FINAL

Report on Carcinogens
Background Document for

Vinyl Fluoride

Meeting of the
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee

Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

US Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Summary Statement

Vinyl Fluoride

CASRN 75-02-5

Carcinogenicity

Vinyl fluoride (VF) is *reasonably anticipated to be a human carcinogen* based on evidence of tumor induction at multiple organ sites in rats and mice. Inhalation exposure of rats to vinyl fluoride resulted in increased incidences of hepatic hemangiosarcomas, hepatocellular adenomas or carcinomas, and Zymbal gland carcinomas; inhalation exposure of mice to VF resulted in increased incidences of hepatic hemangiosarcomas, bronchiolar-alveolar adenomas or adenocarcinomas, hepatocellular adenomas, mammary gland adenocarcinomas, and Harderian gland adenomas (Bogdanffy *et al.* 1995; IARC 1995). The tumor response to VF in laboratory animals is similar to the responses to vinyl chloride, a known human carcinogen (NTP 1998; IARC 1987), and to vinyl bromide, a probable human carcinogen (IARC 1986). A unique feature of vinyl chloride carcinogenicity is the induction of rare hepatic hemangiosarcomas in rats and mice and the causal association in epidemiological studies between vinyl chloride exposure and excess risk of hemangiosarcoma of the liver (NTP 1998). The fact that VF, vinyl chloride, and vinyl bromide induce rare hemangiosarcomas of the liver in experimental animals and induce the formation of similar DNA adducts suggests a possible common mechanism of carcinogenicity for these vinyl halides.

No studies on the potential carcinogenicity of VF in humans have been reported.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

VF is mutagenic in *Salmonella typhimurium* with metabolic activation (Dupont de Nemours and Co 1992a). In addition, VF induces gene mutations and chromosomal aberrations in Chinese hamster ovary cells (with metabolic activation), sex-linked recessive lethal mutations in *Drosophila melanogaster*, and micronuclei in bone marrow cells of female mice (IARC 1995).

The biotransformation pathway for VF is thought to be similar to that of vinyl chloride, that is, cytochrome P-450 mediated oxidation to the haloethylene oxide (fluoroethylene oxide), followed by rearrangement to the haloacetaldehyde (2-fluoroacetaldehyde), which is oxidized to fluoroacetic acid. Human liver microsomes metabolize VF at a rate similar to that of rat or mouse liver microsomes (Cantoreggi and Keller 1997).

VF metabolites form covalent DNA adducts. A dose-related increase in the formation of the promutagenic adduct N^2,3-ethenoguanine was detected in liver DNA of rats and mice exposed to VF by inhalation (Swenberg *et al.* 1995).

No data are available that would suggest that mechanisms thought to account for tumor induction by VF in experimental animals would not also operate in humans.

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1 Introduction

Vinyl fluoride (VF) was nominated for listing in the Report on Carcinogens (RoC) by the National Institute of Environmental Health Sciences (NIEHS) RoC Review Group (RG1) based on review of the 1995 International Agency for Research on Cancer (IARC) monograph (IARC 1995) which indicates that there is sufficient evidence in experimental animals for the carcinogenicity of VF and that it is *probably carcinogenic to humans* (Group 2A).

1.1 Chemical identification

VF is a member of the vinyl halide class. VF and the other vinyl halides are used ubiquitously in industry, especially in manufacture of plastics. They are easily polymerized and copolymerized with various materials, such as acrylonitrile, vinyl acetate, and styrene, to form pliable lightweight plastics or thermoplastic resins (HSDB 1995). VF (C_2H_3F, mol wt 46.044, CASRN 75-02-5) also is known as fluoroethene. It is a colorless gas at ambient temperature and pressure and is highly flammable. VF is used in the production of polyvinyl fluoride and other fluoropolymers and as an intermediate in organic synthesis. The structure of VF is illustrated in Figure 1-1.
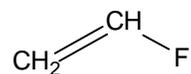
Figure 1-1. Structure of VF

1.2 Physical-chemical properties

VF's RTECS number is YZ7351000, and its UN number for shipping, is 1860. The physical and chemical properties of VF are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of VF

Property	Information	Reference
Molecular weight	46.04	CRC (1993)
Color	colorless	Lewis (1993)
Odor	faint ethereal odor	NIOSH (1994)
Physical state	flammable gas	CRC (1993)
Melting point (°C)	-160.5	CRC (1993)
Boiling point (°C) at 750 mm	-72	CRC (1993)
Specific gravity, at 21°C	0.636 (liquid)	Kirk-Othmer (1991)
Relative vapor density (air = 1)	1.58	HSDB (1995)
Solubility in: Water at 20°C Alcohol Ether Acetone	insoluble soluble soluble soluble	Sax (1979) Sax (1979) Sax (1979) Lide (1994)

1.3 Identification of metabolites

The major metabolites of VF are expected to be fluoroethylene oxide and fluoroacetaldehyde, based on indirect evidence of metabolism similar to that of vinyl chloride (VC) and vinyl bromide (VB); fluoroacetaldehyde can be further metabolized to fluoroacetic acid (Cantoreggi and Keller 1997). In a manner analogous to metabolism of VC (and VB), VF may initially be oxidized by microsomal monooxygenase(s) to fluoroethylene oxide (C_2H_3FO, mol wt 122.95), the structure of which is shown in Figure 1-2 (Bolt 1988; Ballering *et al.* 1996). The structure of fluoroacetaldehyde (C_2H_3FO, mol wt 62.044) is shown in Figure 1-3, and the structure of fluoroacetic acid ($C_2H_3FO_2$, mol wt 78.043, CASRN 144-49-0) is shown in Figure 1-4.
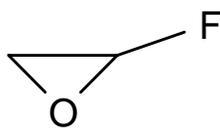
Figure 1-2. Structure of fluoroethylene oxide
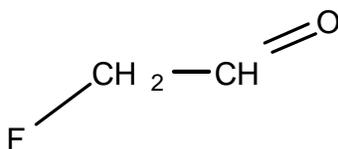
Figure 1-3. Structure of fluoroacetaldehyde
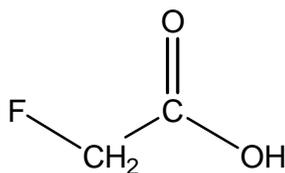
Figure 1-4. Structure of fluoroacetic acid

VF alkylates the prosthetic heme group of cytochrome P-450, and the alkylate has been identified as *N*-(2-oxoethyl)protoporphyrin IX. This observation suggests a reaction of heme with fluoroacetaldehyde (Ortiz de Montellano *et al*. 1982, cited in Cantoreggi and

Keller 1997). It also is likely that incorporation of fluoroacetate into the citric acid cycle disrupts energy metabolism and leads to increased production of mitochondrial acetylcoenzyme A and hence excretion of ketone bodies and free fluoride. Administration of VF has been shown to increase acetone exhalation by rats (Filser *et al*. 1982).

2 Human Exposure

2.1 Use

VF is used mainly in the production of polyvinyl fluoride and other fluoropolymers. Polymers of VF are resistant to weather and have great strength, chemical inertness, and low permeability to air and water. Polyvinyl fluoride is laminated with aluminum, galvanized steel, and cellulosic materials and is used as a protective surface for the exteriors of residential and commercial buildings. Polyvinyl fluoride laminated with various plastics has been used to cover walls, pipes, and electrical equipment and inside aircraft cabins (IARC 1995).

2.2 Production

E.I. du Pont de Nemours & Co. Inc. has been identified as the major manufacturer of VF (HSDB 1995). VF was first prepared in the early 1900s by a reaction of zinc with 1,1-difluoro-2-bromoethane . Modern preparation of VF involves a reaction of acetylene and hydrogen fluoride in the presence of a mercury- or aluminum-based catalyst (IARC 1995). The U.S. Environmental Protection Agency (EPA), through the Office of Pollution Prevention and Toxics, listed VF in the high production volume (HPV) chemical list in 1990. U.S. EPA estimated the annual production volume for 1990 at 3.83 to 6.96 x 10^6 lb (U.S.EPA 1990).

2.3 Analysis

Air samples collected in polytetrafluoroethylene bags can be sampled and analyzed for VF concentrations by gas chromatography (GC) (IARC 1995, HSDB 1995).

2.4 Environmental occurrence

VF is not known to occur naturally (IARC 1995). Industrial release of VF during its use and production may account for its presence in the environment (HSDB 1995).

2.5 Environmental fate

2.5.1 Air

VF exists in the vapor phase in the ambient atmosphere. VF reacts with photochemically produced hydroxyl radicals, with an estimated half-life of about 1.5 days. VF also reacts with atmospheric ozone, leading to its atmospheric degradation (estimated half-life of about 16 days) (HSDB 1995).

2.5.2 Water

The major fate process for VF in water is volatilization. The half-lives for volatilization from a model river (1 m deep) and a model pond (2 m deep) are 2 and 23.5 hours, respectively. VF has a bioconcentration factor (BCF) of 4.7, and therefore is not expected to bioconcentrate in aquatic organisms, as a BCF of greater than 1,000 is required for significant bioaccumulation. Adsorption to sediment is not considered to be an important fate process for VF in water (HSDB 1995).

2.5.3 Soil

Because VF remains a gas under normal conditions, it will evaporate to the atmosphere when released into soil. When dissolved in an aqueous solution, VF is very mobile in soil. There are insufficient data to predict whether biodegradation is an important fate process in soils that preclude evaporation (HSDB 1995).

2.6 Environmental exposure

Environmental exposure to VF occurs via inhalation, because VF released into the environment exists as a gas (IPCS 1993).

2.7 Occupational exposure

Occupational exposure to VF occurs through inhalation (HSDB 1995). Dermal and eye contact can occur among workers handling liquid VF; this type of contact will cause frostbite (IPCS 1993).

VF was sampled in a manufacturing and polymerization plant in the United States. In eight personal air samples taken at the manufacturing plant, concentrations of VF generally were < 2 ppm (3.76 mg/m^3). In one personal sample, however, the concentration of VF was 21 ppm (39.5 mg/m^3). VF concentrations in seven personal samples taken in the polymerization plant ranged from 1 to 4 ppm (1.88 to 7.52 mg/m^3). In four general working areas, the VF concentrations ranged from 1 to 5 ppm (1.88 to 9.4 mg/m^3) (IARC 1995).

The National Institute for Occupational Safety and Health (NIOSH) recommended an exposure limit of 1 ppm (1.88 mg/m^3) as an eight-hour time-weighted average (TWA), with a ceiling value of 5 ppm (9.4 mg/m^3) for short-term (15-minute) exposures (IARC 1995).

2.8 Biological indices of exposure

Simple fluoroalkenes, such as VF, are metabolized in humans, which leads to elevated urinary excretion of free fluoride (the mechanism is discussed in Section 1.3 and 6.1) (HSDB 1995).

2.9 Regulations

VF is regulated by U.S. EPA under the Clean Air Act to prevent accidental releases. It has a threshold reporting quantity of 1,000 lb. VF also is regulated by U.S. EPA under the Toxic Substances Control Act (TSCA), which requires health and safety studies to determine risk of injury to human health or the environment. U.S. EPA regulations are summarized in Table 2-1.

Table 2-1. U.S. EPA Regulations

U.S. EPA Regulations	
Regulatory action	**Effect of regulation and other comments**
40 CFR 68—PART 68—CHEMICAL ACCIDENT PREVENTION PROVISIONS. Promulgated: 59 FR 4493, 01/31/94. U.S. Codes: 42 U.S.C. 7412(r), 7601(a)(1), 7661-7661f.	This part lists regulated substances and thresholds, the petition process for adding or deleting substances, and specific requirements for preventing accidental releases. VF is regulated as a flammable substance with a threshold quantity of 1,000 lb for accidental release prevention.
40 CFR 716—PART 716—HEALTH AND SAFETY DATA REPORTING. Promulgated: 51 FR 32726, 09/15/86. U.S. Codes: 15 U.S.C. 2607(d). The effective date for VF is 10/04/82. The sunset date is 10/04/92.	The provisions of this part require the submission of lists and copies of health and safety studies on chemical substances and mixtures selected for priority consideration for testing rules under section 4(a) of TSCA and on other chemicals for which U.S. EPA requires health and safety information in fulfilling the purposes of TSCA.
40 CFR 799—PART 799—IDENTIFICATION OF SPECIFIC CHEMICAL SUBSTANCE AND MIXTURE TESTING. Promulgated: 49 FR 39817, 10/10/84. U.S. Codes: 15 U.S.C. 2603, 2611, and 2625.	This part identifies VF as a chemical for which data are to be developed to determine the risk of injury to human health or the environment presented by this chemical. All persons who manufacture VF, other than as an impurity, from July 22, 1987, to the end of the reimbursement period shall submit letters of intent to conduct testing or exemption applications, submit study plans, conduct tests in accordance with the TSCA Good Laboratory Practice Standards (40 CFR part 792), and submit data as specified in this section.

Source: These regulations in this table have been updated through the 1998 Code of Federal Regulations 40 CFR, July 1, 1996; 21 CFR, April 1, 1996; 29 CFR, July 1, 1996.

3   Human Cancer Studies

No studies on the relationship of VF exposure to human cancer were available.

4 Studies of Cancer in Experimental Animals

4.1 Carcinogenesis studies of VF in mice

An inhalation study in which Crl:CD-1(ICR)BR mice were exposed to VF at concentrations of 0, 200, 2,000, or 20,000 ppm (equivalent to 0, 376, 3,760, or 37,600 mg/m^3) six hours/day, five days/week for approximately 90 days was used to establish doses for a cancer bioassay in mice. In the range-finding study, results of hematological assessments (after 45 and 90 days) and histopathological assessments revealed no evidence of VF-associated toxicity. On day 93, groups of five mice per sex per exposure level received infusions of [^{3}H]thymidine followed by five additional days of VF exposure for measurement of cell proliferation in liver, kidney, lung, and nasal cavity tissues. Exposure to VF caused hepatocellular proliferation, as indicated by increased incorporation of [^{3}H]thymidine into hepatocytes. The magnitudes of the hepatocellular proliferative responses were similar at 2,000 and 20,000 ppm. Exposure to VF at 20,000 ppm caused increased [^{3}H]thymidine incorporation into nasal olfactory epithelium in male mice (Bogdanffy *et al*. 1990).

In the cancer bioassay (Bogdanffy *et al.* 1995), groups of 80 or 81 male and female Crl:CD-1(ICR)BR mice, approximately 47 days of age, were exposed to VF (purity > 99.94%) by inhalation at concentrations of 0, 25, 250, or 2,500 ppm (0, 47, 470, or 4,700 mg/m^3) for six hours/day, five days/week for up to 18 months. Reduced survival of exposed mice necessitated sacrifice of mice at various times between 375 and 550 days. Animals in the 250- and 2,500-ppm groups were sacrificed when survival of the groups reached approximately 25% (375 and 450 days for high-dose males and females and 412 and 459 days for mid-dose males and females, respectively). Surviving control and low-dose mice of both sexes were sacrificed at the scheduled study termination, 18 months. The approximate survival rates for the control and low-dose groups were 58% and 22%, respectively, for both sexes. All organs of control and high-dose-group animals were examined microscopically; only the nose, lungs, liver, kidneys, gross lesions, and target organs of animals in the other groups were subjected to microscopic evaluation.

The mice were evaluated after necropsy at intervals of 6, 12, 17 to 18, and 19 to 24 months. An early, significant increase in the incidence of lung tumors (bronchioalveolar adenoma) was observed in males in the 250- and 2,500-ppm groups and in females in the 2,500-ppm group sacrificed at 6 months. Statistical analyses of the overall tumor incidences were not conducted because of the varying durations of exposure to VF; however, observations included exposure-related increases in incidences of pulmonary, hepatic, and Harderian gland tumors in both sexes and mammary gland tumors in females. Although the incidence of hepatocellular adenomas did not exhibit dose-dependence, the increased tumor latency, increased multiplicity, and associated increase in putatively preneoplastic basophilic foci led to the conclusion that the tumors observed in males in the 25-ppm group were related to VF exposure.

Overall (aggregate) incidences of tumors in the lungs, liver, Harderian gland, and mammary gland in animals sacrificed or found dead between 7 and 18 months are summarized in Table 4-1.

Table 4-1. Incidences of primary tumors of the liver, lung, mammary gland and Harderian gland in mice exposed to VF gas for up to 18 months

Tumor type	Tumor incidence/number examined			
	Concentration (ppm)			
	0	25	250	2500
Males				
Lungs				
Primary lung tumors	11/81	45/80	52/80	56/81
Bronchioalveolar adenoma	11/81	43/80	48/80	49/81
Bronchioalveolar adenocarcinoma	1/81	1/80	4/80	4/81
Liver				
Hemangiosarcoma	1/81	16/80	42/80	42/81
Hepatocellular adenoma	7/81	15/80	5/80	3/81
Hepatocellular carcinoma	2/81	2/80	1/80	0/81
Harderian gland adenoma	3/80	13/79	12/80	31/80
Females				
Lungs				
Primary lung tumors	9/81	24/80	47/80	53/81
Bronchioalveolar adenoma	9/81	22/80	46/80	49/81
Bronchioalveolar adenocarcinoma	0/81	1/80	1/80	3/81
Liver				
Hemangiosarcoma	0/81	13/81	25/80	32/81
Hepatocellular adenoma	0/81	0/81	1/80	0/81
Mammary gland				
Adenoma	0/79	0/80	0/78	1/79
Adenocarcinoma	0/79	22/80	20/78	19/79
Adenoma, adenocarcinoma, fibroadenoma (combined)	0/77	22/76	20/78	20/77
Harderian gland adenoma	1/81	7/81	6/79	12/81

Source: Bogdanffy *et al.* (1995)

Based on the induction of liver hemangiosarcomas and bronchioalveolar adenomas in both sexes of mice, mammary gland tumors in females, and Harderian gland adenomas in males by VF in this study, IARC concluded that VF is carcinogenic to mice of both sexes (IARC 1995).

4.2 Carcinogenesis studies of VF in rats

An inhalation study in which Sprague-Dawley rats were exposed to VF at concentrations of 0, 200, 2,000, or 20,000 ppm (equivalent to 0, 376, 3,760, or 37,600 mg/m^3) six hours/day, five days/week for 90 days was used to establish doses for a cancer bioassay in rats. In the range-finding study, urinary excretion of fluoride was measured after 45 and 90 days of exposure to VF. Excretion of fluoride, indicative of VF metabolism,

appeared to reach a plateau at an exposure concentration of 2,000 ppm. Urinary excretion of fluoride was greater in both sexes after 90 days of exposure than after 45 days. Results of clinical chemical assessments, hematological assessments (after 45 or 90 days), and histopathological assessments revealed no evidence of VF-associated toxicity. Biochemical evidence of hepatocellular proliferation was observed in rats exposed to VF. On day 93, groups of five rats per sex per exposure level received infusions of [^{3}H]thymidine followed by five additional days of VF exposure for measurement of cell proliferation in liver, kidney, lung, and nasal cavity tissues. Exposure to VF at 2,000 or 20,000 ppm caused statistically significant increases in incorporation of [^{3}H]thymidine into hepatocytes in both sexes. The effect of VF on [^{3}H]thymidine incorporation appeared to plateau between exposure concentrations of 2,000 and 20,000 ppm (Bogdanffy *et al.* 1990).

In the cancer bioassay (Bogdanffy *et al.* 1995), groups of 95 male and 95 female Sprague-Dawley rats (Crl:CD$^®$BR), approximately 40 days of age, were exposed by inhalation to VF (purity > 99.94%) at concentrations of 0, 25, 250, or 2,500 ppm (0, 47, 470, or 4,700 mg/m^3) for six hours/day, five days/week for up to two years. Ten rats per group were sacrificed on test days 275 and 276 for interim examination. Because of high mortality, rats in the 250- and 2,500-ppm groups were sacrificed when the percentage of surviving animals in each group reached approximately 25% (586 days and 657 days for all surviving animals in the 2,500-and 250-ppm groups, respectively). All surviving control and low-dose animals were sacrificed at the scheduled termination of the study (two years). The survival rates for control and low-dose groups at the end of the study were 30% and 25% (males) and 25% and 15% (females), respectively.

The rats were evaluated after necropsy at intervals of 0 to 12, 13 to 18, and 19 to 24 months. An early appearance of VF-induced tumors was observed in the 12 month evaluation. Exposure to VF at 2,500 ppm for up to 12 months was associated with the development of Zymbal gland carcinoma in both sexes. The incidence of Zymbal gland carcinoma in high-dose males was statistically significant (Fisher's exact test). Although the incidence in females (4/18, 22%) was not significantly elevated, it was unusually high for one-year-old rats and was probably due to VF administration.

Exposure of the rats to VF for up to two years caused increased incidence of hemangiosarcomas of the liver, hepatocellular adenomas and carcinomas, and Zymbal gland carcinomas. VF-associated reduced survival and/or saturation of VF metabolism likely limited the induction of neoplasms in high-dose groups. The total tumor incidences in rats exposed to VF for up to two years are summarized in Table 4-2.

Table 4-2. Tumor incidences in rats exposed to VF gas for up to two years

Tumor type	Tumor incidence/number examined			
	Concentration (ppm)			
	0	25	250	2500
Males				
Liver				
Hemangiosarcoma	0/80	5/80	30/80	20/80
Hepatocellular adenoma	1/80	4/80	4/80	4/80
Hepatocellular carcinoma	4/80	6/80	6/80	3/80
Zymbal gland				
Carcinoma, sebaceous/squamous cell	0/80	2/51	3/49	11/80
Females				
Liver				
Hemangiosarcoma	0/80	8/80	19/80	15/80
Hepatocellular adenoma	0/80	4/80	9/80	5/80
Hepatocellular carcinoma	0/80	0/80	0/80	3/80
Zymbal gland				
Carcinoma, sebaceous/squamous cell	0/80	0/50	1/49	12/80

Source: Bogdanffy *et al.* (1995)

Based on the increased incidence of liver hemangiosarcomas and Zymbal gland tumors in both sexes of rats and an increased incidence of hepatocellular adenomas and carcinomas in female rats resulting from exposure to VF in this study, IARC concluded that VF is carcinogenic to rats of both sexes (IARC 1995).

4.3 Additional studies of VF carcinogenicity in animals

Similarly to vinyl chloride, VF induces the formation of preneoplastic ATPase-deficient foci in newborn Wistar rats (Bolt *et al.* 1981).

4.4 Summary

VF is closely related to VC (*known to be a human carcinogen*) and VB (nominated as *reasonably anticipated to be a human carcinogen*). The strong structural similarity among the vinyl halides predicts similar biological effects. That prediction is verified by the similarity of tumor response observed in carcinogenesis experiments conducted with these agents.

Inhalation of VF increases the incidences of hemangiosarcomas in both sexes of mice and rats, tumors of the lung and Harderian gland in male and female mice, and mammary gland tumors in female mice. VF also increases the incidence of Zymbal gland carcinomas and hepatocellular neoplasms in rats of both sexes. The interpretative value of these data is strengthened by the facts that the tumor responses are dose-related and that the spectrum of VF-induced neoplasms closely resembles that reported for VC (Maltoni 1974, as cited in IARC 1979) and VB (IARC 1986). These findings, particularly

the induction of rare hemangiosarcomas of the liver by these three vinyl halides, support the conclusion that VF *is reasonably anticipated to be a human carcinogen*.

5 Genotoxicity

5.1   Prokaryotic systems

5.1.1   Induction of mutation in Salmonella typhimurium

VF was tested for the ability to induce *his* gene mutations in *Salmonella typhimurium.* In a series of five assays, VF was tested in various *S. typhimurium* tester strains at exposure concentrations of 0 to 52% with and without exogenous metabolic activation. VF induced statistically significant increases ($P < 0.01$) in mutation frequency (1.4 to 2.1 times negative control mutation frequencies) in three of the five assays in strain TA1535 with metabolic activation. VF did not induce reverse mutations in strains TA98, TA100, or TA1537 in the presence of metabolic activation, nor in any strain in the absence of metabolic activation (Dupont de Nemours and Co. 1992a).

5.2   Eukaryotic systems

5.2.1   Mutagenicity in Drosophila melanogaster

5.2.1.1   Sex-linked recessive lethal assay

VF caused excessive sex-linked recessive lethal mutations in *Drosophila melanogaster*. Males (N = 198) were exposed to VF at air concentrations of 47.6% for 24 hours and then mated with untreated females. The progeny exhibited a significant increase ($P < 0.01$) in the frequency of sex-linked recessive lethal mutations compared with controls. VF exposure resulted in the production of 100 lethal mutations (2.4%) in the F_2 progeny, compared with 5 lethal mutations (0.08%) among F_2 progeny of flies not exposed to VF. Survivability among the VF-exposed males was 86.4%. Fertility was unaffected in the surviving males (CMA 1988).

In its review of an abstract of a study conducted by Bentley *et al*. (1992, cited in IARC 1995), IARC stated that VF induced sex-linked recessive lethal mutations in *Drosophila melanogaster* at exposure concentrations of 19.1% or 38.8% for six hours.

5.3   Mammalian systems

5.3.1   In vitro *assays*

5.3.1.1   hprt *locus forward mutation test*

VF induced *hprt* forward mutations in Chinese hamster ovary (CHO) cells in the presence of metabolic activation by S9 liver homogenate from rats. CHO cells in uncapped tissue culture flasks were exposed to VF gas concentrations of 0 to 100% in the ambient environment of glass chambers for five hours (with S9 metabolic activation) or for 18 to 19 hours (without S9 metabolic activation). VF was not mutagenic without metabolic activation. However, VF was mutagenic at all concentrations (from 20% to 100%), causing statistically significant dose-related increases in mutant frequencies. Cell survival was 62% in a preliminary non-activated cytotoxicity test at the highest VF concentration tested (100%) (Dupont de Nemours and Co. 1992b).

In its review of an abstract of a study conducted by Bentley *et al*. (1992, cited in IARC 1995), IARC concluded that VF induced gene mutations in Chinese hamster ovary

(CHO) cells with metabolic activation at exposure concentrations of 19.1% or 38.8% for six hours.

5.3.1.2 Chromosomal aberrations

CHO cells were incubated with VF at target concentrations of 0, 10%, 40%, 70%, or 100% for five hours without rat S9 metabolic activation or with VF at target concentrations of 0, 10%, 25%, 50%, or 75% VF for two hours with rat S9 metabolic activation. Statistically significant increases in chromosomal aberrations (CA) occurred at VF levels of 10%, 25%, and 50% with metabolic activation. Significant increases in CA were observed only at the highest concentration without S9 metabolic activation. A second trial used concentrations of VF at 0, 12.3%, 35.4%, 63.3%, or 91.3% with metabolically activated CHO cells. Statistically significant increases in CA frequency were induced at the 35.4% and 63.3% VF concentrations (Dupont de Nemours and Co. 1986).

In its review of an abstract of a study conducted by Bentley *et al*. (1992, cited in IARC 1995), IARC concluded that VF induced CA in CHO cells with metabolic activation at exposure concentrations of 19.1% or 38.8% for six hours.

5.3.2 In vivo *assays*

5.3.2.1 Chromosomal aberrations

Dominant lethal test (rat)

Groups of 40 male Crl:CD$^®$BR rats were exposed by inhalation to VF at concentrations of 0, 200, 2,000, or 20,000 ppm (0, 376, 3,760, or 37,600 mg/m^3) six hours/day for five days and then mated with unexposed females. No statistically significant treatment-related differences in pre- or post-implantation losses were found in females mated with males exposed to VF at any concentration. No males died in the study (Dupont de Nemours and Co. 1988).

Mammalian bone marrow cytogenetic test (metaphase analysis)

VF gave equivocal results in males and positive results in females for induction of micronuclei in bone marrow polychromatic erythrocytes (PCEs) of 43-day-old CD-1 mice. The mice were exposed by inhalation to mean VF concentrations of 0, 50,100, 191,000, or 388,000 ppm (0, 94,348, 359,689, or 730,678 mg/m^3) for 24, 48, or 72 hours. At the 24-hour sampling time, no single test resulted in a significant increase of micronucleated PCEs above control values. However in female mice, a statistically significant trend was observed of concentration-related increases in the proportion of micronucleated PCEs. No statistically significant increases in micronucleated PCEs or concentration-related trends were observed at the 48- and 72-hour sampling times. Increases in the frequency of micronucleated PCEs in males in the 50,100- and 388,000-ppm exposure groups at the 24-, 48-, and 72-hour sampling times were not statistically significant. The frequency of micronucleated PCEs in female mice showed a significant concentration-related increase at the 191,000- and 388,000- ppm exposure levels, confirmed by scoring of additional PCEs. No significant depression of the ratio of PCEs to normochromatic erythrocytes was seen in the VF-exposed mice (Dupont de Nemours and Co. 1987).

In its review of an abstract of a study conducted by Bentley *et al*. (1992, cited in IARC 1995), IARC concluded that VF induced micronuclei in bone marrow cells of female mice at exposure concentrations of 19.1% or 38.8% for six hours. VF did not induce unscheduled DNA synthesis in pachytene spermatocytes or single strand breaks or cross-links in testicular DNA of male mice.

DNA single strand breaks

Testicular-cell DNA from groups of male Sprague-Dawley rats tested by nose-only inhalation exposure to VF at 0 or (2%) 20,000 ppm (37,600 mg/m^3) for six hours/day for one, two, or five consecutive days showed no significantly increased frequencies of single strand breaks or cross-links (Dupont de Nemours and Co. 1991).

In its review of an abstract of a study conducted by Bentley *et al*. (1992, cited in IARC 1995), IARC concluded that VF did not induce unscheduled DNA synthesis in pachytene spermatocytes, nor single strand breaks or cross-links in testicular DNA of male mice.

5.4   Summary

VF is mutagenic and clastogenic. VF, VC, and VB are genotoxic chemicals requiring metabolic activation to similar DNA-reactive intermediates. VF is a base-pair substitution mutagen in *S. typhimurium* strain TA1535. VF induces excessive sex-linked recessive lethal mutations in *D. melanogaster,* induces *hprt* forward mutations in CHO cells with rat S9 metabolic activation, and is clastogenic in CHO cells *in vitro*. In an assay for induction of micronucleated PCEs in female mice *in vivo*, VF gave equivocal results, and it failed to induce single strand breaks or cross-links in DNA when tested *in vivo* in rats.

6 Other Relevant Data

6.1 Absorption, metabolism, and excretion of VF

VF is readily absorbed after inhalation (Filser and Bolt 1980, 1981, cited in IARC 1995).

The metabolic process appears to be saturable and dose-dependent. An early estimate indicated that metabolic saturation occurred at inhalation concentrations in excess of 75 ppm (140 mg/m^3) in rats (Filser and Bolt 1980, cited in IARC 1995). Pharmacokinetic data imply that the rate of biotransformation of VF is about one-fifth that of VC (Bolt *et al.* 1981). VF is metabolized faster than VB, but slower than VC (Bolt *et al.* 1982).

Available evidence suggests that VF is metabolized via the same pathway as VC and VB. VC and VB are metabolized to haloacetaldehydes. Based upon VC metabolism, it also is likely that fluoroacetaldehyde is metabolized to fluoroacetic acid, a potent inhibitor of the Krebs cycle. Incorporation of fluoroacetate into the citric acid cycle disrupts energy metabolism and leads to increased production of mitochondrial acetyl coenzyme A and, hence, excretion of ketone bodies. Administration of VF has been shown to increase acetone exhalation by rats (Filser *et al*. 1982). The results of experiments reported by Cantoreggi and Keller (1997) are consistent with hepatic oxidative metabolism of VF. In the study, mice and rats were exposed to VF in a closed chamber gas uptake system. VF uptake was monitored from the chamber. Mice were observed to have a higher metabolic rate for VF than rats (6.5 vs. 2.2 μmol/hr per kilogram body weight). Inhibition of the cytochrome P-450 enzyme CYP 2E1 by administration of 4-methylpyrazole completely impaired VF metabolism in both species. CYP 2E1 induction in rats (by administration of ethanol) increased metabolic capacity.

The proposed metabolic pathway for VF, similar to the pathways for its vinyl halide analogs VC and VB, is illustrated in Figure 6-1 (Bolt 1988; Ballering *et al*. 1996).
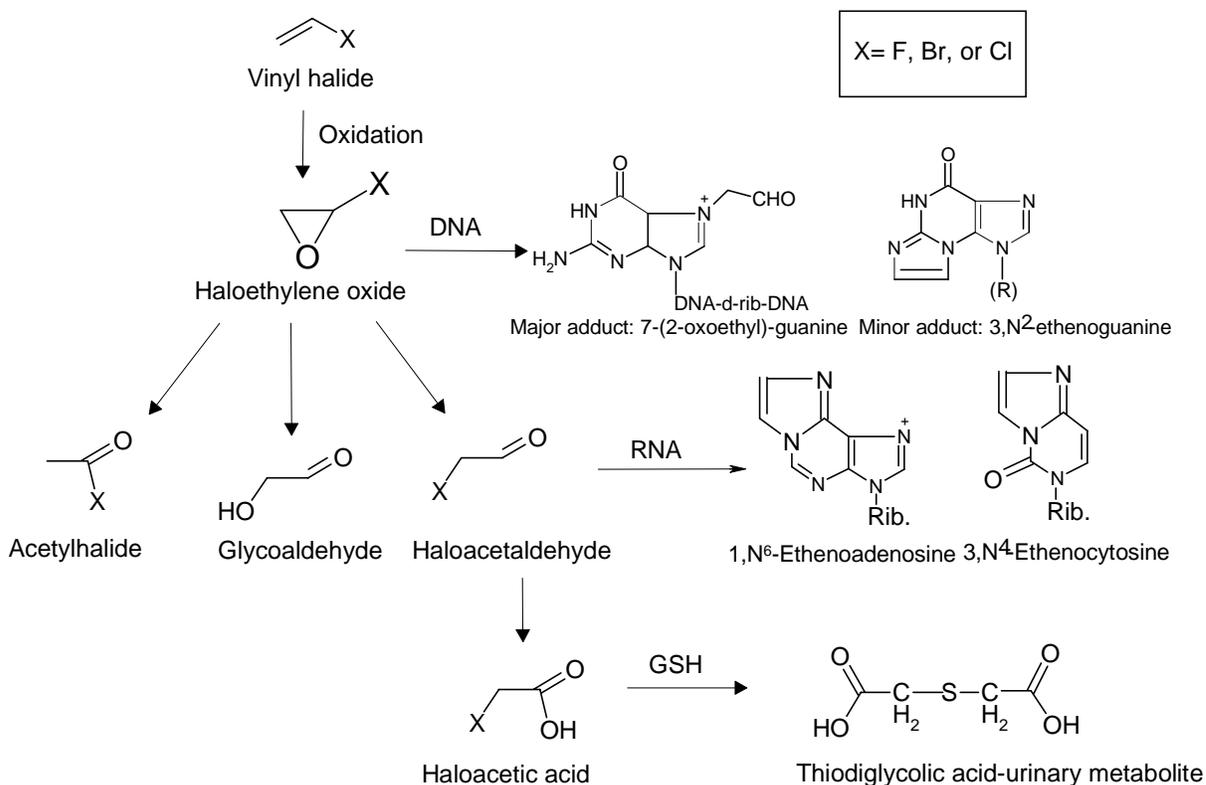
Figure 6-1. Proposed metabolic pathway of VF

Source: Bolt 1986, 1988 and Ballering *et al*. 1996.

VF toxicity is mediated via epoxide formation. Oxidative metabolism of inhaled VF in the presence of Aroclor 1254 (a hepatic cytochrome P-450 inducer) resulted in enhanced toxicity (Conolly *et al.* 1978, cited in Cantoreggi and Keller 1997). In addition, administraton of trichloropropylene oxide (an inhibitor of epoxide hydrolase) also increased VF toxicity (Conolly and Jaeger 1977, cited in Cantoreggi and Keller 1997).

Cantoreggi and Keller (1997) demonstrated that microsomes from mice metabolized VF more rapidly than those from rats (V_{max} = 3.5 and 1.1 nmol/hr per milligram protein, respectively) when rat or mouse liver hepatic microsomes were exposed to VF gas in closed chambers in the presence of an NADPH-regenerating system. Disappearance of VF gas from the incubation headspace was monitored,which was consistent with the results from the *in vivo* experiment. Microsomes from human livers were found to metabolize VF at a rate similar to that for rat or mouse liver microsomes. Among ten human livers tested, V_{max} ranged from 0.57 to 3.3 nmol/hr per milligram protein. V_{max} values were directly related to microsomal content of CYP 2E1.

Urinary excretion of fluoride was determined in rats exposed to 0, 200, 2,000 or 20,000 ppm VF (0, 376, 3,760, or 37,600 mg/m^3) for six hours/day, five days/week, after 45 and 90 days of exposure. Urinary fluoride concentrations were dose-related at both time

periods, but were nonlinear, with a plateau appearing at approximately 2,000 ppm (for both sexes). The appearance of a plateau was taken as evidence of metabolic saturation. It also was noted that urinary fluoride concentrations were consistently higher, after 90 days of exposure to VF than after 45 days. Increased excretion of fluoride after 90 days of VF exposure may reflect hepatic enzyme induction or saturation of deposition sites (Bogdanffy *et al.* 1990).

6.2   Alkylating properties and DNA adduct formation

Although the metabolism of VF is largely unknown, it has been demonstrated that the action of monooxygenase on VF produces fluoroethylene (fluorooxirane, a reactive epoxide intermediate) (see Figure 6-1) (Bolt *et al.* 1982; Cantoreggi and Keller 1997). This is analogous to the mechanism for the metabolic production of chloroethylene (chlorooxirane) from the VF analog VC (Bolt *et al.* 1982).

Comparative molecular orbital studies show that the three-membered ring of fluorooxirane has more tension and is less stable than that of chlorooxirane, although it is less reactive to tissue macromolecules than chlorooxirane. The order of reactivity of vinyl halide-derived haloethylene oxides (halooxiranes) with tissue macromolecules has been proposed to be VC > VF > VB (Bolt *et al.* 1982).

VF alkylates the prosthetic heme group of cytochrome P-450, and the alkylate has been identified as *N*-(2-oxoethyl)protoporphyrin IX. This observation suggests a reaction of heme with fluoroacetaldehyde (Ortiz de Montellano *et al.* 1982, cited in Cantoreggi and Keller 1997).

The VF analog VC induces the formation of DNA adducts 7-(2′-oxoethyl)guanine, N^2,3-ethenoguanine, 3,N^4-ethenocytosine, and 1,N^6-ethenoadenine (Bartsch *et al.* 1994, Guengerich 1994, both cited in La and Swenberg 1996). Similarly, inhaled VF in both rats and mice results in the formation of N^2,3-ethenoguanine in liver DNA. The formation of this adduct reaches a plateau at exposure concentrations of approximately 250 ppm in both species, indicating metabolic saturation at this exposure (Swenberg *et al.* 1995). The transition mutation in the K-*ras* gene, activated by a GC to AT transition at the second base of codon 13 in five of six VC-induced tumors in humans, is consistent with the known miscoding properties of N^2,3-ethenoguanine and 3,N^4-ethenocytosine (La and Swenberg 1996). Accordingly, VF-DNA adducts are analogous to those identified as promutagenic adducts resulting from exposure to VC (Bolt *et al.* 1982). The adduct predominantly formed is 7-(2′-oxoethyl)guanine (over 98%). However, the mutagenic effects of VC may be driven by the formation of the minor DNA adducts N^2,3-ethenoguanine, 3,N^4-ethenocytosine, and 1,N^6-ethenoadenine. These adducts efficiently induce GC to AT, GC to TA, and AT to GC transitions, respectively (Barbin *et al.* 1981, Spengler and Singer 1981, Singer *et al.* 1984, 1987, 1991, Cheng *et al.* 1991, Mroczkowska and Kusmierek 1991, and Basu *et al.* 1993 all cited in La and Swenberg 1996). Although the major DNA adduct, 7-(2′-oxoethyl)guanine, is not involved in base pairing, its removal may result in an apurinic site that can lead to a mutation (La and Swenberg 1996).

6.3   Effects of VF on cell proliferation

Male and female rats and female mice exposed to VF by inhalation exhibited small but significantly increased rates of [^{3}H]thymidine incorporation into hepatocytes. This observation indicates a proliferative response to the chemical (Bogdanffy *et al*. 1990).

In this experiment, animals were exposed to VF in air at concentrations of 0, 200, 2,000 or 20,000 ppm (0, 376.6, 3,766.4, or 37,664 mg/m^3) for six hours/day, five days/week for 90 days. On day 93, the animals were implanted with osmotic mini-pumps containing [^{3}H]thymidine and exposed for five more days. Tritium incorporation into trachea, lungs, nose, liver, and kidneys was measured. High-dose female mice exhibited a significant increase in mean labeling index of hepatocytes whereas high-dose male mice had a significantly elevated labeling index of the olfactory nasal epithelium. Although the mean hepatocyte-labeling index in high-dose male mice was elevated relative to controls, the range of measurements was highly variable, and the difference was not statistically significant. Exposure of male and female rats to VF at 2,000 or 20,000 ppm caused significant increases in hepatocyte labeling. The magnitude of response at 20,000 ppm was similar to that observed at 2,000 ppm.

During assessment of VF carcinogenicity, Bogdanffy *et al.* (1995) attempted to confirm their earlier demonstration of the cellular proliferative effect of VF in mice and rats. In these experiments, a pulse label with 5-bromodeoxyuridine (BrdU) revealed no evidence of cellular proliferation. The researchers concluded that the proliferative response reported earlier might have been too subtle for detection with the less sensitive BrdU pulse labeling technique.

6.4   Structure-activity relationship

The metabolism of VF probably proceeds through the same pathway as that of VC (a known human carcinogen) and the probable human carcinogen VB. VF is less rapidly metabolized than either VC or VB (Bolt *et al*. 1982). The metabolic process appears to be saturable, as observed for VC. Pharmacokinetic data imply that the rate of biotransformation of VF in rats is about one fifth that of VC (Bolt *et al.* 1981).

The spectrum of neoplasms produced by the three vinyl halides in mice and rats is strikingly similar. Table 6-1 summarizes the information available on carcinogenesis, mutagenesis, and metabolism of the three vinyl halides.

Table 6-1. Summary of carcinogenesis, mutagenesis, and pharmacokinetics for VF, VB, and VC

Study	VF	VB	VC
Animal carcinogenicity			
Types of tumors formed			
Hepatic hemangiosarcoma	rats, mice[a]	rats[b]	rats, mice[c]
Extrahepatic hemangiosarcoma	—	—	rats, mice[d]
Hepatocellular carcinoma	rats[e]	rats[b]	—
Hepatocellular adenoma	rats, mice[e]	—	rats[d]
Zymbal gland carcinoma	—	rats[b]	rats[d]
Bronchioalveolar adenoma and adenocarcinoma	rats, mice[e]	—	—
Harderian gland adenocarcinomas	mice[e]	—	—
Mammary gland adenocarcinomas	mice[e]	—	mice[d]
Oncogene activation			
Oncogenicity (formation of ATPase-deficient hepatic foci in newborn rats	positive[f]	positive[g]	positive[h]
Mutagenicity			
Prokaryotic cells *in vitro*	positive[i]	positive[j]	positive[d]
D. melanogaster in vivo	positive[k]	positive[l]	positive[d]
Mammalian cells *in vitro*	positive[m]	na	positive[d]
Mammalian bone marrow test *in vivo*	positive[n]	na	positive[o]
Pharmacokinetics			
Metabolism			
Metabolism by rat liver microsomes	V_{max} = 1.1 nmol/hr-mg protein[p]	na	V_{max} = 280.4 nmol/hr-mg protein[q]
Metabolism by mouse liver microsomes	V_{max} = 3.5 nmol/hr-mg protein[p]	na	na
Metabolism by human liver microsomes	V_{max} = 0.5-3.3 nmol/hr-mg protein[p]	na	na
Detection of free ions in urine	positive[r]	positive[r]	positive[r]
Detection of acetone in exhaled air in rats	positive[s]	positive[s]	positive[s]
Distribution (air partition coefficients)[p]			
Blood (rats)	0.75 ± 0.09	4.05 ± 0.16	1.68 ± 0.18
Liver (rats)	0.83 ± 0.58	3.33 ± 0.38	1.60 ± 0.17
Muscle (rats)	0.54 ± 0.28	2.26 ± 0.13	2.10 ± 0.45
Fat	1.82 ± 0.15	49.2 ± 1.3	20.0 ± 0.7

Study	VF	VB	VC
Alkylating properties			
Reactive intermediates and formation of DNA adducts	7-(2′-oxoethyl)guanine, N^2, 3-ethenoguanine[t]	7-(2′-oxoethyl)guanine; N^2, 3-ethenoguanine[u]	7-(2′-oxoethyl)guanine; N^2, 3-ethenoguanine[v]; 3, N^4-ethenocytosine; 1, N^6-ethenoadenine[u]

—, Not reported; na, Not available

[a]Bogdanffy *et al.* 1995, IARC 1995
[b]IARC 1986
[c]IARC 1979, NTP 1998
[d]IARC 1979
[e]Bogdanffy *et al.* 1995
[f]Bolt *et al.* 1981
[g]Bolt *et al.* 1979
[h]Laib *et al.* 1985
[i]Dupont de Nemours and Co 1992a
[j]IARC 1986, Roldan-Arjona *et al.* 1991
[k]CMA 1988, IARC 1995

[l]Vogel and Nivard 1993, Ballering *et al.* 1996.
[m]Dupont de Nemours and Co 1992b, IARC 1995
[n]Dupont de Nemours and Co 1987, IARC 1995
[o]Richardson *et al.* 1983
[p]Cantonreggi and Keller 1997
[q]el Ghisassi *et al.* 1998
[r]Dilley *et al.* 1974
[s]Filser *et al.* 1982
[t]Swenberg *et al.* 1995
[u]Bolt *et al.* 1988
[v]Swenberg *et al.* 1992

6.5 Summary

The information available on VF metabolism, DNA reactivity of its metabolites, and the spectrum of tumor induction in rats and mice suggests that VF is a genotoxic carcinogen. The metabolism of VF probably proceeds through the same pathway as that of the known human carcinogen VC and the probable human carcinogen VB. Metabolism of VC and VB results in the production of reactive metabolites that bind to proteins and nucleic acids. All three vinyl halide congeners are active in genotoxicity assays. Inhalation exposure to each congener produces unequivocal carcinogenicity in rats and mice of both sexes. The three vinyl halides produce a similar array of neoplasms in mice and rats.

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