82%) and 5/22 (23%) of animals had papillomas and carcinomas of the skin, respectively. In comparison, the same dose of CAA produced papillomas in 3/20 (15%) of animals, which was not different from controls, and there were no carcinomas. The authors concluded from this study that CEO rather than CAA was the proximate carcinogen for VC.

Protein binding of VC following inhalation exposure to 2, 100 or 1000 ppm for 5 hr occurred in the liver, kidney, lung and small intestine, with lesser amounts in other organs and tissues of male Wistar rats (Bolt and Filser, 1977). Binding of VC or its metabolites to protein was partially blocked by reduced glutathione, but binding to DNA was not inhibited. By addition of radiolabeled CAA to the system, it was determined that CAA, or its products of metabolism, readily bind to intracellular protein and that reaction of CAA with DNA was very slow (Guengerich *et al.*, 1981)

In rats, VC metabolism is a dose-dependent saturable process. Watanabe *et al.* (1976a) investigated the fate of radiolabeled VC following oral exposure in male Sprague-

Dawley rats. As the dose was increased from 1 to 100 mg/kg, the proportion of the administered dose expired in the lungs as VC increased from 2 to 67%. Following exposure to air containing 10 ppm VC, urinary and expired radiolabel accounted for 68% and 2%, respectively. Exposure to 1000 ppm VC resulted in urinary and expired radiolabel amounts of 56% and 12%, respectively (Watanabe *et al.*, 1976b). Several 6-hr doses of VC ranging from 1 to 4600 ppm were administered to male Sprague-Dawley rats by inhalation (Watanabe *et al.*, 1978; Gehring *et al.*, 1978). The amount of metabolism and the formation of protein-bound metabolites in the liver were reported to be a sigmoidal function of the log of exposure concentration, which means that binding of $^{14}$C-VC or metabolites was low at exposure concentrations below 50 ppm, linear with respect to log concentration in the range 50–500 ppm, and reached a plateau at 500 ppm. This dose-response relationship was also found to obey apparent Michaelis-Menten kinetics.

## 5. IDENTIFICATION OF DNA ADDUCTS

A number of studies have been performed with the goal of identification of DNA and RNA adducts formed after VC exposure. The adducts of nucleic acids that have been consistently identified are shown in Fig. 3. As described below, *in vitro* adducts were prepared for adenosine, cytosine and guanosine. Also, *in vivo* DNA adducts have been found in mouse liver after i.p. injection and in the liver, lung, kidney and brain of rats after inhalation or drinking water exposure. The major DNA adduct formed *in vivo* was 7-(2-oxoethyl)-deoxyguanosine (7-OEdG); there were smaller amounts of etheno adducts formed from deoxyadenosine, deoxycytosine and deoxyguanosine (Fig. 3).

Osterman-Golkar *et al.* (1977) reported the isolation of $^{14}$C-labeled 7-(2-hydroxyethyl)guanine from hydrolysates of DNA from the livers of BALB and CBA mice exposed to [1,2-$^{14}$C] VC. The authors concluded that the alkylation product originally present in the DNA was 7-OEdG. Green and Hathway (1978) found 3,N$^4$-ethenodeoxycytidine (ϵdC) and 1,N$^6$-ethenodeoxyadenosine (ϵdA) in liver DNA from male and female Wistar rats that had been exposed to VC in their drinking water (250 ppm) for approximately 2 years, beginning on the 27th day of life. These adducts were separated by liquid chromatography and identified by mass spectrometry. Laib *et al.* (1981) assessed the potential of [1,2-$^{14}$C] VC to form *in vitro* and *in vivo* DNA adducts. When calf thymus DNA was incubated with VC, rat liver microsomes and NADPH, ϵdC, ϵdA and 7-OEdG adducts were identified. *In vivo*, they found only the 7-OEdG adduct by liquid chromatography of rat liver DNA after a 5-hr inhalation exposure of the rats to VC. The authors reported that 1,N$^6$-ethenoadenosine (ϵAdo) and 3,N$^4$-ethenocytidine (ϵCyo) were the major alkylation products with RNA, *in vivo* and *in vitro*. In a later study, Laib *et al.* (1985a), using similar procedures, were able to detect the N$^2$,3-ethenoguanine (ϵG) adduct in liver DNA of 12-day-old rats after 2 daily exposures to $^{14}$C-VC. Bergman (1982) identified VC-DNA adducts in liver and detectable amounts of radioactivity in the DNA of spleen, kidney, lung, pancreas and testis of male NMRI mice after a single i.p. injection of 25 μCi $^{14}$C-VC. DNA was hydrolyzed and separated by liquid chromatography. In the liver, which had the greatest amount of VC radioactivity, ϵdA adducts were possibly identified from DNA hydrolysates; a major unknown adduct, probably 7-OEdG, was also detected. Binding to RNA was observed in the spleen, pancreas, liver, kidney, lung and testis, but not in brain. In RNA of liver and kidney, a large part of the radioactivity was incorporated as $C_1$ fragments, but significant amounts of ϵCyo in the kidney and ϵA in liver and kidney were identified. Using specific monoclonal antibodies, Eberle *et al.* (1989) detected ϵdA and ϵdC adducts in liver and lung DNA from Sprague-Dawley rats exposed to 2000 ppm VC by inhalation for 10 days. The DNA was hydrolyzed, and the adducts were separated by HPLC. Ciroussel *et al.* (1990) exposed 7-day-old and 13-week-old BD VI rats to 500 ppm VC 7 hr/day, 7 days/week for 2 weeks. Using HPLC and competitive radioimmunoassay with murine monoclonal antibodies, ϵdA and ϵdC were identified in DNA hydrolysates of liver, lung and brain, but not in the kidneys of the young rats. In the older rats, only the liver was analyzed, and levels of each adduct were 6 times lower than for the young rats. The authors suggested that their data were in good agreement with the organotropism and age-related sensitivity of VC-induced carcinogenicity in rodents. DNA adducts were identified in the liver, lung and kidney of preweanling Sprague-Dawley rats exposed to 600 ppm VC 4 hr/day for 5 days (Fedtke *et al.*, 1990; Swenberg *et al.*, 1992). HPLC and fluorescence detection were used to analyze 7-OEdG. ϵG was determined by isotope dilution mass spectrometry, and ϵdC and ϵdA were determined by competitive radioimmunoassay using monoclonal antibodies, after separation by HPLC. At the end of the 5-day period, the liver had 3- to 8-fold higher amounts of each of the adducts than lung or kidney. No DNA adducts were identified in brain or spleen. The 7-OEdG adduct represented 98% of the adducts detected immediately after dosing; the ϵG and ϵdC adducts were present at about 1% and 0.6%, respectively, and the ϵdA adduct occurred at about 0.1%. The persistence of adducts was determined in livers 0, 3, 7 and 14 days postexposure. The 7-OEdG adduct had a half-life of 62 hr, the ϵG adduct had a half-life of 30 days, and the ϵdC and ϵdA adducts were unrepaired. As a result, at the end of 14 days, all of the detectable adducts were of the etheno type. These data show that the etheno adducts, although formed much less readily, are poorly recognized by repair enzymes and thus accumulate.

Reaction of calf thymus DNA with CEO was found to produce adducts in the order 7-OEdG > ϵAdo > ϵG by analysis of fluorescent properties (Guengerich, 1992). CEO was found to be much more effective than CAA in the production of ϵAdo and ϵG, and no 7-OEdG was produced from CAA. Epoxide hydrolase almost completely blocked the formation of ϵAdo by VC, and either calf thymus DNA

or adenosine, in the presence of rat liver microsomes and NADPH. Glutathione (10 mM) reduced the formation of εAdo in similar conditions by 76% and 61% from adenosine and DNA, respectively. This experiment shows that CEO (and not CAA) is the proximate DNA-reactive species and that protective mechanisms can prevent adduct formation.

## 6. GENETIC ALTERATIONS BY VINYL CHLORIDE-DNA ADDUCTS

Although 7-OEdG is the major DNA adduct formed, etheno adducts have been found to miscode during DNA replication and transcription, and they are presumed to be involved in VC-induced cancer. However, there is conflicting evidence concerning the specific etheno adduct and mutation involved. In an attempt to deduce the importance of the 7-OEdG adduct, Politzer *et al.* (1986) suggested that it might be in equilibrium with a cyclic hemiacetal, and, if this were the case, that hydrogen bonding between guanine and cytosine would be affected. Computational studies of the energies of both the adduct and its hemiacetal suggested a very low probability that hemiacetal formation was a factor in prevention of hydrogen bonding between the bases. Therefore, it was concluded that the 7-OEdG adduct probably would not interfere with G-C base pairing. Krzyzosiak *et al.* (1986) used conformational analyses to determine the mutagenic potential of etheno adducts. The orientation of adenosine to its sugar was more greatly affected by etheno-adduct formation than was the orientation of cytidine to its sugar. In the case of adenosine, εAdo-adduct formation changed the sugar pucker from C3 endo to C2 endo and the glycosyl torsion angle from 10° to 26°. εCyo-adduct formation resulted in essentially no change to the sugar pucker and a change of glycosyl torsion angle from 18° to 10°. This is significant because base mispairing can occur as bases change their glycosyl torsion angles, making hydrogen-bonding sites unavailable.

Singer *et al.* (1991) synthesized the $N^2$,3-ethenodeoxyguanosine (εdG) adduct and found that it was incorporated opposite T at a greater rate than opposite C during DNA replication of a synthetic oligonucleotide template. The frequency of εG to thymine mismatch was similar with all polymerases tested: *Escherichia coli* DNA polymerase I (Klenow fragment), exonuclease-free Klenow, *Drosophila melanogaster* polymerase α and human immunodeficiency virus-I reverse transcriptase. The authors concluded that the replicating enzymes apparently recognize the same structural features, and on replication, G to A transitions would occur, resulting in GC to AT mutations. The mutagenic and genotoxic properties of 1,$N^6$-ethenoadenine (εA), 3,$N^4$-ethenocystine (εC) and a product formed by ring opening of εA, 4-amino-5-(imidazol-2-yl)imidazole, were investigated by Basu *et al.* (1993), who inserted these adducts into acceptor oligonucleotides; they were then ligated at a known site into the genome of the bacteriophage M13-*Nhe*I. After transfection of the adducted phage DNAs into *E. coli*, each of the adducts was found to be genotoxic. The most toxic lesion was 4-amino-5-(imidazol-2-yl)imidazole, which reduced survival of the genome by 97%. εC and εA reduced survival of *E. coli* by 90% and 65%, respectively. In this system, the least mutagenic of the adducts was εA, which caused primarily A→G transitions in 0.1% of survivors. The εA rearrangement product 4-amino-5-(imidazol-2-yl)imidazole induced mutations at a 20-fold higher frequency (~2%). εC induced mutations at a frequency of 1.5-2%; the mutations were mainly C→T transitions, although targeted C→A and −1 deletions were also detected. The mutagenic potency of the exocyclic adduct εdC in *E. coli* and in cultured simian kidney (COS) cells was compared by Moriya *et al.* (1994). A single-stranded shuttle vector containing an εdC lesion was introduced; after replication, the DNA sequence corresponding to the adduct site was analyzed. The mutation frequency for single-stranded DNA that contained εdC was 2% in nonirradiated *E. coli*, 32% in *E. coli* that had been preirradiated with UV light, and 81% in COS cells, indicating that the same lesion in bacteria and mammalian cells causes strikingly different effects. The primary mutations generated in both *E. coli* and COS cells were εdC→A and εdC→T base substitutions. Oligonucleotide duplexes of a defined sequence containing one εAdo were synthesized by Oesch *et al.* (1994) and used as substrates to study the repair of this DNA lesion in cell homogenates of peripheral mononuclear blood cells from workers exposed to VC. The rate of repair of this lesion in preparations from the exposed workers did not differ from the rate of repair of the identical εAdo lesion in cell homogenate preparations from lymphocytes of nonexposed control subjects.

### 6.1. *Studies of Oncogenes and Tumor-Suppressor Genes*

Investigations were performed at codons 12, 13 and 61 of c-Ha-*ras*, c-Ki-*ras* and N-*ras* in angiosarcoma samples taken from VC polymerization plant workers (Marion *et al.*, 1991). A GC to AT transition at base 2 of codon 13 in the c-Ki-*ras* oncogene was the only mutation detected in five of six samples. A mutation of this nature leads to substitution of aspartic acid for glycine in the resulting p21 protein, which is a common amino acid substitution found in many human cancers with mutated codon 13 in the Ki-*ras* oncogene. In a subsequent study from this group, 8 of 9 (89%) VC-exposed workers with ASL were found to have serum positive for immunoblotting with monoclonal antibodies to p21 mutant protein (DeVivo *et al.*, 1994). Also, 22 of 45 (49%) of VC-exposed workers with no evidence of ASL were also positive for p21 mutant protein by this monoclonal antibody assay. Consequently, this particular VC-induced mutation appears to be an early event in the neoplastic process and may be associated with the initiation of carcinogenesis. An investigation of hepatocellular carcinomas and ASL induced in Sprague-Dawley rats by VC inhalation exposure did not have similar oncogene mutations (Froment *et al.*, 1994). Female rats with their pups were exposed 8 hr/day, 6 days/week to 500 ppm VC from days 3 through 42 postpartum. DNA of various oncogenes was analyzed by allele-specific oligonucleotide hybridization, di-

rect sequencing of polymerase chain reaction products and sequencing after cloning. No mutations in codons 12, 13 or 61 of the Ki-*ras* oncogene, in codon 12 of the Ha-*ras* oncogene or in codon 61 of the N-*ras* oncogene were found. However, 2 of 5 resulting ASLs contained codon 13 (GGC to GAC) and codon 36 (ATA to CTA) mutations of the N-*ras* A oncogene, and 2 of 2 hepatocellular carcinomas contained an AT to TA transversion at base 2 of codon 61 in the Ha-*ras* oncogene.

Mutations in the p53 tumor suppressor gene from cancers of factory workers exposed to VC were reported by Hollstein *et al.* (1994). In 2/4 ASL and 1 hepatocellular carcinoma studied, AT to TA transversions were detected in the highly conserved regions at codon 249 and codon 255, respectively.

## 7. GENOTOXICITY STUDIES WITH VINYL CHLORIDE

Genotoxicity tests have shown mostly positive results; most *in vitro* tests required metabolic activation (Table 1). Additionally, some *in vitro* tests without activation were positive, indicating possible direct reactivity of VC with DNA or a low level of oxidation of VC in these systems. DNA damage was found with VC in the *E. coli* polymerase A assay by Rosenkrantz and Leifer (1980) and in the *Bacillus subtilis* assay by Elmore *et al.* (1976). Shimada *et al.* (1985) found that VC induced unscheduled DNA synthesis in primary hepatocytes derived from rats. In general, bacterial reverse mutation assays showed that VC was positive in Ames strains TA100, TA1530, and TA1535, but negative in strains TA1537 and TA1538, which respond only to agents causing frameshift mutations (Table 1). Some of the Ames tests were positive without activation. Other reverse mutation testing, with *E. coli* K12, with CHL/V79 cells at the HPRT and ouabain gene loci and with Chinese hamster ovary cells, was positive provided that there was metabolic activation (Greim *et al.*, 1975; Drevon and Kuroki, 1979; Krahn *et al.*, 1982). Gene conversion testing in *Saccharomyces cerevisiae* was positive with activation in tests that were reported by Loprieno *et al.* (1976) and by Eckardt *et al.* (1981). Recessive lethal testing of *D. melanogaster* was positive in two sets of tests conducted by Verburgt and Vogel (1977) and by Magnusson and Ramel (1978). Negative results were found in dominant lethal *in vivo* testing in the rat and mouse (Anderson *et al.*, 1976; Short *et al.*, 1977; Himeno *et al.*, 1983).

Positive micronucleus results were reported in CBA and $C_{57}$ Bl/6J mice (Jenssen and Ramel, 1980; Richardson *et al.*, 1983) and in human lymphocytes (Fučić *et al.*, 1990). There were nine positive and three negative tests for chromosomal aberrations in lymphocytes from humans exposed to VC, which are listed in Table 1. In the most recent study, Fučić *et al.* (1990) investigated VC-induced chromosomal damage among PVC factory workers who had been employed for an average of 15 years and were matched by age to controls. Chromosomal aberrations, micronuclei and sister chromatid exchange (SCE) frequencies showed statistically significant increases in workers exposed to VC compared with the control group. If the workers were also smokers, SCE were elevated still further, but the micronucleus test and chromosomal aberrations were not affected. Some evidence indicated that the elevated SCE frequencies were present for up to several years after exposure (Fučić *et al.*, 1992). Aneuploidy testing in *D. melanogaster* was negative (Verburgt and Vogel, 1977; Ramel and Magnusson, 1979). SCE in human lymphocytes showed mixed results *in vivo*. Hansteen (1979) found only negative results, while Kučerová *et al.* (1979) reported a weak positive result. Anderson *et al.* (1981) obtained a negative result when the test was done *in vivo* and could get a positive test *in vitro* with activation.

## 8. TOXICITY, CELL PROLIFERATION AND ALTERED FOCI

The sequence of events leading to the development of ASL after exposure to VC and the relationship between hepatocytes and sinusoidal cells have been carefully detailed (Feron *et al.*, 1979; Wisniewska-Knypl *et al.*, 1980; Sokal *et al.*, 1980; Feron *et al.*, 1981; Maltoni *et al.*, 1984). Major proliferative changes occurred in both the hepatocytes and the endothelial cells of the liver after animals were exposed to VC. In the hepatocytes, proliferation of the smooth endoplasmic reticulum, the appearance of swollen, abnormal mitochondria in which the cristae were arranged radially around the periphery of the organelle, or the formation of irregularly shaped mitochondria were the earliest indices of hepatocellular damage after administration of VC (Feron *et al.*, 1979). Proliferation of the endoplasmic reticulum was noted after 3 months' exposure to 50 ppm VC, while swelling of mitochondria with reduction of glycogen deposits in hepatocytes occurred after being exposed to 50 ppm VC 5 hr/day, 5 days/week for 10 months or after 3 months' exposure to 500 ppm VC (Wisniewska-Knypl *et al.*, 1980). The liver parenchymal cells typically became polymorphic, with hyperchromatic, pleomorphic nuclei. Small droplets of fat were deposited in the cytoplasm of parenchymal cells, and the rough endoplasmic reticulum degenerated with loss of ribosomes to the surrounding cytoplasm. Sokal *et al.* (1980) administered VC to male Wistar rats beginning at 2 months of age 5 hr/day, 5 days/week for 10 months. No statistically significant changes in liver cells were found after exposure at 50 ppm. However, at 500 and 20,000 ppm, 13/43 (38%) and 8/17 (47%) of animals, respectively, showed proliferation of the hepatic sinusoidal endothelium. At these levels there was also an increase in nuclear polymorphism of hepatocytes. Hyperplastic changes and dysplasia accompanied the appearance of basophilic preneoplastic foci and liver nodules. The hepatocytes of foci exhibited reduced glucose-6-phosphatase histochemical staining, while the sinusoidal cells located at the periphery of hepatic lobules had increased acid phosphatase (Feron *et al.*, 1979; Sokal *et al.*, 1980). Fatty changes in parenchymal cells and the formation of pockets of cellular necrosis further exacerbated the disruption of the normal cytoarchitecture in tissue sections of liver as sinusoidal cells infiltrated the paren-

TABLE 1. Genotoxicity of Vinyl Chloride

| Species/Strain | Cell/Strain | *In Vivo* test | Results: Without activation | Results: With activation | Reference |
|---|---|---|---|---|---|
| *DNA Interaction* | | | | | |
| DNA Adduct Formation | | | | | |
| Calf | Thymus DNA | no[1] | ND[2] | +[3] | Laib *et al.*, 1981 |
| Mouse/NMRI | Liver | yes[4] | + | ND | Bergman, 1982 |
| Rat/Wistar | Liver | yes | + | ND | Osterman-Golkar *et al.*, 1977 |
| Rat/Wistar | Liver | yes | + | ND | Green and Hathway, 1978 |
| Rat | Liver | yes | + | ND | Laib *et al* , 1981 |
| Rat | Liver, lung | yes | + | ND | Eberle *et al* , 1989 |
| Rat/BD VI | Liver, lung, brain | yes | + | ND | Ciroussel *et al.*, 1990 |
| Rat/Sprague-Dawley | Liver, lung, kidney | yes | + | ND | Swenberg *et al* , 1992 |
| DNA Damage | | | | | |
| *E. coli* | Pol A | no | ND | + | Rosenkrantz and Leifer, 1980 |
| *B. subtilis* | Rec | no | −[5] | ND | Elmore *et al* , 1976 |
| Unscheduled DNA Synthesis | | | | | |
| Rat | Primary hepatocyte | no | + | ND | Shimada *et al* , 1985 |
| *Mutagenicity Assays* | | | | | |
| Reverse Mutation | | | | | |
| *Salmonella typhimurium* | TA1535 | no | − | + | Rannug *et al.*, 1974 |
| *Salmonella typhimurium* | TA1537, 1538 | no | ND | − | Rannug *et al.*, 1974 |
| *Salmonella typhimurium* | TA1530 | no | + | + | Bartsch *et al.*, 1975 |
| *Salmonella typhimurium* | TA1535 | no | ND | + | Bartsch *et al.*, 1975 |
| *Salmonella typhimurium* | TA100, 1535 | no | + | + | McCann *et al.*, 1975 |
| *Salmonella typhimurium* | TA1535 | no | + | + | Andrews *et al.*, 1976 |
| *Salmonella typhimurium* | TA100 | no | − | ND | Elmore *et al.*, 1976 |
| *Salmonella typhimurium* | TA100 | no | + | + | Simmon *et al.*, 1977 |
| *Salmonella typhimurium* | TA100 | no | − | ND | Laumbach *et al.*, 1977 |
| *Salmonella typhimurium* | TA98, 100, 1535, 1538 | no | ND | − | Anderson and Styles, 1978 |
| *Salmonella typhimurium* | TA100, 1535 | no | ND | + | Jones and Hathway, 1978 |
| *Salmonella typhimurium* | TA100 | no | ND | (+)[6] | Bartsch *et al.*, 1979 |
| *Salmonella typhimurium* | TA1530 | no | ND | + | Bartsch *et al.*, 1979 |
| *Salmonella typhimurium* | TA1530 | no | (+) | + | Poncelet *et al.*, 1980 |
| *Salmonella typhimurium* | TA1530 | no | ND | + | Hällström *et al* , 1981 |
| *Salmonella typhimurium* | TA1530 | no | − | + | Duverger-Van Bogaert *et al* , 1982 |
| *E. coli* | K 12 | no | ND | + | Greim *et al.*, 1975 |
| Gene Mutation | | | | | |
| *Saccharomyces cerevisiae* | | no | − | + | Loprieno *et al.*, 1976 |
| *Saccharomyces cerevisiae* | | no | − | + | Eckhardt *et al.*, 1981 |
| Hamster | CHL/V79/HPRT | no | − | + | Drevon and Kuroki, 1979 |
| Hamster | CHL/V79/ouabain | no | − | + | Drevon and Kuroki, 1979 |
| Hamster | CHO | no | − | + | Krahn *et al* , 1982 |
| Dominant Lethal Mutation | | | | | |
| Mouse | | yes | − | ND | Anderson *et al.*, 1976 |
| Mouse | | yes | − | ND | Himeno *et al* , 1983 |
| Rat | | yes | − | ND | Short *et al* , 1977 |
| Sex-Linked, Recessive Lethal | | | | | |
| *D melanogaster* | | yes | + | ND | Verburgt and Vogel, 1977 |
| *D. melanogaster* | | yes | + | ND | Magnusson and Ramel, 1978 |
| *Cytogenetic Assays* | | | | | |
| Chromosomal Aberrations | | | | | |
| Human | Lymphocytes | yes | − | ND | Purchase *et al.*, 1975 |
| Human | Lymphocytes | yes | + | ND | Ducatman *et al.*, 1975 |
| Human | Lymphocytes | yes | + | ND | Funes-Cravioto *et al* , 1975 |
| Human | Lymphocytes | yes | + | ND | Kilian and Picciano, 1976 |
| Human | Lymphocytes | yes | − | ND | Kučerová, 1976 |
| Human | Lymphocytes | yes | + | ND | Szentesi *et al.*, 1976 |
| Human | Lymphocytes | yes | + | ND | Purchase *et al.*, 1978 |

(continued)

TABLE 1. Continued

| Species/Strain | Cell/Strain | *In Vivo* test | Results: Without activation | Results: With activation | Reference |
|---|---|---|---|---|---|
| Human | Lymphocytes | yes | + | ND | Fleig and Theiss, 1978 |
| Human | Lymphocytes | yes | + | ND | Hansteen *et al.*, 1978 |
| Human | Lymphocytes | yes | + | ND | Kučerová *et al.*, 1979 |
| Human | Lymphocytes | yes | − | ND | Anderson *et al.*, 1980 |
| Human | Lymphocytes | yes | + | ND | Fučić *et al.*, 1990 |
| Micronucleus | | | | | |
| Mouse | | yes | + | ND | Jenssen and Ramel, 1980 |
| Mouse | | yes | + | ND | Richardson *et al.*, 1983 |
| Human | Lymphocytes | yes | + | ND | Fučić *et al.*, 1990 |
| SCEs | | | | | |
| Human | Lymphocytes | yes | − | ND | Hansteen, 1979 |
| Human | Lymphocytes | yes | (+) | ND | Kučerová *et al.*, 1979 |
| Human | Lymphocytes | no | − | + | Anderson *et al.*, 1981 |
| Human | Lymphocytes | yes | − | ND | Anderson *et al.*, 1981 |
| Human | Lymphocytes | yes | + | ND | Fučić *et al.*, 1992 |
| Aneuploidy | | | | | |
| *D. melanogaster* | | no | − | ND | Verburgt and Vogel, 1977 |
| *D. melanogaster* | | no | − | ND | Ramel and Magnusson, 1979 |

[1]no indicates test was not done *in vivo*
[2]ND indicates test was not done
[3]+ indicates a positive test result.
[4]yes indicates test was done *in vivo*.
[5]− indicates a negative test result.
[6](+) indicates a weak positive test result.

chyma. The sequence of pathological changes in the endothelial cells lining the sinusoids has been described by Maltoni *et al.* (1984). Initially, there was hyperplasia that may have been accompanied by dysplasia of the endothelial cells. Varying degrees of fibrosis and sinusoidal dilatation may accompany the hyperplasia and dysplasia of the sinusoidal lining cells, leading to a cavernous appearance of some hemangiosarcomas. The angiosarcomas were usually described as multicentric with various patterns, such as cavernous, vascular or anaplastic. At low concentrations such as 1 and 5 ppm by inhalation in Sprague-Dawley rats, none of these proliferative changes were evident in either the parenchymal or sinusoidal cells (Maltoni *et al.*, 1984). At 10 ppm and above the incidence of proliferative changes increased, but it was not noted to be >25% in any of the groups. Because these results were not obtained early in the study, they are difficult to interpret.

Laib *et al.* (1985b) investigated the VC-induced development of phenotypically altered foci in the liver. These authors exposed male and female Wistar rats to 2000 ppm VC 8 hr/day, 5 days/week by inhalation for varying time periods beginning *in utero* (exposure of pregnant females) or beginning at postnatal day 7 or 21. The livers of the rats at 4 months of age were evaluated for ATPase-deficient foci. The number of ATPase-deficient foci was not increased after exposure to VC *in utero* or during postnatal days 1–5, but highly significant increases in foci areas were observed when newborn rats were exposed for 11 or 17 days; the incidence was not further enhanced by a 47- or 83-day exposure. The authors concluded that the period of sensitivity to VC-induced altered foci was characterized by rapid liver growth. This may also indicate the requirement for P450 activation, which is absent in the very young rat. In another study, Laib *et al.* (1985c) studied dose-response of the VC-induced, ATPase-deficient foci in rat liver. Newborn Wistar rats were exposed to 0, 10, 40, 70, 150, 500 and 2000 ppm VC 8 hr/day, 5 days/week for 10 weeks. Also, male and female Sprague-Dawley and Wistar rats were exposed to 2.5–80 ppm VC starting at 3 days of age for 3 weeks and were then maintained for 10 weeks without further dosing. In both sets, there was a linear relationship between dose and area of induced foci expressed as percent of liver area. The authors concluded that within the dose range investigated, there was no evidence for the threshold value of 50 ppm, which had been previously suggested in the literature (Gehring *et al.*, 1978). There was, however, evidence for saturable kinetics; this was seen when the area of foci increased linearly in the range 2.5–500 ppm, with increasing dose until it reached a plateau at 500 ppm in the 10-week-exposure regimen in female Wistar rats. There was a slight increase in the female/male foci-induction ratio in Wistar rats in the dose-response protocol, but there was a much greater increase in this ratio using the Sprague-Dawley rat. No-observed-effect-levels at 10 ppm for Sprague-Dawley rats and at 5 ppm for Wistar rats were found. However, the results appeared to be consistent with a linear dose-response at low doses due to the small number of foci and variability. The authors concluded that there was no threshold. The

relevance of dose-response of liver foci in the pathogenesis of ASL needs to be clarified.

## 9. SUMMARIES

### 9.1. *Rodent Bioassays and Human Studies*

VC offers one of few examples in which both rodent bioassays and human epidemiological studies find the same type of tumor, and this tumor type does not occur appreciably in unexposed rodents or humans. Numerous epidemiological studies have found relatively high incidences of ASL related to VC exposure compared with the rare occurrence of this tumor in the general population. SMRs for ASL in these studies ranged from 400 to several thousand. No other tumor types in humans have been convincingly demonstrated to be associated with VC exposures (Doll, 1988). Brain tumors were found elevated in two studies, and other tumor types have been found in only one study each: malignant melanomas, lympho- and reticulosarcomas, and malignancies of the lymphatic and hematopoietic systems. As reviewed by Doll (1988), one of the studies reporting increased brain cancer incidence (Monson *et al.*, 1975) was included in the larger analysis of the United States cohort, which found no clear trend for latency or the expected inverse relationship for age of first exposure. Also, the inclusion of the study by Byren *et al.* (1976), which found an increase in brain tumors, into the larger European cohort (Simonato *et al.*, 1991) resulted in no increase in brain tumors.

In contrast to humans, the large series of rodent bioassays by Maltoni demonstrated nine types of tumors that were considered by the authors to be correlated with VC dosing in the rat: ASL, extrahepatic angiosarcomas, mammary adenocarcinomas, Zymbal gland carcinomas, nephroblastomas, hepatomas, neuroblastomas, forestomach papillomas and squamous cell carcinomas. For certain types of tumors such as mammary gland, there appeared to be a decrease in incidences at higher doses. A possible explanation, based upon examination of the survival curves from those studies, is that at high doses, the animals succumbed to tumors with shorter latencies such as ASL, thereby decreasing the chance of observing a tumor with a longer latency, such as a mammary tumor.

As shown by Maltoni *et al.* (1984), the route of administration appeared to be a significant factor in the organotropism of VC. When ingested, VC appeared to affect the liver preferentially, producing both hepatocellular carcinomas and angiosarcomas. Hepatocellular carcinomas were only found when VC was incorporated in the diet; VC induced hepatocellular carcinomas at lower doses and a mixture of hepatocellular carcinomas and ASL at higher doses. When given by gavage, VC produced ASL and liver hyperplasia, but no significant increases in adenomas or hepatocellular carcinomas. When injected, VC showed only a borderline tumorigenic effect, probably due to the low concentrations of VC used. In comparison with adults, 12-day-old embryos and newborn rats were much more likely to develop ASL, hepatocellular carcinomas and neuroblastomas (Maltoni *et al.*, 1984; Maltoni and Cotti, 1988).

### 9.2. *Mechanistic Information*

The weight of evidence suggests that CEO, the epoxide of VC formed in the presence of microsomal P450-dependent monooxygenase, is the primary DNA-reactive species (Guengerich, 1992). CEO rapidly rearranges nonenzymatically or enzymatically in the presence of epoxide hydrolase to form CAA. Conversion of CEO, before it reacts with nucleic acids, to CAA converts the DNA alkylating species to a less DNA-reactive compound; CAA also has a higher affinity for protein than for DNA or RNA. This evidence is consistent with the finding that CAA is not tumorigenic (Guengerich, 1992). The detoxifying enzyme epoxide hydrolase and glutathione have substantial protective effects on the rates of etheno-adduct formation by VC (Guengerich, 1992). The preferential reactivity of CEO with DNA and the reactivity of CAA with protein were also reported by Gwinner *et al.* (1983), and they found that precursors of CEO, but not CAA, were capable of forming preneoplastic foci in liver. Reticuloendothelial cells lining liver sinusoids are almost devoid of microsomal mixed-function oxidase, in contrast to parenchymal cells. Guengerich *et al.* (1981) demonstrated that both CEO and CAA leave rat hepatocytes after the cells have been incubated with VC. Guengerich (1986), therefore, proposed that reactive intermediate metabolites of VC are produced in the hepatocytes, which then bind to critical molecules of the sinusoidal cells where they exert their effects, causing cellular transformation. The reason for primary involvement of the sinusoidal cell rather than the hepatocyte in the tumorigenic process may be due to a greater degree of sensitivity to adduct formation or due to poor repair mechanisms of sinusoidal cells.

The DNA adduct formed in the greatest amount during metabolism of VC is 7-OEdG, with minor amounts of εdC, εdA and εdG also being detected after *in vivo* exposure to VC. The 7-OEdG adduct and all three etheno adducts have been identified by Swenberg *et al.* (1992) in rat liver, lung and kidney. Other investigators have also identified the εdC and εdA adducts in the liver, lung and brain (Ciroussel *et al.*, 1990). One difficulty with these experimental studies in liver is that DNA adducts identified may be associated with hepatocytes, endothelial cells or both. Consequently, it is not possible to definitively link one of these adducts to ASL by information on specific DNA-adduct formation.

Studies have implicated the εdG adduct as the likely candidate for the particular mutations found in ASL in humans. The major adduct formed by VC, 7-OEdG, does not cause a direct mutagenic event because the adduct is positioned in the major groove of an undistorted B-DNA helix where no base pairing occurs. In contrast, cyclic etheno adducts require reaction with the exocyclic amino group and an adjacent ring nitrogen on guanine, cytosine or adenine and thus, are hindered from forming when DNA is double-stranded and hydrogen bonded, resulting in less adduct formation. However, these etheno adducts have mutagenic potential because, once formed, they prevent normal base pairing. GC to AT transitions, which have been reported as

VC-induced mutagenic changes, appear to result from miscoding of the εG adduct. This adduct is more likely to base pair with thymine than with its normal pairing partner, cytosine, thus initiating the GC to AT transition. Such transitions in codon 13 of the c-Ki-*ras* oncogene have been found in ASL tumors of VC-exposed workers (Marion *et al.*, 1991). The sera of workers with ASL have been found to contain mutated p21 protein, which is the oncogene product. Also, workers with VC exposure, but no evidence of ASL, have sera containing this mutated oncogene product. Consequently, VC appears to initiate malignant transformations by a mechanism that involves εG-adduct formation, mutation and oncogene activation. Although oncogene activation has also been found in ASL and hepatocellular carcinomas from rats exposed to VC, the types of mutations and oncogenes involved are different from ASL in humans.

Evidence of VC-DNA-adduct formation and genetic alteration is reflected in the positive results of genotoxicity tests, as shown in Table 1. The result of a test for unscheduled DNA synthesis in rat liver was positive. VC-induced mutagenic effects in bacterial systems were found both with and without the presence of a metabolic activation system. This may represent a low level of direct reactivity of VC or a low level of nonenzymatic production of CEO in the test system. The results of strains TA1537 and TA1538 have been negative, which suggests that the VC adducts formed were unable to produce the large conformational changes required for frameshift mutagenesis. In mammalian cells with metabolic activation *in vitro*, positive tests for gene mutation were reported. Peripheral lymphocytes containing chromosomal aberrations, micronucleus and SCE were found among humans exposed in the workplace. However, dominant lethal tests were negative, and most tests for SCE were negative.

Studies of VC-induced cytotoxicity and cell proliferation have shown that a reactive proliferative response in the sinusoidal endothelial cell may play a role in the induction of ASL (Feron *et al.*, 1979; Wisniewska-Knypl *et al.*, 1980; Sokal *et al.*, 1980; Feron *et al.*, 1981; Maltoni *et al.*, 1984). The dose-response of proliferative change occurs within a similar range as the tumorigenic response, in that no significant tumors or proliferative responses are induced up to 10 ppm by inhalation. Proliferative responses occur in the parenchymal cell, and the development of VC-related altered hepatic foci have also been described. However, no significant tumorigenic response occurs in the hepatocyte unless VC has been administered in the diet. In this case, the tumorigenic response is primarily in the hepatocyte at low doses, but it involves the sinusoidal endothelial cells as well at higher doses.

### 9.3. *Species-to-Species Extrapolation*

Incidences of ASL were found by Maltoni *et al.* (1984) to be comparable between different strains of rats and mice, although mice appeared to be more sensitive at lower doses. The hamster was relatively insensitive to VC-induced ASL. Studies by Drew *et al.* (1983) in F344 rats and Swiss mice gave incidences approximately 4–5 times higher than those of Maltoni *et al.* (1984). This may be partially explained by the use of younger animals by Drew *et al.* (1983), which were 8–9 weeks of age vs. the 13-week-old animals used in the experiments by Maltoni *et al.* (1984). Also, the Drew *et al.* (1983) experiments had a 50% greater daily exposure period. The experiments by Lee *et al.* (1978) in CD-1 rats also produced much higher ASL incidences per unit dose than the Maltoni *et al.* (1984) experiments. Almost one-half of these rats showed tumors at the end of 12 months of exposure to 1000 ppm VC. Consequently, the dose-response curve shown in Fig. 2 does not represent the most sensitive strain or species, and at a dose of 100–1000 ppm VC, other studies have shown incidences of ASL approximately 5 times higher.

Neither the mouse nor the hamster had VC-induced Zymbal gland carcinomas, nephroblastomas or neuroblastomas, and, in addition, the hamster did not develop mammary carcinomas or hepatomas. The several mouse strains tested, i.e., Swiss, B6C3F$_1$ and CD-1 (Lee *et al.*, 1978; Hehir *et al.*, 1981; Drew *et al.*, 1983) were much more prone to develop mammary tumors and lung tumors than were rats. Strain variability was also evident, as the Wistar rats had much lower rates of Zymbal gland carcinomas and somewhat lower rates of ASL than the Sprague-Dawley rats. Such variability of tumor types other than ASL from species to species would indicate that extrapolation from animal data to estimates for human populations could be subject to considerable uncertainty. However, ASL was found to be a major tumor type in all three species and in the various strains of these species studied. Most importantly, there appears to be a good correlation between the expected dose-response in rodents compared with humans. There are several possible methods for comparing the results of human and animal studies, which have been reported in the literature. These correlations will be described in the next section.

### 9.4. *Dose-Response Extrapolation*

Maltoni *et al.* (1984) demonstrated that VC was a dose-dependent animal carcinogen, and in inhalation experiments, exposure time, exposure route, animal age, species and strain affected its carcinogenic potential. The ages of animals exposed to VC greatly affect the dose response for ASL. In studies reported by Maltoni *et al.* (1984), newborn animals exposed for only 5 weeks developed approximately the same incidence of ASL as did 11- to 13-week-old animals exposed for 52 weeks. Drew *et al.* (1983) reported total hemangiosarcomas at all sites in rats and found that if 12-month exposures were deferred for 6 or 12 months, the reductions in tumor incidences were about 2- and 10-fold, respectively. In the Maltoni *et al.* (1984) Sprague-Dawley rat experiments, a reduction of total exposure time by two-thirds decreased the incidence of ASL from 12–22% to less than 2%. These authors also found that decreasing the number of exposure periods lowered the incidence of sev-

eral other VC-related tumors, but this apparently had little effect on the incidence of Zymbal gland tumors.

The evaluation of dose-response information necessarily focuses on ASL resulting from inhalation exposure because this is the tumor type and exposure route that occurs in both rodents and humans. Overall, the test results by Maltoni *et al.* (1984) demonstrated a dose-response relationship between concentration and tumor response for ASL. These data are presented in Fig. 2. Data have been plotted on this graph with the dose scale as the log(dose). This dose-response curve shows that there is an apparent linear relationship between log(dose) and ASL tumor incidence. Such a relationship corresponds to a supralinear dose-response curve. At two doses, 1 and 5 ppm, no apparent ASL was found; however, this is consistent with the low probability predicted and the number of test animals.

The studies examining the metabolism and protein binding of VC have been found to follow Michaelis-Menten kinetics, exhibiting a saturation at high doses (Gehring *et al.*, 1978). The dose-response of metabolic activation, therefore, would be represented by a supralinear curve. This data was also found to correlate to the tumor incidence data from the dose-response studies in Sprague-Dawley rats. However, below the saturation point, normal first-order kinetics have also been found to apply. The saturation point has been reported to be approximately 250 ppm in male Wistar rats (Filser and Bolt, 1979). At 250 ppm, the first order rate of clearance of VC in Wistar rats is 11.0 L/hr/kg body weight; the zero order $V_{max}$ at concentrations higher than 250 ppm is 110 μmol/hr/kg body weight. Although several mathematical methods have been used to correlate animal data to human data and to correlate metabolic data to animal data, their usefulness in predicting extrapolation to low doses is limited. Because the relationship of administered dose to delivered dose is linear and first-order below the saturation of 250 ppm, administered dose provides a virtually identical low-dose extrapolation compared with delivered dose (Krewski *et al.*, 1987). Several different mathematical models are capable of fitting the dose-response data in Sprague-Dawley rats, and all provide similar comparisons to the human epidemiological data (Gehring *et al.*, 1979). The use of these various models provides, however, a wide variation in the prediction of human risk at low doses (Purchase *et al.*, 1987).

Several approaches have been used to compare the dose-response for ASL from the Sprague-Dawley VC inhalation studies with epidemiological results. Four models (probit %, linear %, linear forced through origin and one-hit model) were used to empirically fit the dose-related tumor incidence data (Gehring *et al.*, 1979; Schumann *et al.*, 1982). Using the data of Maltoni *et al.* (1984), there was no clear benefit in using any one of the methods to model the dose-response relationship for ASL. All four models were then used to predict the human angiosarcoma incidence from rat angiosarcoma data. In their extrapolations from rat data to predictions of human cases, the investigators assumed that the induction of angiosarcoma was related to the amount of VC-reactive metabolite per unit surface area and that the exposure of rats for 12 months approximates exposure of humans for 35 years (half a lifetime). Presuming a time-weighted average human exposure of 200 ppm, the 8–10 cases predicted by the models compared favorably with the 5 recorded angiosarcoma cases in a cohort of 9677 exposed workers from an unpublished study quoted by these authors. Swaen *et al.* (1987) found that results by Maltoni *et al.* (1984) demonstrated that an incremental increase in ASL of 1% was associated with an increase of 29 ppm VC administered for 52 weeks in Sprague-Dawley rats. These authors also estimated that in the combined epidemiological experiments, an average SMR of 1300 for liver cancer was associated with an exposure duration of 8.7 years to 500 ppm VC. After accounting for differences in the period of exposures, these authors concluded that the death rates for comparable exposures were within a factor of three between the rodents and humans. Humans appeared to be somewhat less sensitive to the effects of VC than rodents. A more elaborate comparative approach was used by Chen and Blancato (1989), which incorporated physiologically based pharmacokinetic models for humans and animals. For this effort, conversion of the inhalation data to a metabolized dose was used. Metabolic constants for rodents were obtained from the literature, and those for humans were derived from primate data. The authors concluded from this analysis that whereas the number of cases predicted by the animal model on a per surface area basis was within a factor of two, the prediction would be 20-fold less on a per body weight basis. However, it should be noted that this analysis was based upon only two cases of ASL reported by Fox and Collier (1977).

Another approach is to use the inhalation dose-specific tumor rate in rodents for comparison with the human tumor rate, based upon the tumor incidence among a derived number of exposure years for exposed workers. The epidemiological study from which such estimates can be made was reported by Theriault and Allard (1981). In this study, there were 451 workers who had exposures for 5 or more years, and during the period of study, 10/451 (2.3%) workers were diagnosed with ASL. Based upon the information reported in this study, using observation period and exposure years, the estimates of numbers of workers exposed in various years can be made (Table 2). From the estimates of exposure by Barnes (1976) during those years, the total number of VC ppm-exposure years can be derived, which when divided by the number of workers and by an exposure duration of 35 years results in an average yearly exposure of 240 ppm/worker. The 35-year-exposure duration has been selected to be one-half of a person's lifetime, which would correspond to the 52-week duration in the rat studies by Maltoni *et al.* (1984). Although the rat studies had only a 4-hr/day exposure period, this duration would be within the range of the workers' exposure, which would probably occur only during part of an 8-hr work day. The dose-response of ASL in these studies has been summarized in Section 2.1.1, and the Maltoni *et al.* (1984) experiments are shown in Fig.

2. The ASL incidence that corresponds to 237 ppm in these studies is about 7%. As indicated previously, a 2.3% ASL incidence in the cohort of Canadian workers was found. However, the average age of the workers at the time of the study was about 54 or 55 years of age, and the lifetime ASL incidence among this group would be expected to be somewhat greater than 2.3%. Consequently, the risk estimates for humans, based upon the Maltoni *et al.* (1984) studies and using various exposure comparison methods, appear to be quite close to those found in human epidemiological studies, even without making adjustments for pharmacokinetic parameters.

Examination of the dose-response of proliferative changes was done by Maltoni *et al.* (1984); however, in most cases, the autopsies were done months after the cessation of VC administration. There was an increase in proliferative changes of sinusoidal cells noted especially at 50 ppm and above in Sprague-Dawley rats. Similar findings were not reported for Wistar rats. However, Sokal *et al.* (1980) examined Wistar rats after 10 months of VC administration and found no increase in proliferation of sinusoidal cells at 50 ppm, but did find an increased incidence of 38% at 500 ppm. This dose-response corresponds to the dose-response of angiosarcomas in Wistar rats reported by Maltoni *et al.* (1984), since there were none at 50 ppm and 10.7% at 500 ppm. Consequently, increased cell proliferation may play a role in the shape of the dose-response for VC-induced ASL. The dose-response study by Laib *et al.* (1985c) demonstrated a plateau at 500 ppm for the formation of altered hepatic foci in hepatocytes, thereby suggesting the operation of saturable metabolic activation of VC.

## 10. USE OF MECHANISTIC DATA IN RISK ASSESSMENT

VC is a DNA-reactive carcinogen that requires bioactivation to the reactive epoxide CEO (Fig. 4). CEO may spontaneously rearrange to an aldehyde, or VC may be metabolized and eliminated by pathways that compete with epoxide formation. Several DNA adducts have been identified in the target organ for ASL. However, the major adduct 7-OEdG does not appear to be mutagenic and, therefore, may not be responsible for tumor formation. The lesser amounts of cyclic etheno adducts, which are also present in the livers of VC-exposed animals, are capable of producing mutations and, therefore, would be involved in the mechanism of tumor formation (Fig. 3). Results shown in Table 1 indicate that VC genotoxicity studies were largely positive. *In vivo* DNA adducts were found in mouse liver after i.p. injection and in liver, lung and brain of rats after inhalation and drinking water exposure. Mutations produced by VC-DNA adducts, especially of genes involving cell cycle regulation, would be expected to produce initiated cells of the sinusoidal endothelium. There is also some evidence that VC induces proliferation of the sinusoidal endothelium, although this response could be the result of mutation rather than an epigenetic effect.

The dose-response data in rats for ASL (Maltoni *et al.*, 1984), the tumor attributed to VC exposure in humans, was used by the United States EPA (1985a) to calculate an upper bound $q_1^*$ of $2.95 \times 10^{-1}$ $(mg/kg/day)^{-1}$ for the cancer risk due to inhalation exposures. The data of Feron *et al.* (1981) from the lifetime feeding study in Wistar rats was utilized by the United States EPA for calculation of a $q_1^*$ for oral exposure of 2.3 $(mg/kg/day)^{-1}$ based on the liver and lung tumor incidence (EPA, 1985b). The development of cancer potency factors derived from animal experiments assume species-to-species extrapolation, which has been the basis for most of the regulation of environmentally occurring carcinogens in the United States. In the case of VC, human mortality studies demonstrating high incidences of ASL associated with inhalation exposure in the workplace provide a basis for comparison with the incidence of ASL from animal studies. Not only does VC exposure produce the same tumor type in both rodents and humans, but as documented in Section 9.4, the dose-related incidences are similar. Compared with the experiments by Maltoni *et al.* (1984) in Sprague-Dawley rats, other rodent species and strains have shown relatively small differences in dose-response relationships for ASL. Some studies of hamsters, rats and mice demonstrated up to a 5-fold, and possibly even a 10-fold, lesser tumorigenic response. On the other hand, studies by other investigators have found up to a 5-fold greater tumor incidence for rats or mice at exposure levels that approximate those of exposed workers in the past, i.e., up to 1000 ppm. These differences between spe-

**TABLE 2. Calculation of Average Exposure Air Level for VC Workers**

| Exposure Years (Yr) | 1943–1947 | 1948–1952 | 1953–1957 | 1958–1962 | 1963–1967 | 1968–1972 | Total |
|---|---|---|---|---|---|---|---|
| Number of workers (N) | 103 | 195 | 304 | 289 | 285 | 169 | — |
| ppm | 1000 | 1000 | 750 | 400 | 300 | 150 | — |
| N × ppm × Yr (in $10^4$) | 51.5 | 97.5 | 114 | 58 | 42.5 | 11.5 | 375 |

Calculation

$$\text{Workers' Average Adjusted Exposure Level} = \frac{3{,}750{,}000 \text{ workers} - \text{ppm} - \text{yr}}{451 \text{ workers} \times 35 \text{ yr}} = 237 \text{ ppm}.$$

*Data from Theriault and Allard (1981).*

cies are relatively small compared with those found for epigenetic carcinogens. The similar DNA-reactive cancer mechanism among species is modulated by variations in various metabolic parameters. Such variations may be accounted for through the use of pharmacologically based pharmacokinetic modeling techniques. Young animals have been found to be more susceptible to VC-induced tumors resulting from DNA adducts, presumably due to greater rates of cell proliferation that would result in higher mutation rates. Although several other target organs for VC-induced cancer have been found in rodents, these have not been demonstrated in humans. Among rodents, the larger differences in species and strain susceptibility for these tumor types may explain the lack of evidence for these other tumor types in humans.

Some of the mathematical models described in Section 4.4 have attempted to calculate dose-response expressions for low doses of VC. However, the dose-response similarities between animals and humans are all in the range of occupational exposures. In other words, although these comparisons show that human incidences of ASL are similar to rats at similar exposure levels, they do not validate the use of a particular mathematical model for low-dose extrapolations. The choice of a particular model for low-dose extrapolation for humans is still based upon mechanistic assumptions that predict a similar dose-response relationship as that found in experimental data on tumor formation. The linear-at-low-dose model used by the United States EPA assumes that at low doses, the dose-response for the supralinear curve is essentially the same as a linear relationship.

In summary, ASL induced by VC has provided an opportunity to examine the extrapolation of animal to human data using a DNA-reactive carcinogen for risk assessment purposes. It has been found that there is relatively good agreement between dose-specific tumor incidences for rodents and humans. Mechanistic information is consistent with a supralinear dose-response relationship, which is effectively linear at low doses. Consequently, although the linear-at-low-dose extrapolation method cannot be validated based upon human data, the well-documented human carcinogenicity of VC justifies the use of this dose-extrapolation method.

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