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LINEAR HALOALKANES AND HALOALKENES

CARCINOGENICITY AND STRUCTURE-ACTIVITY RELATIONSHIPS. OTHER BIOLOGICAL PROPERTIES. METABOLISM. ENVIRONMENTAL SIGNIFICANCE.

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5.2.2.1 Halogenated Linear Alkanes and Alkenes.

5.2.2.1.1 Introduction.

Halogenated hydrocarbons, also known as halocarbons or organohalogen compounds, represent one of the most important classes of synthetic chemicals. First synthesized in the 1820's, a great number of halogenated hydrocarbons are now known. Many of these compounds are produced in enormous quantities and occupy an indispensable role in modern technology. This section focuses on the halogenated aliphatic hydrocarbons, which include the saturated haloalkanes (alkyl halides) and the unsaturated haloalkenes (alkenyl halides).

Haloalkanes and haloalkenes are widely used in chemical manufacturing; as industrial degreasers; in automotive, aircraft, textile, food processing, and various other industries; in anesthesiology; in agriculture; and in innumerable other industrial and commercial applications. Table I summarizes the production data, the major uses and applications of 14 haloalkanes and haloalkenes that have annual production in excess of one million pounds in the United States alone and are known or suspected carcinogens. Other haloalkanes and haloalkenes with limited information on carcinogenicity and with estimated production volumes exceeding one million pounds include (in decreasing order of volume): ethyl chloride, dichlorodifluoromethane (Freon 12), methyl chloride, trichlorofluoromethane (Freon 11), chlorodifluoromethane (Freon 22), 1,2-dichloropropane, trichlorotrifluoroethane, methyl bromide, 1,2-difluoro-

1,1,2,2-tetrachloroethane (Freon 112), and tetrafluoroethylene (40). In addition, halothane (1,1,1-trifluoro-2-bromo-2-chloroethane) is a well known anesthetic agent. A survey of the industrial, commercial, and medical applications of a variety of haloalkenes has been published (41).

As may be expected from the high production volumes and extensive uses, a large number of workers are occupationally exposed to these compounds. The U.S. National Institute for Occupational Safety and Health has published a series of criteria documents on occupational exposure to various haloalkanes and haloalkenes (1, 4, 8, 11, 12, 17, 20, 21, 31, 34, 37, 42-45). Human exposure to halogenated compounds is not confined to occupational setting; emission from industrial production and uses result in the release of large amounts into the environment. Secondary sources such as water chlorination, burning of gasoline, plastics, tobacco, and plant materials also contribute to environmental contamination. Halogenated hydrocarbons have been detected in ambient and indoor atmospheres, in drinking water, in foodstuffs, and in various consumer products (see Section 5.2.2.1.5.2).

The potential health hazard of human exposure to haloalkanes and haloalkenes has been the subject of intensive investigations in recent years. Historically, prior to adequate testing, many halogenated compounds were erroneously assumed to be safe. Chloroform was first introduced as an "ideal" anesthetic agent by Fluorens and Simpson in 1847 and was used for this purpose until the mid 20th century. In the 1920's, carbon tetrachloride was hailed as the drug of choice for the treatment of hookworm. Lambert (46) reported remarkable success in treating 50,000 hookworm patients on Fiji with 3-4 ml of carbon tetrachloride and ascribed several ensuing deaths to impurities. The carcinogenic potential of haloalkanes was first discovered in the 1940's. In 1941, Edwards (47) first reported the induction of liver tumors in the mouse

Table I
Production Volumes, Major Uses, and Recent Health Effects Reviews of Some Important Haloalkanes and Haloalkenes[a]

Compound, Synonyms, CAS No.[b]	Production Volume[c]	Major Uses and Applications[e]	Reviews
Dichloromethane*, methylene chloride, 75-09-2	497 (1975)	Paint remover; degreasing solvent; aerosol propellant; solvent applications in food, pharmaceutical, synthetic fiber and photography industries	NIOSH (1); IARC (2); USEPA (3)
Chloroform*, trichloromethane, 67-66-3	262 (1975)	Production of chlorodifluoromethane (used as refrigreant, propellant); solvent applications in pharmaceutical (e.g., antibotics), dyes, plastics, dry cleaning industries; component of toothpaste, cough medicine, liniments, salves	NIOSH (4); Winslow & Gerstner (5); Reuber (6); IARC (2); USEPA (3)
Carbon tetrachloride*, tetrachloromethane, 56-23-5	907 (1975)	Production of difluorodichloromethane and trichloromethane (used as refrigerant, propellant); solvent; grain fumigant; pesticide; fuel additive	NIOSH (8), IARC (2); Louria and Bogden (9); USEPA (10)
1,2-Dichloroethane*, ethylene dichloride, 107-06-02	7977 (1975)	Production of vinyl chloride and other chlorinated ethenes; lead scavenger in antiknock fuel additive; fumigants	NIOSH (11, 12); Drury & Hammonds (13); IARC (2); Ames et al. (14); Rannug (15)
1,2-Dibromoethane*, ethylene dibromide, 106-93-4	275 (1975)	Lead scavenger in antiknock fuel additive; fumigant; production of vinyl bromide	Kover (16); NIOSH (17); IARC (2, 18); Rannug (15)

Table I (continued)

Compound, Synonyms, CAS No.[b]	Production Volume[c]	Major Uses and Applications[e]	Reviews
1,1,1-Trichloroethane*, methyl chloroform, 71-55-6	459 (1976)	Cold cleaning and vapor degreasing of metals and other materials; production of vinylidene chloride; aerosol propellant; lubricant carrier; coolant in metal cutting oil	Aviado et al. (19); IARC (2)
1,1,2,2-Tetrachloroethane*, acetylene tetrachloride, sym-tetrachloroethane, 79-34-5	37 (1974)	Production of trichloroethylene; industrial solvent	NIOSH (20); IARC (2)
1,2-Dibromo-3-chloropropane, 96-12-8	25 (1975)	Soil fumigant (for protection of field crops, vegetables, fruits, nuts, ornamental plants)	NIOSH (21); IARC (2, 18)
Vinyl chloride*, chloroethene 75-01-4	5736 (1976)	Production of PVC resins (used in plastic pipes, floor tiles, consumer goods, electrical appliances); limited use as aerosol propellant (now banned)	IARC (22, 23); USEPA (24, 25); Bins (26); Wagoner et al. (27); Hopkins (28, 29)
Vinylidene chloride*, 1,1-dichloroethylene, 75-35-4	170 (1975)	Production of copolymers (used mainly for food packaging films and coatings) and modacrylic fibers	IARC (23); USEPA (30)
Trichloroethylene, 79-01-6	303 (1976)	Vapor degreasing of fabricated metal parts; solvent in textile industry, for adhesives and lubricants, and in commercial cleaning solutions; anesthetic agent	NIOSH (31); IARC (2, 32); Aviado et al. (19); USEPA (33)

Table I (continued)

Compound, Synonyms, CAS No.[b]	Production Volume[c]	Major Uses and Applications[e]	Reviews
Tetrachloroethylene*, perchloroethylene, 127-18-4	657 (1976)	Drycleaning and textile industries; industrial metal cleaning; production of fluorocarbons; anthelminthic agent	NIOSH (34); Utzinger & Schlatter (35); IARC (2); USEPA (36)
2-Chloro-1,3-butadiene*, chloroprene, 126-99-8	349 (1975)	Production of polychloroprene (neoprene) elastomers (used in automobile rubber goods, wire, cable, construction and adhesive applications)	NIOSH (37); Haley (38); IARC (23)
Hexachloro-1,3-butadiene*, perchlorobutadiene, 87-68-3	7-14[d] (1972)	Recovery of "snift" (chlorine-containing) gas in chlorine plants; production of lubricants, rubber compounds; gyroscope fluid	IARC (2); USEPA (39)

[a]Major sources of information: IARC Monographs Volumes 19 and 20 (1979); SRI International "A Study of Industrial Data on Candidate Chemicals for Testing," EPA Publ. 560/5-77-006, U.S. Environmental Protection Agency, Washington, D.C., 1977; L. Fishbein [Sci. Total Environ., 11, 111 and 163 (1979)]; NAS, "Nonfluorinated Halomethanes in the Environment," National Academy of Sciences, Washington, D.C., 1978.

[b]Only the most commonly used synonyms are listed. Names with an asterisk are those used in this review. CAS numbers are the Chemical Abstract Services Registry Numbers.

[c]Production volumes in the United States in millions of pounds in the year indicated in parenthesis.

[d]Produced as byproducts or waste products of tetrachloroethylene, trichloroethylene, carbon tetrachloride, and other chlorinated compounds.

[e]Major uses in United States in decreasing order of the volumes of usages.

by carbon tetrachloride. Shortly afterwards, Eschenbrenner and Miller (48) found that chloroform was also hepatocarcinogenic in the mouse. However, very few findings had greater impact on occupational health than the discovery of the carcinogenicity of vinyl chloride. Viola (49) reported first at the 10th International Cancer Congress in 1970 the carcinogenicity of the compound in rats exposed by inhalation. An extensive series of experiments was subsequently undertaken by Maltoni and associates, who not only confirmed the carcinogenicity of vinyl chloride but also showed its high potency and multi-target effect. In 1974, Creech and Johnson (50) reported the development of liver angiosarcoma, a rare form of liver cancer, in 4 vinyl chloride-exposed workers in a vinyl chloride polymerization plant in the United States. A wave of similar findings ensued throughout the world. By October 1977, at least 64 confirmed cases were reported in 12 countries. The cause-effect relationship was established beyond doubt (see Section 5.2.2.1.5.1). The discovery of the carcinogenicity of vinyl chloride stimulated an explosive growth in investigations of the health effects of related compounds. The rapid growth is reflected by a plethora of recent reviews and symposia on haloalkanes and haloalkenes in general (2, 14, 18, 22, 23, 27, 32, 51-60) and reports on individual compounds (see Table I). Some 40-50 haloalkanes and haloalkenes have been tested in various carcinogenesis bioassays and the list is continually growing (see Section 5.2.2.1.3). This section reviews the comparative carcinogenicity of various sub-classes of haloalkanes and haloalkenes, with emphasis on structure-activity relationship, as well as their mutagenicity, metabolism, mechanism of action, and their environmental significance.

5.2.2.1.2 Physicochemical Properties and Biological Effects.

5.2.2.1.2.1 PHYSICAL AND CHEMICAL PROPERTIES.

The physical and chemical properties of haloalkanes and haloalkenes depend largely on the nature of the halogen substituent. The electronegativity of halogens is greater than that of hydrogen and decreases in the order: F > Cl > Br > I (see Section 3.1.2.3, Vol. I). With the exception of iodine, all halogens are more electronegative than carbon, so that the C-X bonds are expected to be partially polarized to yield electron-deficient (partially positively charged) carbon atoms and electron-rich halogen atoms. Yet, despite the partial polarization of the C-F bond, fluorinated compounds are relatively inert as alkylating agents because the strength of the C-F bond is actually higher than that of the C-H bond. As the size of halogen atom increases (in the order F < Cl < Br < I) the bond length increases and the bond energy decreases thus weakening the C-X bond and facilitating the leaving of halogen atom in nucleophilic reactions. The leaving of the halide ion of larger size (e.g., iodide) in an aqueous system may be further facilitated by the lower energy of solvation than that of an ion of smaller size (e.g., fluoride).

The physical and chemical properties of haloalkanes and haloalkenes have been extensively discussed in many reviews (2, 23, 56-58, 61, 62) and standard textbooks. Some important physical properties of several haloalkanes and haloalkenes are summarized in Tables II and III. In general, the volatility of the halogenated compounds decreases with an increase in the number of halogen substituents. With the same degree of halogenation, the volatility decreases in the order: F > Cl > Br > I. For liquid haloalkanes and haloalkenes, solubility in water also tends to decrease with an increase in

halogenation. Comparable chlorinated compounds are generally more soluble than brominated compounds, which are in turn more soluble than iodinated compounds. The partition coefficient of haloalkanes and haloalkenes correlates usually inversely with their water solubility. The partition coefficient of the many haloalkanes and haloalkenes may be calculated, with reasonable agreement with experimental values, by a "fragment method" developed by Hansch and Leo (63). In this method, a given molecule may be "dissected" into small fragments with known assigned constants; the log of the partition coefficient (log P) is then calculated by simply adding up the constants of each fragment. Among comparable halogenated compounds, branching tends to lower the partition coefficient. Multiple halogenation on the same carbon (geminal substitution) or adjacent carbons (vicinal substitution) results in a higher partition coefficient than simple additivity predicts.

Fluorinated alkanes are so stable that they are often referred to as "inert"; nonetheless, fluorocarbons readily react with highly reactive materials such as alkali metals (59). The chemical reactivity of haloalkanes increases in the order: Cl < Br < I. The estimated half-lives of hydrolysis of monohalomethanes in aqueous media are of the order of 10,000, 480, and 8 hours for chloro-, bromo-, and iodomethane, respectively (58). The reactivity of chlorinated methanes decreases with an increase in the degree of chlorination as evidenced by the increase in the half-lives (58). Based on theoretical calculations, 1,2-dihaloalkanes (vicinally substituted) are expected to be more reactive than their 1,1- (geminally substituted) counterpart; this prediction is supported by thermochemical data, microwave spectroscopic data, and quantum mechanical calculations (64). Most haloalkanes are quite resistant to oxidation; some polyhalogenated alkanes are in fact used as flame retardants. Haloalkanes are susceptible to photolysis by high energy u.v. light

Table II
Physical Properties of Some Haloalkanes[a]

Compound	B.P. (°C)	Vapor Pressure (mm Hg)	Solubility in Water (gm/100 ml)	Partition Coefficient[c] (log P)
Chloromethane (Methyl chloride)	-23.7	3,756 at 20°C	0.74 at 25°C	0.91 (octanol)
Iodomethane (Methyl iodide)	42.4	400 at 20°C	1.4 at 20°C	1.69 (octanol)
Dichloromethane (Methylene chloride)	40.1	400 at 24°C	2.0 at 20°C	1.25 (octanol); 1.32 (oil)
Chloroform	61.2	200 at 26°C	0.82 at 20°C	1.97 (octanol); 2.06 (oil)
Iodoform	218[b]	--	0.01 at 25°C	--
Difluorodichloromethane (Freon-12)	-29.8	4,306 at 20°C	0.028 at 25°C	2.16 (octanol)
Fluorotrichloromethane (Freon-11)	23.8	667 at 20°C	0.11 at 20°C	2.53 (octanol)
Carbon tetrachloride	76.7	91 at 20°C	0.078 at 20°C	2.83 (octanol)
1,1-Dichloroethane	57.3	230 at 25°C	0.5 (unspecified)	1.79 (octanol); 1.84 (oil)
1,2-Dichloroethane	83.4	85 at 25°C	0.87 at 20°C	1.48 (octanol); 1.60 (oil)
1,2-Dibromoethane	131.6	11 at 25°C	0.43 at 30°C	--
1,1,1-Trichloroethane	74.1	103 at 20°C	0.03 at 25°C	2.49 (octanol); 2.58 (oil)
1,1,2-Trichloroethane	113.5	16 at 20°C	0.45 at 20°C	2.12 (oil)
1,1,2,2-Tetrachloroethane	146.3	5 at 21°C	0.29 at 25°C	2.57 (oil)
Halothane	50.2	243 at 20°C	0.35 (unspecified)	2.30 (octanol)
Hexachloroethane	188[b]	1 at 32.7°C	0.005 at 22°C	2.49 (octanol)
1,2-Dibromo-3-chloropropane	196	0.8 at 21°C	0.1 (unspecified)	--

[a] Summarized from data compiled by International Agency for Research on Cancer [IARC Monogr. No. 15 (1977), No. 19 (1979), and No. 20 (1979)]; G. McConnell, D.M. Ferguson, and G.R. Pearson [Endeavor 34, 13 (1975)]; National Academy of Sciences, "Nonfluorinated Halomethanes in the Environment," National Academy of Science, 1978; A. Sato and T. Nakajima [Arch. Environ. Health 34, 69 (1979)]; C. Hansch and A. Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology," Wiley, New York, 1979.

[b] Sublimes.

[c] Octanol-water or olive oil-water partition coefficient as indicated.

Table III
Physical Properties of Some Haloalkenes[a]

Compound	B.P. (°C)	Vapor Pressure (mm Hg)	Solubility in Water (gm/100 ml)	Partition Coefficient[b] (log P)
Vinyl chloride	-13.4	2,530 at 20°C	0.11 at 25°C	1.38 (octanol)[c]
1,1-Dichloroethylene (Vinylidene chloride)	32.0	400 at 14.8°C	0.04 at 20°C	2.18 (octanol)
cis-1,2-Dichloroethylene	60.6	208 at 25°C	0.35 at 20°C	1.53 (octanol)[c]; 1.97 (oil)
trans-1,2-Dichloroethylene	47.7	324 at 25°C	0.63 at 20°C	1.53 (octanol)[c]; 1.96 (oil)
Trichloroethylene	87	77 at 25°C	0.1 at 20°C	2.29 (octanol); 2.74 (oil)
Tetrachloroethylene	121	20 at 26.3°C	0.015 at 25°C	2.60 (octanol); 3.65 (oil)
3-Chloropropene (Allyl chloride)	45.1	368 at 25°C	0.1 (unspecified)	--
1,3-Dichloropropene (racemic mixture)	104	--	0.1 at 20°C	--
2-Chloro-1,3-butadiene (Chloroprene)	59.4	215 at 25°C	slightly soluble	--
Hexachloro-1,3-butadiene	210-220	22 at 100°C	insoluble	--

[a]Summarized from data compiled by International Agency for Research on Cancer [IARC Monogr. No. 15 (1977), No. 19 (1979), and No. 20 (1979)]; C.R. Worthing (ed.), "The Pesticide Manual," 6th ed., The British Crop Protection Council, 1979; D.D. Irish, In: "Patty's Industrial Hygiene and Toxicology," 2nd. ed., Vol. II, 1963; A. Sato and T. Nakijima [Arch. Environ. Health 34, 69 (1979)]; C. Hansch and A. Leo, "Substitutent Constants for Correlation Analysis in Chemistry and Biology," Wiley, New York, 1979.

[b]Octanol-water or olive oil-water partition coefficient as indicated.

[c]Calculated values.

and form free radicals (except fluorinated compounds); susceptibility to photolysis follows the order: I > Br > Cl > F.

The ability of haloalkanes to alkylate nucleophiles is of great biological importance. There are two possible mechanisms -- the S_N1 and the S_N2 (for a brief discussion of the S_N1 and S_N2 reactions, see Section 3.2.4 in Vol. I). In the S_N1 type of reaction, electrical effects (hyperconjugation, inductive) on the alkyl group play a determining role in the reactivity of haloalkanes. The relative reactivity follows the order: tert >> sec > pri > CH_3; for example, the relative S_N1 solvolysis rate of tert-butyl bromide is 100,000 times greater than that of sec-propyl bromide, which in turn is 12 times greater than that of ethyl or methyl bromide (65). In a S_N2 type reaction, steric hindrance around the carbon which is in transition state is of greater importance. Under ordinary conditions, haloalkanes with a primary or secondary alkyl group predominantly react by S_N2 mechanism. Reactivity follows the order: CH_3 > pri > sec >> (tert) and the relative S_N2 displacement rates are of the order of 30, 1, 0.4, 0.02 and near zero, for methyl, ethyl, primary alkyl, sec-propyl, tert-butyl halides, respectively (65). The strength of the carbon-halogen bond plays a crucial role in determining the leaving tendency of the halide ion. The bond energy of the C-X bond decreases in the order: F > Cl > Br > I. Moreover, halides with lower solvation energy in aqueous media are also expected to have a higher leaving tendency. The reaction rates of monohalomethanes have in fact been shown to follow the order: I > Br > Cl > F (66).

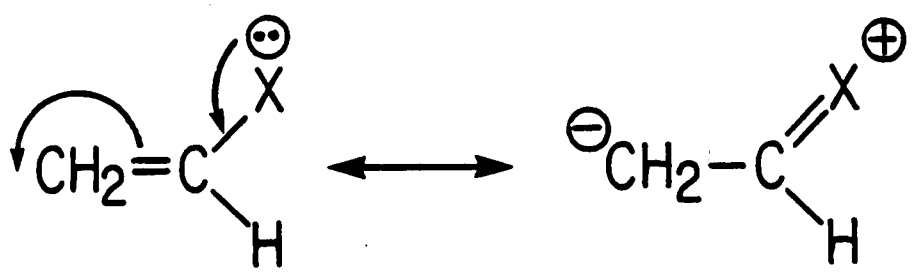
The alkylating activity of several haloalkanes has been measured by the NBP (4-p-nitrobenzyl pyridine) color reaction of Preussmann et al. (67). Methyl iodide has been consistently found to be quite active (68, 69); among the several haloalkanes tested, methyl iodide was the most active alkylating

agent. The relative alkylation rate (expressed as change in absorbance at 560 nm in 60 minutes) is 110, 55, 3, and 0 for methyl iodide, ethyl iodide, 1,2-dibromoethane, and 1,2-dichloroethane, respectively (69). This relative order has also been demonstrated by the use of a biological nucleophile, deoxyguanosine (69). Two primary alkyl chlorides, 1-chloropropane and 1-chlorobutane, have marginal activity and are considered inactive in the NBP reaction (70).

The chemical reactivity of haloalkenes is dependent on the nature, the number, and the position of halogen substitutents, as well as the number and position of the double bond(s). In the ethylene (ethene) series, introduction of the electronegative halogen atoms decreases the electron density in the double bond and exerts a stabilizing effect. The reactivity of ethylene decreases dramatically with the increase in the degree of halogenation. The relative rates of reaction with ozone for ethylene, vinyl chloride (monochloroethylene), trans-1,2-dichloroethylene, cis-1,2-dichloroethylene, 1,1-dichloroethylene, trichloroethylene, and tetrachloroethylene are 25000, 1180, 591, 35.7, 22.1, 3.6, and 1, respectively (71). The C-X bond in vinyl halides is expected to be stronger than that in haloalkanes, because of the possibility of resonance as represented below (56):

[TEXT-FIGURE 1]

Theoretical calculations predict that cis-1,2-dihaloethylenes are more reactive than trans-1,2-dihaloethylenes, which in turn are more reactive than 1,1-dihaloethylenes (64). In the propene series, allyl halides (3-halopropenes) are expected to be much more susceptible to nucleophilic substitution



Text-Figure 1

of the halogen atom than the corresponding haloalkanes, in both the S_N1 and S_N2 reactions. The increase in S_N1 reactivity can be readily explained by resonance stabilization of the carbonium ion,

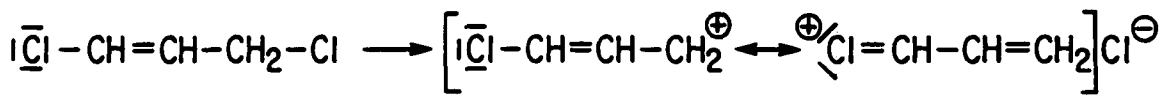
$$CH_2 = CH - CH_2^{\oplus} \longleftrightarrow {}^{\oplus}CH_2 - CH = CH_2 \longleftrightarrow {}^{\frac{1}{2}\oplus}CH_2 \cdots CH \cdots {}^{\frac{1}{2}\oplus}CH_2 .$$

The higher S_N2 reactivity may be due to stabilization of the transition state by delocalization of the π-electrons, lower C-X bond strength, or other factors. In addition, a modified S_N2 reaction (S_N2'), involving nucleophilic (Nu:) attack on the unsaturated γ-carbon with subsequent shift of double bond

[TEXT-FIGURE 2]

and expulsion of halide ion, may proceed concurrently with the S_N2 reaction. The chemical reactivity of halobutenes is also expected to be dependent on the position of the double bond and the halogen atom.

The alkylating activity of a variety of haloalkenes in the NBP reaction has recently been extensively studied by investigators in Germany (70, 72) and in the laboratories of the International Agency for Research on Cancer (73, 74). Table IV summarizes the available data. The Table illustrates the fact that, as expected, vinyl halides are poor alkylators, and metabolic activation (S-9) is needed to bring to fore the alkylating activity. On the other hand, allyl halides are good alkylating agents. The alkylating activity of allyl halide increases in the order: Cl < Br < I (70). In contrast to 3-chloropropene (allyl chloride), the two nonallylic isomers, 1-chloro- and 2-chloropropenes, are completely inactive in the NBP reaction. Substitution of a second chloride atom at the 1-position of allyl chloride (yielding 1,3-dichloro-



Text-Figure 2

Table IV
Relative Alkylating Activity of Haloalkenes (NBP-Reaction)[a]

Compound	Wurzburg Study	IARC Study
A) Haloethenes (Haloethylenes)		
Vinyl chloride		0 (0.03 with S9)[b,c]
Vinyl bromide		0 (0.05 with S9)[b,c]
1,1-Difluoroethylene		0 (with S9)[c]
1,1-Dichloroethylene (Vinylidene chloride)		0 (with S9)[c]
Trichloroethylene		0 (with S9)[c]
B) Halopropenes		
1-Chloropropene	0[d]	
2-Chloropropene	0[d]	
3-Chloropropene (Allyl chloride)[g]	0.285[d,e]	
3-Bromopropene (Allyl bromide)[g]	1.208[e]	
3-Iodopropene (Allyl iodide)[g]	1.828[e]	
3-Chloro-2-methylpropene[g]	0.570[d]	
cis-1,3-Dichloropropene[g]	2.240[d]	
trans-1,3-Dichloropropene[g]	1.933[d]	
2,3-Dichloropropene[g]	0.248[d]	
C) Halobutenes and Halobutadienes		
3-Chloro-1-butene[g]	n.a.[d,f]	
4-Chloro-1-butene	0[d]	
3-Chloro-2-methyl-1-butene[g]	n.a.[d,f]	
3,4-Dichloro-1-butene[g]		0.03[c]
1-Chloro-2-butene[g]	1.035[d]	

Table IV (continued)

Compound	Wurzburg Study	IARC Study
2-Chloro-2-butene	0[d]	
1-chloro-2-methyl-2-butene[g]	2.057[d]	
1,4-Dichloro-2-butene[g]		13[c]
1-Chloro-1,3-butadiene		4.3[c]
2-Chloro-1,3-butadiene[g]		0.2 (0.08 with S9)[c]

[a]The alkylating activity of the compound with 4-(p-nitrobenzyl)-pyridine (NBP) was expressed as ΔE_{560nm} in the Wurzburg study and ΔE at λ_{max} in the IARC study. In some cases (as stated), liver 9,000 x g postmitochondrial fraction (S9) was included in the incubation mixture.

[b]From A. Barbin, H. Bresil, A. Croley, P. Jacquignon, C. Malaveille, R. Montesano, and H. Bartsch [Biochem. Biophys. Res. Commun. 67, 596 (1975)].

[c]From H. Bartsch, C. Mallaveille, A. Barbin, and G. Planche [Arch. Toxicol. 41, 249 (1979)].

[d]From T. Neudecker, D. Lutz, E. Eder, and D. Hsnschler [Biochem. Pharmacol. 29, 2611 (1980)].

[e]From E. Eder, T. Nuedecker, D. Lutz, and D. Henschler [Biochem. Pharmacol. 29, 993 (1980)].

[f]NBP reaction not applicable due to interference (see text).

[g]Contains allylic structure.

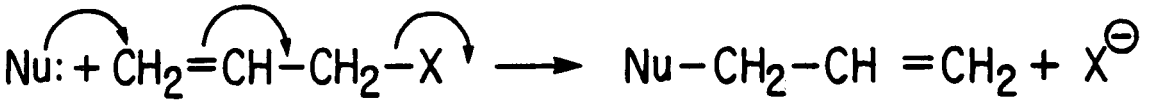
propene) substantially increases the alkylating activity of the compound. Neudecker et al. (72) attributed the increase in activity to the chlorine-induced positive mesomeric (+ M) effect, which exceeds its negative inductive (-I) effect and weakens the allyl C-Cl bond.

[TEXT-FIGURE 3]

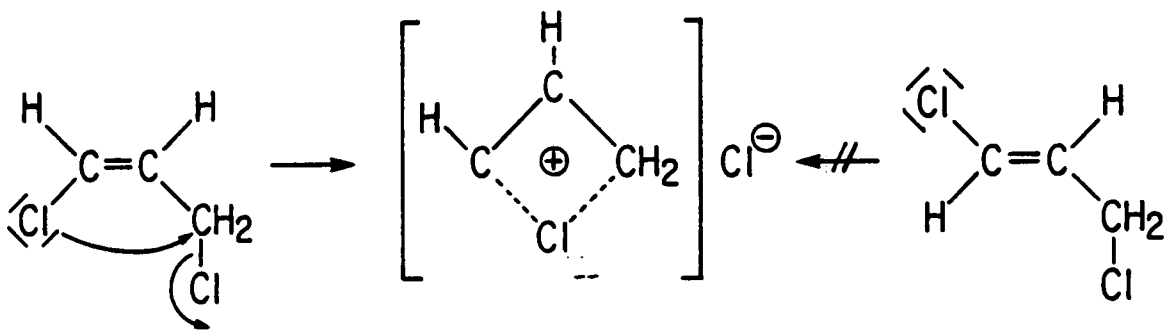
The resulting carbonium ion may be stabilized by resonance. The cis-1,3-dichloropropene is more active than its trans isomer due to a possible neighboring group effect that is absent in the trans isomer.

[TEXT-FIGURE 4]

In contrast to 1,3-dichloropropene, 2,3-dichloropropene is slightly less reactive than allyl chloride. The positive mesomeric effect is absent when the chlorine substituent is at the central carbon of the allylic structure. In this compound, only the negative inductive effect is operative, which stabilizes the allylic C-Cl bond. Halobutenes with allylic structure (e.g., 1-chloro-2-butene) are active in the NBP reaction while those with nonallylic structure (e.g., 2-chloro-2-butene, 4-chloro-1-butene) are not. Substitution in the allylic structure by a methyl group increases the alkylating activity of the haloalkene probably by a combination of positive inductive and hyper-



Text-Figure 3



Text-Figure 4

conjugation effects of the methyl group. Activation is substantial if methyl substitution is on carbon-1 or carbon-3 of the allylic structure (e.g., compare 1-chloro-2-butene with allyl chloride), but relatively small if methyl substitution is on the central carbon (e.g., 3-chloro-2-methyl-propene). 3-Chloro-1-butene and 3-chloro-2-methyl-1-butene, which have allylic structures (Table IV), are expected to be active in the NBP reaction but cannot be tested because of the reagent-induced dehalogenation of the compound (72). Two chlorinated 1,3-butadienes have been tested in the NBP reaction and the results indicate that alkylating activity is not dependent on the allylic structure. The vinylic 1-chloro-1,3-butadiene is considerably more reactive than 2-chloro-1,3-butadiene (chloroprene), which may be considered to have both an allylic and a vinylic structure.

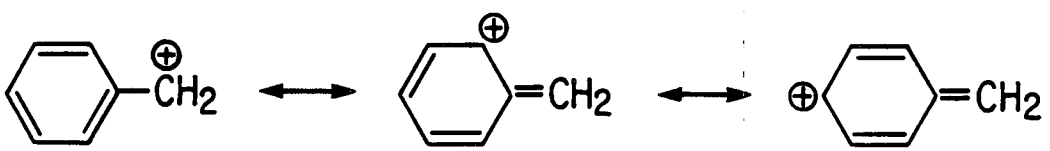
The chemical properties of benzyl chloride, an arylalkyl halide, resemble more closely those of haloalkanes and haloalkenes than those of haloaromatics. Benzyl chloride is more reactive than allyl chloride in both S_N1 and S_N2 type of reactions (65). Resonance stabilization of the benzyl cation

[TEXT-FIGURE 5]

greatly enhances the S_N1 reactivity of the compound. Benzyl chloride has been shown to be highly reactive in the NBP reaction (68, 72).

5.2.2.1.2.2 BIOLOGICAL EFFECTS OTHER THAN CARCINOGENICITY.

Toxic Effects. The toxicology of haloalkanes and haloalkenes has been studied for decades. There are many reviews and monographs (e.g., 9, 19, 57,



Text-Figure 5

58, 61, 62, 75-77) on this subject. Only a brief discussion emphasizing comparative toxicity is presented in this section. Some representative acute toxicity data of haloalkanes and haloalkenes that have been tested for carcinogenicity are summarized in Table V. In general, fluoroalkanes have very low toxicity unless their biotransformation leads to fluoroacetic acid. Fluoroalkenes have not been thoroughly studied; however, the available data suggest that fluoroalkenes are more toxic than fluoroalkanes and should be more carefully investigated (reviewed in 109). Chlorinated alkanes and alkenes are substantially more toxic than their fluorinated counterparts. The principal toxic effects in humans and in mammalian animal species are central nervous system depression (e.g, narcosis), liver and kidney pathology (e.g., fatty changes, necrosis, degeneration), and myocardial sensitization to endogenous epinephrine (resulting in symptoms such as ventricular fibrillation). Inhalation exposure to dichloromethane may also result in hypoxia due to metabolic formation of carbon monoxide which binds to hemoglobin. The position of chlorine substitution on the alkane may play a significant tole in determining the toxicity. 1,1,2-Trichloroethane, for example, is considerably more toxic than its 1,1,1-isomer.

The toxicity of haloalkanes and haloalkenes may be modified by a variety of exogenous and endogenous factors. The toxicity of carbon tetrachloride, the most extensively studied haloalkane, has been shown to be potentiated by a long list of chemical and biological factors. Based on their possible mechanism of action, these factors may be loosely classified as (a) inducers of mixed-function oxidases (MFO), such as phenobarbital, 3-methylcholanthrene, polychlorinated biphenyls, polybrominated biphenyls (110-113), (b) conditions known to deplete tissue glutathione (GSH) level, such as fasting, diurnal

Table V
Acute Toxicity of Haloalkanes and Haloalkenes

Compound	Species & Route	LD_{50} or LC_{50}[a]	Reference
A) Haloalkanes			
Chloromethane (Methyl chloride)	Mouse, inhalation	3,146 ppm for 7 h	(61)
	Rat, inhalation	152 mg/liter for 30 min	(78)
Iodomethane	Mouse, inhalation	5 mg/liter for 57 min	(79)
	Mouse, i.p.	173 mg/kg	(80)
	Rat, inhalation	1.3 mg/liter for 4 h	(80)
	Rat, i.p.	101 mg/kg	(80)
	Rat, s.c.	110 mg/kg	(68)
Dichloromethane (Methylene chloride)	Mouse, inhalation	13,500 ppm for 9-12 h	(81)
	Mouse, i.p.	1.5 ml/kg	(82)
	Rat, oral	2.3 ml/kg	(83)
	Dog, i.p.	0.95 ml/kg	(84)
Chloroform	Mouse, inhalation	4,500 ppm for 9-12 h	(81)
	Mouse, i.p.	1.2 ml/kg	(82)
	Mouse, oral	1,120 mg/kg (M); 1,400 mg/kg (F)	(85)
	Mouse, s.c.	3,283 mg/kg	(78)
	Rat, oral	908 mg/kg (M); 1,117 mg/kg (F)	(86)
	Dog, i.p.	1.0 ml/kg	(84)
Dichlorobromomethane	Mouse, oral	450 mg/kg (M); 900 mg/kg (F)	(85)
	Rat, oral	916 mg/kg (M); 969 mg/kg (F)	(86)
Chlorodibromomethane	Mouse, oral	800 mg/kg (M); 1,200 mg/kg (F)	(85)
	Rat, oral	1,186 mg/kg (M); 848 mg/kg (F)	(86)
Tribromomethane	Mouse, oral	1,400 mg/kg (M); 1,550 mg/kg (F)	(85)
	Mouse, s.c.	1,820 mg/kg	(78)
	Rat, oral	1,388 mg/kg (M); 1,147 mg/kg (F)	(86)
Triiodomethane	Mouse, s.c.	629 mg/kg	(78)
Carbon tetrachloride	Mouse, inhalation	8,500 ppm for 9-12 h	(81)
	Mouse, i.p.	2.7 ml/kg	(84)
	Rat, oral	1.77 ml/kg	(87)
	Dog, i.p.	1.5 ml/kg	(84)
1,2-Dichloroethane	Rat, inhalation	1,000 ppm for 7.2 h	(88)
	Rat, oral	680 mg/kg	(89)

Table V (continued)

Compound	Species & Route	LD_{50} or LC_{50}[a]	Reference
1,2-Dibromoethane	Mouse, oral	420 mg/kg	(90)
	Rat, oral	146 mg/kg (M); 117 mg/kg (F)	(90)
1,1,1-Trichloroethane	Mouse, inhalation	13,500 ppm for 9-12 h	(81)
	Mouse, i.p.	3.8 ml/kg	(82)
	Rat, oral	12,300 mg/kg (M); 10,300 mg/kg (F)	(91)
	Dog, i.p.	3.1 ml/kg	(84)
1,1,2-Trichloroethane	Mouse, inhalation	3,750 ppm for 9-12 h	(81)
	Mouse, i.p.	0.35 ml/kg	(82)
	Rat, oral	0.58 ml/kg	(92)
	Dog, i.p.	0.45 ml/kg	(84)
1,1,2,2-Tetrachloroethane	Mouse, i.p.	820 mg/kg	(93)
	Rat, oral	250 mg/kg	(94)
Halothane	Mouse, inhalation	22,000 ppm for 10 min	(78)
Hexachloroethane	Mouse, i.p.	4,500 mg/kg	(78)
	Rat, oral	6,000 mg/kg	(78)
1,2-Dibromo-3-chloropropane	Mouse, oral	257 mg/kg	(78)
	Rat, inhalation	103 ppm for 8 h	(78)
	Rat, oral	150-370 mg/kg (M); 260-620 mg/kg (F)	(95)
B) Haloalkenes			
Vinyl chloride	Mouse, inhalation	113,000 ppm for 2 h	(96)
	Rat, inhalation	150,000 ppm for 2 h	(96)
Vinylidene chloride[b] (1,1-Dichloroethylene)	Mouse, inhalation	98-105 ppm for 23 h	(97)
	Mouse, oral	194-217 mg/kg	(98)
	Rat, inhalation	10,000-15,000 ppm for 4 h (fed) 500-2,500 ppm for 4 h (fasted)	(99)
	Rat, oral	1,550 mg/kg	(100)
trans-1,2-Dichloroethylene	Mouse, i.p.	3.2 ml/kg	(101a)
	Rat, oral	1.0 ml/kg	(101a)
	Rat, i.p.	6.0 ml/kg	(101a)
Trichloroethylene	Mouse, inhalation	5,500 ppm for 9-12 h	(81)
	Mouse, i.p.	2.2 ml/kg	(82)
	Rat, oral	4.92 ml/kg	(92)
	Dog, i.p.	1.9 ml/kg	(84)

Table V (continued)

Compound	Species & Route	LD_{50} or LC_{50}[a]	Reference
Tetrachloroethylene	Mouse, inhalation	3,700 ppm for 9-12 h	(81)
	Mouse, i.p.	2.9 ml/kg	(84)
	Rat, oral	8.0 ml/kg	(101b)
	Dog, i.p.	2.1 ml/kg	(84)
1-Chloropropene	Rat, oral	1,950 mg/kg	(78)
Allyl chloride (3-Chloropropene)	Rat, oral	700 mg/kg	(102)
1,3-Dichloropropene (cis/trans mixture)	Rat, oral	713 mg/kg (M); 470 mg/kg (F)	(103)
	Rabbit, topical	504 mg/kg	(103)
1,4-Dichloro-2-butene	Rat, inhalation	86 ppm for 4 h	(78)
	Rat, oral	89 mg/kg	(104)
	Rabbit, topical	0.62 ml/kg	(104)
2-Chloro-1,3-butadiene (Chloroprene)	Rat, inhalation	8.2 mg/liter (2,280 ppm) for 4 h	(105)
	Rat, oral	670 mg/kg	(106)
Hexachloro-1,3-butadiene	Mouse, i.p.	76 mg/kg	(107)
	Rat, oral	580 mg/kg (M); 200-400 mg/kg (F)	(108)
	Rat, i.p.	190 mg/kg	(107)

[a]Median lethal concentration (via inhalation) or median lethal dose (via all other routes); (M) = male, (F) = female.

[b]Substantial variation dependent on the condition of animals (see text).

variation (lower GSH at night), diethyl maleate administration (113, 114), (c) ketones such as acetone, Kepone, methyl n-butyl ketone, and 2,5-hexanedione (115 and refs. therein), (d) ketogenic compounds and conditions such as isopropanol, 1,3-butanediol, n-hexane and neonatal or uncontrolled diabetes (115-118), and (e) agents with unclear mechanisms such as ethanol (115, 116, 119 and refs. therein). On the other hand, free radical scavengers (e.g., diethyldithiocarbamate, propyl gallate, GSH, cystamine), antioxidants (e.g., tinoridine), inhibitors of MFO (e.g., SFK 525A), zinc, and cyclohexamide all exhibit protective effect against carbon tetrachloride toxicity (113, 120-122). The toxicity of a number of other haloalkanes and haloalkenes such as chloroform (115, 123-126), vinylidene chloride (97, 127, 128), vinyl chloride (129-131) is also subject to modifiers in an essentially similar manner as carbon tetrachloride. However, there is some evidence that the toxicity of methylene chloride, 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene is not potentiated in certain target organs by some inducers of MFO (110, 112).

Mutagenic Effects. The mutagenicity of haloalkanes and haloalkenes has been extensively studied in a variety of test organisms including bacteria (e.g., 132-136; see also Ames Salmonella test described below), yeasts (137-144), Neurospora (145, 146), higher plants (133, 147, 148), Drosophila (15, 136, 149-152), cultured mammalian cells (133, 137, 153-156), and experimental animals (136, 157-161). A number of studies have been undertaken to evaluate the potential mutagenic effects of vinyl chloride and other haloalkanes and haloalkenes in humans (reviewed in 2, 23, 29, 162). The following discussion focuses only on mutagenicity studies using the Ames Salmonella test, which has been widely employed for the pre-screening of chemical carcinogens.

Close to 80 haloalkanes and haloalkenes have been tested in the Ames Salmonella test. The results of these studies are summarized in Tables VI and VII. With few exceptions, mutagenic haloalkanes and haloalkenes induce mutation predominantly in base-pair substitution mutants (TA100, TA1535). Only dichloromethane (163), trichloroethylene (164), 1,1-difluoro-2-bromo-2-chloroethylene (165), 1,3-dichloropropene (166), and 1,2,3,3-tetrachloropropene (167) display some activity in the frame-shift mutants (TA98, TA1537, TA1538, or TA1978); in all these cases, the activity toward frame-shift mutants is substantially lower than that toward base-pair substitution mutants. For this reason, many of the Ames tests of haloalkanes and haloalkenes are carried out using TA100 and TA1535 only.

It should be emphasized that a number of factors must be considered in the interpretation of mutagenicity data to avoid erroneous conclusions or false negatives: (a) Most low-molecular-weight haloalkanes and haloalkenes are highly volatile and therefore may be lost through evaporation in the standard Ames test. These compounds must be assayed in tightly closed containers to ensure that the bacteria are actually exposed to the level of the chemical added. (b) Some compounds (e.g., allyl chloride) display greater mutagenic activity in suspension assays, in which the chemical is preincubated with the bacteria before plating. The suspension assay should be employed if results from standard assays and assays in closed containers are inconclusive. (c) Some dihalomethanes (e.g., dibromomethane, diiodomethane) and 1,2-dihaloethanes (e.g., 1,2-dichloroethane, 1,2-dibromoethane) are metabolically activated by cytosolic enzymes (S-100 or S-115 fractions) as well as by the microsomal (S-9) fraction (see also Section 5.2.2.1.4). For the 1,2-dihaloethanes, cytosol is actually the better source of activating enzymes. Suspension assays with the inclusion of cytosol should be used for compounds

Table VI
Comparative Mutagenicity of Haloalkanes in the Ames Salmonella Test

Compound	System[a]	Mutagenicity[b] Without Activation[c]	Mutagenicity[b] With Activation[d]
A) Halomethanes			
Chloromethane (methyl chloride)	Enclosed	+ (168-170)	+ (168$^+$, 169, 170)
Bromomethane (methyl bromide)	Enclosed	+ (168, 169)	n.t.
Iodomethane (methyl iodide)	Standard	+ (171)	n.t.
	Enclosed	+ (168, 169)	n.t.
Fluorochloromethane	Unspecified	+ (172)	+ (172$^+$)
Dichloromethane (methylene chloride)	Standard	- (167)	- (167)
	Enclosed	+ (163, 167-169)	+ (163$^+$)
Bromochloromethane	Enclosed	+ (168, 169)	n.t.
Dibromomethane	Enclosed	+ (168, 169)	n.t.
	Suspension	+ (173)	+ (173$^+$) (S9 or S100)
Diidomethane	Suspension	+ (173)	+ (173$^+$) (S9 or S100)
Difluorochloromethane	Enclosed	+ (174)	+ (174)
Trichloromethane (chloroform)	Standard	- (167, 168)	- (167)
	Enclosed	- (168, 169)	- (169)
	Suspension	- (168, 175)	- (175)
Bromodichloromethane	Standard	- (168)	n.t.
	Enclosed	+ (168, 169)	n.t.
Dibromochloromethane	Standard	- (168)	n.t.
	Enclosed	+ (168, 169)	n.t.
Tribromomethane (bromoform)	Standard	- (168)	n.t.
	Enclosed	+ (168, 169)	n.t.
Difluorodichloromethane	Enclosed	- (174)	- (174)
Fluorotrichloromethane	Suspension	- (175)	- (175)
Tetrachloromethane (carbon tetrachloride)	Standard	- (167, 168, 171)	- (167, 171)
	Enclosed	- (168, 169)	- (169)
	Suspension	- (175)	- (175)

Table VI (continued)

Compound	System[a]	Mutagenicity[b] Without Activation[c]	With Activation[d]
B) Haloethanes			
1,1-Dichloroethane	Standard	- (168)	n.t.
1,1-Dibromoethane	Standard	w+ (132)	n.t.
1,2-Dichloroethane (ethylene dichloride)	Standard	- (136, 167, 176)	- (136, 167, 176)
		w+ (132, 177, 178)	w+ (177) + (178^+) (S115)
	Enclosed	w+ (167)	w+ (167)
	Suspension	- (179)	- (179) (M); + (179^+) (S100)
1-Bromo-2-chloroethane	Standard	+ (132)	n.t.
1,2-Dibromoethane (ethylene dibromide)	Standard	+ (132, 171, 177, 178, 180)	+ (177^+, 178^+, 180)
	Suspension	+ (181)	+ (181^-) (M); + (181^+) (S100)
1,1,1-Trichloroethane (methyl chloroform)	Standard	- (167)	- (167)
	Enclosed	w+ (167, 168)	w+ (168)
1,1,2-Trichloroethane	Standard	- (168, 178)	- (178)
1,1,1,2-Tetrachloroethane	Standard	- (168)	n.t.
1,1,2,2-Tetrachloroethane	Standard	- (167)	- (167)
		w+ (132)	
	Enclosed	- (167)	n.t.
1,1,1-Trifluoro-2-bromo-2-chloroethane (halothane)	Enclosed	- (182)	- (182)
	Suspension	- (175, 182)	- (175, 182)
Trifluorotrichloroethane	Standard	- (168)	n.t.
Hexachloroethane	Standard	- (cited in ref. 144)	- (cited in ref. 144)
C) Halopropanes			
1-Chloropropane	Suspension	- (70)	- (70)
2-Chloropropane	Enclosed	+ (168)	+ (168^+)
1,2-Dichloropropane	Standard	- (176); + (166)	- (176); + (166)
1,2-Dibromopropane	Standard	+ (176)	+ (176^+)

Table VI (continued)

Compound	System[a]	Mutagenicity[b] Without Activation[c]	Mutagenicity[b] With Activation[d]
1,3-Dichloropropane	Standard	+ (176)	+ (176)[e]
1,3-Dibromopropane	Standard	± (176)[e]	+ (176)[e]
1,2,3-Trichloropropane	Standard	± (176)	+ (176^{+})
1,2-Dibromo-3-chloropropane (DBCP)	Standard	- (176, 183); + (180)	+ (176^{+}, 180^{+}, 183^{+})
1,2,3-Tribromopropane	Standard	+ (176, 180)	+ (176^{+}, 180)
D) Higher Haloalkanes			
1-Chlorobutane (n-butyl chloride)	Suspension	- (70)	- (70)
1-Bromobutane (n-butyl bromide)	Enclosed	+ (168)	n.t.
1-Bromo-2-methylpropane (i-butyl bromide)	Enclosed	- (168)	n.t.
2-Bromobutane (s-butyl bromide)	Enclosed	+ (168)	n.t.
1,2-Dibromo-2-methylpropane	Standard	+ (132)	n.t.
1,5-Dibromopentane	Standard	+ (132)	n.t.

[a] "Standard" refers to standard Ames Salmonella tests (plate incorporation or spot test); "Enclosed" refers to Salmonella tests carried out in desiccators or closed glass containers; "Suspension" refers to modified Salmonella tests in which bacteria and test chemical were pre-incubated in liquid suspension before plating.

[b] Mutagenicity in base-pair substitution mutants (TA100, TA1535): "+" = positive; "w+" = weakly positive; "±" = marginal; "-" = negative; n.t. = not tested.

[c] Without added mammalian activation system.

[d] Unless specified, the activation system was liver postmitochondrial fraction (S9) plus cofactors. "S100" or "S115" refers to cytosolic fraction plus glutathione. "M" refers microsomal fraction plus cofactors. A superscript "+" or "-" sign over the reference number denotes an increase or a decrease in mutagenicity by the inclusion of the activation system, respectively.

[e] Results obscured by the cytotoxic effects of the chemical.

Table VII
Comparative Mutagenicity of Haloalkenes in the Ames Salmonella Test

Compound	System[a]	Mutagenicity[b]	
		Without Activation[c]	With Activation[d]
A) Haloethenes (Haloethylenes)			
Vinyl chloride	Enclosed	+ or w+ (74, 168, 170, 174, 177, 184-187)	+ (74^+, 168^+, 170^+, 174^+, 177^+, 184-186^+, 187)
Vinyl bromide	Enclosed	+ (74)	+ (74^+)
1,1-Difluoroethylene	Enclosed	- (74)	w+ (74^+)
1,1-Dichloroethylene (Vinylidene chloride)	Enclosed	- (74, 168, 188) + (182)	+ (74^+, 168^+, 182^+, 188^+)
cis-1,2-Dichloroethylene	Standard	- (164, 168)	n.t.
trans-1,2-Dichloroethylene	Standard	- (164, 168)	n.t.
Trichloroethylene	Standard	+ (164)	n.t.
	Enclosed	w+ (168)	+ (168^+); w+ (74^+);
		- (74, 182)	- (182)
	Suspension	- (182)	- (182)
Tetrachloroethylene	Standard	+ (164)	n.t.
	Enclosed	- (74)	- (74)
1,1-Difluoro-2-bromo-2-chloroethylene	Suspension	+ (165)	+ (165)
B) Halopropenes			
1-Chloropropene[e]	Enclosed	+ (168, 169)	+ (168^-)
	Suspension	- (72)	- (72)
1-Bromopropene[e]	Standard	+ (176)	+ (176^-)
2-Chloropropene[e]	Suspension	- (72)	- (72)
2-Bromopropene[e]	Standard	+ (176)	± (176^-)

Table VII (continued)

Compound	System[a]	Mutagenicity[b]	
		Without Activation[c]	With Activation[d]
3-Chloropropene (Allyl chloride)[f]	Standard	- (140, 166); + (189)	- (140, 166); + (189^+)
	Enclosed	+ (169)	n.t.
	Suspension	+ (70, 72, 140)	- (70^-, 72^-); + (140)
3-Bromopropene (Allyl bromide)[f]	Suspension	+ (70)	- (70^-)
3-Iodopropene (Allyl iodide)[f]	Suspension	+ (70)	- (70^-)
3-Chloro-2-methylpropene[f]	Suspension	+ (72)	+ (72^-)
1,3-Dichloropropene[e,f,g]			
cis-isomer	Standard	+ (166)	+ (166)
	Suspension	+ (72)	+ (72^-)
trans-isomer	Standard	+ (166)	+ (166)
	Suspension	+ (72)	+ (72^-)
unspecified	Standard	+ (176)	+ (176^-)
2,3-Dichloropropene[e,f]	Standard	+ (166, 176)	+ (166, 176)
	Suspension	+ (72)	+ (72^+)
2,3-Dibromopropene[e,f]	Standard	+ (176)	+ (176^-)
1,2,3-Trichloropropene[e,f]	Standard	+ (176)	+ (176^+)
1,2,3,3-Tetrachloropropene[e,f]	Standard	+ (167)	+ (167^-)
1,1,2,3,3-Pentachloropropene[e,f]	Standard	+ (167)	+ (167^-)
C) Halobutenes and Halobutadienes			
3-Chloro-1-butene[f]	Suspension	+ (72)	+ (72^-)
4-Chloro-1-butene	Suspension	- (72)	- (72)
3-Chloro-2-methyl-1-butene[f]	Suspension	+ (72)	n.t.
3,4-Dichloro-1-butene[f]	Enclosed	+ (74)	+ (74^+)

Table VII (continued)

Compound	System[a]	Mutagenicity[b]	
		Without Activation[c]	With Activation[d]
1-Chloro-2-butene[f]	Suspension	+ (72)	+ (72^{-})
2-Chloro-2-butene[e]	Suspension	- (72)	+ (72^{+})
1-Chloro-2-methyl-2-butene[f]	Suspension	+ (72)	+ (72^{-})
1,3-Dichloro-2-butene[e,f]	Standard	- (166)	- (166)
1,4-Dichloro-2-butene[f]	Standard	+ (74)	+ (74^{+})
1-Chloro-1,3-butadiene[e]	Enclosed	+ (74)	+ (74^{+})
2-Chloro-1,3-butadiene[e,f]	Enclosed	+ (74)	+ (74^{+})
Hexachloro-1,3-butadiene[e,f]	Suspension	+ (169)	+ (169)

[a] "Standard" refers to standard Ames Salmonella tests (plate incorporation or spot test); "Enclosed" refers to Salmonella tests carried out in desiccators or closed glass containers; "Suspension" refers to modified Salmonella tests in which bacteria and test chemical were pre-incubated in liquid suspension before plating.

[b] Mutagenicity in base-pair substitution mutants (TA100, TA1535): "+" = positive; "w+" = weakly positive; "±" = marginal; "-" = negative; n.t. = not tested.

[c] Without added mammalian activation system.

[d] In most of these studies, the activation system was liver postmitochondrial fraction (S9) plus cofactors. A superscript "+" or "-" sign over the reference number denotes an increase or a decrease in mutagenicity by the inclusion of the activation system, respectively.

[e] Contains a vinylic structure.

[f] Contains an allylic structure.

[g] A purified sample of this compound was reported to be nonmutagenic [R.E. Talcott, The Toxicologist 1, 41 (1981)].

with similar structure. (d) Many haloalkenes are actually more mutagenic in the absence of S-9 fraction that in its presence. The mutagenicity of allyl halides (3-halopropenes) in suspension assay, for example, is completely abolished if S-9 fraction is included. (e) The results of some mutagenicity assays may be obscured by the cytotoxic effect of the chemical (e.g., 1,3-dichloropropane, 1,3-dibromopropane). These considerations suggest that some of the negative findings summarized in Tables VI and VII should be further investigated before firm conclusions can be made.

The data available lead to some interesting structure-activity relationships for mutagenicity of haloalkanes and haloalkenes. Comparison of the relative mutagenic potency of various compounds must be limited to the same laboratories because of inter-laboratory variations and differences in experimental procedures. Among the mutagenic haloalkanes (Table VI), the compounds are either active as such or after metabolic activation. In the fluorochloromethane series both tetrahalomethanes (CF_2Cl_2, $CFCl_3$) are inactive whereas the trihalomethane (CHF_2Cl) and dihalomethane (CH_2FCl) show some mutagenic activity. In the chloromethane series, there is some evidence that mutagenicity declines with an increase in chlorination. Despite extensive studies (see Table VI), carbon tetrachloride and chloroform (which are carcinogenic) have been consistently found inactive in the Ames test. On the other hand, all three monohalomethanes (CH_3Cl, CH_3Br, CH_3I) are active in the Ames test. The mutagenicity of chloromethane is enhanced by metabolic activation by S-9 fraction. All five dihalomethanes are mutagenic. Of the 4 dihalomethanes tested, inclusion of S-9 fraction increases the mutagenicity of the compounds. Consistent with their relative chemical reactivity and metabolic rate, the relative mutagenic potency of bromo- and chloro- compounds in the study of Simmon (169) follows the order: $CH_2Br_2 > CH_2BrCl > CH_2Cl_2$. Dibromo-

and diiodomethanes are activated by microsomal as well as by cytosolic enzymes. Cytosolic glutathione S-transferase is believed to be the activating enzyme for the dihalomethanes. In contrast to the higher chemical reactivity and metabolic rate, diiodomethane appears to be less mutagenic than dibromomethane. Van Bladeren et al. (173) suggested that the reactive intermediate from diiodomethane may be too reactive to reach target macromolecules (i.e., it may be scavenged by water or genetically unimportant nucleophiles). In the trihalomethane group, chloroform has been consistently found to be nonmutagenic in a variety of test conditions. Substitution of either one or two chlorine atoms by either bromine or fluorine yields mutagenic compounds (detected by testing in the closed system). In the tetrahalomethane group, none of the three compounds tested demonstrated any mutagenic activity. Carbon tetrachloride, in particular, has been tested in various systems.

Twelve haloethanes have been tested in the Ames test. With the exception of 1,2-dihaloethanes, haloethanes are either nonmutagenic or weakly mutagenic. 1,2-Dibromoethane has been found to be the most potent mutagen of the group either in the presence or the absence of mammalian activation system. The mutagenicity of 1,2-dibromoethane may be substantially reduced by substitution with chlorine; thus, the mutagenicity of dihaloethane decreases in the order: 1,2-dibromo > 1-bromo-2-chloro > 1,2-dichloro (132). This is consistent with the higher reactivity of bromo compounds as compared to chloro compounds. The position of the halogen may also affect the mutagenicity: 1,2-dibromoethane is considerably more potent than its 1,1-isomer (132). 1,2-Dihaloethanes appear to be more effectively activated by cytosolic than by microsomal enzymes. Guengerich et al. (179) showed that cytosol does activate 1,2-dichloroethane in suspension assay, while microsomes do not. Van Bladeren et al. (173) demonstrated that the inclusion of microsomes actually decreases

the mutagenicity of 1,2-dibromoethane in suspension assay; on the other hand, cytosol increases the mutagenicity of the compound.

A comparative study of the relative mutagenicity of seven halopropanes has been carried out by Stolzenberg and Hine (176). The activities follow the order: 1,2,3-tribromo > 1,2,3-trichloro > 1,2-dibromo-3-chloro > 1,3-dichloro > 1,2-dibromo > inactive 1,2-dichloro. 1,3-Dibromopropane is also mutagenic but its mutagenicity is masked by its cytotoxicity. Blum and Ames (180) have shown that 1,2,3-tribromopropane is more mutagenic than 1,2-dibromo-3-chloropropane. Only limited data are available to compare the mutagenicity of higher haloalkanes. In the study by Brem et al. (132), 1,5-dibromopentane was as potent as 1,2-dibromoethane which, in turn, is more potent than 1,2-dibromo-2-methylpropane.

The mutagenicity of haloalkenes (Table VII) is determined by the position(s) and the number, as well as the nature, of halogen substitutent. In general, there is a good correlation between the alkylating activity (see Table IV) and the mutagenicity of haloalkenes, although exceptions have been noted. Haloalkenes with vinylic structure are either inactive or weak mutagens without activation; inclusion of an S-9 activation system enhances substantially the mutagenicity of most of these compounds. In contrast, most haloalkenes with allylic structure are mutagenic as such and the inclusion of S-9 tends to decrease rather than increase the mutagenicity.

In the haloethene series, vinyl chloride is the most extensively studied compound. Vinyl chloride is a relatively weak mutagen, but its mutagenicity is substantially enhanced by the inclusion of an S-9 activation system. Bartsch et al. (74) have undertaken an extensive study of the mutagenicity of 10 haloalkenes; the relative potency of 6 haloethenes (with metabolic activa-

tion) follows the order: vinyl bromide > 1,1-dichloroethylene > vinyl chloride > trichloroethylene and 1,1-difluoroethylene (which are weakly or marginally active) > tetrachloroethylene (inactive). Compared to the 4 halobutenes and halobutadienes tested in the same study, the haloethenes are significantly less mutagenic (74). Using Escherichia coli as the test organism for mutagenicity, Henschler and associates (135, 190-192) postulated a rule which predicts that chlorinated ethenes with unsymmetric substitution (vinyl chloride, 1,1-dichloroethylene, trichloroethylene) are mutagenic, whereas those with symmetric substitution (1,2-dichloroethylene, tetrachloroethylene) are not (see also Section 5.2.2.1.4.2.1). This rule is partially supported by results obtained using the Ames test (see Table VII). However, exceptions to this rule, such as the lack of mutagenicity of trichloroethylene (182) and the positive result with tetrachloroethylene (164), have been observed by some investigators. In addition, the mutagenicity of cis- and trans-1,2-dichloroethylene has not been adequately tested. 1,1-Difluoro-2-bromo-2-chloroethene, a presumed metabolite of halothane, appears to be an unusual haloethene; it is mutagenic without metabolic activation (165).

In the halopropene series, a very good correlation between alkylating and mutagenic activities of at least 9 compounds has been noted by Eder et al. (70) and by Neudecker et al. (72). In agreement with the expected order of relative chemical reactivity, the mutagenic potency (with and without metabolic activation) of allyl halides follows the order: iodide > bromide >> chloride (70). Similarly, the relative order of potencies (without metabolic activation) -- cis-1,3-dichloro- > trans-1,3-dichloro- >> 3-chloro-2-methyl- > 2,3-dichloro- > 3-chloro- > inactive 2-chloro- and 1-chloropropenes -- is consistent with their relative alkylating activity (72). All the mutagenic halopropenes listed above have allylic structure and are mutagenic as such.

The inclusion of an S-9 system reduces the mutagenicity of all the halopropenes with the exception of 2,3-dichloropropene, which contains a vinylic as well as an allylic structure and whose mutagenicity may be increased by more than 30-fold by metabolic activation (72). The mutagenicity of brominated halopropenes appears to be less predictable from the chemical structure. The data of Stolzenberg and Hine (176) suggest the relative order of potencies (without metabolic activation): 2,3-dibromo- > 1-bromo- > 1,3-dichloro- > 2,3-dichloro- $\geq$ 2-bromo- > 1,2,3-trichloropropene. Mammalian S-9 enhances the mutagenicity of 1,2,3-trichloropropene, has little effect on 2,3-dichloropropene, and greatly reduces the mutagenicity of the other compounds. The potent direct-acting mutagenicity of 1-bromo- and 2-bromopropenes is somewhat unexpected from their vinylic structure.

In the halobutene group, the correlation between direct-acting mutagenicity and allylic structure is also quite evident. In the study of Neudecker et al. (72), the mutagenic potency of 6 halobutenes follows the order (in the absence of metabolic activation): 1-chloro-2-butene > 3-chloro-1-butene > 2-methyl-3-chloro-1-butene $\geq$ 1-chloro-2-methyl-2-butene; 2-chloro-2-butene and 4-chloro-1-butene are inactive. The four mutagenic compounds have allylic structures. The vinylic type compound, 2-chloro-2-butene, may be metabolically activated whereas 4-chloro-1-butene is inactive even in the presence of the S-9 system. No good correlation between mutagenicity and alkylating activity of the four halobutenes and halobutadienes was observed by Bartsch et al. (74). The relative potencies follow the order: 3,4-dichloro-1-butene > 1,4-dichloro-2-butene > 1-chlorobutadiene > 2-chlorobutadiene (without metabolic activation). 3,4-Dichloro-1-butene has a very weak alkylating activity in NBP reaction whereas 1,4-dichloro-2-butene and 1-chlorobutadiene are very strong alkylating agents. The mutagenicity of the four compounds is enhanced

by the S-9 fraction, irrespective of whether the structure is allylic or vinylic. With metabolic activation, 1,4-dichloro-2-butene becomes the most potent mutagen of the group.

The mutagenicity of benzyl chloride has been tested by Neudecker et al. (72). Consistent with its potent alkylating activity, the compound is a strong, direct-acting mutagen. Neudecker et al. (72) have pointed out that benzyl chloride may be considered to have an allylic structure.

Teratogenic Effects. Compared to mutagenicity and carcinogenicity, the teratogenicity of haloalkanes and haloalkenes has not been extensively investigated. A number of these compounds are being actively studied at the time of this writing.

Among halomethanes, dichloromethane (methylene chloride), chloroform, and carbon tetrachloride have been tested. Dichloromethane has been assayed in Sprague-Dawley rats and Swiss Webster mice by Schwetz et al. (193) and in Long-Evans rats by Hardin and Manson (194). In the former investigation rodents were exposed to an atmosphere containing 1,250 ppm dichloromethane for 7 h/day on days 6-15 of gestation, while in the latter study, rats were exposed to 4,500 ppm 6 h/day before and during gestation. Neither study revealed any statistically significant teratogenic effects. However, a critical review of these studies by U.S. Environmental Protection Agency (3) revealed methodological shortcomings which could cast doubt on the conclusion. There is evidence that inhalational exposure of rats to dichloromehane may induce behavioral changes in the offspring, as exhibited by the altered rates of behavioral habituation to novel environments (195).

Chloroform has been tested by inhalational and oral routes. Schwetz et al. (196) exposed Sprague-Dawley rats to air containing 30, 100, or 300 ppm

chloroform for 7 h/day on days 6-15 of gestation. The compound was found to be fetotoxic and teratogenic; the highest dose caused a significant increase in fetal resorption and the conception rate was only 15% (compared to 88% in the control group). Exposure to 100 ppm chloroform led to significant increases in the incidence of malformations including acaudia, imperforate anus, and missing ribs, while exposure to the lowest dose elicited delayed skull ossification and wavy ribs. Thompson et al. (197) administered daily oral doses of 20, 50, or 126 mg/kg to rats or 20, 35, or 50 mg/kg to rabbits on days 6-15 or 6-18 of gestation, respectively; no significant teratogenic or embryocidal effects were observed.

Carbon tetrachloride has no teratogenic effects in Sprague-Dawley rats exposed to 300 or 1,000 ppm of the compound for 7 h/day on days 6-15 of gestation (198). The compound was, however, slightly embryotoxic, inducing some degree of retarded fetal development, such as delayed ossification of sternebrae.

A number of haloethanes have been tested for teratogenicity in mammalian species. 1,1-Dichloroethane is not teratogenic in Sprague-Dawley rats after inhalational exposure to 3,800 or 6,000 ppm for 7 h/day on days 6-15 of gestation (198). 1,2-Dichloroethane is currently being tested in ICR Swiss mice by administration in drinking water in a multi-generation study (199). 1,2-Dibromoethane was tested in Charles River CD rats and CD-1 mice by inhalational exposure at concentrations of 20, 38, and 80 ppm for 23 h/day on days 6-15 of gestation. The compound had little primary effect on fetal development and was not considered teratogenic by the authors (200).

The teratogenicity of 1,1,1-trichloroethane has been tested by several groups of investigators. In the study of Schwetz et al. (193), the incidences

of fetal anomalies in Sprague-Dawley rats and Swiss Webster mice exposed to an atmosphere containing 875 ppm of the compound (7 h/day on day 8-15 of gestation) were not sigificantly higher than the control values. However, the study was considered inconclusive by the U.S. Environmental Protection Agency (201) owing to methodological shortcomings. In a preliminary communication, York et al. (202) reported that 1,1,1-trichloroethane did not induce significant teratogenic effects (neither structural nor behavioral) in Long-Evans rats continuously exposed by inhalation of 2,100 ppm of the haloethane before or during gestation. The teratogenic potential of 1,1,1-trichloroethane is also being investigated in ICR Swiss mice by administration in the drinking water in a multi-generation study (199).

The teratogenicity of halothane (1,1,1-trifluoro-2-bromo-2-chloroethane) has been extensively studied. Basford and Fink (203) exposed Sprague-Dawley rats to an anesthetic concentration (0.8%) of halothane for a prolonged period of time (12 h) on various days of gestation. Significant increases in the incidence of skeletal malformations were observed following exposure on days 8 and 9 of gestation. Even short periods (3 h) of anesthesia with halothane (1 or 1.5%) during organogenesis period were teratogenic in C-57Bl mouse, inducing cleft palate, limb hematomas, and ossification defects (204). However, in Charles River albino rats and New Zealand albino rabbits, brief exposures (1 h/day for 5 days) to anesthetic concentrations (1.35-1.43% for rats; 2.16-2.30% for rabbits) of halothane on days 1-5, 6-10, or 11-15 of gestation did not bring about significant teratogenic effects (205). Neither did prolonged exposures of Sprague-Dawley rats on days 8-12 of gestation to subanesthetic concentrations (50 to 3,200 ppm) of halothane produce no teratogenic effects (206). However, ultrastructural study of the neonatal brain tissues of rats continuously (40 h/week) exposed to 10 ppm halothane through-

out gestation revealed central nervous system damage including focal weakening, vacuoles, myelin figures, and occasional neuronal necrosis. The authors (207, 208) suggested that these anomalies could contribute to behavioral changes and poorer learning ability in the offspring.

Hexachloroethane has been tested in Sprague-Dawley rats by inhalation (15, 48, or 260 ppm) and gavage (50, 100, or 500 mg/kg) from day 6 through day 16 of gestation. The highest dose by each route caused slight maternal toxicity as shown by slight to moderate tremors. There were no significant skeletal or soft tissue anomalies in fetuses indicating a lack of teratogenicity of the compound. Doses that were maternally toxic elicited slight retardation of fetal development (144).

Relatively little information is available on the teratogenicity of haloalkenes. Vinyl chloride has no significant teratogenic effects in CFY rats exposed to air containing 4,000 mg/m^3 of the compound between the 8th and 14th days of gestation (209, 210). Its lack of teratogenicity was also reported by John et al. (211), who exposed CF-1 mice, Sprague-Dawley rats, and New Zealand white rabbits to an atmosphere containing 500 ppm vinyl chloride for 7 h/day during the period of organogenesis. Vinylidene chloride has been tested in Sprague-Dawley rats and New Zealand rabbits by inhalation (20, 80, or 160 ppm) or by ingestion (200 ppm in drinking water) in the study of Murray et al. (212). At doses which were not maternally toxic, no teratogenic effects were noted. Trichloroethylene and tetrachloroethylene were tested in Sprague-Dawley rats and Swiss Webster mice by Schwetz et al. (193). Exposure of the rodents to 300 ppm of either compound for 7 h/day during the period of organogenesis caused no maternal toxicity, fetotoxicity, or teratogenic effects. This study alone is probably inadequate to fully assess the teratogenic potential of trichloro- and tetrachloroethylene. Soviet researchers

have extensively studied the various health effects of 2-chloro-1,3-butadiene (chloroprene). The compound was found embryotoxic and teratogenic in rats by gastric intubation at 0.5 mg/kg or by inhalation at 1.0 ppm (213). However, this finding was not confirmed by Culik et al. (214), who failed to observe significant teratogenic effects in rats exposed to up to 25 ppm of the compound.

5.2.2.1.3 Carcinogenicity and Structure-Activity Relationships.

5.2.2.1.3.1 OVERVIEW.

Since the discovery of the carcinogenicity of carbon tetrachloride and chloroform in the early 1940's, some 40-50 haloalkanes and haloalkenes have been tested for carcinogenic activity. Actually, many of these studies were conducted in recent years in response to the concern which arose from the potent carcinogenicity of vinyl chloride in humans.

Although at least 24 haloalkanes have been investigated in long-term carcinogenicity studies, there is no firm evidence for or against carcinogenic activity. Among the halomethanes, only carbon tetrachloride and chloroform are carcinogenic in rodents by oral administration. Iodomethane, an active alkylating agent, is a locally active carcinogen. There is only a preliminary evidence for the carciongenicity of chlorofluoromethane. If confirmed, the generally held assumption of inactivity of fluorinated alkanes may have to be reassessed. Seven other halomethanes have been tested and found to have no convincing evidence for carcinogenicity; however, with the exception of triidomethane (iodoform), most of these studies are either equivocal or incompletely reported.

The most significant finding among studies of haloethanes is the potent carcinogenicity of 1,2-dibromoethane (ethylene dibromide). This compound is

carcinogenic by topical, oral, or inhalational route. The closely related 1,2-dichloroethane is also carcinogenic (although less potent) by topical or oral administration, but appears to be inactive via inhalation. Besides 1,2-dichloroethane, 6 other chlorinated ethanes have been investigated. The majority of these compounds have been shown to be carcinogenic in $B6C3F_1$ mice but inactive in Osborne-Mendel or F344 rats. All the carcinogenic chlorinated ethanes appear to have a structural similarity -- they are all 1,2-disubstituted (i.e., they have at least one halogen substitution on both carbons). The evidence for noncarcinogenicity of the geminally substituted (1,1-di-, and 1,1,1-tri-) chlorinated ethanes cannot be considered conclusive because of the high incidence of early mortality.

1,2-Dibromo-3-chloropropane (DBCP) is probably the most potent carcinogenic haloalkane thus far discovered. The compound is carcinogenic by topical, oral, or inhalational route, inducing malignant tumors with a high incidence. Significant carcinogenic effects were observed in rodents exposed to as little as 0.6 ppm DBCP in the air or 3 mg/kg/day in the diet. The potent carcinogenicity of DBCP is consistent with the structural requirement of 1,2-dihalogenation observed in haloethanes.

Eleven haloalkenes have thus far been tested in long-term studies. The well-known carcinogenicity of vinyl chloride has been established in at least 16 different studies (mostly by inhalation). The compound induces a variety of tumors in different strains of mice and rats, in the hamster, and in the rabbit. Inhalation appears to be the most effective route. Atmospheric concentration of 5 ppm of vinyl chloride is significantly carcinogenic in the rat. The closely related vinyl bromide is also active. Among the other chlorinated ethenes, vinylidene chloride (1,1-dichloethylene) was found to be a relatively weak carcinogen in early studies. However, more recent investi-

gations do not provide convincing evidence for its carcinogenicity. Similarly, trichloroethylene has been shown to be carcinogenic in two studies but inactive in 7 other studies; the role of possible impurities present in the samples of trichloroethylene used in these studies has not been thoroughly investigated. Technical-grade tetrachloroethylene was reported to induce hepatocellular carcinomas in mice after oral administration but is tentatively considered inactive in the rat. Considerably less information is available on higher haloalkanes. A preliminary report suggests the carcinogenicity of trans-1,4-dichloro-2-butene toward the nasal cavity after inhalation. 2-Chloro-1,3-butadiene (chloroprene) appears to be noncarconigenic whereas hexachloro-1,3-butadiene is carcinogenic at high doses.

To provide a bird's eye view of the relative carcinogenic potency of various haloalkanes and haloalkenes, the mouse bioassay data of U.S. National Cancer Instiute, and the skin carcinogenesis data of Van Duuren and coworkers (215-217) are summarized in Tables VIII and IX, resepctively. It can be assessed from the data in Table VIII that, on a molar basis, 1,2-dibromoethane and 1,2-dibromo-3-chloropropane are the most potent carcinogens, followed by 1,1,2,2-tetrachloroethane, chlorofrom, 1,1,2-trichloroethane, and carbon tetrachloride. Among chloroethanes, 1,1,2,2-tetrachloroethane is most potent; the potency tends to diminish with either an increase or a decreae in chlorination. When tested by the topical route (Table IX), all four 1,2-disubstituted haloalkanes are carcinogenic, inducing "distant" tumors in the lung or forestomach; only 1,2-dibromoethane is locally active. The induction of "distant" tumors may be ascribed to skin absorption or ingestion, through animal grooming, of the haloalkanes. In two-stage skin carcinogenesis studies, only 1,2-dibromo-3-chloropropane, vinylidene chloride and allyl chloride are active as tumor-initiators. Among compounds tested by subcu-

Table VIII
Comparative Carcinogenicities of Haloalkanes and Haloalkenes in $B6C3F_1$ Mice by Oral Administration[a]

Compound	Sex	Dose (mg/kg)	Significant Neoplasm	Incidence (%) Control	Low-dose	High-dose
A) Haloalkanes						
Chloroform[b]	M	138 or 227	Hepatocellular carcinoma	6	36	98
	F	238 or 447	Hepatocellular carcinoma	1	80	95
Iodoform	M or F	47 or 93	None	--	--	--
Carbon tetrachloride[b]	M	1,250 or 2,500	Hepatocellular carcinoma	5	100	98
	F	1,250 or 2,500	Hepatocellular carcinoma	0	100	96
Trichlorofluoromethane	M or F	1,962 or 3,925	None[c]	--	--	--
1,1-Dichloroethane	M	1,442 or 2,885	None[c]	--	--	--
	F	1,665 or 3,331	None[c]	--	--	--
1,2-Dichloroethane[b]	M	97 or 195	Alveolar/bronchiolar adenoma	0	2	31
	F	149 or 229	Alveolar/bronchiolar adenoma	5	14	31
			Mammary adenocarcinoma	0	18	15
			Endometrial stromal polyp or sarcoma	0	10	11
1,2-Dibromoethane[b]	M	62 or 107	Forestomach squamous cell carcinoma	0	90	59
			Alveolar/bronchiolar adenoma	0	9	21
	F	62 or 107	Forestomach squamous cell carcinoma	0	94	56
			Alveolar/bronchiolar adenoma	0	23	13

Table VIII (continued)

Compound	Sex	Dose (mg/kg)	Significant Neoplasm	Incidence (%) Control	Low-dose	High-dose
1,1,1-Trichloroethane	M or F	2,870 or 5,615	None[c]	--	--	--
1,1,2-Trichloroethane	M	195 or 390	Hepatocellular carcinoma	10	37	76
			Adrenal pheochromocytoma	0	0	17
	F	195 or 390	Hepatocellular carcinoma	0	33	89
			Adrenal pheochromocytoma	0	0	28
1,1,2,2-Tetrachloroethane	M	142 or 282	Hepatocellular carcinoma	6	26	90
	F	142 or 282	Hepatocellular carcinoma	0	63	91
Pentachloroethane[d]	M	250 or 500	Hepatocellular carcinoma	8	55	22
	F	250 or 500	Hepatocellular carcinoma	0	60	31
Hexachloroethane	M	590 or 1,190	Hepatocellular carcinoma	15	30	63
	F	590 or 1,190	Hepatocellular carcinoma	10	40	31
1,2-Dibromo-3-chloropropane	M	114 or 219	Forestomach squamous cell carcinoma	0	93	98
	F	114 or 219	Forestomach squamous cell carcinoma	0	100	98
B) Haloalkenes						
Vinylidene chloride (1,1-Dichloroethylene)	M	2 or 10	None	--	--	--
	F	2 or 10	None	--	--	--
Trichloroethylene	M	1,169 or 2,339	Hepatocellular carcinoma	5	52	65
	F	869 or 1739	Hepatocellular carcinoma	0	8	23

Table VIII (continued)

Compound	Sex	Dose (mg/kg)	Significant Neoplasm	Incidence (%)		
				Control	Low-dose	High-dose
Tetrachloroethylene	M	536 or 1,072	Hepatocellular carcinoma	10	65	56
	F	386 or 772	Hepatocellular carcinoma	0	40	40
Allyl chloride	M	172 or 119	None[e]	--	--	--
	F	129 or 258	None[e]	--	--	--

[a]Summarized from National Cancer Institute/National Toxicology Program Carcinogenesis Technical Reports No. 2, 3, 13, 27, 28, 55, 66, 73, 74, 86, 106, 110, and 228.

[b]Also carcinogenic in the rat.

[c]Considered inconclusive because of insufficiently high dose (below maximal tolerated dose) or because inadequate number of mice survived long enough to be at risk from late-developing tumors.

[d]Preliminary data.

[e]Suggestive of positive association with neoplastic lesions of the forestomach.

Table IX
Carcinogenicity of Haloalkanes and Haloalkenes by Topical Application or Subcutaneous Injection

Compound	As Initiator	Repeated Topical Applications		Local Sarcoma After s.c. Injection
		Local Tumor	Distant Tumor	
A) Haloalkanes				
1,2-Dichloroethane	-	-	Lung	n.t.[b]
1,2-Dibromoethane	-	+	Lung	n.t.
1,1,2,2-Tetrabromoethane	-	-	Forestomach	n.t.
1,2-Dibromo-3-chloropropane	+	-	Lung, forestomach	n.t.
B) Haloalkenes				
Vinyl bromide	-	-	None	-
Vinylidene chloride	+	-	None	-
Trichloroethylene	-	-	None	-[c]
Tetrachloroethylene	-	-	None	n.t.
1-Chloropropene	-	-	None	-[d]
Allyl chloride	+	-	None	-
cis-1,3-Dichloropropene	-	-	None	+
trans-1,4-Dichloro-2-butene	-	-	None	+[e]
Hexachlorobutadiene	-	-	None	n.t.

[a] Summarized from B.L. Van Duuren, B.M. Godlschmidt, and I. Seidman [Cancer Res. 35, 2553 (1975)]; B.L. Van Duuren [Environ. Hlth. Persp. 21, 17 (1977)]; B.L. Van Duuren, B.M. Goldschmidt, G. Loewengant, A.C. Smith, S. Melchionne, I. Seidman, and D. Roth [J. Natl. Cancer Inst. 63, 1433 (1979)].

[b] Not tested.

[c] Also inactive by oral administration.

[d] Induced stomach tumors after oral administration.

[e] Inactive by i.p. administration.

taneous administration, only cis-1,3-dichloropropene and trans-1,4-dichloro-2-butene display local carcinogenic activity, inducing local sarcoma at the site of injection.

A number of haloalkanes and haloalkenes have been tested in three short-term assays: pulmonary adenoma (218), in vitro cell transformation (219), and preneoplastic hepatocellular foci (220-222). Of the twenty-two haloalkanes tested in the pulmonary assay (Table X), eleven (mostly iodoalkanes and butyl halides) are active while two (dichloromethane and 1,1,2,2-tetrachloroethane) display marginal activity. With the exception of t-butyl iodide (tested at a very low dose) and iodoethane, all iodoalkanes tested are active. The negative finding of iodoethane is surprising in view of the fact that its lower and higher homologs are all active. Among the butyl halides tested, t- and s-butyl chlorides are both active while their n-isomer is not. This is consistent with the expected higher alkylating activity (via S_N1 mechanism) of tertiary and secondary than of primary alkyl halides. The same relationship may also hold for butyl bromides, although the negative finding of n-butyl bromide is not as convincing because of the low dose administered. In the cell transformation assay of Price et al. (219), dichloromethane, 1,1,1-trichloroethane, trichloroethylene and tetrachloroethylene have been shown to induce phenotypic transformation of F1706 rat embryo cells. Some of the transformed cells grew as undifferentiated fibrosarcomas after innoculation into newborn rats. The transformation activity of dichloromethane is not substantiated by the study of Sivak (cited in ref. 223) using a purified sample of the compound. Bolt and associates (220-222) have tested the ability of seven haloethanes to induce ATPase-deficient preneoplastic hepatocellular foci in newborn rats. The relative activity follows the order: vinyl chloride > vinyl fluoride > vinyl bromide > vinylidene fluoride > vinylidene

Table X
Relative Carcinogenic Potency of Haloalkanes and Haloalkenes
(Pulmonary Adenoma Bioassay)[a]

Compound	Total Dose[b]	No. Lung Tumors/Mouse A/He	No. Lung Tumors/Mouse A/St
Negative controls	--	0.21-0.36	0.19-0.39
Iodomethane (methyl iodide)	0.31	0.55*	
	0.15	0.30	
Dichloromethane (methylene chloride)	151.0		0.50
	32.0		0.94[c]
Trichloromethane (chloroform)	40.2		0.00
	16.2		0.35
Bromodichloromethane	14.6		0.85
Tribromomethane (bromoform)	9.5		0.67
	4.4		1.13*
Bromoethane (ethyl bromide)	55.0	0.35	
Iodoethane (ethyl iodide)	38.4	0.15	
1,2-Dichloroethane (ethylene dichloride)	24.3		0.75
1,1,2,2-Tetrachloroethane	38.1		1.00[c]
1-Iodopropane (n-propyl iodide)	17.6	0.70*	
	7.1	0.22	
2-Iodopropane (i-propyl iodide)	35.2	0.58*	
	17.6	0.44	
	7.0	0.53*	

Table X (continued)

Compound	Total Dose[b]	No. Lung Tumors/Mouse A/He	A/St
1-Chlorobutane (n-butyl chloride)	65.0	0.31	
2-Chlorobutane (s-butyl chloride)	35.0	1.20*	
	17.5	0.67	
2-Chloro-2-methylpropane (t-butyl chloride)	65.0	1.00*	
	32.4	0.73*	
	12.9	0.64	
1-Bromobutane (n-butyl bromide)	1.2	0.14	
1-Bromo-2-methylpropane (i-butyl bromide)	43.7	0.75*	
	21.8	0.64*	
	8.7	0.42	
2-Bromobutane (s-butyl bromide)	43.7	1.15*	
	21.8	1.00*	
	8.7	0.35	
2-Bromo-2-methylpropane (t-butyl bromide)	43.7	0.78*	
	21.8	0.73*	
	8.7	0.53	
1-Iodobutane (n-butyl iodide)	13.1	0.63*	
	6.6	0.60*	
	2.6	0.63*	
2-Iodobutane (s-butyl iodide)	32.6	0.63*	
	16.3	0.33	

Table X (continued)

Compound	Total Dose[b]	No. Lung Tumors/Mouse A/He	A/St
2-Iodo-2-methylpropane (t-butyl iodide)	2.7	0.42	
1-Chlorooctane (n-octyl chloride)	64.4		0.15
Benzyl chloride	15.8	0.25	
1-chloromethyl naphthalene	7.0	0.22	
Tetrachloroethene (tetrachloroethylene)	57.9		0.50
Hexachloro-1,3-butadiene	0.37		0.36
Positive control (urethane)	--	8.1-17.8*	19.6*

[a]Summarized from L. A. Poirier, G. D. Stoner and M. B. Shimkin, Cancer Res. 35, 1411 (1975); J. C. Theiss, G. D. Stoner, M. B. Shimkin and E. K. Weisburger, Cancer Res. 37, 2717 (1977).

[b]Total dose over the 24-week period (mmoles/kg). For negative results, only the highest dose was listed.

[c]Not significant but with p-value close to 0.05.

*Significantly higher than negative controls with $p < 0.05$.

chloride > trichloroethylene and tetrachloroethylene (which are inactive). The difference in activity is believed to be related to the rate of metabolic activation to the presumed epoxide intermediates and the selectivity of the epoxides to react with DNA in target organs.

5.2.2.1.3.2 HALOMETHANES.

5.2.2.1.3.2.1 Carbon Tetrachloride. Investigations on the carcinogenicity of carbon tetrachloride (CCl_4) have been the subject of several recent reviews (2, 9, 10); the major findings are summarized in Table XI. Carbon tetrachloride has also been frequently used in syncarcinogenesis and modification studies discussed in Section 5.2.2.1.3.8. Carbon tetrachloride has been shown to be a liver carcinogen in 3 mammalian species and in the rainbow trout.

The hepatocarcinogenicity of CCl_4 was first reported in 1941 by Edwards (47). Subsequent experiments by Edwards and coworkers (224, 225) showed that CCl_4 was carcinogenic in five strains of mice. These mice received oral administration (gavage) of 0.1 ml of a 40% CCl_4 solution in olive oil 2-3 times per week for various periods of time and were sacrificed around 1 year of age. A slight degree of strain difference in susceptibility of the mice to the hepatocarcinogenic effect of CCl_4 was observed. The respective incidences of liver tumor were: C3H, 88%; A, 98%; Y, 60%; C, 83%; L (male), 47-54%; L (female), 27-38%. The spontaneous liver tumor incidence at the age of around 1 year was below 4% for all five strains. The liver tumors usually emerged following acute necrosis and subsequent cirrhosis.

Eschenbrenner and Miller (226, 227) confirmed the hepatocarcinogenicity of CCl_4 using strain A mice. In addition, they investigated the effects of size and spacing of multiple doses on the incidence of CCl_4-induced hepatomas and the relationship between liver necrosis and tumor induction. Mice were

Table XI
Carcinogenicity of Carbon Tetrachloride

Species & Strain	Route	Principal Organs Affected	References
Mouse, A, C3H, C, L or Y	oral	Liver	(47, 224, 225)
Mouse, A	oral	Liver	(226, 227)
Mouse, C3H	oral	Liver	(228, 229)
Mouse BUB	oral	Liver	(230)
Mouse, C3H	oral	Liver	(231)
Mouse, C3H	rectal instillation	Liver (nodules)	(232)
Mouse, XVII/G	oral	Liver	(233)
Mouse, $B6C3F_1$	oral	Liver	(234)
Rat, albino	inhalation	Liver	(235)
Rat, Buffalo or Wistar	subcutaneous	Liver	(236-238)
Rat, Japanese or Osborne-Mendel	subcutaneous	Liver, thyroid gland	(237)
Rat, --	subcutaneous	Mammary gland	(239)
Rat, Osborne-Mendel	oral	Liver	(234)
Hamster, Syrian golden	oral	Liver	(240)

given 30 graded doses (0.1, 0.2, 0.4, 0.8, and 1.6 ml/kg of body weight) of CCl_4; the interval between consecutive doses ranged from 1 to 5 days. They observed that the tumor incidence and the size of hepatomas progressively increased with increase in dose, as well as with the increase of the interval between the consecutive doses (226). To investigate whether liver necrosis is a prerequisite for tumor induction, Eschenbrenner and Miller (227) divided carcinogenic "necrotizing" doses into smaller "nonnecrotizing doses" and administered the smaller doses more frequently to provide the same total doses. Hepatoma induction was observed in mice with no signs of liver necrosis. The authors concluded that: "While it was found that a correlation exists between the degree of liver necrosis and the incidence of hepatomas in relation to dose, the use of a graded series of necrotizing and nonnecrotizing doses indicated that repeated liver necrosis and its associated chronic regenerative state are probably not necessary for the induction of tumors with carbon tetrachloride" (227).

The hepatocarcinogencity of CCl_4 in the mouse has also been confirmed in several other studies using C3H, BuB, XVII/G, and $B6C3F_1$ strains (see Table XI). In a NCI study (234), nearly all the mice (see Table VIII) developed hepatocellular carcinomas by the end of the bioassay. A study by Confer and Stenger (232) showed that CCl_4 was also hepatocarcinogenic by intrarectal administration. Thirteen of the 25 male C3H mice that received biweekly doses of 0.1 ml of a 40% solution of CCl_4 in olive oil for 20-26 weeks developed liver tumors described as nodular hyperplasia. No such tumors occurred in 10 vehicle-treated control mice.

The carcinogenicity of CCl_4 in the rat has been tested by inhalational, subcutaneous, and oral routes. Costa et al. (235) exposed a group of albino rats to an atmosphere containing CCl_4 for 7 months. Among the 30 survivors,

10 had liver nodules diagnosed histologically as early or established liver carcinomas. The details of the study and the type of control were not given. Reuber and Glover (236-238) investigated the carcinogenicity of CCl_4 in 6 different strains (Buffalo, Japanese, Osborne-Mendel, Wistar, Black, Sprague-Dawley) of rats following biweekly subcutaneous injections of 1.3 ml/kg of a 50% solution of CCl_4 in corn oil. A low incidence of small hepatomas was observed in Buffalo strain rats of both sexes treated at the age of 24 or 52 weeks (236). The hepatocarcinogenicity of CCl_4 in Buffalo strain rats was enhanced by simultaneous administration of 3-methylcholanthrene (238). Japanese, Osborne-Mendel, and Wistar rats appeared to be considerably more susceptible to the hepatocarcinogenic action of CCl_4; their respective incidences of hepatocellular carcinomas were 80% (12/15), 62% (8/13), and 33% (4/12). Black and Sprague-Dawley rats died shortly after treatment. This strain difference was found to be due to the hepatotoxicity of CCl_4. As shown in Table XII, Black and Sprague-Dawley rats developed severe liver cirrhosis and survived an average of 11 and 13 weeks, respectively. The susceptible strains had milder cirrhosis and survived long enough for the development of tumors (237). In addition to liver tumors, there were carcinomas of the thyroid gland in three Osborne-Mendel and three Japanese rats (237). The mammary gland appeared to be the principal target organ of an unspecified strain of white female rats in the study of Alpert et al. (239). Of the thirty rats that received biweekly subcutaneous injections of 1 ml/kg CCl_4 for 2 years, eight developed mammary adenocarcinomas, one had a mammary fibroadenoma. No such tumors were observed in fifteen untreated control rats. In sharp contrast to the potent hepatocarcinogenicity in mice, CCl_4 appears to be inactive or at most marginally active in the Osborne-Mendel rat by oral administration (234).

Table XII
Strain-dependent Induction of Liver Cirrhosis, Nodules and Carcinomas in Male Rats by Carbon Tetrachloride[a]

Strain	No. of rats at start	No. of rats with cirrhosis				No. of rats with Hyperplastic Nodules	No. of rats with Carcinoma	Average Survival (weeks)
		Mild	Moderate	Severe	Total			
Japanese	15	9	5	1	15	3	12 (80%)	47
Osborne-Mendel	13	2	7	4	13	4	8 (62%)	44
Wistar	12	0	6	6	12	7	4 (33%)	33
Black	17	0	4	13	17	7	0 (0%)	11
Sprague-Dawley	16	0	0	16	16	2	0 (0%)	13

[a]Summarized from M. D. Reuber and E. L. Glover [J. Nat. Cancer Inst. 44, 419 (1970).] All rats were 12 weeks old at the start of the experiment. They were given subcutaneous injections of a 50% solution of CCl_4 in corn oil twice a week and were sacrificed when they became moribund.

In addition to rats and mice, CCl_4 has been shown to be hepatocarcinogenic in the hamster and the rainbow trout. Della Porta et al. (240) administered 30 weekly oral doses of 6.25-12.5 µl CCl_4 in 5% corn oil solution to 20 (10 of each sex) 12-week-old Syrian golden hamsters. All 10 (5 of each sex) animals that survived 10 or more weeks beyond the end of treatment developed liver-cell carcionmas. Halver (241) fed rainbow trout diets containing 3.2 and 12.8 ppm CCl_4. After 20 months, 4/44 low dose group and 3/34 high dose group developed hepatomas; none were found in controls.

5.2.2.1.3.2.2 Chloroform. The carcinogenicity of chloroform ($CHCl_3$) has been tested in the mouse, the rat, and the dog. These bioassay studies have been reviewed at some preliminary stages by Winslow and Gerstner (5), Reuber (6), and IARC (2); the final reports on some of the data have been published (242-245). Table XIII summarizes these findings. Chloroform was first reported to be hepatocarcinogenic in the mouse by Eschenbrenner and Miller (48) in 1945. Groups of 5 strain A mice were given 30 oral doses of 0.1, 0.2, 0.4, 0.8, or 1.6 ml/kg (body weight) $CHCl_3$ in olive oil. The high doses were toxic and killed many mice early in the experiment. A high incidence of hepatomas and liver cirrhosis was observed among the survivors. No hepatomas were found in the low dose groups and the control group. Rudali (233) administered by gavage 0.1 ml of a 40% solution of $CHCl_3$ in oil twice a week for 6 months to 24 NLC mice. Of the five animals that survived 297 days, three had hepatomas. No hepatomas were observed in several other groups of mice given a number of tetrahalomethanes other than CCl_4 (see Section 5.2.2.1.3.2.3). The hepatocarcinogenicity of $CHCl_3$ in the mouse has been confirmed in a NCI study (242). Groups of 50 $B6C3F_1$ mice of each sex were given (by gavage) a 2-5% solution of $CHCl_3$ in corn oil 5 times/week for 78 weeks. The average doses were 138 and 277 mg/kg for males and 238 and 477 mg/kg for females. The mice

Table XIII
Carcinogenicity of Chloroform

Species & Strain	Route	Principal Organs Affected	References
Mouse, A	oral	Liver	(48)
Mouse, NLC	oral	Liver	(233)
Mouse, $B6A2F_1$	subcutaneous	No significant effect	(246)
Mouse, $B6C3F_1$	oral	Liver	(242)
Mouse, ICI-Swiss	oral	Kidney (males only)	(243)
Mouse, C57BL, CBA or CF-1	oral	None	(243)
Rat, Osborne-Mendel	oral	Kidney (males only)	(242)
Rat, Sprague-Dawley	oral	No significant effect	(244)
Dog, beagle	oral	No significant effect	(245)

were sacrificed at 92-93 weeks. Significant hepatocarcinogenic effect of $CHCl_3$ were observed (see Table VIII); nearly all the mice in the high dose group had hepatocellular carcinomas. In contrast to these studies, Roe et al. (243) observed no significant carcinogenic effects of $CHCl_3$ in 3 strains (C57BL, CBA, or CF-1) of male mice that had received $CHCl_3$ by gavage at doses up to 60 mg/kg/day, 6 days/week for 80 weeks. In a fourth strain (ICI-Swiss), males (but not females) in 60 mg/kg/day group had significantly higher incidence of epithelial tumors of the kidney. No significant effects were noted in mice exposed to a lower dose (17 mg/kg/day) of $CHCl_3$. Besides oral administration, Roe et al. (246) also tested $CHCl_3$ injected subcutaneously as a single dose of 0.2 mg in arachis oil, or 8 daily doses of 0.2 mg to newborn $(C57 \times DBA2)F_1$ mice in the intrascapular region. After 77-80 weeks, no evidence of significant carcinogenic effects of $CHCl_3$ was found. The significance of this study may be questionable because of the low dose.

The carcinogenicity of $CHCl_3$ in the rat has also been evaluated. In an NCI study (242), Osborne-Mendel rats were given 90 and 180 mg/kg (males) or 100 and 200 mg/kg (females) $CHCl_3$ in corn oil 5 days/week for 78 weeks and were then sacrificed after 111 weeks. The most significant finding was the induction of kidney epithelial tumors in male rats with incidences of 0, 8, and 24% in the control, low dose, and high dose groups, respectively. A statistically significant increase in thyroid tumors in treated female rats was also observed; however, this finding was not considered to be "biologically significant" (242). Reuber (6) has recently re-examined the NCI data; combining the data on cholangiofibromas, hyperplastic nodules, and carcinomas of the liver, he concluded that $CHCl_3$ was hepatocarcinogenic in the rat with the females being more susceptible than the males. In another study in the rat, Palmer et al. (244) found no significant carcinogenic effects of

$CHCl_3$ in Sprague-Dawley rats of both sexes which received oral doses of 60 mg/kg/day $CHCl_3$ 6 days/week for 80 weeks and then observed for up to a total of 95 weeks. The tumor incidence was 39% in $CHCl_3$-treated rats and 38% in vehicle-treated controls.

The potential carcinogenicity of $CHCl_3$ has also been evaluated using beagle dogs. Heywood et al. (245) gave beagle dogs $CHCl_3$ equivalent to 15 or 30 mg/kg/day, 6 days/week for 7.5 years and the animals were observed for an additional 20-24 weeks. A number of macroscopic and microscopic tumors (mostly testicular and mammary) were found in both the $CHCl_3$-treated and the control groups. No tumors were seen in the liver and kidney. Despite relatively high incidences of testicular and mammary tumors in the low dose group the authors (245) did not attribute tumor induction to $CHCl_3$ treatment because of the long duration of the study and the lack of dose-dependence. Heywood et al. (245) concluded that exposure of beagle dogs to $CHCl_3$ had no effect on the incidence of tumors in beagle dogs.

5.2.2.1.3.2.3 Halomethanes Other than Carbon Tetrachloride and Chloroform. Besides carbon tetrachloride and chloroform, 12 other halomethanes have been tested for carcinogenicity and the results of these studies are summarized in Table XIV. Only one monohalomethane has thus far been studied. Druckrey et al. (68) reported that iodomethane (methyl iodide) induces local sarcomas at the site of subcutaneous injection. Groups of BD rats were given either a single dose of 50 mg/kg or weekly injections of 10 or 20 mg/kg iodomethane in vegetable oil for one year; the incidences of local sarcomas after lifetime observation were 4/14, 9/12, and 6/6, respectively. The average latent period was 580-610 days. No local sarcomas were found in the vehicle-treated control rats. Iodomethane appears to be also carcinogenic by intraperitoneal injections. Moreover, in the pulmonary adenoma bioassay study by Poirier et al.

Table XIV
Carcinogenicity of Halomethanes Other Than Chloroform and Carbon Tetrachloride

Compound	Species & Strain	Route	Principal Organs Affected	Reference
Iodomethane (Methyl iodide)	Mouse, A/He	i.p.	Lung[b]	(247)
	Rat, BD	s.c.	Local sarcoma	(68)
Chlorofluoromethane	Rat, --	unspecified	unspecified	(172)
Dichloromethane	Rat, Sprague-Dawley	inhalation	None[c]	(248-250)
(Methylene chloride)	Hamster, --	inhalation	None	(248-250)
Bromodichloromethane	Mouse, XVII/G, NLC or RIII/f	oral	None[d]	(233)
Dibromochloromethane	Mouse, XVII/G, NLC or RIII/f	oral	None[d]	(233)
Tribromomethane (Bromoform)	Mouse, A/St	i.p.	Lung[b]	(251)
Triiodomethane (Iodoform)	Mouse, $B6C3F_1$	oral	None	(252)
	Rat, Osborne-Mendel	oral	None	(252)
Trichlorofluoromethane	Mouse, Swiss	s.c.	None	(253)
(Freon 11)	Mouse, $B63F_1$	oral	None	(254)
	Rat, Osborne-Mendel	oral	None (inconclusive)[e]	(254)
Trichlorobromomethane	Mouse, XVII/G, NLC or RIII/f	oral	None[d]	(233)
Dibromodichloromethane	Mouse, XVII/G, NLC or RIII/f	oral	None[d]	(233)
Tetrabromomethane	Mouse, XVII/G, NLC or RIII/f	oral	None[d]	(233)

[a] In addition to the studies listed above, dichloromethane, and bromodichloromethane have been tested in pulmonary adenoma assay and found to have no significant effect (see Table X).

[b] Lung adenomas (see Table X).

[c] A possible increase in the incidence of benign mammary tumors was noted (cited in ref. 3).

[d] It is not certain whether the duration of the experiment was sufficiently long (see text).

[e] Inadequate number of rats survived long enough to be at risk from late-developing tumors.

(247), iodomethane caused a significant increase in the number of tumors/mouse (see Table X).

Very little information is available on the carcinogenicity of dihalomethanes. In an industry-sponsored study published in the trade literature (248-250), it was reported that dichloromethane (methylene chloride) is not carcinogenic in rats and hamsters of both sexes. In these studies, approximately 2,000 animals were exposed via inhalation to 0, 500, 1,500, and 3,500 ppm dichloromethane for 6 hr/day, 5 days/week for 2 years; details of the methodology have not been given. With the exception of benign mammary tumors in rats of both sexes, there was no increase in the incidence of malignancies in exposed animals. The increase in benign mammary tumor was attributed to spontaneous incidence in this strain (Sprague-Dawley) of rats. However, the U.S. Interagency Regulatory Liaison Group suggested that the observation may be an indication of the oncogenic potential of the compound (3). Dichloromethane slighly increased the number of lung tumors/mouse (see Table X) in the pulmonary adenoma assay by Theiss et al. (251); the increase had marginal statistical significance ($p = 0.054$). In a recent abstract, Green (172) has claimed that chlorofluoromethane is carcinogenic in the rat. Chlorofluoromethane was found to be mutagenic in the Ames Salmonella test.

Bromodichloromethane and dibromochloromethane were reported to be noncarcinogenic in a brief report by Rudali (233). In this study, groups of mice were given oral doses of 0.1 ml of a 40% solution of the trihalomethanes in oil. Although the duration of the treatment and the length of the observational period were not reported, in the same study chloroform was hepatocarcinogenic after 297 days. In the pulmonary adenoma assay by Theiss et al. (251), bromodichloromethane was inactive while tribromomethane (bromoform) had a significant effect (see Table X). Triiodomethane (iodoform) has been tested

in Osborne-Mendel rats and $B6C3F_1$ mice of both sexes in a NCI bioassay (252); no significant carcinogenic effects were observed.

Besides carbon tetrachloride, four tetrahalomethanes have been bioassayed for carcinogenicity. Trichlorofluoromethane (Freon 11) has no significant carcinogenic effects one year after repeated subcutaneous injections into neonatal Swiss ICR/Ha mice (253). An NCI study (254) confirmed the lack of carcinogenicity of trichlorofluoromethane in the mouse; $B6C3F_1$ mice that received average oral doses of 1,962 and 3,925 mg/kg/day, 5 days/week for 78 weeks did not develop tumors attributable to the treatment. Osborne-Mendel rats were also used in the study. However, the doses administered (488 and 977 mg/kg/day for males; 538 and 1,077 mg/kg/day for females) caused a high rate of early deaths so that an insufficient number of rats survived long enough to exclude the possibility of late-developing tumors. Three tetrahalomethanes (trichlorobromomethane, dibromodichloromethane, and tetrabromomethane) were tested by Rudali (233). Groups of mice received 0.1 ml of either a 10% solution of trichlorobromomethane or dichlorodibromomethane or a 40% solution of tetrabromomethane for an unspecified period of time. None of these compounds was carcinogenic. The two chlorinated compounds (CCl_3Br and CCl_2Br_2) were hepatotoxic, while tetrabromomehane was not. In the same study, oral administration of a 40% and a 25% solution of carbon tetrachloride (used as positive control) led to hepatoma incidences of 91% and 5%, respectively.

5.2.2.1.3.3 HALOETHANES.

Over a dozen haloethanes have been tested for carcinogenicity. The relative carcinogenic potency of various haloethanes tested orally in $B6C3F_1$ mice is given in Table VIII (Section 5.2.2.1.3.1). More detailed information on the conditions of carcinogenicity testing of haloethanes is tabulated in Table XV.

Table XV
Carcinogenicity of Haloethanes[a]

Compound	Species & Strain	Route	Principal Organs Affected	Reference
A) Dihaloethanes				
1,1-Dichloroethane	Mouse, $B6C3F_1$	oral	None (inconclusive)[b]	(255)
	Rat, Osborne-Mendel	oral	None (inconclusive)[b]	(255)
1,2-Dichloroethane (Ethylene dichloride)	Mouse, $B6C3F_1$	oral	Lung, mammary gland, uterus	(256, 257)
	Mouse, Swiss ICR/Ha	topical	Lung	(216)
	Mouse, Swiss	inhalation	None	(258)
	Rat, Osborne-Mendel	oral	Forestomach, mammary gland, circulatory system, subcutaneous tissues	(256, 257)
	Rat, Sprague-Dawley	inhalation	None	(258)
1,2-Dibromoethane	Mouse, $B6C3F_1$	oral	Forestomach, lung	(259, 260)
		inhalation	Lung, subcutaneous tissue, nasal cavity, mammary gland	(261)
	Mouse, Swiss ICR/Ha	topical	Skin, lung	(216)
	Rat, Osborne-Mendel	oral	Forestomach, circulatory system, liver	(259, 260)
	Rat, F344	inhalation	Nasal cavity, circulatory system, pituitary gland, genital tract, lung, mammary gland	(261)
	Rat, Sprague-Dawley	inhalation	Spleen (preliminary)[c]	(262)

Table XV (continued)

Compound	Species & Strain	Route	Principal Organs Affected	Reference
B) Trihalomethanes				
1,1,1-Trichloroethane (Methyl chloroform)	Mouse, $B6C3F_1$	oral	None (inconclusive)[b]	(263)
	Rat, Osborne-Mendel	oral	None (inconclusive)[b]	(263)
	Rat, Sprague-Dawley	inhalation	None (1-year study only)	(Quast et al 1978, cited in ref. 201)
1,1,2-Trichloroethane	Mouse, $B6C3F_1$	oral	Liver, adrenal gland	(264)
	Rat, Osborne-Mendel	oral	None	(264)
C) Tetrahaloethanes				
1,1,2,2-Tetrachloroethane	Mouse, $B6C3F_1$	oral	Liver	(265)
	Rat, Osborne-Mendel	oral	None (inconclusive)[b]	(265)
1,1,2,2-Tetrabromoethane	Mouse, Swiss ICR/Ha	topical	Forestomach	(216)
D) Pentahaloethane				
Pentachloroethane	Mouse, $B6C3F_1$	oral	Liver (preliminary)	J. Mennear, NCI/NTP, personal communication
	Rat, F344	oral	None (preliminary)	J. Mennear, NCI/NTP, personal communication
1,1,1-Trifluoro-2-bromo-2-chloroethane (Halothane)	Mouse, Swiss ICR (perinatal exposure)	inhalation	None	(266)

Table XV (continued)

Compound	Species & Strain	Route	Principal Organs Affected	Reference
E) Hexahaloethanes				
1,1,2-Trichloro-1,2,2-trifluoroethane (Freon-113)	Mouse, Swiss	s.c.	None	(253)
1,1,2,2-Tetrachloro-1,2-difluoroethane (Freon-112)	Mouse, Swiss	s.c.	None	(253, 267)
Hexachloroethane	Mouse, $B6C3F_1$	oral	Liver	(268)
	Rat, Osborne-Mendel	oral	None	(268)

[a] In addition to the studies listed above, bromoethane, iodoethane, 1,2-dichloroethane, and 1,1,2,2-tetrachloroethane have been tested in pulmonary adenoma assay and found to have no significant effect (see Table X).

[b] Considered inconclusive either because of insufficiently high doses or because of early mortality.

[c] As a part of a synergism study with Disulfiram (see Section 5.2.2.1.3).

With the exception of the inactivity on bromoethane (ethyl bromide) and iodoethane (ethyl iodide) in the pulmonary adenoma assay by Poirier et al. (247) (see Table X), there is no information on monohaloethanes. The inactivity of iodoethane is surprising in view of the fact that iodomethane, iodopropanes, and 1- and 2-iodobutanes were all tumorigenic in the same study. Iodoethane is about 50% as active as iodomethane as an alkylating agent in the NBP test (69).

Of the three dihaloethanes tested, the results on technical grade 1,1-dichloroethane (255) were inconclusive due to poor survival rate. The survival rate was 32-80% for the mice and only 4-40% for the rats; pneumonia occurred in 80% of the rats. There was suggestive evidence of an increase in the incidence of mammary adenocarcinomas and in hemangiosarcomas among female rats and of endometrial stromal polyps among female mice. A retesting of the compound is needed before conclusions can be made.

Technical grade 1,2-dichloroethane (ethylene dichloride) is carcinogenic by oral administration. Significant increase in the incidence of alveolar/bronchiolar adenoma in mice of both sexes, and of mammary adenocarcinoma and endometrial stromal polyp or sarcoma in female mice was observed (see Table VIII). In Osborne-Mendel rats (dosed 47 and 95 mg/kg/day, 5 days/week for 78 weeks), 1,2-dichloroethane brought about significant increase in the incidence of squamous cell carcinomas of the forestomach (control 0%, low dose 6%, high dose 18%), hemangiosarcoma, and subcutaneous fibromas in males and of mammary adenocarcinoma (control 0%, low dose 2%, high dose 36%) in females (256, 257). Also, by topical route, 1,2-dichloroethane (126 mg/application, 3 times/week, 440-594 days) was carcinogenic, inducing lung tumors in 26/30 female Swiss ICR/Ha mice; however, no local tumors were observed (216). In contrast to oral and topical administration, Maltoni et al. (258) observed no

significant carcinogenic effects in Sprague-Dawley rats and Swiss mice after exposing the animals to atmospheres containing 5, 10, 50, or 150-200 ppm of a relatively pure (> 99.8%) sample of 1,2-dichloroethane. The nature for the discrepancy is not clear. In an assessment of the above data, Hooper et al. (269) suggested that the apparent discrepancy may be due to differences in the route, the strain of the animal, or to the statistical consideration of the effect of "intercurrent mortality."

Whereas the carcinogenicity of 1,2-dichloroethane may be debatable or is dependent on the route of administration, there is little doubt that its bromo analog, 1,2-dibromoethane (ethylene dibromide) is a potent carcinogen. At least six studies concur on the carcinogenicity of the compound. By oral administration, 1,2-dibromoethane (technical grade) induced squamous cell carcinoma of the forestomach in over 90% of the mice in the low dose group (see Table VIII) with some tumors appearing as early as the 24th week of treatment. Alveolar/bronchiolar adenomas were also noted. No such tumors were observed in the control mice. In Osborne-Mendel rats of both sexes, which received 37-41 mg/kg/day, 5 days/week for 78 weeks, the incidences of forestomach squamous cell carcinomas were 58-90% with the first tumor appearing as early as the 12th week of treatment. Increases in hemangiosarcomas and hepatocellular carcinomas were also noted in male and female rats, respectively. By topical route, 1,2-dibromoethane (25 or 50 mg/application, 3 times/week for 440-594 days) induced lung tumors in 50 and papillomas (5 progressing to squamous cell carcinomas) in 10 of 60 Swiss ICR/Ha mice (216). By inhalational route, 1,2-dibromoethane is a multi-potential carcinogen in $B6C3F_1$ mice, F344 rats, and possibly in Sprague-Dawley rats. In a NCI inhalation study (261), groups of 50 $B6C3F_1$ mice and F344 rats of each sex were exposed to atmospheres containing 10 or 40 ppm of 1,2-dibromoethane

(> 99.3% pure) for 78-103 weeks. In $B6C3F_1$ mice, significant increases in the incidence observed were: alveolar/bronchiolar carcinomas or adenomas in males (control 0%, low dose 6%, high dose 50%) and females (8%, 22%, 82%); and hemangiosarcomas of the circulatory system (0%, 22%, 46%), fibromas of the subcutaneous tissue (0%, 8%, 22%), tumors of the nasal cavity (0%, 0%, 24%), and adenocarcinomas of the mammary gland (4%, 28%, 16%) in females. In F344 rats, 1,2-dibromoethane caused significant increases in the incidence of: tumors (many malignant) of the nasal cavity (males, 0%, 78%, 82%; females, 2%, 68%, 86%), hemangiosarcomas of the circulatory system (males, 0%, 2%, 30%; females 0%, 0%, 10%), adenomas of the pituitary gland (males, 0%, 15%, 4%; females, 2%, 37%, 9%) in both sexes; and mesotheliomas in the tunica vaginalis (0%, 14%, 50%) in males; and alveolar/bronchiolar carcionmas or adenomas (0%, 0%, 11%), and fibroadenomas of the mammary gland (8%, 58%, 48%) in females. In the Sprague-Dawley rat, preliminary results (262) indicate increase in the incidence of hemangiosarcomas in the spleen of rats exposed to 20 ppm 1,2-dibromoethane for 18 months. Simultaneous administration of 1,2-dibromoethane and the apparently innocuous disulfiram led to dramatic increases in incidences of tumors in the liver, spleen, kidney, and omentum (see Section 5.2.2.1.3.8).

1,1,1-Trichloroethane (methyl chloroform) was found in two studies to be not carcinogenic in rodents (Table XV); however, neither bioassay can be considered adequate because of insufficient duration of the experiment. In the NCI oral study (263), groups of 50 $B6C3F_1$ mice and Osborne-Mendel rats of each sex were given technical grade 1,1,1-trichloroethane in corn oil 5 days/week for 78 weeks. A large number of animals had short life spans due to the toxicity of the compound or pneumonia (only 31% of the mice and 3% of the rats survived to the end of the experiment). At the time of this writing, the

compound is being retested by the U.S. National Toxicology Program. In an inhalation study (Quast et al., 1979, cited in ref. 201), in which groups of 92-94 Sprague-Dawley rats of each sex were exposed to atmosphere containing 875 or 1,750 ppm of the compound 6 hr/day, 5 days/week for 52 weeks, no significant effects were noted. The animals survived an average of 628-677 days. The U.S. Environmental Protection Agency (201) considered the study inadequate because of insufficient duration of exposure. However, it should be noted that the study was specifically designed to simulate the proportion of the total life span to which an average human would be occupationally exposed.

In contrast to the 1,1,1-isomer, 1,1,2-trichloroethane was found to be carcinogenic in $B6C3F_1$ mice (264). As seen in Table VIII, the compound caused significant increase in the incidence of hepatocellular carcinomas in mice of both sexes; the high dose was also associated with the induction of pheochromocytoma of the adrenal gland. However, in Osborne-Mendel rats of either sex, no tumors were observed following oral doses of 46 or 92 mg/kg/day, 5 days/week for 78 weeks (Table XV).

Two isomers of tetrachloroethane have been tested by the NCI by oral administration. The data on the 1,1,1,2-isomer are under final review at the time of this writing. The 1,1,2,2-isomer (technical grade) was hepatocarcinogenic in $B6C3F_1$ mice, but noncarcinogenic in Osborne-Mendel rats (265). The incidence of hepatocellular carcinomas was over 90% in the mice of the high dose group (see Table VIII). The doses that the rats received were 62 and 108 mg/kg/day for males and 43 and 76 mg/kg/day for females. The carcinogenicity of 1,1,2,2-tetrabromoethane was also reported (216). Topical application of 15 mg of the compound to the dorsal skin of female Swiss ICR/Ha mice 3 times/week for 440-594 days led to the induction of stomach tumors in 4/30 animals; no local carcinogenic effects were observed, however (Table XV).

Pentachloroethane (270) appears to have the same carcinogenic effects as 1,1,2,2-tetrachloroethane. As shown in Table VIII, pentachloroethane causes a significant increase in the incidence of hepatocellular carcinomas in $B6C3F_1$ mice. The compound is also noncarcinogenic in the rat (Table XV). The strain used in this study was F344; the dosages were 75 and 150 mg/kg/day. Halothane (1,1,1-trifluoro-2-bromo-2-chloroethane), an anesthetic agent, has been suspected for some time to be carcinogenic. This compound has recently been tested by Eger et al. (266). Perinatal exposures of Swiss ICR mice to 1/32, 1/8, and 1/2 maximum allowable concentrations (MAC) of halothane 2 hr/day on days 11, 13, 15, and 17 of gestation and 2 hr/day, 3 days/week for 8 weeks post-partum elicited no significant carcinogenic effects. The investigators emphasized that the doses administered (up to 1/2 MAC), at an age of rapid growth (a period of high susceptibility to carcinogenesis), were sufficient to have revealed a potent carcinogen.

Three fully halogenated ethanes (hexahaloethanes) have been tested for carcinogenicity. Both 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113) and 1,1,2,2-tetrachloro-1,2-difluoroethane (Freon 112) were found to have no significant carcinogenic effects by subcutaneous injections into the neck of neonatal Swiss ICR/Ha mice (253, 267). In these studies, doses of 0.1 ml of 10% solution of either Freon in tricaprylin were injected into 1- and 7-day-old mice, and 0.2 ml into 14- and 21-day-old mice. The animals were allowed to survive until the experiments ended after one year. It is interesting to note that while the Freons tested in this study were noncarcinogenic, simultaneous administration of either Freon and piperonyl butoxide led to induction of liver tumors (see Section 5.2.2.1.3.8). Hexachloroethane was tested by the NCI (268) in rodents by oral administration. Like its lower homologs (1,1,2,2-tetrachloro- and pentachloroethanes), hexachloroethane was hepatocar-

cinogenic in the B6C3F$_1$ mouse (see Table VIII). Osborne-Mendel rats given doses of 212 and 423 mg/kg/day did not develop any tumors attributable to the treatment (Table XV).

5.2.2.1.3.4 HALOPROPANES AND HIGHER HALOALKANES.

There is a paucity of data regarding the carcinogenicity of halopropanes although one compound in this group, 1,2-dibromo-3-chloropropane, may prove to be the most potent carcinogenic haloalkane. The information available on halopropanes is summarized in Table XVI. Two iodopropanes have been tested in the pulmonary adenoma assay by Poirier et al. (247). Both compounds led to significant increases in tumor incidence in the lung adenoma assay (see Table X).

1,2-Dibromo-3-chloropropane (DBCP), a soil fumigant, was first reported to be a potent carcinogen in a preliminary communication of NCI data by Olson et al. (259). A final report of the study was subsequently published (271). Virtually all the B6C3F$_1$ mice that received oral doses of DBCP developed squamous cell carcinomas of the forestomach (see Tables VIII and XVI). Osborne-Mendel rats which received oral doses of 15 and 29 mg/kg/day, 5 days/week for 64-78 weeks also developed the same tumor with high incidence (94% for both low and high dose males; 76 and 59% for low and high dose females, respectively). Some of these carcinomas were accompanied by pulmonary metastases. In addition, female rats had significantly increased incidences of adenocarcinomas of the mammary gland (control 10%, low dose 48%, high dose 62%). Palpable mammary tumors were noted already after 14 weeks of treatment. The carcinogenicity of DBCP has been confirmed by a chronic feeding study conducted for the Dow Chemical Company (272). Groups of 50 Charles River albino rats and HAM/ICR Swiss mice of each sex were fed diets

Table XVI
Carcinogenicity of Halopropanes

Compound	Species & Strain	Route	Principal Organs Affected	Reference
1-Iodopropane	Mouse, A/He	i.p.	Lung[a]	(247)
2-Iodopropane	Mouse, A/He	i.p.	Lung[a]	(247)
1,2-Dibromo-3-chloro-propane	Mouse, $B6C3F_1$	oral	Forestomach	(259, 271)
	Mouse, Swiss ICR/Ha	topical	Lung, forestomach[b]	(216)
	Mouse, Swiss HAM/ICR	oral	Forestomach	(272)
	Mouse, $B6C3F_1$	inhalation	Nasal cavity, lung	(273-275)
	Rat, Osborne-Mendel	oral	Forestomach, mammary gland	(259, 271)
	Rat, Charles River albino	oral	Forestomach, liver, kidney	(272)
	Rat, F344	inhalation	Nasal cavity, tongue pharynyx, adrenal gland	(275)

[a]Lung adenomas, see Table X.

[b]Also active as an initiator.

containing DBCP (equivalent to 0.3, 1.0, and 3.0 mg/kg/day) for 104 or 78 weeks, respectively. The statistically significant increases in tumors include the following histological types: squamous cell carcinomas and papillomas of the forestomach in rats and mice of either sex, renal tubular adenomas and carcinomas in rats of either sex, and hepatocellular carcinomas in male rats. Dibromochloropropane was also carcinogenic by topical applications to female Swiss ICR/Ha mice. Van Duuren et al. (216) showed that repeated applications (3 times/week for 440-594 days) of 11.7 or 35 mg DBCP led to the induction of lung tumors in 52/60 mice and of tumors of the forestomach in 35/60 mice, including 15 squamous cell carcinomas. Interestingly, no skin tumors were observed, although the compound was active in an initiation-promotion study. Considering the possibility of inhalational exposure, the NCI (275) has recently retested DBCP by this route. Groups of 50 $B6C3F_1$ mice and F344 rats of each sex were exposed to an atmosphere containing 0.6 or 3.0 ppm DBCP 6 hr/day, 5 days/week for 103 weeks. Significant increases in the incidences of tumors of the nasal cavity (control 0%, low dose 2%, high dose 44% in males; 0%, 22%, 76% in females) and alveolar/bronchiolar carcinomas or adenomas (0%, 8%, 16% for males; 8%, 10%, 28% for females) were observed in mice of both sexes. Increased incidence of tumors of the nasal cavity (0%, 80%, 88% in males; 2%, 54%, 84% in females) were also noted in the rat. In addition, rats of both sexes had higher incidences of squamous cell carcinomas or adenomas of the tongue (0%, 2%, 22% in males; 0%, 8%, 18% in females) and females developed squamous cell papillomas or carcinomas of the pharynx (0%, 0%, 12%) and cortical adenomas of the adrenal gland (0%, 14%, 10%).

In addition to halopropanes, a number of higher haloalkanes have been tested in the pulmonary adenoma assay by Poirier et al. (247) and Theiss et

al. (251). The results of these studies are shown in Table X in Section 5.2.2.1.3.1. As previously discussed, the pulmonary adenoma assay should be considered as a limited test for carcinogenicity; a positive result is strongly indicative of potential carcinogenicity whereas a negative result is of little predictive value. The haloalkanes found positive in the assay include s-, t-butyl chloride, i-, s-, and t-butyl bromide, and n- and s-butyl iodide.

5.2.2.1.3.5 HALOETHENES.

5.2.2.1.3.5.1 Vinyl Chloride. Vinyl chloride (VC) has attracted a great deal of attention since the discovery of its carcinogenic action in humans. The extensive carcinogenicity bioassays of VC have been reviewed in a number of publications (22, 23, 25, 276, 277). Only a brief account of these studies will be presented in this section; the major findings are summarized in Table XVII. Vinyl chloride has been found to be carcinogenic in at least 4 animal species and in humans (see Section 5.2.2.1.5.1). It is a highly potent multi-target carcinogen in rodents by inhalation. The histopathological types of tumors are: liver angiosarcoma, carcinoma of the Zymbal glands (in ear duct), nephroblastoma, neuroblastoma, mammary adenocarcinoma, forestomach papilloma, lung tumor, vascular tumor, and epithelial tumor of the skin.

The carcinogenicity of VC was first discovered by Viola et al. (285) in 1970. Male Ar/IRE rats exposed to an atmosphere containing 30,000 ppm of VC, 4 hr/day, 5 days/week for 52 weeks, developed tumors (reported to be skin tumors) of the submaxillary parotid region, and tumors of the lung and bones. Maltoni and Lefemine (286) examined the slides from this experiment and concluded that the cutaneous tumors actually arose from Zymbal glands and that the pulmonary tumors were most likely metastases from Zymbal gland car-

Table XVII
Carcinogenicity of Vinyl Chloride

Species & Strain	Route	Principal Organs Affected	Reference
Mouse, Swiss	inhalation	Lung, mammary gland, liver, vascular system, skin	(278, 279)
Mouse, CDI Swiss/ChR	inhalation	Lung, liver, mammary gland	(280)
Mouse, NMRI	inhalation	Lung, various sites	(281)
Mouse, CD-1	inhalation	Lung, mammary gland, liver	(282-284)
Rat, Ar/IRE Wistar	inhalation	Ear duct (Zymbal gland)[a]	(285, data re-examined in ref. 286)
Rat, Sprague-Dawley	inhalation	Ear duct, liver, kidney, brain, mammary gland, forestomach	(278, 279, 287, 288)
	oral	Liver	(279, 287)
	s.c.	Kidney? (preliminary)	(279)
	i.p.	Kidney?, subcutaneous tissue? (preliminary)	(279)
	inhalation[b]	Ear duct, subcutaneous tissue	(279)
	inhalation	Liver[c] (preliminary)	(289)
Rat, Wistar	inhalation	Liver, kidney, brain, ear duct	(279)
Rat, CD	inhalation	Liver, lung	(282, 283)
Hamster, Golden	inhalation	Forestomach, skin	(279)
Rabbit, --	inhalation	Skin, lung	(290)

[a]Originally reported as skin tumors of the submaxillary parotid region, and tumors of the lungs and bones (see text).

[b]Prenatal exposure from 12th to 18th day of gestation.

[c]Markedly potentiated by simultaneous administration of ethanol.

cinomas.. Beginning in 1971, an extensive series of experiments were undertaken by Maltoni and associates (278, 279, 286-288) to investigate the effects of dose, length of treatment, route of administration, and species, strain, sex, and age of animals on VC-induced carcinogenesis. The most pertinent and interesting findings of these studies are summarized below.

A clear-cut dose-response relationship has been observed in the induction of liver angiosarcomas in Sprague-Dawley rats (287). Exposure (4 hr/day, 5 days/week for 52 weeks) of rats to air containing 1, 5, 10, 25, 50, 100, 150, 200, 250, 500, 2,500, 6,000, 10,000, or 30,000 ppm VC led to tumor incidences of 0, 0, 0.8, 4.2, 4.8 or 1.7 (2 groups exposed to 50 ppm), 0.8, 5.0, 10.0, 5.1, 10.0, 21.7, 22.0, 11.7, and 30.0%, respectively. The average latent period progressively decreased from 79-135 weeks for low doses (below 50 ppm) to less than 54 weeks for the highest dose. By oral administration (5 days/week for 52 weeks), doses of 0.03, 0.30, 1.0, 3.33, 16.65, or 50.0 mg/kg/day produced incidences of 0, 0.7, 2.0, 0, 12.0, and 21.0%, respectively. The lowest doses capable of inducing statistically significant incidences in various types of tumors were: Zymbal gland carcinoma, 10,000 ppm; liver angiosarcoma, 200 ppm or 50 mg/kg for males, and 50 ppm or 16.65 mg/kg for females; nephroblastoma, 100 ppm for males and 250 ppm for females; neuroblastoma, 10,000 ppm for females; mammary gland adenocarcionma, 5 ppm for females; forestomach papilloma, 30,000 ppm (287).

A reduction in the length of treatment may markedly decrease the carcinogenicity of VC. Maltoni (288) showed that Sprague-Dawley rats exposed to 6,000 ppm VC for 52, 17, or 5 weeks had liver angiosarcoma incidences of 22, 0.6, and 0%, respectively.

The route of administration may significantly affect the carcinogenicity of VC. In contrast to the multi-target carcinogenicity of VC by inhalation exposure, the induction of liver angiosarcoma seemed to be the only significant carcinogenic effect of VC by oral administration (287). The carcinogenicity of VC by intraperitoneal or subuctaneous injection may be doubtful. Maltoni (279) reported preliminary data showing that one nephroblastoma and one subcutaneous angiosarcoma were found among 240 rats which received 1-4 intraperitoneal injections of 4.25 mg VC. One nephroblastoma was observed among 75 rats that received a single subcutaneous injection of 4.25 mg VC.

Significant species- and strain-differences in VC carcinogenesis have been reported by Maltoni (279, 288). Swiss mice are quite susceptible to the carcinogenic action of VC. Exposure to air containing 50-10,000 ppm for 30 weeks caused high incidences of lung tumors, mammary carcinomas, vascular tumors, and liver angiosarcomas. Epithelial tumors of the skin were also occasionally observed. Marked increases in the incidence of mammary and vascular tumors were evident even at the lowest dose (279). Golden hamsters appeared to be considerably less susceptible than Swiss mice. Exposure to 50-10,000 ppm VC in air for 30 weeks led to the induction of forestomach epithelial tumors, skin trichoepitheliomas, and occasional liver angiosarcomas and melanomas (279). Wistar rats had a similar carcinogenic response to VC as Sprague-Dawley rats; however, the relative response of different organs or tissues differed. The most notable difference was the considerably higher incidence of Zymbal gland carcinomas in Sprague-Dawley than Wistar rats (279, 288).

The influence of age on VC carcinogenesis is striking. Newborn Sprague-Dawley rats seem to be extremely susceptible to the hepatocarcinogenic action of VC. Exposure of 1-day-old rats (a group of 43 rats to 6,000 ppm, another

group of 46 rats to 10,000 ppm) to VC in air, 4 hr/day, 5 days/week for only 5 weeks resulted in the induction of 20 liver angiosarcomas and 28 hepatomas after 104 weeks. Only one hepatoma was found among 240 rats similarly terated starting at the age of 13 weeks (279, 288). Vinyl chloride is also an active transplacental carcinogen. Exposure of pregnant Sprague-Dawley rats to 6,000 or 10,000 ppm VC in air, 4 hr/day from 12th to 18th day of gestation, was sufficient to induce tumors in a number of offspring (279).

The influence of sex on VC carcinogenesis depends on the target organ involved. The data of Maltoni et al. (287) indicate that the lowest effective carcinogenic dose for the induction of liver angiosarcoma is lower in females that in male Sprague-Dawley rats. On the other hand, male rats may be more susceptible to the induction of nephroblastoma by VC. Excess mammary gland adenocarcinomas were observed in female rats exposed to as low as 5 ppm VC in air.

In addition to the studies summarized above, the carcinogenicity of VC has also been demonstrated in several other strains of mice and rats and in rabbits by the inhalational route. In agreement with Maltoni's results on Swiss mice, the most affected organ in CDI Swiss/ChR (280), NMRI (281), and CD-1 (282-284) was the lung. Angiosarcomas of the liver and various other sites, and mammary gland adenocarcinomas were also found in most of these studies. Preliminary data of Radike et al. (289) confirmed the hepatocarcinogenic action of VC in Sprague-Dawley rats; in addition, there is some evidence that ethanol potentiates the carcinogenicity of VC (see Section 5.2.2.1.3.8). Lee et al. (282, 283) exposed groups of 36 CD strain rats of each sex to 50, 250, or 1,000 ppm VC in air, 6 hr/day, 5 days/week for 12 months. Exposure of rats to 250 or 1,000 ppm VC induced hemangiosarcomas of the liver (12/58 in 250 ppm group; 22/51 in 1,000 ppm group) and the lung

(3/58; 13/51). Caputo et al. (290) exposed a group of 40 rabbits to 10,000 ppm VC in air, 4 hr/day, 5 days/week for 12 months. After 15 months of observation, 12 skin acanthomas and 6 lung adenocarcinomas were seen; no such tumors occurred in 20 controls.

5.2.2.1.3.5.2 Haloethenes Other Than Vinyl Chloride. The discovery of the carcinogenicity of VC has spurred great interest and concern about the potential health hazards of related compounds. A number of haloethenes have been tested for carcinogenicity; the major findings of these studies are summarized in Table XVIII.

Vinyl Bromide. The data on vinyl bromide are sparse or incomplete at the time of this writing. Van Duuren (217) reported that vinyl bromide was completely inactive as an initiator (15 mg as initiating dose plus phorbol myristate acetate as promotor) or complete carcinogen (tested by repeated applications of 15 mg vinyl bromide, 3 times/week for 60 weeks) on mouse skin. Weekly subcutaneous injections of 25 mg vinyl bromide for 60 weeks also failed to elicit any tumor. In a short-term assay described in Section 5.2.2.1.3.1, Bolt et al. (220, 222) found that vinyl bromide induced preneoplastic lesions in newborn rats; consistent with their relative rates of metabolism, the potency of vinyl bromide was lower than that of vinyl chloride. Preliminary or unpublished results cited by Bahlman et al. (291) and Infante and Marlow (60) indicated that inhalation exposure of Sprague-Dawley rats to vinyl bromide induced tumors in the liver, ear duct, and possibly also in the lung, lymphatic system, and mammary gland. The details of the study were not given.

Vinylidene Chloride (1,1-Dichloroethylene). The question of the carcinogenicity of vinylidene chloride (VDC) was first raised by Viola at the 11th

Table XVIII
Carcinogenicity of Haloethenes Other Than Vinyl Chloride

Compound	Species & Strain	Route	Principal Organs Affected	Reference
Vinyl bromide[a]	Mouse, Swiss ICR/Ha	topical	None	(217)
		s.c.	None	(217)
	Rat, Sprague-Dawley	inhalation	Liver, ear duct, lung, lymphatic system, mammary gland, (preliminary)	(Huntingdon Res. Ctr., cited in ref. 291; W. Busey, cited in ref. 60)
Vinylidene chloride (1,1-Dichloro-ethylene)	Mouse, Swiss	inhalation	Kidney	(288, 292)
	Mouse, CD-1	inhalation	No significant effect[b]	(282, 283)
	Mouse, Swiss ICR/Ha	topical	None[c]	(216)
		s.c.	None	(216)
	Mouse, $B6C3F_1$	oral	None	(293)
	Rat, Sprague-Dawley	inhalation	Mammary gland	(292)
		inhalation	None (preliminary)	(294)
		oral	None (preliminary)	(294)
		inhalation	None (preliminary)	(295)
	Rat, CD	inhalation	No significant effect[d]	(282, 283)
	Rat, BD IV	oral[e]	No significant effect[f]	(296)
	Rat, F344	oral	None	(293)
	Hamster, Chinese	inhalation	None (preliminary)	(288)
Trichloroethylene	Mouse, NLC	oral	None[g]	(233)
	Mouse, $B6C3F_1$	oral	Liver	(234)
	Mouse, Swiss ICR/Ha	topical	None	(216)
		s.c.	None	(216)
		oral	None	(216)

Table XVIII (continued)

Compound	Species & Strain	Route	Principal Organs Affected	Reference
Trichloroethylene (cont'd)	Mouse, NMRI	inhalation	Lymphatic system	(297)
	Rat, Osborne-Mendel	oral	None	(234)
	Rat, WIST	inhalation	None	(297)
	Hamster, Syrian	inhalation	None	(297)
Tetrachloroethylene[h] (Perchloroethylene)	Mouse, Swiss ICR/Ha	topical	None	(216)
	Mouse, $B6C3F_1$	oral	Liver	(298)
	Rat, Osborne-Mendel	oral	None (inconclusive)[i]	(298)

[a]Vinyl bromide has also been shown to induce preneoplastic lesions in newborn rats [H.M. Bolt, Arbeitsmed. Sozialmed. Praventivmed. 15, 49 (1980)].

[b]Three mice developed hemangiosarcoma of the liver and two mice developed skin keratoacanthomas after exposure to 55 ppm of vinylidene chloride.

[c]Active as an initiator.

[d]Two rats developed hemangiosarcomas in the mesenteric lymph node or subcutaneous tissue.

[e]Prenatal and lifetime exposure.

[f]There was a significant increase in the incidence of liver hyperplastic nodules.

[g]It is not certain whether the duration of the experiment was sufficiently long.

[h]Tetrachloroethylene has been tested in pulmonary adenoma assay and found to have no significant effect (see Table X).

[i]Due to high incidence of early death among treated animals.

International Cancer Congress in 1974. The compound has since been tested in at least 13-14 studies and was found to be a relatively weak carcinogen in some studies, but inactive in others. Maltoni and coworkers (288, 292) exposed Swiss mice to 25 ppm (maximum tolerable dose), Sprague-Dawley rats to 10-200 ppm, and Chinese hamsters to 25 ppm VDC in air, 4 hr/day, 4-5 days/week for 52 weeks. Several additional groups of rats were given oral doses of 0.5 mg/kg/day VDC in water or 5, 10, or 20 mg/kg/day VDC in olive oil at the same schedule. Preliminary data after 98 weeks indicated that the most significant carcinogenic effect was the induction of kidney adenocarcinomas in mice. Males (16% incidence) were considerably more susceptible than females (0.7% incidence) (288). In rats exposed by inhalation, an increase in the incidence of mammary tumors was noted; however, no dose-response relationship was observed. One Zymbal gland carcinoma was found in a rat in the 100 ppm group. No increase in mammary tumors was observed among rats exposed by ingestion. One Zymbal gland carcinoma occurred in a rat in the 10 mg/kg group (292). In the hamsters, no tumors were found after 74 weeks (292).

A low incidence of tumors in rodents following VDC exposure was also noted by Lee et al. (282, 283) in a 1-year inhalation study. Among 70 CD-1 mice that survived exposure to 55 ppm VDC for 1-3, 4-6, 7-9, or 10-12 months, 3 developed hemangiosarcomas of the liver and 2 had skin keratoacanthomas. In CD rats, 2/36 male rats exposed to 55 ppm VDC in air for 12 months developed angiosarcomas -- one in the mesenteric lymph node and one in subcutaneous tissue. There were no tumors in exposed females.

Vinylidene chloride has also been tested by other routes of administration (see Table XVIII); none of these studies gave evidence of significant carcinogenicity. Van Duuren et al. (216) did not find any carcinogenic effect after repeated topical applications of 40 or 121 mg VDC (3 times/week for 440-

594 days) or subcutaneous injections of 2.0 mg VDC (weekly for 518-694 days) to female Swiss ICR/Ha mice. The compound (125 mg) was, however, active as an initiator in a two-stage skin carcinogenesis bioassay, inducing 1 squamous cell carcinoma and 9 papillomas in 8/30 mice. Rampy et al. (294) exposed Sprague-Dawley rats to VDC orally (68-220 ppm in drinking water) or by inhalation (10-75 ppm in air) for 18 months. Preliminary data indicated that the tumor incidence in VDC-exposed rats was not greater than in the controls. The same conclusion was reached by Viola and Caputo (295) from their preliminary data of an inhalation study in which Sprague-Dawley rats were exposed to 75 or 100 ppm VDC.

Additional bioassay studies support the lack of carcinogenicity of VDC. In the study of Ponomarkov and Tomatis (296), pregnant female BD IV rats were given an oral dose of 150 mg/kg body weight of VDC on day 17 of gestation and their offspring were administered orally 50 mg/kg/week VDC from weaning to the end of the life span. There was an increase, although not statistically significant, of liver and meningeal tumors. However, the increase in the incidence of liver hyperplastic nodules was significant. In a U.S. National Toxicology Program carcinogenesis bioassay (293), groups of 50 B6C3F_1 mice and 50 F344 rats of each sex were given VDC orally for 104 weeks. The doses were 2 or 10 mg/kg for mice and 1 or 5 mg/kg for rats. No significant increase in tumor incidence was observed in mice and rats of either sex.

Trichloroethylene. Information on the carcinogenicity of trichloroethylene (TCE) is summarized in Table XVIII. Trichloroethylene was found noncarcinogenic in a limited study by Rudali (233). Twenty-eight NLC mice were given orally 0.1 ml of a 40% TCE solution, twice a weekly for 6 months. No tumors were found at the termination of the experiment of unspecified duration. The carcinogenicity of TCE (industrial grade, epoxide-stabilized,

> 99%) was first detected in $B6C3F_1$ mice (234; see also Table VIII). Significant increase in the incidence of hepatocellular carcinoma was observed in mice of both sexes, the males being more susceptible. In male mice of the high dose group, the first tumor was seen at the 27th week, 9 other tumors were found by the 78th week. In contrast to $B6C3F_1$ mice, Osborne-Mendel rats were not susceptible to TCE. The tumor incidence in rats given 549 or 1,097 mg/kg/day of TCE, 5 times/week for 78 weeks was not significantly different from those in control rats.

The carcinogenicity of TCE has also been tested in female Swiss ICR/Ha mice by Van Duuren et al. (216) by skin painting, subcutaneous injection, or by oral administration. None of these treatments brought about any significant increase in tumor incidence.

Henschler et al. (297) have investigated more recently the carcinogenicity of TCE in 3 animal species via inhalation exposure. Groups of NMRI mice, WIST rats, and Syrian hamsters of each sex were exposed to 100 or 500 ppm purified TCE, 6 hr/day, 5 days/week for 18 months. The only significant effect observed was an increase in the incidence of malignant lymphomas in NMRI mice: 9/29 in controls, 17/30 in 100 ppm group, 18/28 in 500 ppm group. Since this strain of mice is known to have a relatively high spontaneous incidence of malignant lymphoma, the authors did not consider the effect highly significant. In rats and hamsters, no carcinogenic effects were observed. The authors concluded from their findings that purified TCE is not carcinogenic. However, they stressed that their conclusions may apply only to pure TCE stabilized by an amine base instead of epoxide-stabilizers used in industrial grade TCE.

Tetrachloroethylene. The carcinogenicity of tetrachloroethylene (USP grade) has been tested by oral administration (298; see also Table VIII). Significant increases in the incidence of hepatocellular carcinomas were observed in $B6C3F_1$ mice of both sexes. The first tumor was detected in a male animal (in the low dose group) that died during the 27th week. In the same study, no significant increase in the tumor incidence was observed in Osborne-Mendel rats receiving 471 and 941 mg/kg/day (for males) and 474 and 949 mg/kg/day (for females), respectively. However, since a high incidence of early death occurred among treated rats due to toxic nephropathy, the negative finding in rats was not considered conclusive. Tetrachloroethylene has also been tested by Van Duuren et al. (216) by skin painting; repeated applications (3 times 18 or 54 mg/week for 426-576 days) did not elicit any significant increase of local or distant tumors.

5.2.2.1.3.6 HALOPROPENES.

Only three halopropenes have thus far been tested for carcinogenicity (see Table XIX). Van Duuren et al. (216) showed that repeated skin painting (3 times/week for at least 342 days) of 1-chloropropene (2.5 mg/application), allyl chloride (31 or 94 mg/application), or cis-1,3-dichloropropene (41 or 122 mg/application) to female Swiss ICR/Ha mice did not induce any significant carcinogenic effects. Only allyl chloride (99 mg) was weakly active as an initiator in the 2-stage skin carcinogenesis assay, inducing 10 papillomas in 7/30 mice. By subcutaneous injection (once a week for over 538 days), both 1-chloropropene (1.0 mg/injection) and allyl chloride (1.5 mg/application) were inactive, while cis-1,3-dichloropropene (3.0 mg/injection) was significantly carcinogenic, inducing local sarcomas in 6/30 mice. Only 1-chloropropene was tested by oral administration; weekly intragastric administration of 1.0 mg of the compound led to significant increase in the incidence of fore-

Table XIX
Carcinogenicity of Halopropenes

Compound	Species & Strain	Route	Principal Organs Affected	Reference
1-Chloropropene	Mouse, Swiss ICR/Ha	topical	None	(216)
		s.c.	None	(216)
		oral	Forestomach	(216)
Allyl chloride (3-Chloropropene)	Mouse, $B6C3F_1$	oral	None[a]	(299)
	Mouse, Swiss ICR/Ha	topical	None[b]	(216)
		s.c.	None	(216)
	Rat, Osborne-Mendel	oral	None	(299)
cis-1,3-Dichloropropene	Mouse, Swiss ICR/Ha	topical	None	(216)
		s.c.	Local sarcoma	(216)

[a]There was some suggestive evidence of positive association with neoplastic lesions of the forestomach.

[b]Active as an initiator.

stomach tumors (13/30, including 3 squamous cell carcinomas) in female mice. Four out of 30 male mice also developed forestomach tumors but the increase in the incidence was not statistically significant.

In addition to the above study, a bioassay for possible carcinogenicity of technical-grade allyl chloride was conducted by NCI (299) using $B6C3F_1$ mice and Osborne-Mendel rats. The time-weighted average doses were: 172 and 199 mg/kg/day for male mice; 129 and 258 mg/kg/day for female mice; 57 and 77 mg/kg/day for male rats; and 55 and 83 mg/kg/day for female rats. The animals were dosed orally 5 times/week for 78 weeks and observed for an additional 14 weeks for mice and 30-33 weeks for rats. The survival rate of high dose male mice and high dose rats of both sexes was extremely poor. Based on observations on the surviving animals, some "suggestive" evidence of a relatively weak carcinogenicity of the compound was noted in mice of both sexes. Low incidence of squamous-cell carcinomas (2/46 low dose males; 2/47 low dose females) or papillomas (1/47 low dose males; 3/45 low dose females) of the forestomach were observed. In addition, proliferative nonneoplastic lesions (e.g., acanthosis and hyperkeratosis) occurred in the stomach of many treated mice but not in controls. No convincing evidence for the carcinogenicity of allyl chloride was found in Osborne-Mendel rats of both sexes.

5.2.2.1.3.7 HALOBUTENES, HALOBUTADIENES AND ARYLALKYL HALIDES.

One halobutene and two halobutadienes have been bioassayed for carcinogenicity; the results of these studies are summarized in Table XX. Van Duuren et al. (215) tested trans-1,4-dichloro-2-butene by topical, subcutaneous, or intraperitoneal route in female Swiss ICR/Ha mice. By subcutaneous injection (1 dose of 0.05 mg/week for 537 days), the compound was weakly carcinogenic, inducing local sarcomas in 3/30 mice. By intraperitoneal administrations

Table XX
Carcinogenicity of Halobutenes and Halobutadienes

Compound	Species & Strain	Route	Principal Organs Affected	Reference
trans-1,4-Dichloro-2-butene	Mouse, Swiss ICR/Ha	topical	None	(215)
		s.c.	Local sarcoma	(215)
		i.p.	No significant effect	(215)
	Rat, --	inhalation	Nasal cavity (preliminary)	(B.C. McKusick cited in ref. 51)
2-Chloro-1,3-butadiene (Chloroprene)	Mouse, random-bred	topical	None	(300, 301)
	Rat, random-bred	oral	None	(300, 301)
		s.c.	None	(300, 301)
		intratracheal	None	(300, 301)
	Rat, BD IV	oral[a]	None	(296)
Hexachloro-1,3-butadiene[b]	Mouse, Swiss ICR/Ha	topical	None	(216)
	Rat, Sprague-Dawley	oral	Kidney	(108, 302)

[a]Prenatal and lifetime exposure.

[b]Inactive in pulmonary adenoma assay (see Table X).

(1 x 0.05 mg/week for 537 days), the compound induced local sarcomas in 2/30 mice; however, the increase in the tumor incidence was not statistically significant. By topical route, the compound was inactive both as a complete carcinogen (3 x 1.0 mg/week for 537 days) or as an initiator (one initiating dose of 1.0 mg followed by promotion with phorbol myristate acetate). The compound was subsequently tested by inhalation and a significant incidence of malignant nasal tumors in rats exposed to 5 ppm was reported (McKusick, cited in ref. 51).

2-Chloro-1,3-butadiene (chloroprene) has been tested in randombred albino mice and rats by Zil'fyan et al. (300, 301) by several routes of administration. Their data have been reviewed by an IARC Study Group (23). The compound was noncarcinogenic in the mouse tested by repeated skin applications (twice-weekly applications of a 50% solution of chloroprene in benzene for 25 weeks). In the rat, the compound did not induce tumors after oral (2 x 200 mg/kg/week for 25 weeks), intratracheal (5 x 200 mg/kg at 20-day intervals), or subcutaneous (10 x 400 mg/kg) administration. However, the IARC Study Group (23) pointed out the incomplete reporting of the experimental details. A long-term ingestion study of chloroprene has recently been described by Ponomarkov and Tomatis (296). Pregnant female BD IV rats were given an oral dose of 100 mg/kg body weight of chloroprene on day 17 of gestation. Their offspring were given by stomach tube 50 mg/kg/week of the compound from the time of weaning to the end of the lifespan. The test yielded no evidence for the carcinogenicity of the compound.

Kociba et al. (108, 302) tested hexachloro-1,3-butadiene (HCBD) in Sprague-Dawley rats. The rats were fed, for up to 2 years, diets containing sufficient HCBD to maintain daily intakes of 0.2, 2.0, or 20 mg/kg body weight. Ingestion of the highest dose produced a signficant increase in the

incidence of renal tubular adenomas and adenocarcinomas. Males (18% incidence) were more susceptible than females (7.5% incidence). No renal tumors were observed in rats fed lower doses of HCBD. In the pulmonary adenoma assay of Theiss et al. (251), HCBD was inactive (see Table X). Van Duuren et al. (216) have tested HCBD by skin application in female Swiss ICR/Ha mice. The compound was inactive in tests as a complete carcinogen (3 x 2.0 or 6.0 mg/week for over 440 days) or as an initiator (a single dose of 15 mg followed by phorbol myristate acetate).

Benzyl chloride ($C_6H_5CH_2Cl$), an arylalkyl halide, has been included for discussion in this section because its chemical properties (see Section 5.2.2.1.2.1) resemble those of allyl halides more closely than those of aryl halides. Druckrey et al. (68) reported that benzyl chloride induces local sarcomas at the site of subcutaneous injections into BD strain rats. The tumor incidences were 3/14 and 6/8 in two groups of rats that received weekly injections of 40 and 80 mg/kg benzyl chloride, respectively. The mean latent period was 500 days. The carcinogenic potency of benzyl chloride was comparable to that of iodomethane. In the pulmonary adenoma assay, Poirier et al. (247), found benzyl chloride inactive (see Section 5.2.2.1.3.1).

5.2.2.1.3.8 MODIFICATION OF CARCINOGENESIS.

Considering the importance of this class of compounds, surprisingly little is known on the potential role of environmental factors in modifying the carcinogenicity of haloalkanes and haloalkenes. However, carbon tetrachloride has been extensively used in syncarcinogenesis studies and in studies on the modification of the carcinogenicity of other compounds. Carbon tetrachloride acts synergistically with a variety of synthetic or naturally occurring carcinogens, such as 2-N-fluorenylacetamide (303), 3-methylchol-

anthrene (238), 2,7-bis(acetamide)fluorene (304), aflatoxin B_1 (305), and flower stalk of a type of Japanese coltsfoot, Petasites japonica (306) in the induction of liver tumors in rodents. In addition, combined treatment of β-naphthylamine and carbon tetrachloride induces tumors in both the liver and the urinary bladder in the dog (307). Carbon tetrachloride has also been shown to potentiate the carcinogenic action of N-nitroso compounds (such as dimethyl-, diethyl-, and methylethylnitrosamine, N-butyl-N-nitrosourea, and dimethylnitrosamine precursors) (308-312; see also Section 5.2.1.2.3.7), polycyclic aromatic hydrocarbons such as benzo[a]pyrene (313), azo dyes such as 3'-methyl-4-dimethylaminoazobenzene (314), and aromatic amines such as 2-N-fluorenylacetamide (315, 316). In most of these studies, hepatonecrotic doses of carbon tetrachloride were required and for this reason the potentiation is generally considered to be due to the promoting, co-carcinogenic or "chemical traumatic" (a form of "chemical hepatectomy") effects of carbon tetrachloride. With the N-nitroso compounds, maximum potentiation was observed when carbon tetrachloride was administered shortly (1 day) before the N-nitroso compounds (309, 311). However, carbon tetrachloride also potentiates the carcinogenicity of diethylnitrosamine when given repeatedly after the administration. There is some evidence that the two potentiating effects (i.e., before and after diethylnitrosamine) are additive and independent of each other (310). Not all carcinogens are potentiated by carbon tetrachloride, however. For example, administration of carbon tetrachloride before urethan reduces the incidence of lung adenomas by 32-47% in CC57Br mice (317).

The carcinogenicity of vinyl chloride is modified by ethanol and disulfiram. A preliminary report by Radike et al. (289) suggests potentiation of carcinogenicity of vinyl chloride by ethanol. Male Sprague-Dawley rats receiving the combined treatment (73%) developed more liver tumors than those

receiving vinyl chloride (38%) or ethanol (0%) alone. Winston et al. (318) reported that disulfiram protects against the carcinogenic effects of vinyl chloride in CD-1 mice. Disulfiram delayed the induction of bronchiolo-alveolar adenomas and reduced the incidence of hepatic hamangiosarcomas and mammary gland tumors.

In sharp contrast to the protective effect of disulfiram against vinyl chloride and a number of other carcinogens (see Section 5.2.1.6.3.9), disulfiram has been shown to enhance the carcinogenicity of 1,2-dibromoethane in Sprague-Dawley rats. Rats receiving the combined long-term treatment had significantly higher incidences of hepatic, splenic, and renal tumors than those receiving 1,2-dibromoethane alone and developed moreover hemangiosarcomas of the omentum (Midwest Research Institute, 1979, cited in ref. 262). The effect is believed to be related to the inhibition of alcohol dehydrogenase by disulfiram (262; see also Section 5.2.2.1.4.1.2).

Another unusual case of synergism was noted by Epstein et al. (253) in a study on fluoroalkanes (Freon 112, Freon 113) and piperonyl butoxide. When administered singly, neither fluoroalkane nor piperonyl butoxide was carcinogenic in neonatal Swiss ICR/Ha mice; however, combined treatment of Freon 112 (1,1,2,2-tetrachloro-1,2-difluoroethane) and piperonyl butoxide led to the induction of hepatomas in 31% of male mice. Three female mice developed malignant lymphomas. Combined treatment of Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) and piperonyl butoxide also enhanced the incidence of hepatomas in male mice. The mechanism of the synergism is not known. It was suggested (253) that piperonyl butoxide may modify a presumed in vivo dehalogenation of the fluoroalkanes.

5.2.2.1.4 Metabolism and Mechanism of Action.

The metabolism and mechanisms of action of haloalkanes and haloalkenes have been extensively studied. Depending on the chemical structure, haloalkanes and haloalkenes may either interact directly with cellular macromolecules or may first undergo metabolic activation to reactive intermediates to initiate carcinogenesis and mutagenesis.

5.2.2.1.4.1 METABOLISM AND MECHANISM OF ACTION OF HALOALKANES.

5.2.2.1.4.1.1 Halomethanes. A comparative metabolic study of haloalkanes and haloalkenes has been conducted by Nakajima and Sato (319) in Wistar rats. Among chlorinated halomethanes, the in vivo relative rates of metabolism follow the order: dichloromethane > chloroform >> carbon tetrachloride. Male rats display higher metabolic activity than female rats. Food deprivation increases the metabolic rates in both sexes.

Monohalomethanes. Very little information is available on the metabolism of monohalomethanes. The U.S. Environmental Protection Agency (320) reviewed, in 1980, the available information on chloromethane. Several reports indicate the presence of methanol, formaldehyde or formate in the blood or urine of animals or humans exposed to chloromethane. However, these findings have not been consistently confirmed. Monohalomethanes may be capable of reacting directly with nucleophiles. Iodomethane (methyl iodide) is a well known methylating agent. Among the haloalkanes tested in the NBP reaction, methyl iodide is the most active alkylator (see Section 5.2.2.1.2.1). Iodomethane, bromomethane and chloromethane are all mutagenic in the Ames test without activation. There is some evidence that chloromethane may react with tissue nucleophiles by an enzyme-catalyzed reaction. Redford-Ellis and Gowenlock (321, 322) detected the presence of S-methyl-cysteine, S-methyl-glutathione

and traces of methylated histidine and methionine after *in vitro* reaction of ^{14}C-chloromethane with human blood or tissue homogenates. Covalent binding was substantially reduced when the blood or tissue homogenates were heated.

Dihalomethanes. The metabolism of dihalomethanes has been extensively investigated by Anders, Ahmed, Kubic and coworkers (*reviewed in* 323, 324). *In vitro* studies indicate that the rates of metabolism of dihalomethanes ranks as follows: $CH_2I_2 > CH_2Br_2 > CH_2BrCl > CH_2Cl_2$ (325-328). The two major metabolic pathways of dihalomethanes are shown in Fig. 1. In the first pathway, dihalomethane is hydroxylated by mixed function oxidases to yield hydroxydihalomethane, which spontaneously decomposes to give formyl halide and carbon monoxide. This pathway is supported by the increase in carboxyhemoglobin in humans and animals exposed to dihalomethanes. The production of carbon monoxide and carboxy-hemoglobin from the dihalomethane was confirmed using ^{13}C-labeled dichloromethane (329). The involvement of mixed function oxidases is indicated by the requirement of NADPH, and molecular oxygen. Agents (*e.g.*, phenobarbital, 3-methylcholanthrene, SKF-525A) that modify the activity of cytochrome P-450 also bring about a corresponding change in the metabolism of dihalomethanes to carbon monoxide (325). Metabolic studies using deuterated dihalomethanes and $^{18}O_2$ substantiate the view that the insertion of oxygen (from O_2) in the C-H bond is the rate-limiting step (330). The formyl halide intermediate is a potential acylating agent and has been postulated to be a reactive intermediate responsible for covalent binding of dichloromethane to tissue nucleophiles. The covalent binding of dichloromethane to microsomal lipids and proteins has been demonstrated and shown to have the same requirement for NADPH and oxygen, the same response to phenobarbital pretreatment and similar kinetic properties as its metabolism to carbon monoxide (323).

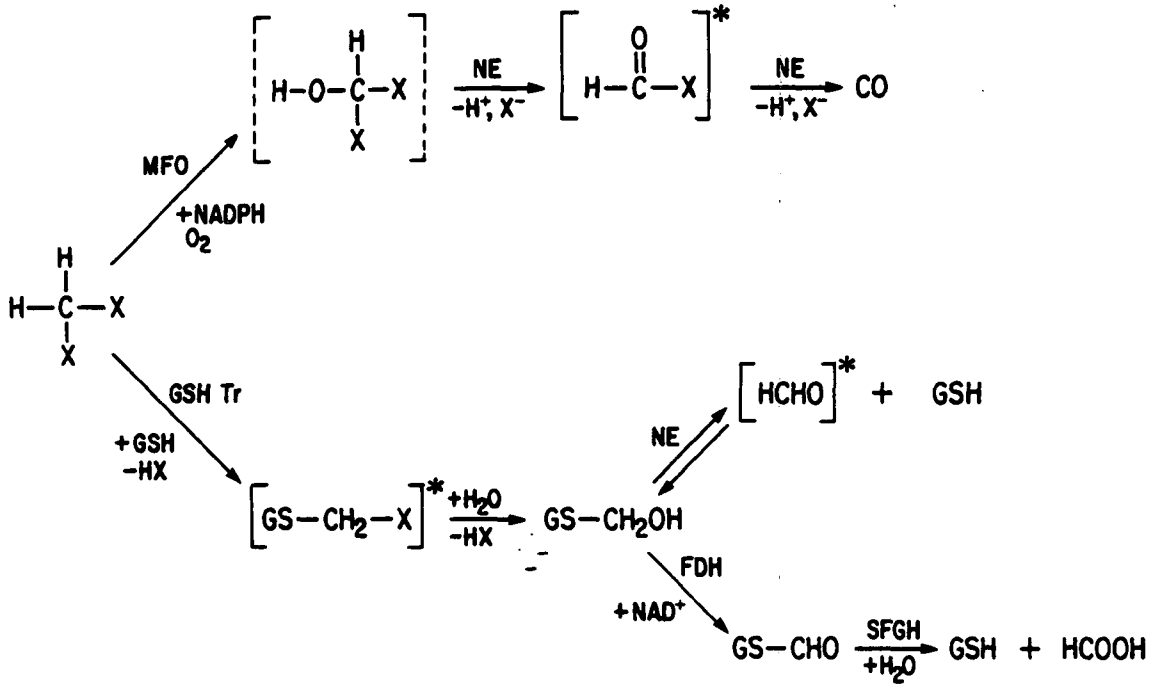


Fig. 1. Proposed metabolic pathways of dihalomethanes. The abbreviations used are: MFO = mixed function oxidases; NE = nonenzymic process; GSH Tr = glutathione transferase; FDH = formaldehyde dehydrogenase; SFGH = S-formyl glutathione hydrolase. Compounds with an asterisk are potential reactive intermediates. [Adapted from A.E. Ahmed, V.L. Kubic, J.L. Stevens, and M.W. Anders: Fed. Proc. 39, 3150 (1980)]

In addition to the above pathway, dihalomethanes are also metabolized to formaldehyde and inorganic halide. Ahmed and Anders (326, 331) have studied this pathway in detail (see Fig. 1). The reaction is catalyzed by enzymes present in hepatic cytosolic fraction and requires reduced glutathione (GSH) as a co-factor. The reaction may be inhibited by sulfhydryl reagents (e.g., p-chloromercuribenzoate, diethyl maleate) and by known substrates of glutathione transferase (e.g., iodomethane, 1-chloro-2,4-dinitrobenzene) indicating the involvement of the enzyme in the metabolism of dihalomethane. Similar metabolic rates have been observed with the use of dibromomethane, deuterated dibromomethane and bromochloromethane suggesting that nucleophilic attack on the carbon with subsequent displacement of halide is the initial, rate-limiting step. S-Halomethyl glutathione conjugate has been postulated to be the reaction intermediate, which is expected to undergo nonenzymic hydrolysis to S-hydroxymethyl glutathione, which in turn may be converted to formaldehyde and glutathione. The production of formaldehyde may be substantially decreased in the presence of NAD^+; instead, formic acid is produced. It has been suggested (324) that cytosolic formaldehyde dehydrogenase may oxidize S-hydroxymethyl glutathione to S-formyl glutathione, which is then hydrolyzed by cytosolic S-formyl glutathione hydrolase to formic acid and glutathione. The biological significance of this metabolic pathway is not clear. Although the pathway appears to be detoxifying in nature, it should be pointed out that the S-halomethyl glutathione intermediate is an α-halomethyl thioether, which may possess reactivity similar to that of the potent carcinogen, bis(chloromethyl)ether (see Section 5.2.1.1.2). As expected, the mutagenicity of dibromo- and diiodomethane is also enhanced by the inclusion of microsomal or cytosolic fraction (see Section 5.2.2.1.2.2).

Trihalomethanes (haloforms). Comparative *in vivo* and *in vitro* studies by Anders and associates (324) showed that the relative metabolic rate of trihalomethanes follows the order: $CHI_3 > CHBr_3 > CHBr_2Cl > CHBrCl_2 = CHCl_3$. Substantial species differences have been noted in the *in vivo* metabolism of chloroform. Brown *et al.* (332) showed that after the administration of a single oral dose (60 mg/kg) of ^{14}C-labeled chloroform, mice, rats, and monkeys excreted 6%, 20%, and 79% of the dose unchanged and 80%, 66%, and 18% of the dose as CO_2 in expired air, respectively. A study by Fry *et al.* (333) indicated that in humans orally administered chloroform is largely expired unchanged.

The possible metabolic pathways of trihalomethanes are depicted in Fig. 2. Several investigations concur that microsomal hydroxylation of the C-H bond is the rate-limiting initial step. The hydroxytrihalomethane thus formed is highly unstable and may decompose to dihalocarbonyl intermediate, which may (a) be hydrolyzed to carbon dioxide, (b) react with cysteine to form 2-oxothiazolidine-4-carboxylic acid (OTZ), (c) react with sulfhydryl compounds to yield disulfide and carbon monoxide, or (d) covalently bind to tissue macromalecules. In the study of Mansuy *et al.* (334), aerobic incubation of chloroform in the presence of rat liver microsomes and NADPH yielded a reactive intermediate which reacted with cysteine to form OTZ. The intermediate was deduced to be dichlorocarbonyl, more familiarly known as phosgene. A subsequent study by the same investigators (335) showed that phosgene was responsible for the covalent binding of chloroform to microsomal macromolecules and that human microsomes also metabolize chloroform to phosgene.

In vitro and *in vivo* studies by Pohl and coworkers (336-340) support the conclusion that phosgene is the reactive intermediate of chloroform. The production of phosgene was significantly decreased when deuterated chloroform

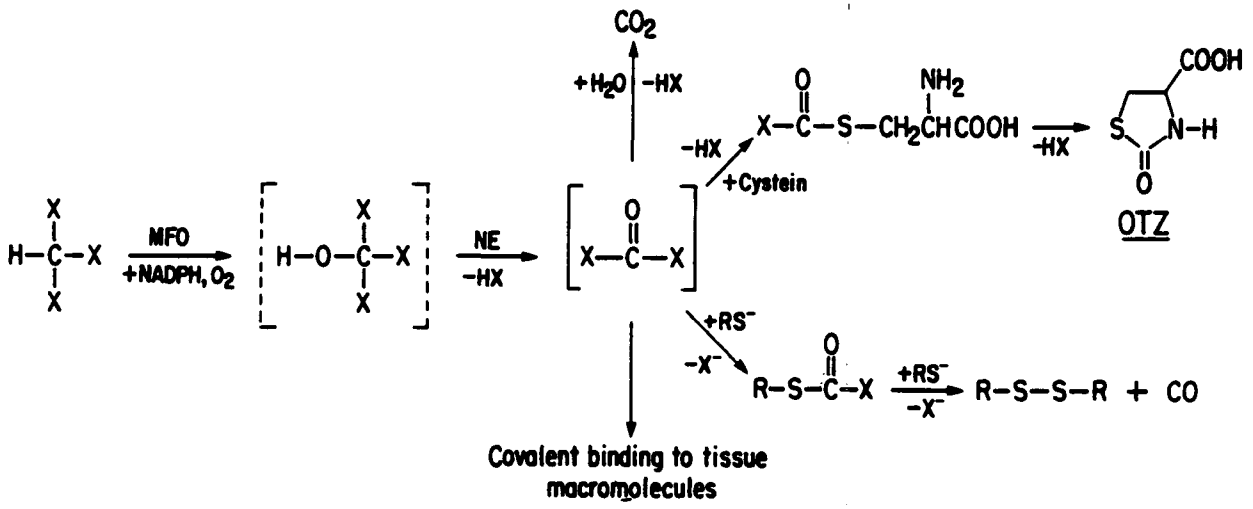


Fig. 2. Proposed metabolic pathways of trihalomethanes. The abbreviations used are: MFO = mixed function oxidases; NE = nonenzymic process; RS^- = glutathione or other sulfhydryl compounds; OTZ = 2-oxothiazolidine-4-carboxylic acid.

was used, supporting the view that the breakage of the C-H bond is the rate-limiting step (338, 340). Binding studies with the use of ^{14}C-, ^{3}H-, or ^{36}Cl-labeled chloroform indicate that only the ^{14}C-label becomes appreciably bound by covalence to microsomal proteins. Covalent binding is proportionately decreased when phosgene is trapped as OTZ by the addition of cysteine. These results led Pohl et al. (339) to suggest that phosgene is the major, if not the only, reactive intermediate formed from chloroform. Phosgene produced from chloroform may also react with glutathione; diglutathionyl dithiocarbonate (GS-CO-SG) has been demonstrated to be a final metabolite of chloroform (341).

Like dihalomethanes, trihalomethanes are also metabolized to carbon monoxide; this pathway has been investigated in detail by Anders and associates (323, 324, 342-345). In vitro production of carbon monoxide from trihalomethanes requires the presence of active microsomes, NADPH and molecular oxygen. Pretreatment of animals with phenobarbital or 3-methylcholanthrene increases whereas cobaltous chloride or SKF-525A decreases the activity. Addition of glutathione or sulfhydryl compounds greatly increases the production of carbon monoxide, although glutathione alone was found ineffective without NADPH and molecular oxygen (343). The use of ^{13}C-labeled tribromomethane and molecular $^{18}O_2$ showed that the oxygen of carbon monoxide originated from molecular oxygen (343) whereas the carbon was from tribromomethane (342). The lower metabolism of deuterated tribromomethane supports the view that breakage of C-H bond is the rate-limiting step (342, 345). The presence of dibromocarbonyl, the bromo analog of phosgene, as a reactive intermediate of tribromomethane has also been demonstrated by the trapping of the intermediate by cysteine as OTZ (345). Anders' group (324, 345) concluded that tribromomethane is first hydroxylated by microsomal mixed function oxi-

dase. The successive attacks by two molecules of sulfhydryl compounds on dibromocarbonyl yield a disulfide compound and carbon monoxide (see Fig. 2).

The covalent binding of chloroform to microsomal proteins and lipids has been demonstrated by various investigators (175, 335, 339, 340, 346-350). There is ample evidence to suggest that the covalent binding may be related to the toxic action of chloroform (e.g., 340, 349, 351). However, there is no evidence of covalent binding of chloroform to RNA (175, 348, 350) or DNA (350). Diaz Gomez and Castro (350) have recently demonstrated significant covalent binding of chloroform to hepatic histones and to nonhistone proteins; they suggested that the covalent binding to nuclear protein may be related to chloroform-induced hepatocarcinogenesis. This epigenetic mechanism is consistent with the lack of mutagenic action of chloroform in the Ames test (see Section 5.2.2.1.2.2).

Tetrahalomethane. Carbon tetrachloride is the only tetrahalomethane that has been extensively studied. The possible metabolic pathways of carbon tetrachloride are depicted in Fig. 3. It is now generally accepted that the first metabolic step involves reductive dehalogenation through interaction with cytochrome P-450 with the formation of the extremely short-lived trichloromethyl free radical (reviewed in 111, 113, 352, 353). Formation of the free radical has recently been demonstrated in vitro (352, 354) and in vivo (355) by electron spin resonance studies with the use of "spin-trapping" compounds although the exact form of the free radical is uncertain. Under anaerobic conditions, the trichloromethyl free radical may undergo a variety of reactions including (a) dimerization to hexachloroethane (356, 357), (b) addition of a proton and an electron to form chloroform (356-359), (c) binding to microsomal proteins and lipids (175, 348, 357, 360, 361), and (d) further reductive dehalogenation to carbon monoxide probably via a dichlorocarbene

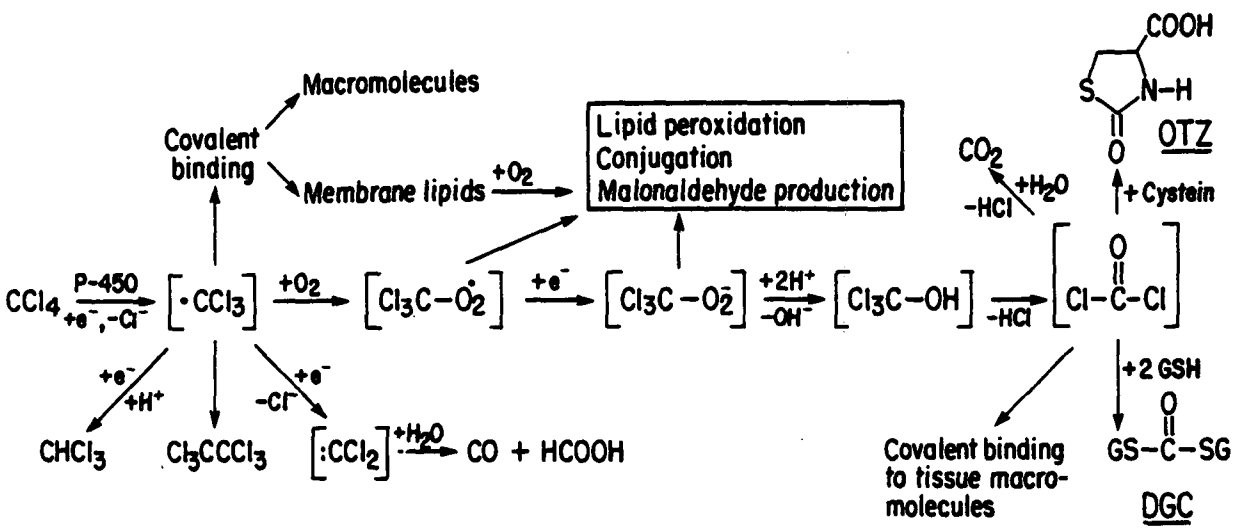


Fig. 3 Proposed metabolic pathways of carbon tetrachloride. The abbreviations used are: P-450 = cytochrome P-450; GSH = glutathione; OTZ = 2-oxothiazolidine-4-carboxylic acid; DGC = diglutathionyl carbonate.

($:CCl_2$) intermediate (362). Under aerobic condition, however, it appears that the trichloromethyl free radical is predominantly oxygenated, with the formation of carbon dioxide as the final product (353, 363, 364). Shah et al. (353), and Kubic and Anders (365) have recently identified phosgene (dichlorocarbonyl or carbonylchloride) as an intermediate in the aerobic metabolism of carbon tetrachloride, by trapping the intermediate with cysteine to yield 2-oxothiazolidine-4-carboxylic acid. Further evidence of phosgene as the intermediate has been provided by Pohl et al. (341), who isolated diglutathionyl carbonate (GS-CO-SG) as a final metabolite of carbon tetrachloride indicating the interaction of phosgene with 2 molecules of glutathione. The production of phosgene requires the presence of molecular oxygen and NADPH, is not affected by glutathione, and may be inhibited by carbon monoxide or SKF-525A, suggesting the involvement of cytochrome P-450 (365). Shah et al. (353) postulated the formation of hydroxytrichloromethane as the precursor of phosgene.

Despite extensive studies, the mechanism of carcinogenic action of carbon tetrachloride remains obscure. At least two possible mechanisms have been proposed. It is well known that carbon tetrachloride binds covalently to microsomal proteins and lipids following metabolic activation. Using ^{36}Cl-labeled carbon tetrachloride, Reynold (360) showed the incorporation of ^{36}Cl into microsomal lipids and proteins; he regarded trichloromethyl free radical ($\cdot CCl_3$) as the reactive intermediate. The evidence for covalent binding of carbon tetrachloride to nucleic acids appears to be less convincing. A number of investigators (175, 348, 356, 357, 360, 362) were unable to detect significant levels of covalent binding of carbon tetrachloride to nucleic acids. Only two groups of investigators reported positive findings. Rocchi et al. (366) did not observe any covalent binding in untreated animals; however,

after 3-methylcholanthrene pretreatment, *in vivo* covalent binding to mouse liver DNA or rat liver RNA was detected. In *in vitro* studies, covalent binding was observed only if hepatic microsomes from 3-methyclcholanthrene-pretreated rodents were used along with "pH 5 enzyme"; significant level of covalent binding to nuclear proteins also occurred (366). The covalent binding of carbon tetrachloride to mouse hepatic DNA and nuclear proteins has recently been confirmed by Diaz Gomez and Castro (367), who suggested that both processes could be relevant to the hepatocarcinogenic action of the compound. The recent identification of phosgene as a reactive intermediate of carbon tetrachloride metabolism invites further investigations. Phosgene has two highly reactive chlorines and may well act as a bifunctional alkylating agent.

The peroxidation of microsomal lipids by free radicals originating from carbon tetrachloride has been suggested to play a major, if not obligatory, role in its toxic and possibly carcinogenic action (113, 368). Considering the extremely short half-life (estimated to be around 1 microsecond) of the $\cdot CCl_3$ radical, Slater (368) postulated a cascade type of events in which $\cdot CCl_3$ first reacts with microsomal polyunsaturated fatty acids to form fatty acid radicals and peroxy radicals, which then break down to yield diffusible hydroperoxides and unsaturated hydroxy-aldehydes such as malonaldehyde (which has been reported to be a carcinogen, *see* Section 5.2.1.7.1.) Alternatively, peroxidation of membrane lipids which is known to bring about a spectrum of pathological changes leading to fatty liver, loss of protein synthesizing capability, structural disorganization of the endoplasmic reticulum, and eventually cell necrosis (113). It is possible that some of these pathological changes represent aspects of epigenetic mechanism for carcinogenesis.

Besides carbon tetrachloride, the metabolism of fluorotrichloromethane (Freon 11), difluorodichloromethane (Freon 12), and bromotrichloromethane has been studied. The presence of a fluorine atom increases while the presence of the bromine atom decreases the stability against reductive dehalogenation. Thus, Cox et al. (369) were unable to detect reductive dehalogenation of fluorotrichloromethane by microsomes from phenobarbital-pretreated mice, rats, guinea pigs or hamsters. No evidence of free radical or fluorodichloromethane was found. An in vivo study by Blake and Mergner (370) provided no firm evidence of metabolism of fluorotrichloromethane in dogs. For difluorodichloromethane, at most, only about 1% of the compound appeared to be metabolized in dogs. Nonetheless, in vitro binding study of fluorotrichloromethane by Uehleke and his associates (175, 348) showed that the fluorocarbon does bind covalently to microsomal proteins and lipids; however, the extent of binding was substantially lower than that of carbon tetrachloride. Pohl et al. (341) have shown subsequently that bromotrichloromethane is probably metabolized in a similar manner as carbon tetrachloride but higher amounts of dihalocarbonyl were produced from bromotrichloromethane.

5.2.2.1.4.1.2 Haloethanes. Several comparative metabolic studies have been undertaken on haloethanes. In a series of reports, Yilner (371-375) studied the metabolism of 5 chloroethanes; his major findings are summarized in Table XXI. It is evident from the Table that both the metabolic rate and the metabolic fate of chloroethanes are dependent on the number and the position(s) of chlorine substituent(s). 1,1,2,2-Tetrachloroethane is metabolized much faster than its 1,1,1,2-isomer. The major metabolite is S-carboxymethyl cysteine for 1,1-di- and 1,1,1-trichloroethane, whereas for 1,1,1,2-tetra- and pentachloroethanes, trichloroethanol and trichloreacetic acid appear to be the only urinary metabolites. Trace amounts of chlorinated ethylenes may be detected

Table XXI
Comparative Metabolism of ^{14}C-Labeled Chloroethanes in Mice[a]

Chloroethane	Dose (g/kg)	Expired Unchanged (% of dose)	Metabolites (% of dose)		Major Identified Urinary Metabolite (% of urinary ^{14}C activity)
			Expired	Urinary	
1,2-Di-	0.05-0.17 (i.p.)	10-45	12-15 (as CO_2)	51-73	S-Carboxymethylcysteine (44-46%, free) (0.5-5%, conjugated) Thiodiacetic acid (33-34%) Chloroacetic acid (6-23%)
1,1,2-Tri-	0.1-0.2 (i.p.)	6-9	10-13 (as CO_2)	73-87	S-Carboxymethylcysteine (29-46%, free) (3-10%, conjugated) Thiodiacetic acid (38-42%) Chloroacetic acid (6-31%)
1,1,1,2-Tetra-	1.2-2.0 (s.c.)	21-62	< 0.02 (as $CHCl=CCl_2$)	18-56	Trichloroethanol (89-94%) Trichloroacetic acid (6-12%)
1,1,2,2-Tetra-	0.21-0.32 (i.p.)	< 4	45-61 (as CO_2) 0.2-0.4 (as $CHCl=CCl_2$) 0.2-0.4 (as $CCl_2=CCl_2$)	23-34	Dichloroacetic acid (20-34%) Trichloroacetic acid (2-8%) Trichloroethanol (3-15%) Oxalic acid (5-10%) Glyoxylic acid (0.4-1.4%) Unidentified (approx. 50%)
Penta-	1.1-1.8 (s.c.)	12-51	2-16 (as $CHCl=CCl_2$) 3-9 (as $CHCl=CCl_2$)	25-50	Trichloroethanol (64%) Trichloroacetic acid (36%)

[a]Summarized from the data of S. Yilner [Acta Pharmacol. Toxicol. 30, 257 (1971); 30, 248 (1971); 29, 471 (1971); 29, 499 (1971); 29, 481 (1971).] The excretion of radioactivity was followed by 3 days.

in the expired air of mice given 1,1,1,2-tetra-, 1,1,2,2-tetra-, or pentachloroethanes. A comparative study of Nakajima and Sato (319) shows that the relative *in vivo* metabolic rate of chloroethanes follows the order: 1,2-di- > 1,1,2-tri- > 1,1-di- > 1,1,2,2-tetra- > 1,1,1,2-tetra- > 1,1,1-tri- in male Wistar rats, and 1,1,2,2-tetra- > 1,2-di- > 1,1-di- > 1,1,2-tri- > 1,1,1,2-tetra- > 1,1,1-tri- in female rats. The relative *in vitro* metabolic rate (as measured by percent ^{36}Cl enzymatically removed from ^{36}Cl-labeled haloethane by rat liver microsomes) was shown by Van Dyke and Wineman (376) to follow the approximate order: 1,1-di- > 1,1,2-tri- > 1,1,2,2-tetra- > penta- > mono- = 1,2-di- = 1,1,1-tri-. Among the haloethanes, only 1,2-dihaloethane and halothane have been extensively studied; these are discussed below.

1,2-Dihaloethanes. Owing to their mutagenic and carcinogenic properties and industrial uses, 1,2-dihaloethanes (ethylene dihalides) have attracted much attention. Rannug (15) and Anders and Livesey (377) have reviewed, in 1980, the metabolic studies of these compounds. Several subsequent studies (179, 181, 378) have since been reported. The known metabolites of 1,2-dichloro- and 1,2-dibromoethanes are: inorganic halides (379, 380), S-carboxymethylcysteine, thiodiacetic acid, chloroacetic acid (375), N-acetyl-S-(2-hydroxyethyl)-cysteine and its S-oxide (381), S-(2-hydroxyethyl)-cysteine (381, 382), S-(2-hydroxyethyl)-glutathione and its S-oxide, S,S'-ethylene-bis-(glutathione) (380), bromoacetaldehyde (383), and ethylene (384). At least two major routes of metabolism have been proposed (see Fig. 4). In the first route, oxidative metabolism of 1,2-dihaloethane (i) by microsomal cytochrome P-450-dependent mixed-function oxidase yields the highly unstable hydroxy intermediate (ii), which spontaneously decomposes to haloacetaldehyde (iii). Haloacetaldehyde is highly reactive and may (a) covalently bind to nucleophilic macromolecules, (b) react with glutathione to form a conjugate which

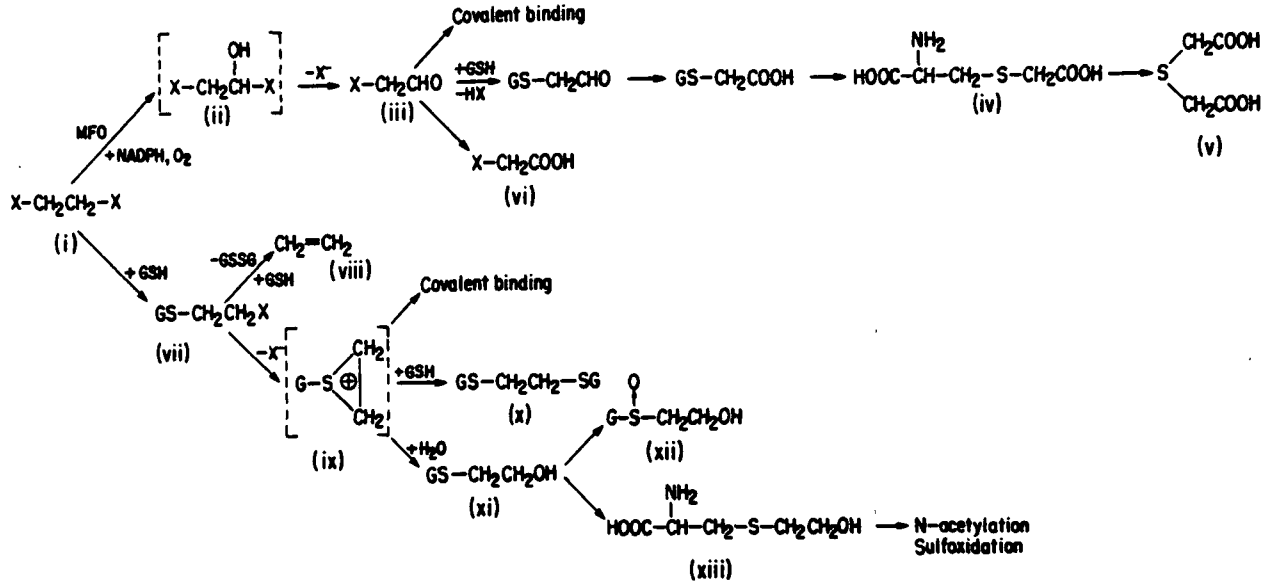


Fig. 4. Proposed metabolic pathways of 1,2-dihaloethanes. The chemical names of the compounds are: i = 1,2-dihaloethane; ii = hydroxy-1,2-dihaloethane; iii = haloacetaldehyde; iv = S-carboxymethyl-cysteine; v = thiodiacetic acid; vi = haloacetic acid; vii = S-(2-haloethyl)-glutathione; vii = ethylene; ix = episulfonium ion intermediate; x = S,S'-ethylene-bis-(glutathione); xi = S-(2-hydroxyethyl)-glutathione; xii = S-(2-hydroxyethyl)-glutathione sulfoxide; xiii = S-(2-hydroxyethyl)-cysteine. [Modified from M.W. Anders, and J.C. Livesey: In "Ethylene Dichloride: A Potential Health Risk?" (B. Ames, P. Infante, and R. Reitz, eds.), Cold Spring Harbor Laboratory, New York, 1980, p. 331]

gives rise to S-carboxylmethyl cysteine (iv), and thiodiacetic acid (v) after further metabolism by dehydrogenase, peptidase and deaminase, or (c) be oxidized to halocetic acid (vi). In the second route, nucleophilic attack of 1,2-dihaloethane by reduced glutathione catalysized by glutathione transferase yields S-(2-haloethyl)-glutathione (vii), which may be attacked by a second molecule of reduced glutathione to yield ethylene (viii). Alternatively, since S-(2-haloethyl)-glutathione is actually a half-sulfur mustard, it may cyclize to the highly reactive episulfonium ion (ix), which may (a) be attacked by reduced glutathione to form S,S'-ethylene-bis-(glutathione) (x), (b) hydrolyze to S-(2-hydroxyethyl)-glutathione (xi), or (c) possibly act as an alkylating agent. The S-(2-hydroxethyl)-glutathione (xi) may either be oxidized to its sulfoxide or hydrolyzed by peptidase to yield S-(2-hydroxyethyl)-cysteine (xiii), which can be further N-acetylated to N-acetyl-S-(2-hydroxyethyl)-cysteine and sulfoxidized to its sulfoxide. In addition to the above routes, several other possibilities have been proposed (179, 385). The formation of an extremely reactive 1-chloroso-2-chloroethane ($ClCH_2CH_2\overset{\oplus}{Cl}\overset{\ominus}{=}O$) intermediate by microsomal oxidation of 1,2-dichloroethane has been suggested (179). This intermediate is impossible to detect directly due to its high reactivity; it is expected to (a) rearrange to a hypochlorite ($ClCH_2CH_2OCl$), which may give rise to chloroacetaldehyde or 2-chloroethanol, or (b) react with glutathione to form S-(2-chloroethyl)-glutathione. Theoretically, 1,2-dihaloethanes may also undergo reductive dehalogenation to yield chloroethyl free radical or dehydrohalogenation to vinyl chloride; however, there appears to be no sufficient experimental evidence to support these pathways (179).

The role of metabolism in the generation of mutagenic or carcinogenic intermediates from 1,2-dihaloethanes has been extensively investigated but still remains unresolved. Haloacetaldehyde and the episulfonium intermediate have been regarded as the principal reactive intermediates. Hill et al. (383) identified bromoacetaldehyde as a microsomal metabolite of 1,2-dibromoethane, demonstrated that bromoacetaldehyde was capable of binding directly (without metabolic activation) to nucleophiles, and suggested the role of this intermediate in the macromolecular binding of 1,2-dibromoethane. This finding was confirmed by Banerjee, Van Duuren and coworkers (385, 386). The microsomal mixed function oxidases-mediated covalent binding to macromolecules has also been shown with 1,2-dichloroethane (378, 385). There is preliminary evidence for a correlation between the microsome-mediated binding and species and organ susceptibility to 1,2-dichloroethane-induced carcinogenesis (378, 385). The observation that disulfiram enhances the carcinogenicity of 1,2-dibromoethane (262) is consistent with the proposed role of haloacetaldehyde; indeed, the inhibition of aldehyde dehydrogenase by disulfiram is expected to block further oxidation of bromoacetaldehyde and this results in increased tissue level of this intermediate. In contrast to the above findings, there is sufficient evidence to indicate that the mutagenic activity of 1,2-dihaloethanes is mainly due to cytosol-catalyzed activation via conjugation with reduced glutathione (GSH). Studies by Rannug et al. (178), Van Bladeren et al. (181), and Guengerich et al. (177) all indicate that cytosol is the better source of the activating enzyme (GSH transferase) for 1,2-dihaloethanes in the Ames test. Metabolic activation of 1,2-dihaloethanes by the commonly used S-9 fraction (9000 x g supernatant which contains both cytosol and microsomes) is most likely due mainly to cytosol component because the inclusion of microsomes alone decreases rather than increases the mutagenic activity of 1,2-di-

haloethanes (in suspension assay, see Section 5.2.2.1.2.2). The episulfonium ion (ix) has been suggested to be the most likely mutagenic intermediate. Episulfonium ions are active electrophiles capable of readily reacting with nucleophiles (reviewed in 387). The above observations may imply a possible bifurcation of the metabolic activation of 1,2-dihaloethanes into pathways leading to carcinogenic and to mutagenic intermediates. A major discrepancy between the binding studies of Banerjee et al. (378, 385) and Guengerich et al. (179) has been noted. The former group showed that the covalent binding of 1,2-dichloroethane to DNA was not catalyzed by cytosol and was inhibited by glutathione whereas the latter group demonstrated that glutathione actually enhanced the microsome-mediated binding to DNA and that cytosol catalyzed the covalent binding in the presence of glutathione.

Halothane. The metabolism of halothane has been extensively studied because of the widespread use of the compound as an anesthetic agent. This topic has been thoroughly reviewed in 1976 and 1977 (44, 59, 388) and will not be further elaborated in this section because of the lack of evidence of carcinogenicity or mutagenicity of the compound. It is important to point out, however, that covalent binding of halothane metabolites to proteins and lipids (but not RNA, DNA) does occur (e.g., 175, 389) and that 1,1-difluoro-2-bromo-2-chloroethylene, a "presumed" metabolite of halothane has been shown to be mutagenic (165).

5.2.2.1.4.1.3 Halopropanes. Little information is available on halopropanes and higher haloalkanes. Nakajima and Sato (319) reported that 1-chloropropane is metabolized in the rat at a rate higher than most halomethanes, haloethanes, and haloethenes. Hutson et al. (390) noted rapid metabolism of ^{14}C-labeled 1,2-dichloropropane in the rat; 80-90% of the radioactivity is

excreted via the exhaled air and the urine within the first 24 hours. About 45% of the radioactivity in the exhaled air is in the form of carbon dioxide. The identity of other exhaled and urinary metabolites has not been investigated. The metabolism of the soil fumigant, 1,2-dibromo-3-chloro-propane (DBCP) in the rat has recently been studied by Jones et al. (391). Figure 5 depicts its possible metabolic pathways. The initial step is presumed to be the dehalogenation of the central bromine atom of DBCP (i), yielding the reactive carbonium ion (ii), which readily reacts with water to form 1-bromo-3-chloro-propan-2-ol (iii). Dehydrohalogenation of the intermediate (iii) readily occurs, especially under alkaline condition, with the formation of either epibromohydrin (iv, X = Br) or epichlorohydrin (iv, X = Cl). Glutathione conjugation of these epoxides, followed by hydrolysis by peptidase, and then N-acetylation, produces the mercapturic acid intermediate (v). Dehydrohalogenation of the intermediate (v) yields the epoxide (vi), which may either be conjugated by a second molecule of glutathione to eventually yield 1,3-(bis-N-acetylcysteinyl)-propan-2-ol (vii) or be hydrolyzed to S-(2,3-dihydroxypropyl)-mercapturic acid (viii). Hydrolysis of epihalohydrin (iv) produces α-halohydrin (ix), which may yield compound (viii) via epoxide (x) or be oxidized to β-halolactate (xi) and eventually to oxalic acid (xii). Compounds (vii) and (viii) have actually been isolated from the urine of rats given DBCP. The production of α-halohydrins and oxalate may explain the antifertility and renal toxicity of DBCP, whereas the epoxides are the potential mutagenic or carcinogenic intermediates. The metabolism of 1,2,3-tribromopropane (391) and 1,2-dichloropropane (393) has also been studied and found to be similar to that of DBCP. Oxalic acid (xii), β-bromolactate (xi, X = Br), and the mercapturic acid conjugates (vii) and (viii) have been identified as the metabolites of 1,2,3-tribromopropane. For 1,2-dichloropropane,

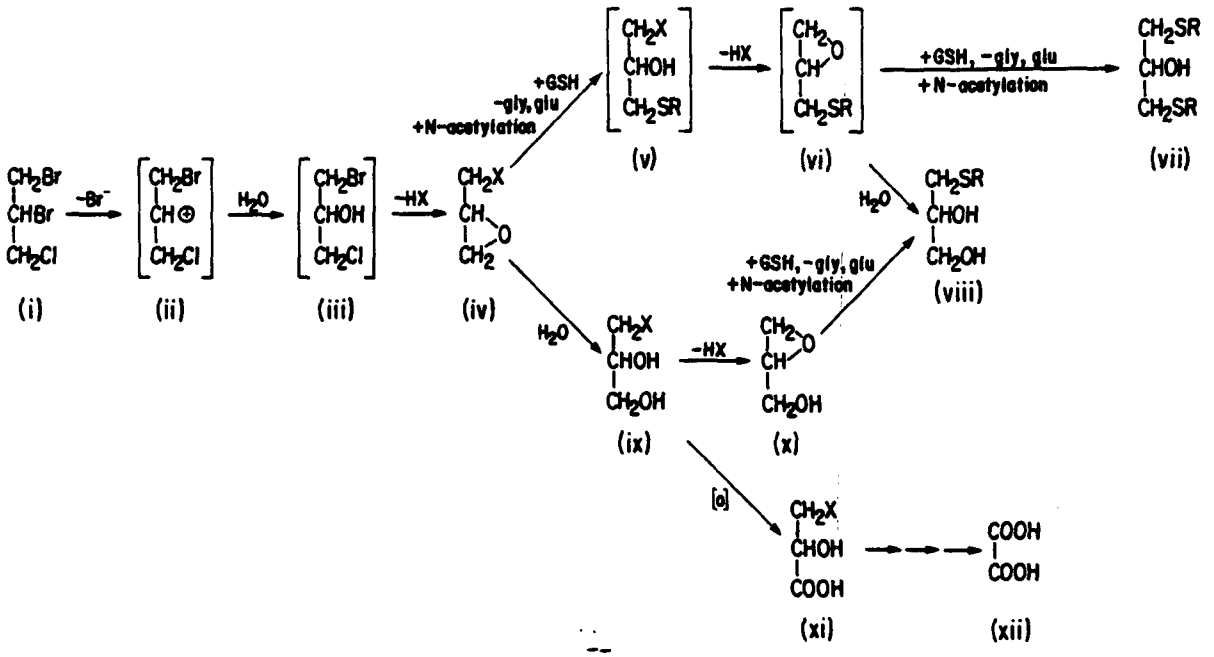


Fig. 5. Proposed metabolic pathways of 1,2-dibromo-3-chloropropane. In the formulas, R = $-CH_2\underset{\underset{NHCOCH_3}{|}}{CH}COOH$; X = Cl or Br. The chemical names of the compounds are: i = 1,2-dibromo-3-chloropropane; ii = carbonium ion intermediate; iii = 1-bromo-3-chloropropan-2-ol; iv = epihalohydrin; v = S-(2-hydroxyl-3-halopropyl)-mercapturic acid; vi = S-(2,3-epoxypropyl)-mercapturic acid; vii = 1,3-(bis-N-acetylcysteinyl)-propan-2-ol; viii = S-(2,3-dihydroxypropyl)-mercapturic acid; ix = 3-halo-1,2-propanediol; x = glycidol; xi = β-halolactic acid; xii = oxalic acid. [Modified from A.R. Jones, G. Fakhouri, and P. Gadiel, Experientia 35, 1432 (1979)]

the metabolites include S-(2-hydroxypropyl)-mercapturic acid (major metabolite), β-chlorolactate (xi, X = Cl), and S-(2,3-dihydroxypropyl)-mercapturic acid (viii).

5.2.2.1.4.2 METABOLISM AND MECHANISM OF ACTION OF HALOALKENES.

5.2.2.1.4.2.1 Haloethenes (Haloethylenes). Haloethenes are the most extensively studied haloalkenes because of their economic importance and because of the potent carcinogenicity of vinyl chloride in this class. The role of metabolism in the activation of chlorinated ethenes has been reviewed by Henschler (135, 191) and Leibman and Ortiz (392). The general metabolic scheme of these compounds is shown in Fig. 6. It is generally believed that microsomal oxidation of chlorinated ethenes to their respective epoxide is the first and obligatory step in the metabolic activation of the whole class. The epoxide of chlorinated ethene is highly reactive and may undergo a variety of reactions including (i) covalent binding to cellular macromolecules, (ii) conjugation with soluble nucleophiles such as glutathione, (iii) enzymatic (by epoxide hydrase) or nonenzymatic hydrolysis to chlorinated ethylene glycol, and (iv) intramolecular rearrangement (Cl shift) to chlorinated acetaldehyde (reaction a, X_4 = H) or acetyl chloride (reaction b). The conjugation is generally regarded to be a detoxification reaction. Chlorinated ethylene glycol is unstable and is expected to undergo further decomposition (392). The intramolecular rearrangement plays a predominant role in the metabolism. Theoretical considerations and thermal rearrangement studies by Henschler and coworkers (135, 190-192) suggest that, depending on the number and position(s) of chlorine substituent(s), two types of products -- acyl chlorides (tri-, di-, or monochloroacetyl chlorides for tetra-, tri-, or 1,1-dichloroethylenes, respectively) or aldehydes (di- or monochloroacetaldehyde for cis/trans 1,2-dichloroethylene and vinyl chloride, respectively) -- may arise. The

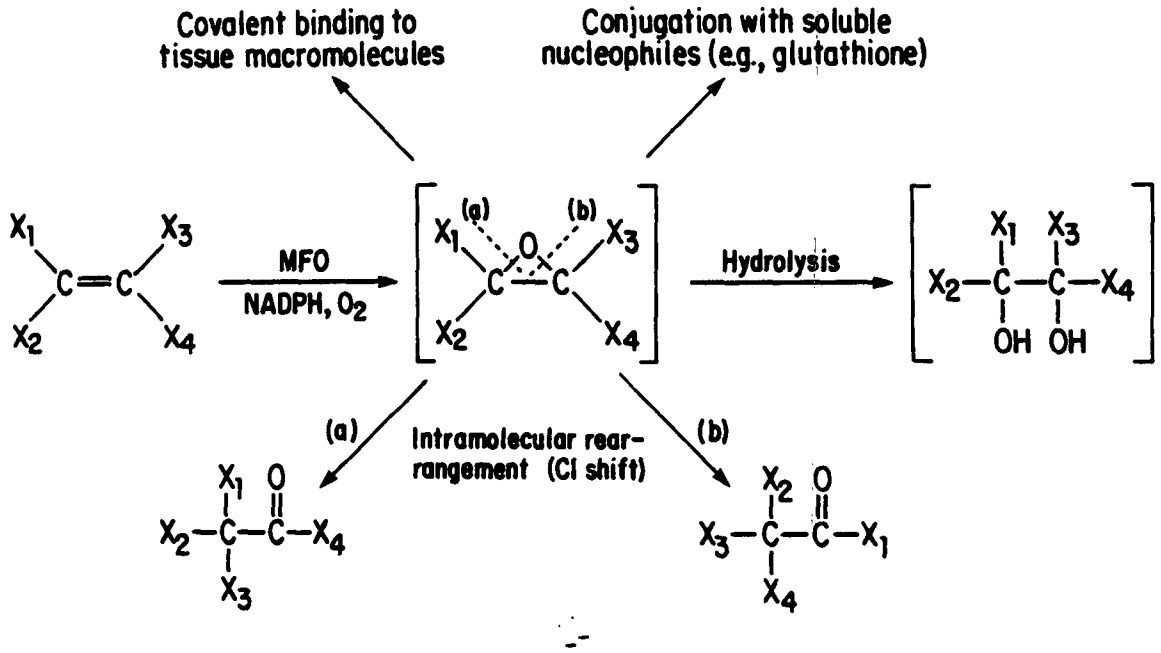


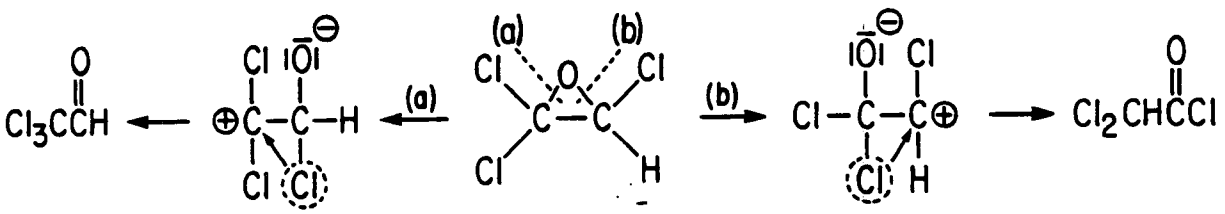
Fig. 6. General metabolic scheme of chlorinated ethenes. (In the formula, X_1 = Cl; X_2, X_3, X_4 = Cl or H.)

aldehydes may be further reduced or oxidized to alcohols or acids, respectively, whereas the acyl chlorides may act as acylating agent or be hydrolyzed to acids. These predictions have been supported by metabolic studies of various chlorinated ethenes (see below) with trichloroethylene as the only exception. Assuming the formation of ketocarbenium ion intermediate after C-O heterolysis as the first step, trichloroethylene epoxide is expected to yield

[TEXT-FIGURE 6]

dichloroacetyl chloride [reaction (b)] because the ionized carbon is more stable with one chlorine than with two chlorine substitutions. Metabolic studies have, however, shown that reaction (a) with the formation of trichloroacetaldehyde (or its hydrated form, chloral hydrate) is the preferred route.

The epoxide of chlorinated ethene has been regarded as one of the principal reactive intermediates responsible for the potential mutagenic or carcinogenic action of the parent compound. Henschler and his group (135, 190-192) have postulated that epoxides of unsymmetrically substituted chlorinated ethenes (vinyl chloride, 1,1-dichloroethylene, trichloroethylene) are less stable and more electrophilic than those with symmetric chlorine substitutions (cis or trans 1,2-dichloroethylene, tetrachloroethylene). Using a mutagenicity test with Escherichia coli K12, a relationship between instability of the epoxide and the mutagenicity of the parent compound has been noted: the unsymmetric chlorinated ethenes are all mutagenic whereas the symmetric ones are not. It is not known to what extent this rule may apply to other



Text-Figure 6

systems. This molecular rule is partially supported by the results obtained using the Ames test (see Table VII). In addition, tetrachloroethylene appears to be at least as carcinogenic as trichloroethylene in $B6C3F_1$ mice. However, a recent theoretical computational study by Politzer et al. (394) indicates that there is no significant difference in the calculated stabilities of epoxides of various symmetric and unsymmetric chlorinated ethenes.

A number of comparative metabolic studies of haloethenes have been carried out. Using isolated perfused rat liver preparations Bonse et al. (395) showed that, in general, an inverse relationship exists between the number of chlorine substituents and the metabolic rate of chlorinated ethenes. This is supported by the in vivo study of Nakajima and Sato (319), who showed that the metabolic rate in Wistar rats of three chlorinated ethenes follows the order: 1,1-dichloro > trichloro >> tetrachloro. A more extensive study by Filser and Bolt (396) is also in agreement with this correlation (with the exception of trichloroethylene). The estimated zero-order maximal metabolic rates (V_{max}) of six chlorinated ethenes follow the order: trichloro > monochloro (i.e., vinyl chloride) > 1,1-dichloro > cis-1,2-dichloro > trans-1,2-dichloro > tetrachloro. Fluorinated alkenes are substantially less susceptible than chlorinated alkenes to biotransformation. The zero-order V_{max} of 1,1-difluoroethylene is nearly 100 times lower than that of 1,1-dichloroethylene (396). In the vinyl halide series, the zero-order V_{max} follows the order: vinyl chloride > vinyl bromide > vinyl fluoride (396). Although Filser and Bolt (396) have cautioned that substantial differences in metabolic rates may exist among different species and strains so that the above pharmacokinetic data may be valid only for the Wistar rats used, a pharmacokinetic study by Monster (397) with trichloroethylene and tetrachloroethylene in human subjects shows that (in agreement with rat studies) trichloroethylene is

indeed rapidly metabolized (75% metabolized) whereas tetrachloroethylene is very resistant to metabolism (2% metabolized). Many metabolic and mechanism studies on the individual haloethenes have been conducted.

Vinyl Chloride. The metabolism of vinyl chloride has been thoroughly reviewed by Plugge and Safe (398), IARC (23), and Fishbein (54). The major metabolic pathways are depicted in Fig. 7. Vinyl chloride (i) is believed to be oxidized to its epoxide (chloroethylene oxide, ii), which may undergo intramolecular rearrangement (Cl shift) to generate chloroacetaldehyde (iii). Oxidation of compound (iii) by aldehyde dehydrogenase yields chloroacetic acid (iv). Compounds (ii), (iii), and (iv) may be conjugated with glutathione (GSH) to glutathione conjugates (v and vi), which give rise to S-formylmethyl-cysteine (vii) and S-carboxymethyl-cysteine (viii) after hydrolysis by peptidase. Compound (viii) may be converted to thiodiglycolate (thiodiacetate, ix) after deamination and decarboxylation, while compound (vii) may be reduced to S-(2-hydroxyethyl)-cysteine (x) and then N-acetylated to the mercapturic acid conjugate, N-acetyl-S-(2-hydroxyethyl)-cysteine (xi). Compounds (iv), (viii), (ix), (x), and (xi) have all been detected as urinary metabolites (see ref. 398). The generation of $^{14}CO_2$ and a number of minor metabolites in animals given ^{14}C-labeled vinyl chloride was postulated to occur via the tricarboxylic acid cycle or one-carbon or two-carbon pools with chloracetic acid (iv) or chloroethylene glycol (xii) as the starting intermediates (398). In addition to above pathways, Green and Hathway (399) detected S-(2-chloroethyl)cysteine and its N-acetylated derivative as urinary metabolites and proposed a possible direct interaction between glutathione and vinyl chloride per se. This pathway is, however, not supported by a 1979 study of Guengerich and Watanabe (400) using ^{36}Cl-labeled vinyl chloride; they concluded that any chemical mechanism for activation and binding of vinyl chloride involves release of the chlorine atoms as chloride ions.

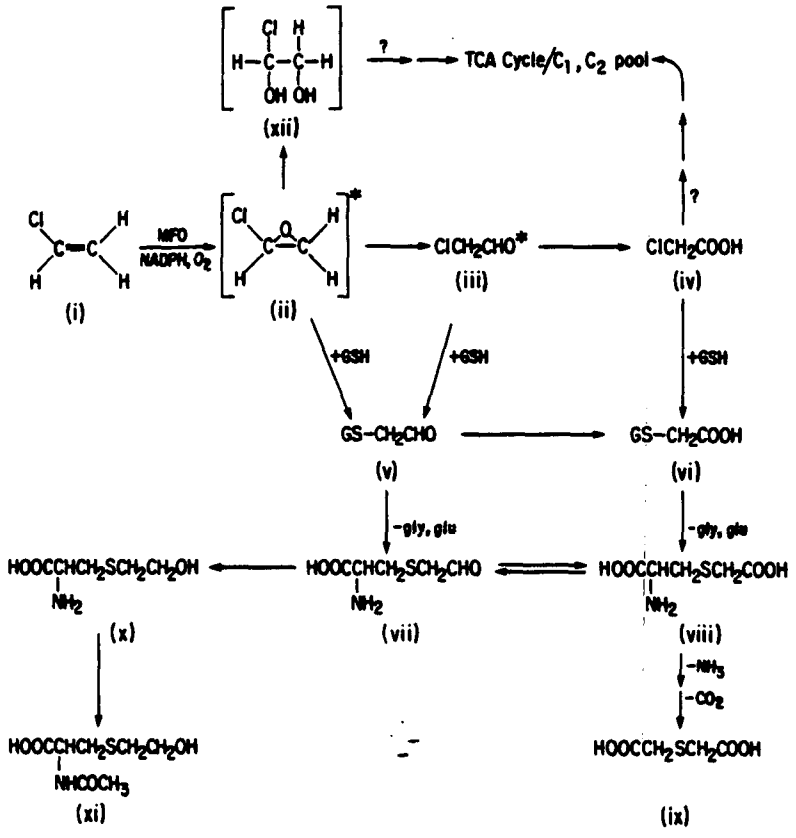


Fig. 7. Major metabolic pathways of vinyl chloride. The chemical names of the compounds are: i = vinyl chloride; ii = chloroethylene oxide; iii = chloroacetaldehyde; iv = chloroacetic acid; v = S-formylmethyl glutathione; vi = S-carboxymethyl glutathione; vii = S-formylmethyl-cysteine; viii = S-carboxymethyl-cysteine; ix = thiodiacetic acid; x = S-(2-hydroxyethyl)-cysteine; xi = S-(2-hydroxyethyl)-mercapturic acid; xii = chloroethylene glycol. Compounds with an asterisk are potential reactive intermediate. [Adapted from H. Plugge and S. Safe: Chemosphere 6, 309 (1977)]

The pharmacokinetics of the metabolism of vinyl chloride has been extensively investigated (396, 401-405). Since the metabolism of vinyl chloride appears to be a saturable process, the incorporation of a pharmacokinetic model in the risk assessment of low dose exposure to vinyl chloride has been proposed (406, 407). It is interesting to point out that considerable species differences in vinyl chloride metabolism have been observed. Buchter et al. (408, 409) reported that the first order metabolic clearance rate (in liter/h/kg body weight) for vinyl chloride in various animal species decreases in the order: mouse (25.6) > gerbil (12.5) > rat (11.0 for Wistar strain) > monkey (3.55) > rabbit (2.74) > man (2.02). They stressed that this species difference should be taken into account in risk assessment.

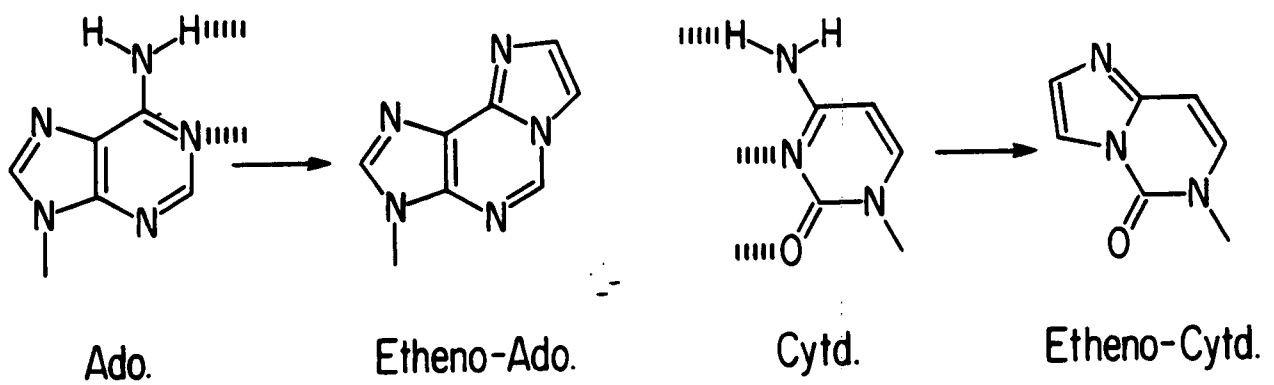
The covalent binding of vinyl chloride metabolites to cellular macromolecules has been the subject of intensive investigations (400, 410-417) because of its implication in the initiation of mutagenesis and/or carcinogenesis. Both chloroethylene oxide (vinyl chloride epoxide) and chloroacetaldehyde have been regarded as the possible "ultimate" mutagen or carcinogen of vinyl chloride. Both compounds are highly reactive (with chloroethylene oxide being much more so) (73, 412) and may react directly with adenosine to form 3-β-ribofuranosyl-imidazo-[2,1-i] purine (1,N^6-etheno-adenosine) (73). Both chloroacetaldehyde (154, 177, 418, 419) and chloroethylene oxide (154, 178, 418) are mutagenic in bacterial and V79 Chinese hamster cell test systems, although the latter compound is much more potent and is also mutagenic in yeast. A recent study by Zajdela et al. (412) shows, moreover, that chloroethylene oxide is a potent local carcinogen by subcutaneous injection and an active tumor-initiator by skin painting. Chloroacetaldehyde is inactive as a tumor-initiator; however, its potential complete carcinogenicity cannot be evaluated because of its potent necrotizing activity. Most investigators

(410-412, 414) consider chloroethylene oxide as the principal reactive intermediate, although some (400, 413) regard the less reactive chloroacetaldehyde to be a more effective biological alkylating agent. The nature of covalent binding between vinyl chloride metabolite and DNA (414, 415) or RNA (416, 417) has been investigated. 9-β-D-2'-Deoxyribofuranosyl-imidazo-[2,1-i] purine (1,N^6-etheno-deoxyadenosine), 1-β-D-2'-deoxyribofuranosyl-1,2-dihydro-2-oxo-imidazo-[1,2-c] pyrimidine (3,N^4-etheno-deoxycytidine), 1,N^6-etheno-adenosine, and 3,N^4-etheno-cytidine have been identified as reaction products.

[TEXT-FIGURE 7]

The introduction of such etheno groupings into DNA bases is expected to interfere with the normal Watson-Crick type base pairing (412). Besides vinyl chloride, vinyl bromide has also been shown to alkylate (after metabolic activation) polyadenylic acid, polycytidylic acid, or RNA to yield 1,N^6-etheno-denosine and 3,N^4-etheno-cytidine (421).

Vinylidene Chloride (1,1-Dichloroethylene). The metabolic fate of vinylidene chloride has been investigated in several studies (98, 422-426). The major metabolic pathways proposed are summarized in Fig. 8. Vinylidene chloride (i) is expected to be oxidized to 1,1-dichloroethylene oxide (ii) which rearranges to chloroacetyl chloride (iii) and is then oxidized to chloroacetic acid (iv). The epoxide (ii) may also conjugate with glutathione and eventually yield the N-acetyl-S-cysteinyl-acetyl derivative (v) as a final metabolite (98, 425). Reichert et al. (426) detected methyl-thio-acetylamino-ethanol (vi) as a major metabolite and postulated the interaction of chloro-



Text-Figure 7

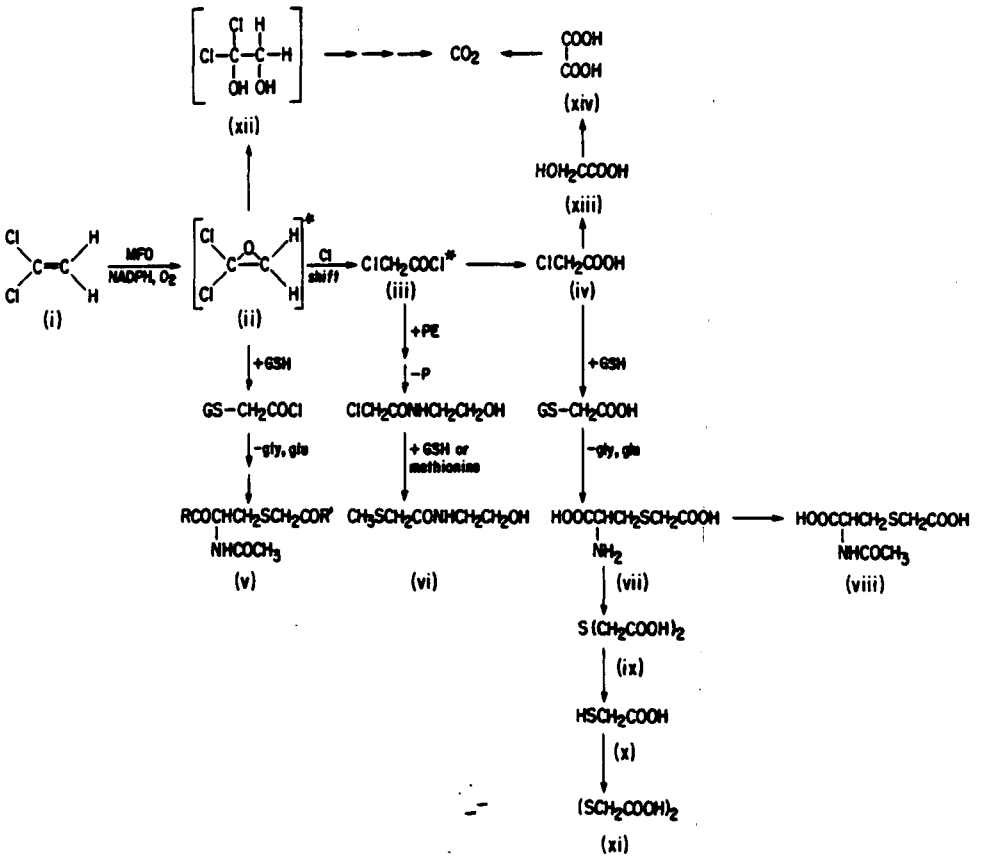


Fig. 8. Proposed metabolic pathways of vinylidene chloride. The abbreviations used are: MFO = mixed function oxidases; GSH = glutathione; PE = phosphatidyl ethanolamine; P = phosphatidyl group. The chemical names of the compounds are: i = vinylidene chloride; ii = 1,1-dichloroethylene oxide; iii = chloroacetyl chloride; iv = chloroacetic acid; v = S-(N-acetylcysteinyl)-acetyl derivative; vi = methylthioacetylaminoethanol; vii = S-carboxymethyl-cysteine; vii = S-carboxymethyl-mercapturic acid; ix = thiodiglycolic acid; x = thioglycolic acid; xi = dithioglycolic acid; xii = 1,1-dichloroethylene glycol. Compounds with an asterisk are potential reactive intermediates.

acetyl chloride (iii) with phosphatidyl ethanolamine in membrane lipid followed by nucleophilic attack by a methylthio-containing compound (e.g., methionine) or glutathione, as the reaction mechanism. Thiodiglycolic acid (ix) has been identified as one of the predominant metabolites (98, 423-426); its formation may be accounted for by glutathione conjugation of chloroacetic acid (iv), followed by hydrolysis of the glycine and glutamate moieties, transamination, and decarboxylation. N-Acetyl-S-(carboxymethyl)cysteine (viii), another major metabolite (426), may arise by N-acetylation of intermediate (vii). Hydrolysis of thiodiglycolic acid by β-thionase yields thioglycolic acid (x) and dithioglycolic acid (xi) which have been detected as minor metabolites (98, 422-425). In addition to the above metabolites, the formation of N-acetyl-S-(2-hydroxyethyl)cysteine has been reported (423, 424); no reaction mechanism has been proposed. The generation of carbon dioxide as the major exhaled metabolite may be accounted for by the degradation of dichloroethylene glycol (xii) or chloroacetic acid (iv) via glycolic acid (xiii) and oxalic acid (xiv) (98, 425). A recent study by Andersen et al. (128) suggests, however, that the epoxide hydratase pathway may be of minimal significance in the metabolism of vinylidene chloride.

Both 1,1-dichloroethylene oxide and chloroacetyl chloride are regarded as potential "ultimate" mutagenic or carcinogenic intermediates (98, 422, 425). Reitz et al. (427) investigated the potential of vinylidene chloride in covalent binding to cellular macromolecules. Alkylation of DNA was observed in the liver and kidney of both rats and mice exposed to 50 ppm ^{14}C-labeled vinylidene chloride. The level of binding was, however, quite low (about two orders of magnitudes less than that reported for dimethylnitrosamine in rats). Extensive tissue damage was associated with the administration of carcinogenic doses of vinylidene chloride. The authors (427) are of the view

that epigenetic mechanism(s) related to cytotoxicity may play a more important role in the carcinogenic action of vinylidene chloride.

Trichloroethylene. The metabolism of trichloroethylene has been reviewed by Kelley and Brown (428), Van Duuren (217), Leibman and Ortiz (392), Vaughan et al. (388), IARC (2), and Fishbein (54). Several studies (429-432) have since then been published. The metabolic pathways of trichloroethylene are depicted in Fig. 9. Like all chlorinated ethenes, trichloroethylene (i) is expected to be metabolized to its epoxide (ii) by microsomal mixed function oxidases. Intramolecular rearrangement of the epoxide yields trichloroacetaldehyde (iii) which is readily hydrated to chloral hydrate (iv). Subsequent reduction and oxidation of (iii) or (iv) give rise to the final major urinary metabolites, trichloroethanol (v) (and its glucuronide) and trichloroacetic acid (vi), respectively. The formation of compounds (iii), (iv), (v), and (vi) is regarded to be detoxification. The enzymes involved in the reduction and oxidation, and their subcellular distribution, have been described in a recent study by Ikeda et al. (432). Another route of detoxification is the enzymatic (epoxide hydrase) or nonenzymatic hydrolysis of the epoxide (ii) to trichloroethylene glycol (ix). The identification of a small amount of dichloroacetic acid (vii) as a "new" metabolite of trichloroethylene in the mouse by Hathway (430) suggests a possible activating route. The intermediate, dichloroacetyl chloride (vii) may be expected to react with nucleotides in DNA to form cyclized products the same way as chloroacetyl chloride (422). Hathway (430) proposed that this minor pathway is significant only when mice are given massive doses of trichloroethylene resulting in a buildup of trichloroacetaldehyde and reversion to the epoxide.

Trichloroethylene epoxide is generally regarded to be the principal reactive intermediate of the parent compound. Henschler et al. (431) have

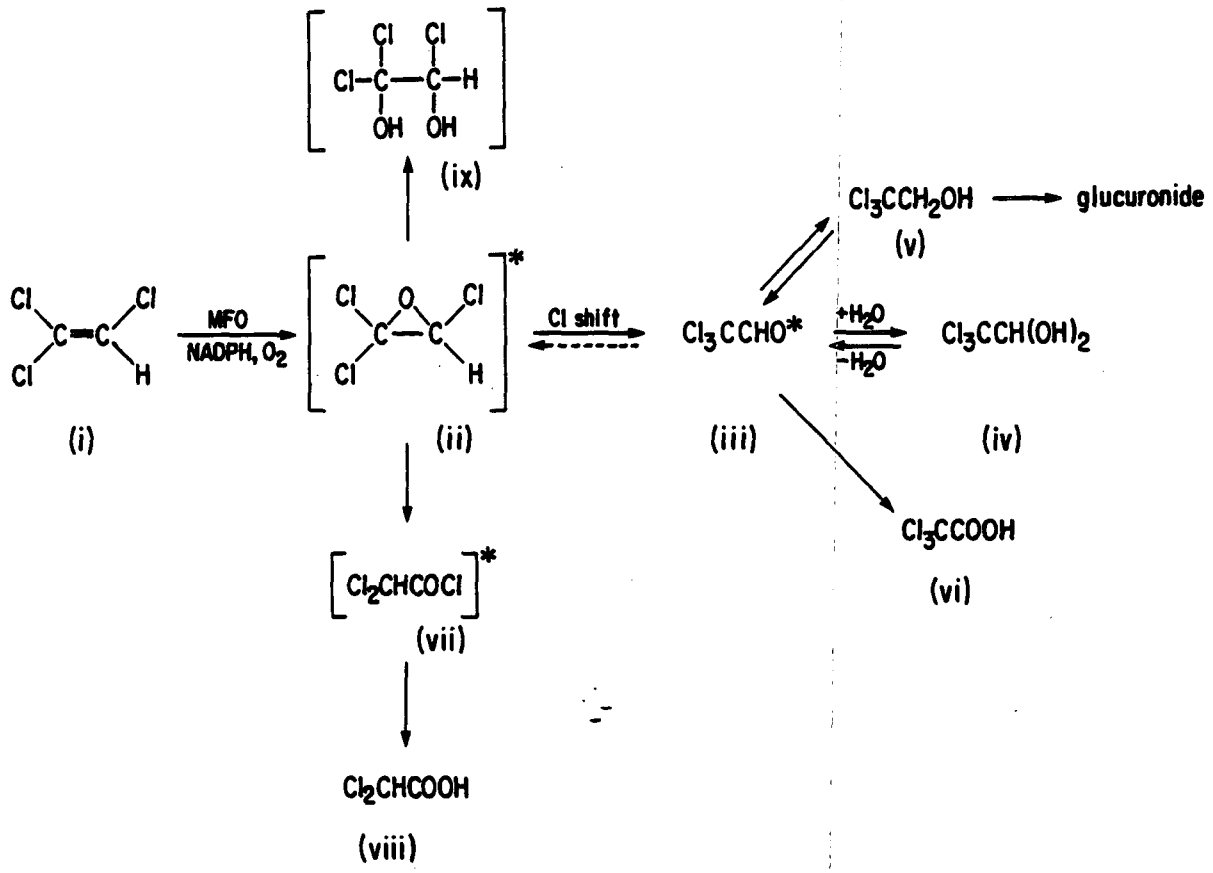


Fig. 9. Proposed metabolic pathways of trichloroethylene. The chemical names of the compounds are: i = trichloroethylene; ii = trichloroethylene oxide; iii = trichloroacetaldehyde; iv = chloral hydrate; v = trichloroethanol; vi = trichloroacetic acid; vii = dichloroacetyl chloride; viii = dichloroacetic acid; ix = trichloroethylene glycol. Compounds with an asterisk are potential reactive intermediates.

synthesized the epoxide and studied its reactivity in aqueous systems. The decomposition pattern of the epoxide appears to be quite different from that derived from in vivo metabolism of trichloroethylene. Henschler et al. (431) suggested that, under normal in vivo conditions, the highly reactive epoxide (produced from trichloroethylene) may be confined within the hydrophobic milieu of microsomes and less likely to undergo reactions observed from the synthetic epoxide in aqueous system. Nonetheless, covalent binding (although relatively low) of trichloroethylene metabolite to cellular macromolecules has been demonstrated in in vitro and in vivo studies (175, 429, 433, 434). The covalent binding is modified correspondingly by inducers and inhibitors of microsomal mixed function oxidases and is enhanced by 3,3,3-trichloropropene oxide, a typical inhibitor of epoxide hydrase (429, 434). Trichloroethylene metabolites appear to bind to more nucleophilic sites of proteins than do vinyl chloride metabolites which mainly bind to sulfhydryl groups (433). A substantial level of covalent binding of trichloroethylene to exogenously added DNA in the presence of microsomes from male $B6C3F_1$ mice rather than from female mice was observed (429). This observation is in good agreement with the substantially lower carcinogenicity of the compound in female mice (234).

Tetrachloroethylene. The metabolism of tetrachloroethylene has been reviewed by Leibman and Ortiz (392) and subsequent studies (435-437) have been reported. Figure 10 depicts the major metabolic pathways. Like other chlorinated ethenes, tetrachloroethylene (i) is believed to be metabolized to tetrachloroethylene epoxide (ii), which rearranges to trichloroacetyl chloride (iii), which in turn is hydrolyzed to form trichloroacetic acid (iv). Trichloroacetic acid has indeed been detected as the major urinary metabolite of tetrachloroethylene by many investigators (392 and refs. therein, 436). The epoxide (iii) may also be hydroxyzed to tetrachloroethylene glycol (v), which

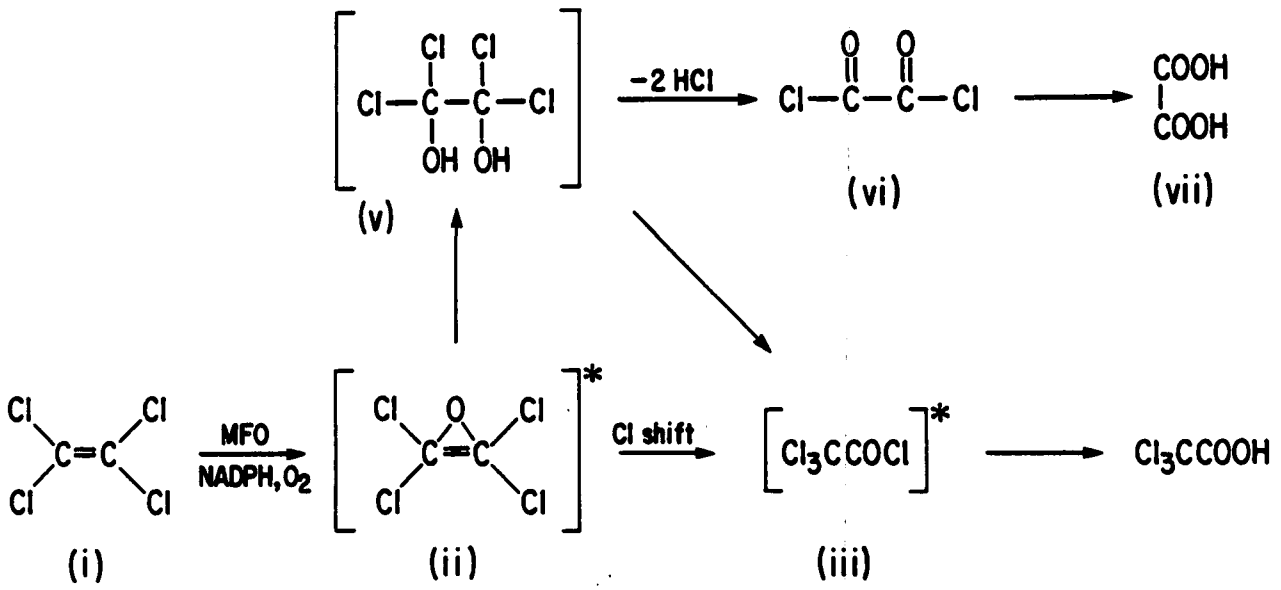


Fig. 10. Major metabolic pathways of tetrachloroethylene. The chemical names of the compounds are: i = tetrachloroethylene; ii = tetrachloroethylene oxide; iii = trichloroacetyl chloride; iv = trichloroacetic acid; v = tetrachloroethylene glycol; vi = acyl chloride intermediate (oxalyl chloride); vii = oxalic acid. Compounds with an asterisk are potential reactive intermediates.

may readily rearrange to yield trichloroacetyl chloride (iii) or decompose to oxalic acid (vii) via the acyl chloride intermediate (vi). Pegg et al. (435) recently identified oxalic acid as the major urinary metabolite and suggested that the hydrolysis of the epoxide (ii) to the diol (v) may be a major pathway in the metabolism of tetrachloroethylene. It is interesting to point out that reaction with glutathione does not seem to be a significant route of metabolism; the glutathione pool in rat liver is not depleted following tetrachloroethylene exposure (435).

Tetrachloroethylene epoxide and trichloroacetyl chloride are the presumed reactive intermediates of tetrachloroethylene. Bonse et al. (395) detected the in vitro covalent binding of some tetrachloroethylene metabolite in perfused rat liver and postulated that trichloroacetyl chloride reacted with cell constituents resulting in acylation. The acylation of hepatic microsomes has also been demonstrated by Costa and Ivanetich (436). Schumann et al. (437) reported the lack of evidence of covalent binding of tetrachloroethylene to hepatic DNA of $B6C3F_1$ mice, a strain of mice that is susceptible to the carcinogenic action of the compound. They have suggested that epigenetic mechanisms involving cytotoxicity are probably involved in the tumor induction by tetrachloroethylene.

5.2.2.1.4.2.2 Halopropenes, Halobutenes, and Halobutadienes, Very little information is available on higher haloalkenes. As discussed in Section 5.2.2.1.2, haloalkenes with vinylic structure differ significantly from those with allylic structure regarding metabolic activation. In general, vinylic haloalkenes require metabolic activation (most likely, epoxidation) whereas allylic haloalkenes may react directly with tissue nucleophiles. The mutagenic activity of vinylic and allylic haloalkenes (Table VII) reflects this difference. It is not known to what extent the direct-acting alkylating

activity of allylic haloalkenes may contribute to their potential carcinogenic activity. Highly reactive compounds may react with the first available nucleophile (including soluble tissue nucleophiles, non-essential proteins, etc.) before they can reach the critical target site(s).

Allyl Halides (3-Halopropenes). The metabolism of allyl halides in the rat was studied by Kaye et al. (438). S-Allyl-mercapturic acid and its sulfoxide are the major metabolites of allyl chloride, while S-allyl-cysteine has been shown to be a metabolite of allyl bromide and iodide. These metabolites can be accounted for by glutathione conjugation followed by hydrolysis by peptidase (yielding cysteine derivatives) and N-acetylation (yielding mercapturic acid derivatives). S-(3-Hydroxypropyl)-mercapturic acid has also been detected; it is not known whether hydroxylation occurs before or after glutathione conjugation. The above metabolism appears to be mainly detoxification. Allyl halides are direct-acting mutagens in the Ames test and their mutagenicity is reduced by inclusion of the S-9 mix (see Table VII). Van Duuren (217) hypothesized two possible activating metabolic pathways for allyl halides. Allyl halides may be converted to allyl alcohols and then oxidized to acrolein (which is mutagenic; Section 5.2.1.7.1), and acrylic acid. Alternatively, allyl halides may be epoxidized to epihalohydrin (which is carcinogenic; Section 5.2.1.1.5), and then converted to glycidol, glycidaldehyde (carcinogenic; Section 5.2.1.1.5), and epoxyproprionic acid.

1,3-Dichloropropenes. Hutson et al. (390) reported that 1,3-dichloropropenes are rapidly metabolized and excreted by the rat. Of the ^{14}C-labeled 1,3-dichloropropene administered, 80-90% of the radioactivity was eliminated in the urine, expired air, and feces within 24 hours. The trans isomer yielded more $^{14}CO_2$ (23.6% of the dose) in the expired air while the cis isomer yielded less $^{14}CO_2$ (3.9% of the dose) but correspondingly more radioactivity

in the urine. Climie et al. (439) characterized the urinary metabolite of cis-1,3-dichloropropene. Nearly all (92%) of the urinary radioactivity was present as a mercapturic acid derivative, S-(cis-3-chloroprop-2-enyl)-N-acetyl-cysteine. The same metabolite can be produced by in vitro incubation with glutathione in the presence of rat liver cytosol. Thus, like allyl halides, 1,3-dichloropropenes are probably detoxified by glutathione conjugation. 1,3-Dichloropropenes are direct-acting mutagens in the Ames test and their mutagenic activity appears to be reduced by inclusion of the S-9 mix (see Table VII).

1,4-Dichloro-2-butene. The metabolic fate of 1,4-dichloro-2-butene has not been investigated. Van Duuren et al. (215) hypothesized that the compound may be biotransformed to its epoxide which could be its reactive intermediate. However, Bartsch et al. (74) have synthesized this putative metabolite, 1,4-dichloro-2,3-epoxybutane, and tested it for mutagenicity and alkylating activity; at equimolar concentrations, the epoxide showed lower mutagenicity in Ames test and lower alkylating activity in NBP test than the parent compound, suggesting that other reactive intermediate(s) may be involved. One such possibility is dechlorination as well as epoxidation of the parent compound to a monochlorinated epoxide resembling in structure to epichlorohydrin, a potent mutagen and carcinogen.

2-Chloro-1,3-butadiene (Chloroprene). The biotransformation of chloroprene has been postulated by Haley (38) to involve microsome-catalyzed epoxidation and subsequent glutathione conjugation to form a mercapturic acid derivative. This hypothesis is supported by a 1980 study by Summer and Greim (440), who showed that administration of chloroprene to rats leads to the depletion of hepatic glutathione and increased excretion of thioethers (presumably glutathione conjugates and mercapturic acid derivatives) in the

urine. In vitro study revealed that the glutathione conjugation of chloroprene appears to require the presence of microsomes, suggesting the invovlement of an epoxide intermediate. Bartsch et al. (74) showed that incubation of chloroprene in the presence of mouse liver microsomes and cofactors yielded a volatile alkylating intermediate (presumably an epoxide) that reacted with 4-(p-nitrobenzyl)pyridine to form an NBP adduct.

Hexachlorobutadiene. The disposition of hexachlorobutadiene in the rat has been studied by Davis et al. (441). Rats given a tracer dose (0.1 mg/kg) of ^{14}C-labeled hexachlorobutadiene excreted 40% of the dose in feces (indicating biliary excretion) and 30% in urine within 48 hours. Rats given a nephrotoxic dose (300 mg/kg) of the compound only excreted 7% in feces and 6% in urine. All of the radioactivity in bile and 87% of that in urine was water soluble indicating the biotransformation of hexachlorobutadiene (which is lipophilic) to polar metabolites. The identity of the metabolites has not been determined. There is some evidence that glutathione conjugation may be involved, since the hepatic glutathione of the rat was depleted following hexachlorobutadiene administration (442).

5.2.2.1.5 Environmental Significance.

As may be expected from the extensive production and widespread use of haloalkanes and haloalkenes (see Section 5.2.2.1.1), human exposure to these compounds is virtually inevitable. With the spread of halocarbons into the environment and consumer products, the general population, especially those living in the vicinity of emission sources, may be exposed to low levels of halocarbons via the air, the drinking water, and the food. In the wake of the discovery of human carcinogenicity of vinyl chloride, the potential insidious health hazard of low level exposure to halocarbons has been the focus of great

concern. This subsection discusses the epidemiologic evidence for or against carcinogenicity of halocarbons (Section 5.2.2.1.5.1). The sources and occurrence of halocarbons in the ambient and indoor air, the drinking water, and in foodstuffs are discussed (Section 5.2.2.1.5.2). Human exposure in the occupational environment (reviewed in 1, 4, 8, 11, 12, 17, 20, 21, 31, 34, 37, 42-45) and environmental problems related to fluorocarbons in the stratosphere are not touched upon.

5.2.2.1.5.1 EPIDEMIOLOGIC EVIDENCE.

With the exception of vinyl chloride, there is insufficient epidemiologic evidence to unequivocally establish or refute the human carcinogenicity of haloalkanes and haloalkenes. The major problems encountered in epidemiologic studies of these compounds include: (a) insufficient latent period, (b) small cohort size, (c) lack of accurate quantitative exposure data, and (d) presence of confounding factors (such as other chemicals, cigarette smoking, alcohol usage, etc.). A brief review of available epidemiologic evidence is presented below.

Methylene Chloride (Dichloromethane). Only one epidemiological study with long-term follow-up of exposed workers has thus far been published in the open literature. Friedlander et al. (443) used several approaches (proportionate mortality ratio, standardized mortality rate, and survivorship analyses) to assess the health effects of chronic exposure of workers to between 30 and 125 ppm of the solvent. There was no evidence of human carcinogenicity of the compound. A critique of the above study has been presented by U.S. Environmental Protection Agency (3).

Chloroform and Other Trihalomethanes. Two epidemiologic studies of occupational exposure to $CHCl_3$ have been reported. In the first study, Bomski

et al. (444) found no evidence of liver cancer among exposed workers. However, the study was considered "uninformative" by the International Agency for Research on Cancer work group (2) with respect to $CHCl_3$ carcinogenicity because of the small number of workers and short follow-up time since first exposure. Since $CHCl_3$ was used as an inhalation anesthetic during the latter half of the 19th cenury and the early 20th century, anesthesiologists of that era were likely to be occupationally exposed to the compound. A retrospective epidemiologic study of this particular occupational group has recently been conducted by Linde and Mesnick (445). The evidence of this study does not suggest that $CHCl_3$ is carcinogenic in humans. However, because of the small population, the small number of cancer deaths involved, the different age disributions, and the lack of quantitative data, this study cannot definitely refute the human carcinogenicity of $CHCl_3$.

Chloroform and a number of other trihalomethanes (THMs) have been detected in the drinking water of many U.S. cities (see Section 5.2.2.1.5.2). A preliminary survey by U.S. Environmental Protection Agency suggested positive correlation between THMs level in water supplies (measured in 1975) and cancer mortality rates (recorded in 1969-1971). Various epidemiologic studies (e.g., 446, 447) have since been conducted using data with indirect or direct evidence of the presence of THMs in water supplies. The U.S. National Academy of Sciences (448) has reviewed these studies and concluded that:

> "The conclusions drawn in the second group of studies (i.e., studies with direct measurement of THMs), in which many cancer sites were examined, suggest that higher concentrations of THMs in drinking water may be associated with an increased frequency of cancer of the bladder. The results do not established causality,

and the quantitative estimates of increased or decreased risk are extremely crude. The effects of certain potentially important confounding factors, such as cigarette smoking, have not been determined."

Carbon Tetrachloride. There appears to be no epidemiologic studies directly involving CCl_4. However, at least three cases of liver cancer in humans exposed to CCl_4 have been reported. In the first case (449), a woman with previous history of periodic jaundice developed cirrhosis followed by cancer of the liver shortly after several exposures. She died 3 years after the first exposure. A fireman with a long history of CCl_4 exposure from fire extinguishers developed cirrhosis and an "epithelioma" of the liver 4 years after an acute intoxication by the haloalkane (450). A 59-year-old man, who 7 years earlier had had an episode of CCl_4-induced acute renal failure and liver damage, succumbed with the development of a hepatocellular carcinoma and concomitant cirrhosis (451).

1,2-Dibromoethane (Ethylene Dibromide). An epidemiologic study of the cancer mortality of 161 workers at two 1,2-dibromoethane manufacturing plants has been conducted by Ott et al. (452). Cancer mortality was significantly higher in one plant (5 observed vs. 2.2 expected) but lower (2 observed vs. 3.6 expected) in the other plant. The findings of this study neither establish nor rule out 1,2-dibromoethane as a human carcinogen.

Halothane (1,1,1-trifluoro-2-bromo-2-chloroethane). Halothane has been extensively used in combination with other compounds as anesthetic agent in contemporary medicine. Many epidemiologic studies of the potential carcinogenicity of anesthetic gases have been carried out. These studies were thoroughly reviewed by the National Institute for Occupational Safety and

Health (44). It appears that no unequivocal conclusion can be drawn from these studies.

Vinyl Chloride. The human carcinogenicity of vinyl chloride has been well established; this subject has been extensively reviewed (23, 25, 27, 28, 277). In 1974, Creech and Johnson (50) were the first to report the development of liver angiosarcoma, a rare form of liver cancer, in 4 vinyl chloride-exposed workers. As of October, 1977, a total of 64 cases of liver angiosarcoma were reported in 12 countries (453). A summary of these cases is presented in Table XXII. The latent period for tumor induction ranged from 9 to 38 with a median of 21 years (453). Several studies (454, 455) indicated that the tumor incidence was dependent on the intensity and duration of exposure. Among the more heavily exposed workers, the incidence of liver cancer may be as much as 4-5 (456) or 11 (457) times higher than that expected from spontaneous incidence. In addition to the liver, vinyl chloride has been found by some (but not all) investigators to increase significantly the incidence of brain tumors (457, 458), lung tumors (457-459), and pancreatic tumors (456). Besides occupational exposure, there is also some evidence that individuals living near vinyl chloride polymerization plants may have a higher cancer risk than the general population. Several cases of liver angiosarcoma in individuals, whose only apparent exposure was that of living near PVC plants, have been reported (460, 461).

Vinylidene Chloride (1,1-Dichloroethylene). One epidemiologic study of the cancer mortality of 138 workers exposed to vinylidene chloride has been reported (462). Only one death from respiratory cancer was noted in a worker after a lapse of more than 15 years following the initial exposure; the expected lung cancer rate for this group was 0.2. Since 40% of this small cohort had less than 15-year lapse after first exposure, it would be premature to draw any firm conclusion from this study.

Table XXII
Summary of Case Reports of Liver Angiosarcoma
in Vinyl Chloride/PVC Workers[a]

Country	No. of Cases	Years of Exposure	Latency (years)	Age at Diagnosis
Belgium[b]	1	--	--	--
Canada	10	5-26	11-28	41-61
Czechoslovakia	2	15, 16	15, 16	40, 46
Fed. Rep. Germany	9	10-22	12-22	38-58
France	8	10-29	10-29	38-63
Great Britain	2	4, 22	9, 28	37, 71
Italy	2	6, 21	15, 22	43, 55
Japan	1	22	22	52
Norway	1	21	22	56
Sweden	3	18-31	19-31	43-65
U.S.A.	23	4-28	12-38	37-67
Yugoslavia	2	18, 20	20, 23	42, 59
Total Reported	64	4-31	9-38	37-71

[a]Summarized from R. Spirtas and R. Kaminski [J. Occup. Med. 20, 427 (1978)].
[b]Data not available.

Trichloroethylene. Two epidemiologic studies on the possible cancer hazard from trichloroethylene exposure have been conducted. In a Swedish study, Axelson et al. (463) examined a cohort of 518 men and found no excess cancer deaths (11 observed versus 14.5 expected). However, because of the short follow-up period, a cancer hazard could not be ruled out. In fact, in the subcohort with high exposure and > 10 years of latency time, there were 3 cancer deaths observed versus 1.8 expected. In a Finnish study, Tola et al. (464) followed a cohort of 2117 workers and also observed no excess of cancer deaths. However, the investigators cautioned that because of the short follow-up (6-13 years), the carcinogenicity of trichloroethylene cannot be excluded at this stage of the study.

Tetrachloroethylene and Other Chlorinated Solvents. A number of chlorinated solvents such as carbon tetrachloride, trichloroethylene, and tetrachloroethylene (perchloroethylene) have been extensively used as dry cleaning fluids. Tetrachloroethylene, in particular, has been the predominant solvent in use since the 1950s. An epidemiologic study of laundry and dry cleaning workers is being carried out at the time of this writing by the U.S. National Cancer Institute. A preliminary report (465) indicates slight excess of liver cancer and leukemia among exposed workers and underscores the need for additional study of this occupational group.

2-Chloro-1,3-butadiene (Chloroprene). Two epidemiologic studies of occupational exposure to 2-chloro-1,3-butadiene yielded contradictory findings. In a Soviet study, Khachatryan (466, 467) reported excess of skin and lung cancer among exposed workers. In a more recent U.S. study, Pell (468) concluded that there was no significant excess of cancers associated with 2-chloro-1,3-butadiene exposure. It has been pointed out, however, that both studies have a number of methodological shortcomings (296, 469). The

former study failed to distinguish prevalent from incident causes, did not adjust the effect of sex and age, and did not consider the importance of latency. The latter study had incomplete follow-up, small number of persons/years of exposure, and inadequate consideration of potential confounding variables. There is one confirmed case of liver angiosarcoma in a worker who had extensive exposure probably only to the finished polychloroprene product manufacured (Herbert, 1976, cited in ref. 469).

5.2.2.1.5.2 ENVIRONMENTAL SOURCES, OCCURRENCES AND EXPOSURE.

5.2.2.1.5.2.1 Haloalkanes and Haloalkenes in the Air.

Sources. There are three principal categories of emission sources of haloalkanes and haloalkenes in the air: (a) environmental losses during manufacturing, processing, distribution, use, and disposal of products, (b) emission from secondary formation reactions or as incidental byproducts of anthropogenic activities, and (c) production of natural origins.

(a) Environmental losses from product manufacture, distribution, and use represent the most important source of most haloalkanes and haloalkenes in the ambient air. Owing to their high volatility and extensive uses (some of which are dispersive, e.g., degreaser, solvent, fumigant, fuel additive), substantial amounts of low-molecular-weight halogenated compounds are released into the atmosphere. Since the passage of Clean Air Act, the U.S. Environmental Protection Agency has been monitoring and assessing the environmental losses of selected haloalkanes and haloalkenes into the ambient air. Table XXIII lists the estimated annual U.S. emission of these compounds in recent years. In addition to the compounds listed, substantial emission of 1,1,1-trichloroethane (methyl chloroform), 1,2-dibromoethane (ethylene dibromide), and fluorocarbons is expected. Lovelock (481) estimated that the worldwide annual

Table XXIII
Estimated Annual U.S. Emissions of Some Nonfluorinated Haloalkanes and Haloalkenes from Industrial Sources into the Atmosphere

Compound	Emission (million lb/year)	Year	Reference
Dichloromethane	438[a,b]	1973	(A.D. Little, Inc. cited in ref. 58)
Chloroform	14[b,c]	1973	(A.D. Little, Inc. cited in ref. 58)
Carbon tetrachloride	99	1973	(470)
	91	1973	(471)
	65	1973	(472)
	75	1973	(58)
	84	1974	(58)
	66	1975	(58)
	61	1976	(58)
	13	1977	(473)
1,2-Dichloroethane	189	1973	(474)
	163	1974	(475)
	110	1977	(476)
Vinyl chloride	242	prior to 1975	(24)
	40	1977	(473)
Vinylidene chloride	4	1974	(477)
	2.5	1975	(478)
Trichloroethylene	258[d]	1974	(479)
Tetrachloroethylene	553[e]	1974	(480)
Chloroprene	11	1977	(473)

[a]Equivalent to 84.2% total production

[b]Uncorrected for emissions not reaching the atmosphere

[c]Equivalent to 5.6% total production

[d]Equivalent to 60% total production

[e]Equivalent to 85% total domestic consumption

emission of 1,1,1-trichloroethane, difluorodichloromethane (Freon 12), and trichlorofluoromethane (Freon 11) from chemical industry was of the order or 0.5, 0.33, and 0.38 megatons, respectively. 1,2-Dibromoethane may be readily released into the atmosphere through its dispersive use as a gasoline additive. The U.S. Environmental Protection Agency (cited in ref. 16) estimated emission factors of 0.008 and 0.31 gm 1,2-dibromoethane/gm lead/gallon gasoline from automobile exhaust in the most likely and the worst cases, respectively. Assuming lead content of 1.9 gm/gallon gasoline and annual consumption of 100 billion gallons in the United States in 1973 (cited in ref. 16) the corresponding crude estimates of annual emission into the atmosphere would be 1.5 and 59 million kg. There is some evidence (see Table XXIII) that the environmental emission of some haloalkanes and haloalkenes is diminishing as a result of implementation of new control mechanisms, substitution of alternative processes or fuel, or leveling off of demand for production.

(b) A variey of anthropogenic activities may give rise to secondary formation and subsequent emission of haloalkanes and haloalkenes into the atmosphere, although most of these sources are difficult to quantify. Combustion and chlorination are the two principal processes contributing to incidental formation.

Incineration of plastic solid wastes is a potentially important source of atmospheric halocarbons. Several investigators (e.g., 482-484) have found evidence that atmospheric methyl chloride originates from the thermal decomposition of polyvinyl chloride (PVC). The yield depends on the composition of PVC and the type of combustion process and may range from 0.31 to 3.75 mg/gm of PVC. Palmer (485) estimated that the U.S. annual emission of methyl chloride originating from the combustion of PVC is 84 million kg. Thrune (486) identified methyl chloride, methyl bromide, and methylene bromide as

minor decomposition products of epoxy resins cured with methylene dianiline. Boettner et al. (487) found that vinyl chloride is a combustion product in the incineration of plastics. Ahling et al. (488) showed that the amount of vinyl chloride released from the combustion of PVC is higher than was expectable from the residual monomer present in the plastic, suggesting pyrolytic formation. The emission factor (mg vinyl chloride/gm PVC) was 53.6 at combustion temperature of 140°C, 69.9 at 420°C, but decreased to 9.7 at 600°C and 1.7 at 790°C. The authors (488) concluded that incineration of PVC at high temperature is not a major emission source of vinyl chloride.

Automobile exhaust has been suggested to be a potential source of methyl bromide and chloroform in the urban atmosphere. Harsch and Rasmussen (cited in ref. 58) detected 18-55 ppb methyl bromide in the exhaust of automobiles burning "leaded" gasoline; automobiles burning "unleaded" gasoline emitted only 1-2 ppb in the exhaust. Thermal decomposition of the gasoline additive, 1,2-dibromoethane, is believed to be the source. Harsch et al. (489) also showed that the urban atmospheric concentration of chloroform was higher during heavy traffic and adverse meterological conditions. The exhaust gases from vehicles (burning "leaded" gasoline), in which pollution was not controlled, contained 5.6-6.8 ppb chloroform while those from pollution-controlled vehicles had substantially lower (0.066-0.091 ppb) levels of chloroform.

Cigarette smoking may be one of the most important sources of halocarbons in the indoor atmosphere. The presence of methyl chloride in cigarette smoke has been demonstrated by various authors (reviewed in 58). Chopra and Sherman (490) detected methyl chloride in the smoke of various types of tobacco and chloroform if the tobacco was previously fumigated with p,p'-DDT. The yield of methyl chloride was 11.6 mg/pack smoked. On the basis of 1974 world

tobacco production of 5.23 megaton/year and the data of Chopra and Sherman, it was estimated (58) that the annual emission of methyl chloride to the atmosphere from cigarette smoking was in the order of 10.5 million kg. Besides halomethanes, trace amounts (up to 30 ppb) of vinyl chloride were reported to be present in tobacco smoke (491).

Chlorination of waste water and drinking water is another potential secondary anthropogenic source of halomethanes in the atmosphere. Keith (cited in ref. 58) found that the use of chlorine in the treatment of waste water from paper mills results in very high concentrations of methylene chloride and chloroform in the effluent. A portion of these compounds may conceivably escape into the atmosphere because of their volatility. It is now generally accepted that chlorination of drinking water leads to the formation of trihalomethanes (see Section 5.2.2.1.5.2.2). Barcelona (492) estimated that a substantial amount (about 78.5 kg) of chloroform in the municipal water supplies of the Los Angeles area may escape into the atmosphere every day. Such escape may boost the atmospheric level of chloroform by about 79 ng/m^3 air and may account in part for the difference in chloroform levels in urban and rural atmosphere. Consistent with the above finding, Batjer et al. (493) found that the air in eight covered public swimming pools (with chlorinated water) in Bremen, Germany contained significant amounts of chloroform.

(c) Natural production (either biosynthetic, pyrogenic, or photochemical) is the most important source of a number of halomethanes in the environment. Biosynthesis by marine algae is virtually the exclusive source of iodomethane (methyl iodide) in the environment. Lovelock et al. (494) estimated a world-wide annual production of 40 megatons (36 billion kg) of iodomethane in the ocean by this source. A fraction of the iodomethane may escape into the atmosphere above the ocean. Methyl iodide may react with sodium chloride in

the ocean to yield methyl chloride (495), which is considerably more volatile than methyl iodide. The evaporation of methyl chloride from the ocean is believed to be one of the two major sources (both natural) of the compound in the air (481). Singh et al. (496) found significant concentration gradients of methyl halides between marine and continental (non-urban) air masses supporting the view that the methyl halides originate from the ocean. The smoldering combusion of plant materials (e.g., forest, grass) is another natural source of methyl chloride in the environment. Roughly 10% of the chlorine content of smoldering vegetation is believed to be converted to methyl chloride (481). Palmer (485) estimated that the U.S. annual emission of methyl chloride from forest fires and agricultural burning to be around 126 million kg. Lovelock (481) estimated an annual worldwide emission rate of 10 megatons (9.1 billion kg) as a result of marine algal biosynthesis and grass and forest fires.

Atmospheric photochemical reaction has been suggested to be a possible source of carbon tetrachloride (481, 497). Under laboratory conditions, Lovelock (481, 497) was able to produce small but significant amounts of carbon tetrachloride by irradiating methyl chloride with sunlight. Graedel and Allara (498), however, considered the reaction too slow to be of any major significance. Photochemical reactions may convert the relatively unstable chlorinated ethylenes into the more stable halomethanes. Singh et al. (499) simulated tropospheric irradiation of synthetic mixtures of tetrachloroethylene in air and obtained carbon tetrachloride with an average yield of about 8% by weight. Similarly, Appleby et al. (500) detected chloroform as a solar-induced photochemical reaction product of trichloroethylene.

Occurrence in Ambient Air. As may be expected from the extensive emissions discussed above, ambient air is contaminated by some haloalkanes and

haloalkenes. The extent of contamination is dependent on the intensity of emission, the distance from emission sources, the stability of the individual halocarbons in the atmosphere, the geographic location, and the metereologic conditions.

The U.S. Environmental Protection Agency has monitored the ambient air for a number of halocarbons in the vicinity of selected industrial sites. In 1975, the Agency (24) reported the detection of vinyl chloride in the ambient air of residential areas near VC/PVC plants. The concentration exceeded 1 ppm in less than 10% of the time. The maximum concentration was 33 ppm in a grab (instantaneous) sample collected at a distance of 0.5 km from the center of a plant. For vinylidene chloride (501), the highest concentration was 14 ppb at the property line downwind of a monomer production plant. Vinylidene chloride was still detectable at a station 1.5 miles away from the production facilities. Environmental monitoring of trichloroethylene (502) and 1,1,1-trichloroethane (503) showed considerable variations with concentrations ranging from 1 ppb (limit of detection) to 270 ppb and 0.03 ppb (limit of detection) to 155 ppb, respectively. The atmospheric concentration of 1,2-dichloroethane near point sources was reported to be very low, although the methodology used may not be appropriate (13). A high concentration of 75 ppb 1,2-dichloroethane was detected in the air near a vinyl chloride plant in the Netherlands (504). The maximum atmospheric concentrations of 1,2-dibromoethane at downwind locations near the property line of two major manufacturers were reported (505) to be 90 and 115 $\mu g/m^3$ (0.011 and 0.014 ppb). The urban air at locations near major streets and gasoline stations in three western U.S. cities (Phoenix, Los Angeles, Seattle) contained 0.008, 0.014, and 0.010 ppb 1,2-dibromoethane (505). Close to 20 different brominated alkanes and alkenes were identified in the ambient air surrounding bromine industrial plants in the state of Arkansas (506).

A number of halocarbons have been consistently detected in the ambient air at various locations in the United States and around the world. Reviews on the environmental data on trichloroethylene (507), 1,1,1-trichloroethane (508), and several halomethanes (58) have been published. Information on atmospheric occurrences of various haloalkanes and haloalkenes has also been summarized in several IARC monographs (2, 18, 23) and in the reviews of Fishbein (54, 55). Only a selection of representative studies is given in Table XXIV to illustrate the general trend. All the numbers shown are either typical or average values and considerably higher concentrations may be found in ambient air near emission sources under adverse metereological conditions. The most commonly detected compounds are chloroform, carbon tetrachloride, 1,1,1-trichloroethane, trichloroethylene and tetrachloroethylene. In general, halocarbons with anthropogenic origins are found in substantially higher concentration in urban areas than in rural or oceanic areas. For compounds with natural origin (marine algal biosynthesis), only methyl chloride showed distinctly higher concentrations in oceanic air than in either urban or rural air.

Occurrence in the Air of Indoor Environments. Indoor or enclosed environment represents a significant but often neglected source of human exposure to air pollutants. Depending on the size of the enclosed space and the ventilation, even a relatively minor emission may generate alarmingly high atmospheric levels of pollutants. There is a paucity of monitoring data on halocarbons in the nonindustrial indoor environment. The data available have been reviewed by Bridbord et al. (515) and the U.S. National Academy of Sciences (58). The most commonly detected halocarbons are difluorodichloromethane (Freon 12), trichlorofluoromethane (Freon 11), methyl chloride, methylene chloride, chloroform, carbon tetrachloride, and 1,1,1-trichloro-

Table XXIV
Representative Atmospheric Concentrations of Some Haloalkanes and Haloalkenes (ppt)[a]

Compound	Rural Area	Oceanic Area	Urban Area		Reference
Methyl chloride	713 ± 51	1260 ± 434	834 ± 40	(Los Angeles)	(496)
Methyl bromide	15 ± 10	93 ± 100	108 ± 138	(Los Angeles)	(496)
Methyl iodide	9 ± 5	7 ± 7	24 ± 20	(Los Angeles)	(496)
	<1	16	6	(New York)	(509)
Methylene chloride	36 ± 11	--	<20 - 144		(Pierotti & Rasmussen, cited in ref. 58)
	--	35 ± 19	--		(510)
Chloroform	25 ± 8	40 ± 38	102 ± 102	(Los Angeles)	(496)
	7	--	25	(Bremen, Germany)	(493)
Carbon tetrachloride	240	280	380	(New York)	(509)
	116 ± 6	128 ± 16	134 ± 20	(Los Angeles)	(496)
	180	--	220	(Los Angeles)	(511)
	--	--	1400	(Tokyo)	(512)

Table XXIV (continued)

Compound	Rural Area	Oceanic Area	Urban Area		Reference
1,1,1-Trichloroethane	83	180	280	(New York)	(509)
	50	--	370	(Los Angeles)	(511)
	--	--	800	(Tokyo)	(512)
Trichloroethylene	<20	180	110	(New York)	(509)
	2	1	156	(Liverpool)	(513)
	--	--	1200	(Tokyo)	(512)
Tetrachloroethylene	9	73	1200	(New York)	(509)
	<125	--	1250	(Los Angeles)	(511)
	590	--	880	(Munich)	(514)
	--	--	1200	(Tokyo)	(512)

[a]In parts per trillion (ppt) by volume. The numbers shown are either typical, average, or average ± standard deviations. The conversion factors (1 ppt = x ng/m^3 at 25°C, 760 mm Hg) are: CH_3Cl, 2.07; CH_3Br, 3.88; CH_3I, 5.80; CH_2Cl_2, 3.5; $CHCl_3$, 4.9; CCl_4, 6.3; CH_3CCl_3, 5.4; $CHClCCl_2$, 5.45; Cl_2CCCl_2, 6.78.

ethane. These compounds may escape into the atmosphere from their uses as refrigerants, aerosol propellants or ingredients, or solvents. Methyl chloride may be produced during the smoldering combustion of tobacco. Hester et al. (516) measured fluorocarbon levels in air samples taken from homes in the Los Angeles area. Levels of Freon 11 and Freon 12 are generally higher indoors than outdoors and, in a few cases, may exceed 12 ppb and 500 ppb, respectively. Beauty shops, supermarkets, department stores, and drug stores also have substantially higher levels of Freons than the outside air. The effect of cigarette smoking on the indoor concentration of methyl chloride may be quite dramatic; Harsch (cited in ref. 58) reported that the atmospheric concentration of methyl chloride in an apartment increased from the usual level of 0.5-0.7 ppb to 20 ppb after a cigarette had been smoked. A high concentration of 23.4 ppb methylene chloride in a beauty shop was attributed to the use of hair spray aerosol (58). The extensive use of methylene chloride as a paint remover in indoor spaces may also be a significant source of exposure. The concentration of chloroform and carbon tetrachloride in the indoor air in the study by Harsch was relatively low. However, Batjer et al. (493) found that air collected 0.2-1 m away from the water surface in several covered public swimming pools contained an average of 7.5-49 ppb chloroform with a maximum of 78 ppb. The presence of chloroform was attributed to the formation during chlorination. In the chemistry building at the University of Montana, Taketomo and Grimsrud (517) detected high concentrations of chloroform (1.76 ppm) and carbon tetrachloride (14.3 ppb) and it is very likely that similar levels may be found at university chemistry laboratories around the world. A high concentration of 21 ppb 1,1,1-trichloroethane was also detected by Taketomo and Grimsrud (517) in a grocery store. The air of some unventilated new automobiles may contain measurable amounts of vinyl chloride emitted

from the plastic interior. Hedley et al. (518) reported that of the seven different models of new 1975 automobiles tested, only 2 had vinyl chloride ranging from 0.4-1.2 ppm. The other five were below the detection limits of 0.05 ppm.

5.2.2.1.5.2.2 Haloalkanes and Haloalkenes in the Water. The U.S. Environmental Protection Agency completed, in 1980, a series of Ambient Water Criteria Documents on carbon tetrachloride (10), chloroform (7), halomethanes (219), chlorinated ethanes (519), dichloropropanes and dichloropropenes (520), vinyl chloride (25), dichloroethylenes (30), trichloroethylene (33), tetrachloroethylene (36), and hexachlorobutadiene (39). The readers are referred to the documents for aquatic toxicity and risk assessment of health hazards on these compounds. The following discussion focuses on sources and occurrence of halocarbons in the drinking water.

Sources and occurrences in surface water. The sources of halocarbons present in the water are essentially the same as those present in the atmosphere, with the exception that less volatile compounds have a greater chance of entering the water than the atmosphere. Natural production probably plays a negligible role in directly contributing halocarbons to surface waters that are used as the principal source of drinking water.

Discharges of waste or byproducts represents one of the most important sources of contamination of surface waters. Relatively few estimates of the extent of such discharges are available. Neufeld et al. (478) estimated that between 2,600 and 3,000 lb of vinylidene chloride are discharged into wastewater effluent every year. In a 1974 report, U.S. Environmental Protection Agency (521) reckoned that two chemical plants in the Long Beach, California area discharged a total of 12.3 kg/day vinyl chloride into waste water. Mumma

and Lawless (522) estimated that 7.3-14.5 million pounds of hexachlorobutadiene were produced in 1972 mainly as incidental waste product or byproduct of tetrachloroethylene, trichloroethylene, and carbon tetrachloride. It is not known what proportion of the waste product was discharged into the water. The waste water effluents and surface water in the vicinity of a number of industrial plants were monitored. The concentrations ranged from 0.05 ppm to 20 ppm (typically 2-3 ppm) for vinyl chloride (523); < 1 ppm to 550 ppb (typically 200-400 ppb) for trichloroethylene (502); < 1 ppb to 16 ppm (typically around 200 ppb) for 1,1,1-trichloroethane (503). A study by Keith (cited in ref. 58) showed concentrations of 5 ppb to 132 ppm methylene chloride, 0.05-650 ppb chlorodibromomethane, 5 ppb to 22 ppm chloroform, and 10 ppb to 5 ppm carbon tetrachloride in waste water effluents. Hexachlorobutadiene was found in water samples collected from inland sites bordering the lower Mississippi River; a landfill pond near an industrial source was found to contain 4.49 ppb of the compound (524). The surface waters from fourteen heavily industrialized U.S. river basins contained 1-90 ppb of 1,2-dichloroethane (525).

A number of large spills have been recorded by U.S. Coast Guard (cited in ref. 58). These include a spill of 8,327 liters of methyl chloride into Chesapeake Bay in June 1973, a spill of 1,892 liters of carbon tetrachloride into the Kanawha River, West Virginia in April 1975, and a spill of 242,240 liters of chloroform into Lower Mississippi in September 1973. A second massive spill into Mississippi of 1.75 million pounds of chloroform occurred when tow barge tanks ruptured at Baton Rouge, Louisiana. Concentrations up to 300 ppb were found at two points 16.3 and 121 miles downstream, up to 7.5 days after the spill (526).

A variety of other factors such as atmospheric fallout, agricultural and roadway runoff may also contribute to surface water contamination by halocarbons. However, no quantitative data on these potential sources are available.

Sources and occurrence in finished drinking water. A variety of haloalkanes and haloalkenes have been detected in finished drinking water. The majority of these compounds originate most likely from contaminated raw water supply. Chlorination of water supplies contributes to the formation of trihalomethanes. Polyvinyl chloride pipe used in water distribution systems may also be a source of low levels of vinyl chloride in drinking water.

The environmental impact and health effects of the chlorination of water have attracted much attention in recent years and an annual conference has been devoted to the study of this problem (527-529). The production of chloroform and other trihalomethanes by chlorination of water containing organic matters was first discovered in 1974 by Rook (530) and Bellar et al. (531). The finding has since been confirmed by various investigators (532-534). The formation of haloform is dependent on the presence of precursor organic matter such as humic acid, phenols, aromatic amines, and simple aliphatic carbonyl compounds. The reaction mechanism involves a series of base-catalyzed halogenation and hydrolysis reactions. The ratio of brominated trihalomethanes to chloroform is related to the concentration of bromine in the water supply. Chloroform and trihalomethanes have now been detected in most municipal water supplies.

The use of polyvinyl chloride pipe in water distribution systems provides a potential source of low levels of vinyl chloride in finished drinking water. Dressman and McFarren (535) have studied five water distribution

systems using PVC pipes of different age, length, and size. Three of these five systems contained water with measurable amounts of vinyl chloride. The highest concentration (1.4 ppb) was found in water from the most recently installed and the longest pipe system. Traces (0.03 and 0.06 ppb) of vinyl chloride were still detected in the water from the two oldest systems about 9 years after installation. A voluntary standard of < 10 ppm residual monomer in finished PVC pipe has been adopted.

The occurrence of halocarbons in finished drinking water has been closely monitored by U.S. Environmental Protection Agency. Table XXV summarizes the monitoring data in a 10-city study (536). At least 29 haloalkanes and haloalkenes have been identified. The compounds that were detected with high frequencies are: all the trihalomethanes, methylene chloride, carbon tetrachloride, and tetrachloroethylene. The concentrations ranged from undetectable to the highest for chloroform at 301 ppb. A similar trend has been observed in monitoring studies at other U.S. cities or regions (537-540). Monitoring studies of German drinking water yielded qualitatively comparable results (541). However, the concentrations of trihalomethanes in Germany were considerably lower than those in U.S. drinking water. The investigators (541) attributed the quantitative differences to the use of different amounts of chlorine in water treatment. Well water (539) and ground water (540) were found to yield considerably less or no trihalomethanes after chlorination probably because of lesser amounts of organic matters. However, wells are susceptible to contamination by leaching. A well water supply on Long Island, New York was reported (539) to contain 50 ppb vinyl chloride, 500 ppb tetrachloroethylene, and 65 ppb trichloroethylene due to leaching from a nearby air base.

Table XXV
Haloalkanes and Haloalkenes Detected in U.S. Drinking Water[a]

Compound	Number of Cities[b]	Concentration Range (μg/liter)
Chloromethane	5	n.q.[c]
Iodomethane	1	n.q.
Dichloromethane	9	0.1-1.6
Bromochloromethane	1	n.q.
Dibromomethane	1	n.q.
Fluorodichloromethane	2	n.q.
Chloroform	10	0.08-301.0
Bromodichloromethane	9	0.1-73.0
Dibromochloromethane	9	0.01-32.0
Tribromomethane	5	0.2-3.0
Dichloroiodomethane	7	n.q.
Fluorotrichloromethane	4	n.q.
Carbon tetrachloride	8	0.1-0.13
Chloroethane	5	n.q.
Bromoethane	2	n.q.
1,1-Dichloroethane	2	n.q.
1,2-Dichloroethane	3	n.q.
1,1,1-Trichloroethane	3	n.q.
1,1,2-Trichloroethane	1	n.q.
Trichlorotrifluoroethane	2	n.q.
Hexachloroethane	1	0.07-0.5
2-Chloropropane	1	n.q.
Vinyl chloride	2	0.27-5.6
Vinylidene chloride	4	0.1
cis-1,2-Dichloroethylene	3	0.1-16.0
trans-1,2-Dichloroethylene	1	1.0
Trichloroethylene	5	0.1-0.5
Tetrachloroethylene	8	0.01-0.46
Hexachlorobutadiene	1	<0.01

[a]Summarized from USEPA: "Preliminary Assessment of Suspected Carcinogens in Drinking Water. Report to Congress," U.S. Environmental Protection Agency, Washington, D.C., 1975

[b]Number of cities with positive findings (in a 10-city survey)

[c]Not quantified

5.2.2.1.5.2.3 Haloalkanes and Haloalkenes in Foodstuffs. In contrast to the extensive monitoring of haloalkanes and haloalkenes in the air and the drinking water, there is a paucity of data on their occurrence in foodstuffs. An extensive study by McConnell et al. (542) suggests that several haloalkanes and haloalkenes occur ubiquitously in various foodstuffs in England.

Sources and occurrence. A variety of sources may contribute to the occurrence of halocarbons in foodstuffs. These include: (a) biosynthesis, (b) environmental contamination, (c) food fumigation, (d) food processing, and (e) food packaging and storage.

(a) With the exception of lower marine plant organisms, biosynthesis of halocarbons is virtually nonexistent. Nonetheless, human exposure to naturally-occurring halocarbons in foodstuffs has been noted. Limu kohu (Hawaiian for supreme seaweed), a highly prized edible seaweed in Hawaii, has been found to contain a variety of halocarbons. Burreson et al. (543) identified 42 organohalogen compounds in the essential oil of the seaweed. The principal (80% by weight) constituent of the oil is tribromomethane (bromoform). Other prevalent haloalkanes and haloalkenes are dibromoiodomethane (5%), bromodiiodomethane (2%) and 1,1,3,3-tetrabromopropene (2%). The presence of these and other halogenated compounds suggests that limu kohu should be a poisonous seaweed to eat; however, to the knowledge of the authors (543), not a simple case of illness has so far been attributed to ingestion of the alga. A prospective epidemiological study should probably be undertaken to assess the long-term health hazard of the seaweed.

(b) Indirect environmental contamination probably plays a major contributory role in the occurrence of halocarbons in foodstuffs. McConnell et al.

(542) and Pearson and McConnell (544) have surveyed the extent of contamination by chlorinated aliphatic hydrocarbons in various foodstuffs in England and edible marine organisms obtained from Liverpool Bay. The results of their studies are summarized in Table XXVI. Consistent with their occurrence in the atmosphere and the water, chloroform, carbon tetrachloride, 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene are frequently detected; the highest concentrations observed were: 33 ppb (in Cheshire cheese), 19.7 ppb (in black grapes), 47 ppb (in fish), 60 ppb (in packeted coffee), and 41 ppb (in fish), respectively.

Hexachloro-1,3-butadiene (HCBD) contamination of foodstuffs appears to be universal (see Table XXVI). In England, HCBD residues (in ppb) were detected in milk (0.08), butter (2.0), vegetable cooking oil (0.2), light ale (0.2), tomatoes (0.8), and imported black grapes (3.7) in the study of McConnell et al. (542). The tomatoes were harvested from plants grown on a reclaimed lagoon contaminated with HCBD. The presence of HCBD in grapes was attributed to its use as an insecticide for vineyards in some countries. In Germany, Kotzias et al. (545) found HCBD in milk, egg yolk, and margarine at levels of 4,242, and 32 ppb, respectively. In a study of foodstuffs collected from Lower Mississippi River basin within a 25-mile radius of trichloroethylene and tetrachloroethylene producers (who also produce HCBD as waste product), no HCBD residues were consistently found in samples of milk, egg, and vegetables (546). However, in the same area, freshwater fish were found to contain as much as 4.65 ppm (547) and 1.2 ppm HCBD (546) while crayfish had 11-70 ppb HCBD (524). Freshwater fish fed by the Rhine Water also contained HCBD ranging from 110 ppb to 2.04 ppm (548).

The bioconcentration of haloalkanes and haloalkenes has not been thoroughly investigated. Based on comparison of the concentrations of halo-

Table XXVI
Concentrations of Haloalkanes and Haloalkenes Frequently Detected in Foodstuffs (ppb)[a]

Foodstuff	$CHCl_3$	CCl_4	CH_3CCl_3	$CHCl=CCl_2$	$CCl_2=CCl_2$	HCBD[b]
Dairy produce	1.4-33[c]	0.2-14[c]	--	0.3-10[c]	ND-13[c]	ND-2[c]; ND[d]; 4-42[e]
Meat	1-4[c]	7-9[c]	3-6[c]	12-22[c]	0.9-5[c]	ND[c]
Oils & Fats	2-10[c]	0.7-18[c]	5-10[c]	ND-19[c]	0.01-7[c]	ND-0.2[e]; 33[e]
Beverages	0.4-18[c]	0.2-4[c]	7[c]	ND-60[c]	ND-3[c]	ND-0.2[c]
Fruits & vegetables	ND-18[c]	3-19.7[c]	1-4[c]	ND-7[c]	ND-2[c]	ND-3.7[c]; ND[d]
Bread	2[c]	5[c]	2[c]	7[c]	1[c]	ND[c]
Fish & shellfish	3-180[f]	0.1-6[f]	0.03-47[f]	--	0.05-41[f]	10-1200[d]; ND-7[f]; trace-4650[g]; 11-70[h]; 110-2040[i]

[a]In parts per billion, equivalent to μg/liter or μg/kg

[b]Abbreviations used: HCBD = Hexachloro-1,3-butadiene; ND = not detectable

[c]In England; summarized from G. McConnell, D.M. Ferguson, and C.R. Pearson [Endeavour, 34 13 (1975)]

[d]In U.S.A.; summarized from G. Yip [J. Assoc. Off. Anal. Chem., 59, 559 (1976)]

[e]In Germany, summarized from D. Kotzias, J.P. Lay, W. Klein, and F. Korte [Chemosphere, 4, 247 (1975)]

[f]In U.K.; summarized from C.R. Pearson and G. McConnell [Proc. R. Soc. Lond. B., 189, 305 (1975)]

[g]In U.S.A.; summarized from M.P. Yurawecz, P.A. Dreifuss, and L.R. Kamps [J. Assoc. Off. Anal. Chem., 59, 552 (1976)]

[h]In U.S.A.; summarized from J.L. Laseter et al., "An Ecological Study of Hexachlorobutadiene," EPA 560/6-76-010, Environmental Protection Agency, 1976

[i]In Europe; R.W. Goldback, H. van Genderen, and P. Leeuwangh [Sci. Total Environ., 6. 31 (1976)]

carbons in aquatic organisms and in water, McConnell et al. (542) concluded that the bioconcentration of most haloalkanes and haloalkenes is at least a thousand times lower than that of DDT or polychlorinated biphenyls. The U.S. Environmental Protection Agency's calculated "weighted average" bioconcentration factors into the "edible portion of all freshwater and estuarine aquatic organisms consumed by Americans" are: chloroform, 3.75 (7); carbon tetrachloride, 18.75 (10); 1,2-dichloroethane, 1.2 (519); 1,1,1-trichloroethane, 5.6 (519); vinyl chloride, 1.17 (25); vinylidene chloride, 5.6 (30); trichloroethylene, 10.6 (33); tetrachloroethylene, 30.6 (36); and hexachlorobutadiene, 2.78 (39). The calculation of most of these values is based on their partition coefficients and have not been experimentally confirmed.

(c) Fumigation of foodstuffs may be a possible source of halocarbon contamination. Halocarbons used (either alone or in mixture) in the fumigation of grain, fruit, or soil are methyl bromide, methyl chloride, chloroform, carbon tetrachloride, 1,2-dichloroethane, 1,2-dibromoethane, 1,1,1-trichloroethane, 1,2-dibromo-3-chloropropane, trichloroethylene, tetrachloroethylene, and 1,3-dichloropropene (549). In general, the amount of fumigant residues depends on the nature of the fumigant, the fumigant dosage, storage conditions, length of aeration, and extent of processing (58, 549). A National Academy of Sciences panel (58) has recently reviewed the literature on the use of halomethanes as fumigants. Usually, proper storage and aeration reduce fumigant residues to trace levels; however, some fumigants (e.g., carbon tetrachloride) may persist at low levels for as much as a year. For example, Scudamore and Heuser (550) found that the residues in wheat after 3-6 days of fumigation with 80 mg/l CCl_4 decreased from 200 to 400 ppm initially to 1-10 ppm after 6 months of aeration, but persisted with levels up to 4.7 ppm after 12 months. Berck (551) detected CCl_4 in fumigated wheat in concentrations

ranging from 72.6 ppm (1 week aeration) to 3.2 ppm (7 weeks aeration). Bread made from the latter sample had residues of about 0.02 ppm (the figure of 0.2 ppm in the original article appears to be a misprint) in the lower crust and 0.01 ppm in the crumb. In apples fumigated with 12 or 24 mg/l 1,2-dibromoethane, the respective residues after 1 day were 36 and 75 ppm and decreased to 1.2 and 1.6 ppm after 6 days (552). Newsome et al. (553) reported that 1,2-dibromo-3-chloropropane was found in radish and carrot crops after application of the fumigant to soil at a rate of 12.3 lb/acre. Residues were higher in carrots (up to 1.5 ppm), most of it in the root, and persisted for 16 weeks when applied at seeding. One-third of the residues still remained after cooking unpeeled carrots in boiling water for 5 minutes.

(d) Chlorinated aliphatic hydrocarbons have been used as extraction solvents in food processing. Trichloroethylene was widely used to decaffeinate coffee until its removal from use in 1977. To replace trichloroethylene, dichloromethane is now being used; the maximum level of residue allowed is 10 ppm. Dichloromethane, 1,2-dichloroethane, and trichloroethylene have been employed as extractants in the preparation of spice oleoresins. Page and Kennedy (554) analyzed 17 different samples of spice oleoresins from three different manufacturers. No trichloroethylene residues were detected; the residues of dichloromethane and 1,2-dichloroethane ranged from 1-83 ppm and 2-23 ppm, respectively.

(e) Migration of residual vinyl chloride or vinylidene chloride monomer from plastic food packaging film or containers into foods is another potential source of contamination. Polyvinyl chloride (PVC) products may contain vinyl chloride monomer ranging from 5-800 ppm. Depending on the type of food and storage time, some migration has been observed. As an indication of the extent of migration, Van Esch and Van Logten (555) reported that from PVC

bottles containing 30 ppm residual monomer, migration into water, soft drink and blood after 40 days produced vinyl chloride concentrations of 20-50 ppb, 0.2 ppb, and 14-80 ppb, respectively. A sample of beer was found to contain 2 ppm vinyl chloride after 6 years storage in PVC bootle containing 70 ppm residual monomer. Williams and Miles (556) detected vinyl chloride in alcoholic beverages, peanut oil, and vinegar in PVC bottles at levels of < 0.025-1.6 μg/ml, 0.3-3.29 ppm, and 0-8.4 μg/ml, respectively. Migration of vinylidene chloride monomer from household or industrial food packaging film is also a potential problem. U.S. Environmental Protection Agency (501) reported findings of 4.9 and 58 ppm vinylidene chloride in two samples of Saran Wrap. Birkel et al. (557) analyzed several lots of plastic food packaging films (Saran Wrap type) and detected vinylidene chloride with concentrations ranging from 6 to 30 ppm. The extent of migration of the monomer from the packaging film into foods has not been assessed.

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Notes Added After Completion of Section 5.2.2.1

Many new findings on haloalkanes and haloalkenes have been reported since the completion of Section 5.2.2.1. The vigorous ongoing research is reflected by a plethora of recent reviews or monographs on the toxicological, metabolic and environmental aspects of chloroform (1), carbon tetrachloride (2), dibromochloropropane (3), vinyl chloride (4-6), tetrachloroethylene (7), and various haloalkanes and haloalkenes (8-12).

MUTAGENICITY

As an update to Tables VI and VII, Ames mutagenicity data of a variety of haloalkanes and arylalkyl halides are summarized in Update Table I and compared to their *in vitro* cell transformation and *in vivo* carcinogenic activities. In agreement with previous findings, the data indicate that all fully halogenated alkanes and most highly halogenated alkanes are not mutagenic in the Ames test; most are also inactive in the cell transformation assay. There is a poor correlation between bacterial mutagenicity and animal carcinogenicity of highly halogenated alkanes (*e.g.*, chloroform, 1,1,1-trifluoro-2-chloroethane, 1,1,1,2-/1,1,2,2-tetrachloroethane, pentachloroethane). The bacterial mutagenicity of chloromethane and bromomethane has been confirmed; the former compound is also positive in cell transformation assay while the latter is a potent carcinogen in the rat. With the exception of difluoromethane, all dihalomethanes are mutagenic. A comparative study by Osterman-Golkar *et al.* (19) showed that diiodomethane and dibromomethane are approximately equipotent in mutagenicity and are about 3 times more active than chlorobromomethane which, in turn, is about 20 time more potent than dichloromethane. In contrast to the lack of mutagenicity of difluoromethane, chlorofluoromethane (FC-31) is an active mutagen (13, 17); FC-31 is more mutagenic than vinyl

Update Table I

Mutagenicity of Haloalkanes and Arylalkyl Halides in the Ames Test and Comparison to Their In Vitro Cell Transformation and In Vivo Carcinogenic Activities

Compound	Ames Test[a]	C.T.[b]	Chronic Bioassay	
			Mouse	Rat
(A) Halomethanes				
Chloromethane	+ (13)	+ (13)		
Bromomethane[c]	+ (14, 15)			+
Iodomethane	+[d]	- (16)	+	+
Difluoromethane (FC-32)	- (13)	- (13)		
Chlorofluoromethane (FC-31)[c]	+ (13, 17)	+ (13)		+
Dichloromethane[c]	+ (13, 17-20)	+ (13)	-	(+)
Chlorobromomethane	+ (19)			
Dibromomethane	+ (19)			
Diiodomethane	+ (19)			
Trifluoromethane (FC-23)	- (13)			
Chlorodifluoromethane (FC-22)[c]	+ (13)	- (13)		-/(+)
Dichlorofluoromethane (FC-21)	- (13)	- (13)		
Chloroform	- (13, 21)	- (13)	+	+
Chlorodibromomethane[c]	- (22)		+/±	-
Tribromomethane	- (22, 24); + (24)		(+)	
Triiodomethane	+ (24)		-	-
Chlorotrifluoromethane (FC-13)	- (13)			
Dichlorodifluoromethane (FC-12)	- (13)	- (13)		
Trichlorofluoromethane (FC-11)	- (13)		-	-
Tetrabromomethane	- (23)		(-)	

Update Table I (continued)

Compound	Ames Test[a]	C.T.[b]	Chronic Bioassay	
			Mouse	Rat
(B) Haloethanes				
Bromoethane	- (24)			
1,1-Difluoroethane (FC-152a)	- (13)			
1,1,1-Trifluoroethane (FC-143a)[c]	+ (13)	- (13)		-
1,1,2-Trifluoroethane (FC-143)	+ (13)			
1-Chloro-1,1-difluoroethane (FC-142b)	+ (13)	+ (13)		
1,1,1-Trichloroethane[c]	- (13, 24); + (18, 26)	- (13)	+/±	-
1,1,1,2-Tetrafluoroethane[c] (FC-134a)	- (13)	- (13)		-
1,1,2,2-Tetrafluoroethane (FC-134)	- (13)			
1,1,1-Trifluoro-2-chloroethane[c] (FC-133a)	- (13)	- (13)		+
1,1,1,2-Tetrachloroethane[c]	- (24)		+	-
1,1,2,2-Tetrachloroethane	- (24)		+	(-)
Pentafluoroethane (FC-125)	- (13)			
1,1,1,2-Tetrafluoro-2-chloroethane (FC-124)	- (13)			
1,1,1-Trifluoro-2,2-dichloroethane (FC-123)	- (13)	- (13)		
Pentachloroethane[c]	- (24)		+	-
1,1,1,2,2-Pentafluoro-2-chloroethane (FC-115)	- (13)	- (13)		
1,1,2,2-Tetrafluoro-1,2-dichloroethane (FC-114)	- (13)			
1,1,2-Trifluoro-1,2,2-trichloroethane (FC-113)	- (13)			

Update Table I (continued)

Compound	Ames Test[a]	C.T.[b]	Chronic Bioassay	
			Mouse	Rat
(C) Higher Haloalkanes				
1,2-Dichloropropane[c]	+ (24); ± (27)		+	±
1,2,3-Trichloropropane	+ (24)			
(D) Arylalkyl Halides				
Benzyl chloride[c]	- (28); + (29, 30)	- (28)	+	
p-Methylbenzyl chloride	+ (29)			
p-Nitrobenzyl chloride	+ (29)			
p-Phenylbenzyl chloride	+ (28)	+ (28)		
Benzyl bromide	+ (29)			
Benzal chloride[c]	+ (30)		+	
Benzotrichloride[c]	+ (30)		+	
p-Chlorobenzotrifluoride	- (31)			

[a]Update to Tables VI and VII. Additional mutagenicity data on some of the compounds listed here have been summarized in Table VI.

[b]*In vitro* cell transformation assay using BHK or 3T3 cells.

[c]Recent carcinogenicity data on these compounds are discussed in this Update (see Update Table II).

[d]See Table VI.

chloride and is about 25-30 times more active than dichloromethane (13). Green (17) attributed the higher mutagenicity of FC-31 (after mammalian metabolic activation) to the formation of reactive intermediates (e.g., formyl fluoride), stable enough to bind to cellular macromolecules (before being hydrolyzed). In accord with bacterial mutagenicity data, FC-31 is active in cell transformation assay and is a potent carcinogen.

Mixed results have been obtained with trihalomethanes. There is some evidence for the mutagenicity of triiodomethane and tribromomethane, but trichloromethane and trifluoromethane are clearly inactive. Chlorodifluoromethane (FC-22) is a weak to moderately active mutagen whereas dichlorofluoromethane (FC-21) is inactive. Both compounds are negative in cell transformation assay. In carcinogenicity bioassays, FC-22 is negative in one study but active (weakly) in another. No convincing evidence of bacterial mutagenicity of chlorodibromomethane has been found in a recent study by the U.S. National Toxicology Program (22); however, there is some evidence that the compound is carcinogenic in mice.

Among haloethanes, three fluorinated compounds were shown to be mutagenic in the Ames test (13). The mutagenicity of 1,1,1-trifluoroethane (FC-143a) and 1,1,2-trifluoroethane (FC-143) is of particular concern in view of the generally held assumption that fluorinated compounds are biologically almost inert by virtue of their strong C-F bonds. Despite the positive mutagenicity of FC-143a, however, the compound is not carcinogenic in the rat. In contrast, the nonmutagenic 1,1,1-trifluoro-2-chloroethane (FC-133a) is a potent carcinogen in the rat (see Update Table II). 1-Chloro-1,1-difluoroethane (FC-142b) gives positive response in both the Ames test and the cell transformation assay; its carcinogenicity remains to be tested. The mutagenicity of 1,1,1-trichloroethane has been confirmed (18, 26); its potency is, however,

quite low (26). A variety of haloethanes with more than four halogens has been tested and found negative in the Ames test and the cell transformation assay. For these highly halogenated alkanes, short-term mutagenicity tests appear to have little value in predicting potential carcinogenicity.

A series of arylalkyl halides has been tested for mutagenicity. Yasuda et al. (30) reported that the mutagenic potency of three arylalkyl halides follows the order: benzotrichloride > benzal chloride > benzyl chloride. The same ranking of carcinogenic potency has been observed for these compounds (see Update Table II). Ashby et al. (28) showed that ring substitution with a phenyl group at the para position of benzyl chloride yields a highly potent, direct-acting mutagenic compound (p-phenylbenzyl chloride or 4-chloromethylbiphenyl) which is also positive in the cell transformation assay. The introduction of phenyl ring is believed to enhance the mutagenicity of benzyl chloride by increasing its lipophilicity, diminishing its enzymatic detoxification and helping to stabilize the reactive carbonium ion derived from it. Hemminki et al. (29) compared the bacterial mutagenicity, and the sister chromatid exchange (SCE) inducing activity of four arylalkyl halides with their chemical reactivity. Using 4-(p-nitrobenyl)pyridine (NBP) as the nucleophile, the aralkylating activity follows the order: benzyl bromide > p-methylbenzyl chloride > benzyl chloride > p-nitrobenzyl chloride. The mutagenic potency in the Ames test follows the order: p-nitrobenzyl chloride >> benzyl bromide > benzyl chloride = p-methylbenzyl chloride while the SCE-inducing activity follows the order: benzyl bromide > benzyl chloride = p-nitrobenzyl chloride > p-methylbenzyl chloride. The particularly high bacterial mutagenicity of p-nitrobenzyl chloride is attributed to reactions other than direct aralkylation whereas p-methylbenzyl chloride appears to be exceptionally weak because of its preferential binding to the N-2 position of guanine in DNA.

TERATOGENICITY

Barlow and Sullivan (32) have recently reviewed the data on reproductive hazards of a number of industrial chemicals which include 14 haloalkanes and haloalkenes. The final results of a teratogenicity study of 1,1,1-trichloroethane in Long-Evans rats by York et al. (33) have been published. No significant malformations or neurobehavioral abnormalities have been found in the offspring of female rats exposed to vapor containing 2,100 ppm of the compound either before or during gestation. Ruddick and Newsome (34) gave pregnant rats daily doses of 12.5, 25 or 50 mg/kg 1,2-dibromo-3-chloropropane (DBCP) orally from day 6 through 15 of gestation. No teratogenic effects were observed. The two higher doses were slightly maternally toxic and reduced fetal body weight. John et al. (35) found vinyl chloride (VC) not teratogenic in the offspring of CF-1 mice exposed to 50 or 500 ppm VC and Sprague-Dawley rats and New Zealand rabbits exposed to 500 or 2,500 ppm VC. Exposure of the pregnant animals simultaneously to VC (by inhalation) and 15% ethanol (in drinking water) failed to elicit additional fetal effects other than those normally associated with ethanol consumption. Reevaluation by Clemmesen (6) of the epidemiological evidence of teratogenicity or embryotoxicity of vinyl chloride in humans casts doubt on earlier conclusions. Eliminating parental age as a confounding factor, there seems to be no convincing evidence that vinyl chloride may present a significant reproductive hazard to workers employed in PVC-producing facilities.

CARCINOGENICITY

Recent carcinogenicity studies of haloalkanes and haloalkenes are summarized in Update Table II. The highlights of these are discussed below.

Update Table II

Recent Carcinogenicity Studies on Haloalkanes and Haloalkenes[a]

Compound	Species and strain	Route	Dose and duration[b]	Significant neoplasm	Incidence	References
Bromomethane	Rat, Wistar	Oral	0.4, 2, 10, or 50 mg/kg/ for 13 wk	Forestomach carcinoma	65% at 50 mg/kg	(36)
Dichloromethane	Mouse, $B6C3F_1$	Oral	60-250 mg/kg for 2 yr	None	--	(37)
	Rat, S.-D.	Inhalation	500, 1,500 or 3,500 ppm for 2 yr	Benign mammary tumor S.c. carcinoma	--[c] 11.3% at 3,500 ppm	(38)
	Hamster, Syrian	Inhalation	500, 1,500 or 3,500 ppm for 2 yr	None	--	(38)
Chlorofluoro-methane (FC-31)	Rat, Alpk/Ak	Oral	300 mg/kg for 52 wk; observed 73 wk	Forestomach carcinoma	93%	(13)
Dichlorofluoro-methane (FC-22)	Mouse, --	Inhalation	50,000 ppm for 2 yr	None	--	(cited in 13)
	Rat, Alpk/Ak	Oral	300 mg/kg for 52 wk; observed 73 wk	None	--	(13)
	Rat, --	Inhalation	50,000 ppm for 2 yr	S.c. carcinoma	"low"	(cited in 13)
Chlorodibromo-methane	Mouse, $B6C3F_1$	Oral	50 or 100 mg/kg for 105 wk	Hepatocellular carcinoma Hepatocellular carcinoma or adenoma	M: 38% at 100 mg/kg F: 12%, 20%, 38%[d]	(22)

Update Table II (continued)

Compound	Species and strain	Route	Dose and duration[b]	Significant neoplasm	Incidence	References
Chlorodibromomethane (cont'd)	Rat, F344/N	Oral	40 or 80 mg/kg for 104 wk	None	--	(22)
1,2-Dibromoethane	Rat, S.-D.	Inhalation	20 ppm for 78 wk	Spleen hemangiosarcoma	M: 21%; F: 13%	(39)
				Adrenal tumors	M: 23%; F: 13%	
				S.c. mesenchymal tumors	M: 23%	
				Mammary tumors	F: 52%	
1,1,1-Trifluoroethane (FC-143a)	Rat, Alpk/Ar	Oral	300 mg/kg for 52 wk; observed 73 wk	None	--	(13)
1,1,1-Trichloroethane	Mouse, $B6C3F_1$	Oral	1,500 or 3,000 mg/kg for 103 wk	Hepatocellular carcinoma	F: 6%, 10%, 20%[d]	(40)
	Rat, F344/N	Oral	375 or 750 mg/kg for 103 wk	None[e]	--	(40)
1,1,1,2-Tetrafluoroethane (FC-134a)	Rat, Alpk/Ak	Oral	300 mg/kg for 52 wk; observed 73 wk	None	--	(13)
1,1,1-Trifluoro-2-chloroethane (FC-133a)	Rat, Alpk/Ak	Oral	300 mg/kg for 52 wk; observed 73 wk	Uterine carcinoma	F: 43%	(13)
				Interstitial cell adenoma of testis	M: 81%	

Update Table II (continued)

Compound	Species and strain	Route	Dose and duration[b]	Significant neoplasm	Incidence	References
1,1,1,2-Tetrachloroethane	Mouse, $B6C3F_1$	Oral	250 or 500 mg/kg for 65-103 wk	Hepatocellular carcinoma Hepatocellular adenoma	F: 2%, 11%, 13%[d] M: 13%, 30%, 42%[d] F: 8%, 17%, 50%[d]	(41)
	Rat, F344/N	Oral	125 or 250 mg/kg for 103 wk	None[e]	--	(41)
Pentachloroethane	Mouse, $B6C3F_1$	Oral	250 or 500 mg/kg for 41-103 wk	Hepatocellular carcinoma	M: 8%, 59%, 16%[d,e] F: 2%, 67%, 29%[d,e]	(42, 43)
	Rat, F344/N	Oral	75 or 150 mg/kg for 103 wk	None[e]	--	(42, 43)
1,2-Dichloropropane	Mouse, $B6C3F_1$	Oral	125 or 250 mg/kg for 103 wk	Hepatocellular adenoma	M: 14%, 20%, 32%[d] F: 0%, 8%, 10%[d]	(27)
	Rat, F344/N	Oral	62 or 125 mg/kg for 103 weeks	Mammary adenocarcinoma (marginal)	F: 2%, 4%, 10%[d]	(27)
Vinyl chloride	Mouse, ICR or A/J	Inhalation	50-50,000 ppm for 1 hour, single or repeated	Pulmonary tumors	Low incidences	(44)
	Mouse, CD-1	Inhalation	1-600 ppm for 4 wk	Pulmonary tumors	See text	(45)
	Rat, Wistar	Oral	1.7, 5.0 or 14.1 mg/kg for up to 2.7 yr	Hepatocellular carcinoma Liver angiosarcomas	M: 14% at 14.1 mg/kg F: 32% at 5.0 mg/kg; 57% at 14.1 mg/kg M: 11% at 5.0 mg/kg; 46% at 14.1 mg/kg	(46)

Update Table II (continued)

Compound	Species and strain	Route	Dose and duration[b]	Significant neoplasm	Incidence	References
Vinyl chloride (cont'd)	Rat, S.-D.	Inhalation	600 ppm for 1 yr	Hepatocellular carcinoma Liver angiosarcoma	44% 23%	(47)
Vinyl bromide	Rat, S.-D.	Inhalation	9.7-1,235 ppm for 2 yr	Liver, Zymbal gland tumors	See text	(48)
Vinylidene fluoride	Rat, S.-D.	Oral	4.12 or 8.25 mg/kg for 52 wk; observed 89 wk	Fat tissue tumors	8.6% at 8.25 mg/kg	(49)
Trichloroethylene (epichlorohydrin-free)	Mouse, $B6C3F_1$	Oral	1,000 mg/kg for 103 wk	Hepatocellular carcinoma Harderian gland adenoma	M: 60%; F: 27% M: 8%; F: 6%	(50)
	Rat, F344/N	Oral	500 or 1,000 mg/kg for 103 wk	Renal tubular-cell adenocarcinoma	M: 0%, 0%, 6%[d]	(50)
Benzyl chloride ($C_6H_5CH_2Cl$)	Mouse, ICR	Topical	2.3 µl/mouse, 2x/week for 50 wk	Skin carcinoma	15%	(51)
Benzal chloride ($C_6H_5CHCl_2$)	Mouse, ICR	Topical	2.3 µl/mouse, 2x/week for 50 wk	Skin carcinoma	58%	(51)
Benzotrichloride ($C_6H_5CCl_3$)	Mouse, ICR	Topical	2.3 µl/mouse, 2x/week for 50 wk	Skin carcinoma Lung tumors Digestive tract tumors	68% 58% 100%	(51)
		Topical	5 µl/mouse, 2 or 3x/week for 9.8 months	Skin carcinoma Lung tumors Lymphoma	70% 100% 30%	(51)

Update Table II (continued)

Compound	Species and strain	Route	Dose and duration[b]	Significant neoplasm	Incidence	References
Benzotrichloride (cont'd)	Mouse, ICR-SLC	Topical	5 µl/mouse, 2x/week for 30 wk; observed 10 wk	Skin carcinoma or sarcoma	48%	(52)
				Lung tumors	14%	
				Digestive tract tumors	10%	
p-Chlorobenzotrichloride ($ClC_6H_4CCl_3$)	Mouse, ICR-SLC	Oral	0.05-2 µl/mouse, 2x/week for 17.5 wk; observed 78 wk	Forestomach, lung, skin cancer, lymphoma, thymoma	See text	(52)
		Topical	5 µl/mouse, 2x/week for 30 wk; observed 10 wk	Skin carcinoma or sarcoma	64%	(52)
				Digestive tract tumors	36%	

[a]Update to Tables VII, XIV, XV, XVI, XVII and XVIII.

[b]Except where indicated, the doses were administered daily, 5 days per week for the period specified.

[c]Increase in the number of tumors per rat.

[d]Tumor incidences for control, low-dose and high-dose groups.

[e]Early mortality in the high-dose group might have reduced the sensitivity of the bioassay to detect a carcinogenic response.

Five halomethanes have been shown to be carcinogenic in at least one animal species. In a 90-day subchronic toxicity study of a widely used soil fumigant, bromomethane (methyl bromide), Danse et al. (36) unexpectedly found squamous cell carcinomas of the forestomach in 13 of 20 rats fed 50 mg/kg bromomethane in arachis oil. All 20 animals showed marked diffuse hyperplasia of the epithelium of the forestomach. Lower doses produced no tumors and much less pronounced or no hyperplasia within this short period of time. Dichloromethane (methylene chloride) has been tested in three different animal species. A preliminary communication by Serata et al. (37) indicated no evidence of carcinogenicity in $B6C3F_1$ mice ingesting dichloromethane via drinking water. Burek et al. (38) found some evidence of a weak or marginally active carcinogenic activity in rats exposed to dichloromethane vapor. In female rats, only a dose-dependent small increase in the multiplicity of benign mammary tumors (spontaneously occurring in this strain) was observed. On the other hand, male rats exposed to 3,500 ppm dichloromethane had a significant increase in the incidence of sarcomas (11.3% vs. 1% control) in the ventral neck region located in or around salivary glands. In contrast to rats, Syrian golden hamsters exposed to the same concentrations of dichloromethane showed no evidence of carcinogenicity. Longstaff et al. (13) found two fluorinated halomethanes carcinogenic in the rat. Fluorochloromethane (FC-31) is a highly active carcinogen by oral administration inducing squamous cell carcinoma and/or fibrosarcoma of the forestomach in 67 of 72 dosed rats compared with only 1 of 208 controls. Dichlorofluoromethane (FC-22) is not carcinogenic in rats by oral administration but induces a low incidence of subcutaneous fibrosarcomas in the region of the salivary gland in male rats exposed to 50,000 ppm FC-22 vapor for 2 years (Litchfield and Longstaff, cited in ref. 13). A 2-year carcinogenicity bioassay of chlorodibromomethane by the

U.S. National Toxicology Program (22) showed no evidence of carcinogenicity in rats. There was equivocal evidence of carcinogenicity for male mice in which chlorodibromomethane caused a significant increase in the incidence of hepatocellular carcinomas in the high dose group. Some evidence of carcinogenicity was observed for female mice since chlorodibromomethane caused a significant increase in the combined incidence of hepatocellular adenomas and carcinomas.

Wong et al. (39) provided additional data for the potent carcinogenicity of 1,2-dibromoethane (ethylene dibromide). Sprague-Dawley rats exposed to vapor containing only 20 ppm 1,2-dibromoethane for 18 months developed a variety of tumors (see Update Table II). A combined treatment of 1,2-dibromoethane vapor and disulfiram in diet showed a marked potentiation of the carcinogenic effects. Significant increases in the incidences of hepatocellular, splenic, mesentary, renal and thyroid tumors were observed. Disulfiram, an inhibitor of aldehyde dehydrogenase, is believed to potentiate the carcinogenic action of 1,2-dibromoethane by prolonging the lifetime of its putative reactive intermediate, bromoacetaldehyde (see Section 5.2.2.1.4.1.2). Three fluorinated haloethanes have been tested for carcinogenic activity in the rat by Longstaff et al. (13). Both 1,1,1-trifluoroethane (FC-143a) and 1,1,1,2-tetrafluoroethane (FC-134a) are not carcinogenic. In contrast, 1,1,1-trifluoro-2-chloroethane (FC-133a) has been found to be a fairly potent carcinogen. Female rats dosed with FC-133a showed an increased incidence of uterine carcinomas whereas males had a significantly higher incidence of benign interstitial cell tumors of the testis. The demonstration of carcinogenicity of FC-133a is somewhat surprising in view of its lack of activity in the Ames test and the in vitro cell transformation assay. The carcinogenicity studies of three chlorinated derivatives (1,1,1-tri-, 1,1,1,2-tetra- and penta-) of ethane have been completed by the U.S. National Toxicology

Program (40-43). Consistent with previous findings of the refractoriness of rats to chlorinated ethanes, none of the three chlorinated ethanes showed any evidence of carcinogenicity in the rat. However, in $B6C3F_1$ mice, all three chlorinated ethanes showed some evidence of carcinogenicity. 1,1,1-Trichloroethane was active in female mice causing a significant increase in the incidence of hepatocellular carcinoma. The compound also increased the incidence of hepatocellular carcinomas in male mice but the evidence was considered equivocal. In the study on 1,1,1,2-tetrachloroethane, the survival rate of high-dose groups was poor because of toxicity. Nevertheless, 1,1,1,2-tetrachloroethane clearly increased the incidence of hepatocellular carcinomas in female mice and of hepatocellular adenomas in mice of either sex. Technical grade pentachloroethane (contains 4.2% hexachloroethane) significantly elevated the incidence of hepatocellular carcinoma in all groups of dosed mice. There was also a significant dose-related increase in hepatocellular adenoma in female mice. Thus, among the eight chloroethanes tested, six are hepatocarcinogenic in female mice inducing hepatocellular carcinoma. Based on comparison of dosage and tumor incidence in the low-dose groups, the relative hepatocarcinogenic potency follows the order: 1,1,2,2-tetra- > penta- > 1,1,2-tri- > hexa- > 1,1,1,2-tetra- > 1,1,1-tri- (see Update Table III).

The finding of potent carcinogenicity of 1,2-dibromo-3-chloropropane has prompted the U.S. National Toxicology Program to investigate the carcinogenic potential of other halopropanes. A carcinogenesis bioassay (27) of 1,2-dichloropropane showed some evidence of carcinogenicity in mice of either sex as indicated by an increased incidence of hepatocellular adenomas. There was equivocal evidence of carcinogenicity in female F344/N rats as 1,2-dichloropropane caused a slight dose-related increase in the incidence of adenocarcinoma of the mammary gland. No increases in spontaneous tumor incidences

Update Table III

Relative Potency of Chloroethanes in the Induction of Hepatocellular Carcinoma in Female F6C3F$_1$ Mice[a]

Chloroethane	Dosage (mmol/kg)		% Incidence		Relative potenency	
	Low dose	High dose	Low dose	High dose	Low dose	High dose
1,1,1-tri-	11.2	22.4	10	20	1.2	1.6
1,1,2-tri-	1.46	2.91	33	89	30	56
1,1,1,2-tetra-	1.49	2.98	11	(13)[b]	10	(8.0)[b]
1,1,2,2-tetra-	0.85	1.68	63	91	100	100
penta-	1.23	2.46	67	(29)[b]	74	(22)[b]
hexa-	2.49	5.02	40	31	22	11

[a]Calculated from U.S. National Cancer Institute/National Toxicology Program data (see Table VII and Update Table II).

[b]Early mortalities in these high dose groups precluded an accurate evaluation of the lifetime incidence of hepatocellular carcinoma.

[c]Calculated from the ratio of the percent incidence to dosage and assigning a value of 100 to 1,1,2,2-tetrachloroethane.

were observed in male F344/N rats. Another halopropane, 1,2,3-trichloropropane, was being tested at the time of this writing.

A number of carcinogenicity studies of vinyl chloride (VC) have recently been published. Hehir et al. (44) reported that a single 1-hour exposure to 50,000 ppm VC was sufficient to elicit a positive carcinogenic response in mice; lower concentrations brought about borderline (at 5,000 ppm) or negative (at 50 or 500 ppm) responses. For repeated short-term exposures, the concentration of VC appeared to play a more important role than the cumulative dose. Mice subjected to 10 1-hour exposures to 500 ppm VC had a positive carcinogenic response whereas those subjected to 100 1-hour exposures to 50 ppm VC did not. Suzuki (45) exposed CD-1 mice to low concentrations of VC (1, 10, 100, 300 and 600 ppm) for 4 weeks; at 41 weeks after exposure, a dose-response relationship in the incidence of alveologenic tumors (11.1, 33.3, 66.7, 71.4 and 85.7%, respectively, compared with 0% in controls) was observed. The latency period was 10 weeks in the 600 ppm group, 12 weeks in the 300 ppm group and 40 weeks in the 100, 10 and 1 ppm groups. Feron et al. (46) maintained Wistar rats on diets containing VC-contaminated polyvinyl chloride powder giving daily intakes of 1.7, 5.0 and 14.1 mg VC/kg body weight. Significant increases in the incidences of hepatocellular carcinoma and angiosarcoma (see Update Table II) were observed. Even a low dose of 1.7 mg/kg was potentially carcinogenic as evidenced by an increased incidence of neoplastic nodules in female mice. Further studies on even lower doses were being conducted by the investigators at the time of this writing. Radike et al. (47) found that rats exposed to 600 ppm VC in air for 1 year developed hepatocellular carcinomas (44%) and liver angiosarcomas (23%). Simultaneous exposure of the VC-treated rats to ethanol significantly enhanced the incidences of hepatocellular carcinomas (60%) as well as angiosarcomas (50%). The

authors (47) suggested that ethanol potentiates VC carcinogenesis by generating acetaldehyde which prolongs the half-life of one of the putative reactive intermediates of VC (chloroacetaldehyde) by competing for oxidation by aldehyde dehydrogenase.

The final results of an inhalation study of vinyl bromide have been published (48). Sprague-Dawley rats were exposed for 2 years to air containing 9.7, 52, 247 and 1,236 ppm vinyl bromide. Angiosarcomas, primarily of the liver, were induced in both male (5.8%, 30%, 51%, 36% compared with 0% controls) and female (8.3%, 42%, 51%, 34% compared with 1% controls) rats. A significant increase in the number of Zymbal's gland carcinoma was seen in males exposed to 247 and 1,235 ppm and females exposed to 1,235 ppm vinyl bromide. An increased incidence of hepatocellular neoplasms (carcinomas or "neoplastic nodules") was also found in males exposed to 247 ppm and females exposed to 9.7, 52 and 247 ppm vinyl bromide. These results indicate that vinyl bromide is at least as potent as or possibly more potent than vinyl chloride as a carcinogen.

A long-term carcinogenicity bioassay (49) on vinylidene fluoride (1,1-difluoroethylene) showed a slight but significant increase in the incidence of tumors in fat tissue (lipomas and liposarcomas) in a high-dose group (8.7% vs. 1.8% controls). Trichloroethylene (epichlorohydrin-free) was retested by U.S. National Toxicology Program (50) because a previous bioassay used a technical grade (epoxide-stabilized) sample. In agreement with the previous study, trichloroethylene induced hepatocellular carcinomas in $B6C3F_1$ mice. In addition, an increase in the incidence of Harderian gland adenomas was observed. There was also some evidence that trichloroethylene is carcinogenic in male (but not female) F344/N rats, inducing adenocarcinomas in renal tubular cells.

Four arylalkyl halides have been tested for carcinogenic activity in ICR mice. Fukuda et al. (51) found benzotrichloride to be a potent, multi-target carcinogen. Topical application of 2.3 µl benzotrichloride to the skin of female ICR mice led to high incidences of skin carcinomas, lung tumors and tumors of the upper digestive tract. Topical application of higher amounts of benzotrichloride induced lymphomas as well. The induction of digestive tract tumors was attributed to a direct action of the chemical ingested as a result of licking the treated skin. Two closely related compounds, benzal chloride and benzyl chloride, were found to be less carcinogenic than benzotrichloride (see Update Table II). The relative order of carcinogenic potency of these three compounds correlates well their mutagenic activity in the Ames test (30). The carcinogenicity of benzotrichloride was confirmed in a study by the Hooker Chemical Co. (52). In addition, p-chlorobenzotrichloride is carcinogenic both by topical and oral administration; its potency appears to be higher than that of benzotrichloride (see Update Table II). Oral administration (0.05, 0.13, 0.32, 0.8 or 2 µl, twice weekly for 17.5 weeks) led to dose-related increases in the incidences of tumors of the forestomach, lung, skin and lymphatic systems. The overall tumor incidences were 6/22, 10/28, 17/22, 27/29 and 25/29 compared with 2/26 controls.

METABOLISM AND MECHANISM OF ACTION

The metabolism and mechanism of action of haloalkanes and haloalkenes have been reviewed in several recent publications (2, 3, 7, 9, 11, 12). Among halomethanes, Dodd et al. (53) reported that chloromethane (methyl chloride) is metabolized mainly by GSH-S-alkyltransferase-catalyzed conjugation with reduced GSH. Preliminary data indicate that the chloromethane-GSH conjugate thus formed may be further metabolized to a toxic product (probably formaldehyde) suggesting that this pathway represents a toxication rather than detoxi-

cation metabolic route. Green (17) observed that rat liver S9 mix substantially enhances the mutagenic activity of chlorofluoromethane (FC-31) but has no effect on dichloromethane. The difference was attributed to differential stability of formyl halide, the postulated reactive intermediate in the oxidative metabolism of dihalomethane (see Fig. 1 in Section 5.2.2.1.4.1.1). Formyl fluoride is apparently stable enough to reach and interact with target macromolecules to exert its mutagenic action, whereas formyl chloride is not.

Dichloromethyl carbene ($:CCl_2$) was postulated as a possible reactive intermediate in the reductive metabolism of carbon tetrachloride (see Fig. 3, Section 5.2.2.1.4.1.1). Direct evidence for a carbene intermediate was recently provided by Pohl and George (54) who trapped the intermediate generated during the reductive metabolism of carbon tetrachloride with 2,3-dimethyl-2-butene to form 1,1-dichloro-2,2,3,3-tetramethylcyclopropane. Using 2,6-dimethylphenol as a nucleophilic trapping agent, Pohl, Mico and coworkers (55, 56) found evidence for another reactive intermediate, electrophilic halogen, in the metabolism of carbon tetrachloride, trichlorobromomethane and tetrabromomethane. They (56) postulated a reductive-oxygenation pathway in which tetrahalomethane is first reductively dehalogenated to yield a trihalomethyl radical, which reacts with oxygen to form a trihalomethylperoxyl radical; this, in turn, decomposes to yield a dihalocarbonyl and an electrophilic halogen. The carbene, the trihalomethyl radical, the peroxyl radical, the electrophilic halogen and the dihalocarbonyl are all potentially toxic metabolites. There is some evidence that trichloromethyl radical, chemically produced from carbon tetrachloride by benzoyl peroxide catalysis, may interact with bases of DNA (57). DiRenzo et al. (58) showed that microsomes from phenobarbital-pretreated rats can bioactivate bromotrichloromethane (0.51 nmol/mg DNA/h), chloroform (0.46) and carbon tetrachloride (0.39) to bind

covalently to calf thymus DNA, a substantially lower level of binding (0.11) was found for dichloromethane. There is growing evidence that a disturbance in hepatocellular Ca^{++} homeostasis may be involved in triggering the hepatotoxic actions of carbon tetrachloride (2); this new direction of research is worthy of further exploration.

The structure-activity relationship in the microsomal dechlorination of a series of 12 chloroethanes (including some fluorochloroethanes) was studied by Salmon et al. (59). Comparison of the dechlorination rate with electron densities showed poor correlation. However, substantially better correlation was achieved if the compounds were separated into three structural classes (RCH_2Cl, $RCHCl_2$ and $RCCl_3$). Among these three classes, the dechlorination rate and reactivities decrease in the order: $RCHCl_2 >> RCH_2Cl > RCCl_3$. The kinetic parameters of microsomal dechlorination of five chloroethanes were measured; their respective V_{max} (in nmol/min/mg protein) and Km (in mM) were: 1,1-dichloroethane (41.7; 0.36), 1,1,2,2-tetrachloroethane (18.2; 0.55), 1,2-dichloroethane (0.24; 0.14), 1,1,1-trichloroethane (0.2; 0.273) and hexachloroethane (0.91; 2.73). Consistent with the above data, McCall et al. (60) found that 1,1-dichloroethane was dechlorinated by rat hepatic microsomes at an approximately 10-fold greater rate than was 1,2-dichloroethane. The microsomal metabolism of 1,1-dichloroethane appeared to be mainly detoxifying, with acetic acid as the major metabolite. On the other hand, microsomal metabolism of 1,2-dichloroethane yielded chloroacetaldehyde as the major metabolite, a mutagenic and potentially carcinogenic intermediate. DiRenzo et al. (58) have compared the extent of microsome-catalyzed covalent binding of several haloethanes to calf thymus DNA. Both 1,2-dibromoethane and 1,1,2-trichloroethane are bound to an appreciable extent whereas low level of binding was noted for halothane, 1,2-dichloroethane and 1,1,1-trichloroethane. The

nature of covalent binding of 1,2-dibromoethane to DNA was investigated (61); the compound binds to DNA probably as a monofunctional intermediate (possibly as episulfonium ion) rather than as a bifunctional intermediate (such as bromoacetaldehyde).

A quantum chemical structure-activity study intended to correlate oxidative metabolism with carcinogenic potency of chloroethanes has been carried out by Loew et al. (62). Assuming chlorinated aldehydes and acylchlorides as the ultimate carcinogen(s), several molecular properties -- possibly useful as indicators of the relative oxidative metabolism of chloroethanes to reactive intermediates and of the electrophilicity of reactive intermediates -- were calculated and compared to the known hepatocarcinogenic potency of seven chloroethanes in mice. The molecular properties that gave the best correlation were: the enthalpy of hydroxylation of chloroethanes by radical oxygen, the heat of formation of chlorinated aldehydes and the energy of the lowest unoccupied molecular orbital (LUMO; an indicator of electrophilicity) in chlorinated aldehydes. Based on these considerations, 1,1,1,2-tetrachloroethane was predicted to be an active carcinogen of similar potency as its 1,1,2,2-isomer, whereas monochloroethane was predicted to be inactive. A recent carcinogenesis bioassay by U.S. National Toxicology Program (41) confirmed the carcinogenicity of 1,1,1,2-tetrachloroethane in mice; however, its potency appeared to be substantially lower than that of its 1,1,2,2-isomer (see Update Table III).

The role of epoxides, generated during the metabolic activation, in the mechanism of genotoxic action of haloalkanes has been further investigated. Scherer et al. (63) showed that chloroethylene oxide (produced by UV-induced chlorination of ethylene oxide by t-butyl hypochlorite) binds covalently to deoxyguanosine yielding 7-(2-oxoethyl)guanine as the major adduct. The ter-

minal aldehyde group of the 7-N-(2-oxoethyl)guanine reacts reversibly with the oxygen at the 6-position of guanine to form a hemiacetal, O^6,7-(1'-hydroxy-ethano)guanine, which is expected to cause faulty base pairing during DNA replication. 7-(2-Oxoethyl)guanine is the major product of base alkylation in liver DNA of rats exposed to vinyl chloride (64). Van Duuren et al. (65) showed that the cis and trans isomers of epoxides of 1-chloropropene and 1,3-dichloropropene are all carcinogenic in mice after topical or subcutaneous administration, consistent with their putative role as the carcinogenic intermediates of the parent chloropropenes. On the other hand, the epoxides of trichloroethylene and tetrachloroethylene are both inactive in these skin carcinogenesis studies casting doubt on the carcinogenicity of the parent compounds (see Section 5.2.2.1.3.5.2) or suggesting alternative carcinogenic intermediates.

As discussed in Section 5.2.2.1.4.2.1, the stability and reactivity of the epoxides of halogenated ethylenes have a profound effect in determining the carcinogenic potential of the parent compounds. The key factor is an optimum balance between the stability and the reactivity of the epoxide to both reach and react with the DNA target (66). Several quantum chemical structure-activity studies have been conducted using different computational methods to address this issue (67-70). Jones and Mackrodt (67, 68) calculated the "two-center bond energy" of the weakest C-O bond of the epoxides of a series of halogenated ethylenes and compared to their mutagenicity (in E. coli K12) and oncogenicity (ability to induce preneoplastic hepatocellular foci). A striking similarity between the patterns of these two activities was observed. The observed "threshold band" for oncogenicity ranged from -14.1 to -12.9 eV, compared with -14.5 to -12.8 eV for mutagenicity. It was suggested that epoxides which fall within these limits are potentially hazardous while

those outside may be either too unstable to reach target or too stable and therefore not reactive enough. Politzer and Proctor (69) found that oxygen protonation weakens the C-O bonds of chloroethylene oxide facilitating ring opening and carbocation, the effect being substantially greater for the C-O bond involving the carbon bearing the chlorine. Correlative studies with an extended series of epoxides of haloalkenes (P. Politzer, personal communication) suggested that the ease of protonation (as measured by the electrostatic potential around the oxygen of the epoxide group) may prove to be a useful predictive tool in assessing the carcinogenic potential of epoxides and their parent haloalkenes. Considering chlorinated aldehydes and acylchlorides as well as epoxides as possible ultimate carcinogens, Loew et al. (70) calculated various molecular properties that can serve as indicators of the relative metabolism of six chlorinated ethylenes to reactive intermediates and of the electrophilicity of reactive intermediates and compared to the known carcinogenic potency of four of these compounds. The results suggested that the chlorinated aldehydes and acylchlorides may be the more likely ultimate carcinogens. The relative extent of metabolism of these carbonyl products, rather than their electrophilicity, is a determinant of the relative carcinogenic activity of the parent compound. 1,2-Dichloroethylene was predicted to be carcinogenic with an activity intermediate between vinylidene chloride and tetrachloroethylene.

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