FINAL

Report on Carcinogens Background Document for

Vinyl Bromide

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

Prepared for the:
U.S. Department of Health and Human Services
Public Health Services
National Toxicology Program
Research Triangle Park, NC 27709

Prepared by:
Technology Planning and Management Corporation
Canterbury Hall, Suite 310
4815 Emperor Blvd
Durham, NC 27703
Contract Number NOI-ES-85421

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

US Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

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

Reasonably Anticipated to be Human Carcinogens:

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

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

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

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

Summary Statement

Vinyl Bromide

CASRN 593-60-2

Carcinogenicity

Vinyl bromide (VB) is *reasonably anticipated to be a human carcinogen* based on evidence of tumor induction at multiple organ sites in rats. Inhalation exposure of rats to VB resulted in increased incidences of hepatic hemangiosarcomas, Zymbal gland carcinomas, and liver neoplastic nodules and hepatocellular carcinomas (Benya *et al*. 1982, IARC 1986). The biological activity of VB is similar to that of its vinyl halide analogs, vinyl chloride (VC), a known human carcinogen (NTP 1998; IARC 1987), and of vinyl fluoride (VF), a probable human carcinogen (IARC 1995). A unique feature of VC carcinogenicity is the induction of rare hepatic hemangiosarcomas in animals and the causal association in epidemiological studies between VC exposure and excess risk of angiosarcoma of the liver (NTP 1998). VB appears to be a more potent inducer of liver angiosarcomas in rats than VC. The fact that VB and VF also induces rare hemangiosarcomas of the liver in rats and induced the formation of similar DNA adducts suggests a possible common mechanism of carcinogenicity for these three vinyl halides.

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

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

VB is genotoxic in *Salmonella typhimurium* (IARC 1986) and *Drosophila melanogaster* (Ballering *et al*. 1996). VB also induces DNA damage in several organs of mice (Sasaki *et al*. 1998). The biotransformation pathway for VB is similar to that of VF and VC. All three compounds undergo cytochrome P-450 mediated oxidation to the corresponding haloethylene oxide (bromoethylene oxide, fluoroethylene oxide, and chloroethylene oxide). These intermediates may rearrange to the corresponding haloacetaldehydes (2-bromoacetaldehyde, 2-fluoroacetaldehyde, and 2-chloroacetaldehyde) which, in turn, are oxidized to haloacetic acids. The K_m for VB metabolism is about an order of magnitude lower than that for VC (Bolt *et al*. 1978), which implies that the greater carcinogenic potency of VB, may be related to kinetic differences in metabolism.

The metabolism of VB generates products that bind covalently to DNA and to protein; 2-bromoethylene oxide is the major DNA binding agent, and 2-bromoacetaldehyde is the major protein alkylating agent (Guengerich *et al*. 1981). After exposure to vinyl chloride, the major DNA adduct formed is 7-(2-oxoethyl)guanine (constituting approximately 98% of all adducts) (Bolt 1988). By analogy, the 7-position of guanine is considered to be the preferential site of DNA alkylation by bromoethylene oxide, the primary metabolite of VB biotransformation (Bolt 1988). Chloroacetaldehyde and bromoacetaldehyde can react with adenine or cytosine bases in DNA or RNA to produce cyclic etheno-DNA/RNA

adducts (1,N^6-ethenoadenosine and 3,N^4-ethenocytosine). Etheno-DNA adducts can cause miscoding as a consequence of their modification of base-pairing sites. Because the cyclic etheno-adducts have a longer half-life than 7-(2-oxoethyl)guanine, they have a greater potential to accumulate with chronic exposure (Swenberg *et al*. 1992).

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

Table of Contents

List of Tables

List of Figures

1 Introduction

Vinyl bromide (VB) was nominated for listing in the Report on Carcinogens (RoC) by the National Institute of Environmental Health Sciences (NIEHS) RoC Review Group (RG1) based on the 1999 International Agency for Research on Cancer IARC monograph (IARC 1999), which indicates that there is sufficient evidence in experimental animals for the carcinogenicity of VB and that it is *probably carcinogenic to humans* (Group 2A).

1.1 Chemical identification

VB is a member of the vinyl halide class. The vinyl halides are easily polymerized and copolymerized with various materials, such as acrylonitrile, vinyl acetate, and styrene, to form pliable, lightweight plastics or thermoplastic resins. Vinyl bromide (C_2H_3Br, mol wt 106.95, CASRN 593-60-2) also is known as bromoethylene, monobromoethylene, and bromoethene. It is a colorless gas at ambient temperature and pressure. VB has widespread industrial use, especially in the plastics industry. It is used in the production of polyvinyl bromide and other bromopolymers. A common intermediate in organic synthesis, it is used in the chemical, plastic and plastic products, leather and leather products, and metal fabrication industries. The structure of VB is illustrated in Figure 1-1.

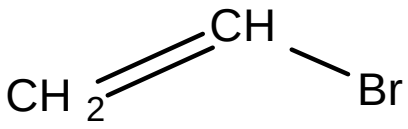

Figure 1-1. Structure of VB

1.2 Physical-chemical properties

VB is incompatible with strong oxidizing agents, copper, copper alloys, and plastics. It is a highly flammable gas under normal atmospheric conditions and a colorless liquid under pressure (IARC 1986). Its RTECS number is KU8400000. The physical and chemical properties of VB are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of VB

Property	Information	Reference
Molecular weight	106.95	Budavari *et al*. (1996); CRC (1998)
Color	colorless	Budavari *et al*. (1996); CRC (1998)
Odor	characteristic pungent odor pleasant odor	IARC (1986) NIOSH (1994)
Physical state	flammable gas	Budavari *et al*. (1996); CRC (1998)
Melting point (°C)	- 139.5	Budavari *et al*. (1996); CRC (1998)
Boiling Point (°C) at 750 mm	15.8	Budavari *et al*. (1996); CRC (1998)
Flash point (°C)	5	Chemfinder (1999)

Property	Information	Reference
Specific gravity	1.4933	Chemfinder (1999)
Relative vapor density (air = 1)	3.8	Physchem (1999)
Vapor pressure (mm Hg)	1,033	HSDB 1996
Solubility in:		
Water at 20°C	insoluble	Budavari *et al*. (1996)
Chloroform	soluble	IARC (1986)
10% Ethanol	soluble	CRC (1998)
10% Ethyl Ether	soluble	CRC (1998)
10% Acetone	soluble	CRC (1998)
10 % Benzene	soluble	CRC (1998)

1.3 Identification of metabolites

The major metabolites of VB are bromoethylene oxide, bromoacetaldehyde, and bromoacetic acid. VB is initially oxidized by microsomal monooxygenase(s) to bromoethylene oxide (Bolt 1988; Ballering *et al*. 1996). The structure of bromoethylene oxide is illustrated in Figure 1-2.

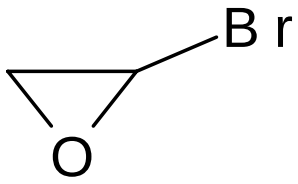

Figure 1-2. Structure of bromoethylene oxide

Bromoethylene oxide is deactivated by an epoxide hydrolase or gluthione transferase. It also may rearrange to form bromoacetaldehyde (Bolt 1988; Ballering *et al*. 1996). The structure of bromoacetaldehyde (C_2H_3BrO, mol wt 122.95, CASRN 17157-48-1) is shown in Figure 1-3.

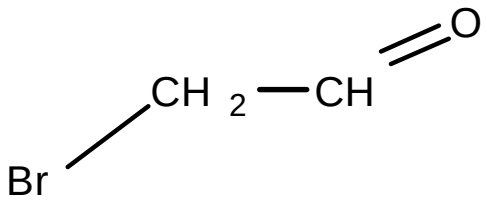

Figure 1-3. Structure of bromoacetaldehyde

Bromoacetic acid ($C_2H_3BrO_2$, mol wt 138.95, CASRN 79-08-3) is detected as a metabolite in VB-treated experimental animals. It probably is formed as a result of oxidation of bromoacetaldehyde (Bolt 1988; Ballering *et al*. 1996). The structure of bromoacetic acid is illustrated in Figure 1-4.

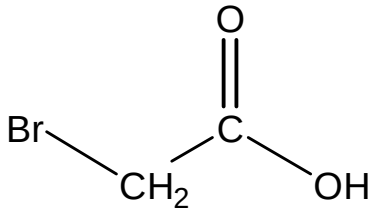

Figure 1-4. Structure of bromoacetic acid

2 Human Exposure

2.1 Use

VB is used predominantly as an intermediate in the production of polymers and copolymers. It is used in polymers as a flame retardant and in the production of monoacrylic fibers for carpet-backing material. As a comonomer with acrylonitrile, it is used in the production of fabrics and fabric blends used in sleepwear (mostly children's) and home furnishings. Copolymerized with vinyl acetate and maleic anhydride, VB is used to produce granular products. VC-VB copolymers are used for preparing films, for impregnating or laminating fibers, and as rubber substitutes. VB also is used in leather and fabricated metal products (HSDB 1996). Polyvinyl bromide, made from VB, is a polymer of little commercial value because it is unstable at room temperature. VB also is used in the production of pharmaceuticals and fumigants (IARC 1986).

2.2 Production

VB was first produced in the United States in 1968. In 1982, U.S. production was estimated to be 51 million lb (HSDB 1996). Currently, one producer, Monsanto Co., is identified by the U.S. Environmental Protection Agency (EPA) (TRI 1996). In 1994, U.S. EPA reported VB output levels to be < 1 million pounds and VB was not listed as a high production volume (HPV) chemical (U.S. EPA 1994).

2.3 Environmental exposure

2.3.1 Routes of exposure

The primary routes of potential human exposure to VB are inhalation and dermal contact. VB is not known to occur naturally in the environment. It is assumed that most, if not all, VB environmental exposure occurs as a result of industrial contamination (IARC 1986).

2.3.2 Industrial releases into the environment

In 1996, the most recent year for which information is available, only one facility reported environmental releases of VB. Monsanto Co. reported releasing a total of 5,840 lb of VB into the air, 240 lb in non-point source releases and 5,600 lb is point source releases (TRI 1996).

2.4 Occupational exposure

The National Institute for Occupational Safety and Health (NIOSH) has identified the following industries in which VB exposure occurs: chemicals and allied production, rubber and plastic production, leather and leather product production, and fabricated metal production for wholesale trade (NIOSH 1978).

The NIOSH National Occupational Exposure Survey (NOES) estimated that 1,821 workers potentially were exposed to VB from 1981 to 1983 (NIOSH 1990).

Median eight-hour time-weighted average (TWA) exposures were calculated for a VB manufacturing plant. They ranged from 0.4 to 27.5 mg/m^3 (0.1 to 6.3 ppm), depending upon jobs and areas surveyed. Personal air samples (one hour) were taken for various employees at this VB manufacturing plant. A plant operator was exposed to VB concentrations of 0.4 to 1.7 mg/m^3 (0.09 to 0.4 ppm), a laboratory technician to concentrations of 1.3 to 2.2 mg/m^3 (0.3 to 0.5 ppm),

and two loading crewmen to concentrations of 5.2 to 27.5 mg/m^3 (1.2 - 6.3 ppm) (Bales 1978, Oser 1980, both cited by IARC 1986).

2.5 Biological indices of exposure

No biomarkers of VB exposure are known. Air sampling is the method of choice for determining VB exposure levels. Table 2-1 identifies procedures used for VB air analysis.

Table 2-1. Methods for the analysis of VB in air

Sample preparation	Assay procedure	Limit of detection	Reference
Adsorb (charcoal tube); desorb (ethanol)	GC/FID	1.3 mg/m^3	Spafford and Dillon (1981); Taylor (1981)
Adsorb (charcoal tube); desorb (heat), purge (helium), dry (calcium sulphate tube), and adsorb (Tenax tube); desorb (thermal) and trap (liquid nitrogen); vaporize (heat) onto capillary GC column	GC/MS	8 ng/m^3	Pellizzari *et al.* (1978)
Adsorb (Tenax-GC); desorb (heat), purge (helium), trap (liquid nitrogen cooled nickel capillary); vaporize (heat) directly onto capillary GC column	GC/MS	250 ng/m^3	Pellizzari *et al.* (1978); Krost *et al.* (1982)

Source: IARC (1986).

GC: gas chromatography, FID: flame ionization detection, MS: mass spectrometry

2.6 Environmental fate

VB is found in nature as a result of industrial spills and discharges. About 99.8% of all polluting VB eventually dissipates in the air, and the rest contaminates water (U. S. EPA 1986). VB also may occur as a degradation product of 1,2-dibromoethane (HSDB 1996). Although VB concentrations were detected in the air in two communities of Arkansas with VB industries, exact levels were not reported (IARC 1986).

2.6.1 Air

Based upon the high vapor pressure of VB, it is most likely to exist in vapor phase in the atmosphere. VB is expected to react with hydroxyl radicals produced photochemically. Its major reactions are with the OH^- radical and ozone (O_3), which remove it from the air. The reported atmospheric lifetimes of VB range from less than one day to five days (HSDB 1996).

2.6.2 Water

VB is not prevalent in water, because it is water insoluble and highly volatile. VB has a half-life of less than two days in water (U. S. EPA 1986). Its volatilization half-life in a model river is estimated to be three hours (HSDB 1996). Bioaccumulation in aquatic organisms is thought to be insignificant, because the concentration of VB detected in fish tissues is not expected to exceed that of the habitat (U.S. EPA 1986). VB has a bioconcentration factor (BCF) of 9; a BCF greater than 1,000 is required for significant bioaccumulation in aquatic organisms (HSDB 1996).

2.6.3 Soil

VB has a high mobility in soil and only slightly adsorbs to suspended solids and sediments in water. When released into wet soil, VB rapidly evaporates or undergoes extensive leaching. Upon release into dry soil, VB rapidly evaporates (HSDB 1996).

2.7 Regulations

U.S. EPA regulates VB under the Clean Air Act (CAA), and the Occupational Safety and Health Administration (OSHA) regulates VB under the Hazard Communication Standard as a chemical hazard in laboratories. U.S. EPA regulations are summarized in Table 2-2, and OSHA regulations are summarized in Table 2-3.

Table 2-2. U.S. EPA regulations

U.S. EPA Regulations	
Regulatory action	**Effect of regulation and other comments**
40 CFR 63 – PART 63 – NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Codes: 7401 *et seq*.; CAA.	Standards that regulate specific categories of stationary sources that emit (or have potential to emit) one or more hazardous air pollutants are listed in this part pursuant to section 112(b) of the CAA.
40 CFR 63.800ff. – Subpart JJ – National Emission Standards for Wood Furniture Manufacturing Operations. Promulgated: 60 FR 62936, 12/07/95.	The provisions of this subpart apply to each facility that is engaged in the manufacture of wood furniture or wood furniture components and that is a major source as defined in 40 CFR 63.2. This subpart details which applications VB is prohibited from use. It also lists VB as a volatile, hazardous air pollutant.

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

Table 2-3. OSHA regulations

OSHA Regulations	
Regulatory action	**Effect of regulation and other comments**
29 CFR 1910.1200—Sec. 1910.1200. Hazard Communication. Promulgated 62 FR 42018, 08/04/97.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication program to include labels, material safety data sheets, and worker training.
29 CFR 1910.1450. Promulgated 1/31/90. Amended 58 FR 40191, 7/27/93. OSHA Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As select carcinogen (IARC Group 2A, *possibly carcinogenic to humans*), VB is included as a chemical hazard in laboratories. Employers are required to provide employee information and training and to provide Chemical Hygiene Plan.

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

3 Human Cancer Studies

There are no reports of an association between exposure to VB and cancer in humans.

4 Studies of Cancer in Experimental Animals

An IARC Working Group reviewed studies of the carcinogenic potential of VB (IARC 1986, 1999). These reviews catalogued carcinogenesis studies conducted via inhalation, subcutaneous, and dermal routes of administration. The results of the reviews and evaluations are summarized in Section 4. A thorough search of the peer-reviewed scientific literature did not reveal any additional animal studies of the carcinogenic potential of VB.

4.1 Inhalation exposure

Rats exposed to VB by inhalation developed tumors at multiple sites. In this study, 9- to 10-week-old Sprague-Dawley rats (120 of each sex) were exposed to VB in air at concentrations of 10, 50, 250, or 1250 ppm (corresponding to 44, 219, 1093, or 5463 mg/m^3) six hours/day, five days/week, for 104 weeks. Hydroquinone methyl ether (0.02%, used as stabilizer), ethylene oxide (0.03%), acetylene (0.0007%), and aldehydes and ketones (0.008%) were present in the VB used. The animals in the highest dose group were sacrificed at 72 weeks because of 50% mortality (Benya *et al*. 1982, cited in IARC 1986).

VB caused statistically significant dose-related increases in the incidences of angiosarcomas of the liver and squamous cell carcinomas of the Zymbal gland in both sexes. In addition, the incidences of neoplastic nodules of the liver and hepatocellular carcinomas were significantly increased in males and females exposed to 250 ppm, but not in those exposed to 50 or 1250 ppm. Failure of the highest dose to increase the incidence of hepatocellular tumors was most likely a consequence of the reduced survival and early sacrifice of those animals. Tumor incidences in rats exposed to VB are summarized in Table 4-1.

Table 4-1. Tumor incidences in Sprague-Dawley rats exposed to VB by inhalation for up to 104 weeks

Tumor type	Tumor response/number examined				
	Inhalation concentration of vinyl bromide in air (ppm)[a]				
	0	10	50	250	1250
Males					
Liver: angiosarcoma	0/144	7/120*	36/120***	61/120***	43/120***
Liver: neoplastic nodules and hepatocellular carcinoma	4/143	5/103*	10/119*	13/120*	5/119*
Zymbal gland: squamous cell carcinoma	2/142	1/99	1/112	13/114**	35/116**
Females					
Liver: angiosarcoma	1/144	10/120***	50/120***	61/120***	41/120***
Liver: neoplastic nodules and hepatocellular carcinoma	7/142	18/101**	12/113	21/118**	9/112
Zymbal gland: squamous cell carcinoma	0/139	0/99	3/113	2/119	11/114***

Source: Benya *et al*. (1982, cited in IARC 1986)
[a]Logistic regression test for trend $^{*}P < 0.025$, $^{**}P < 0.005$, $^{***}P < 0.001$.

Based on the increased incidence of angiosarcomas of the liver and squamous cell carcinomas in both sexes of the rats in this study, IARC concluded that there is sufficient evidence of

carcinogenicity of VB in experimental animals and classified VB as *probably carcinogenic to humans* (Group 2A) (IARC 1986).

4.2 Dermal exposure

VB failed to induce skin tumors in mice when applied dermally (15 mg in 0.1 mL of acetone) three times a week for 60 weeks (Van Duuren 1977, cited by IARC 1986). There was no evidence of initiator activity when VB was tested in a two-stage skin carcinogenesis test. Groups of 30 ICR/Ha Swiss mice received a single topical treatment of VB (15 mg in 0.1 mL of acetone), followed by application three times a week for 60 weeks of 2.5 μg of 12-*0*-tetradecanoylphorbol-13-acetate (TPA) in 0.1 mL of acetone. Additional groups of 30 mice received a single application of 7,12-dimethylbenz[*a*]anthracene followed by treatment with TPA (positive controls), treatment with TPA only, or no treatment at all.

One of 30 mice dosed with VB followed by TPA had a skin papilloma at 412 days, and one skin carcinoma was observed in TPA-treated controls after 44 days. The positive control group had a high incidence of skin tumors (incidence not specified). No tumors were found in the untreated mice. Systemic carcinogenesis was not assessed. The IARC Working Group noted that because the skin application sites were not covered, the mice may have received less than the nominal dose, as VB is volatile (Van Duuren 1977, cited by IARC 1986).

4.3 Subcutaneous exposure

Female mice administered VB by subcutaneous injection, did not develop tumors at the injection site. Groups of female ICR/Ha Swiss mice were administered 25 mg VB in 0.5 mL trioctanoin once a week for 48 weeks and were observed for up to 420 days. No tumors were noted in VB-treated mice or in vehicle or untreated control mice. Systemic carcinogenesis was not assessed (Van Duuren 1977, cited in IARC 1986).

4.4 Summary

VB failed to induce skin tumors or to show any evidence of initiator activity when applied dermally, three times weekly for 60 weeks, to mice. Subcutaneously injected VB failed to induce injection-site tumors in female mice treated weekly for 48 weeks and observed for up to 420 days. (Systemic carcinogenesis was not assessed in these studies.) Rats exposed to VB by inhalation developed tumors at multiple sites, including angiosarcomas of the liver and squamous cell carcinomas of the Zymbal gland in both sexes. Hepatic neoplastic nodules and hepatocellular carcinomas also were significantly increased in males and females exposed to VB at a concentration of 250 ppm, but not at the highest concentration tested. Based on the increased incidence of angiosarcomas of the liver and squamous cell carcinomas in both sexes of the rats in this study, IARC concluded that there is sufficient evidence of carcinogenicity of VB in experimental animals and classified VB as *probably carcinogenic to humans* (Group 2A). The spectrum of these VB-induced neoplasms closely resembled that produced in Sprague-Dawley rats by inhalation exposure to vinyl chloride.

5 Genotoxicity

5.1 Prokaryotic systems

5.1.1 Induction of mutation in Salmonella typhimurium

A number of studies have shown that exposure to vapors of VB (0.2% to 20% v/v in air for various time periods) is mutagenic to *Salmonella typhimurium* strains TA1530 and TA100 in the presence or absence of a metabolic activation system (S9 liver homogenate from Aroclor-induced rats or phenobarbital-induced mice) (Bartsch 1976; Bartsch *et al*. 1976, 1979; Lijinsky and Andrews 1980, all cited in IARC 1986).

VB was assayed for mutagenicity with the ara forward-mutation test in *S. typhimurium,* with or without an exogenous metabolizing system (S9 rat liver homogenate) (Roldan-Arjona *et al.* 1991). Because VB is volatile, its bacterial mutagenic activity is difficult to assay by the standard plate incorporation or preincubation mutagenesis test. To avoid a false negative result, VB was tested in a liquid test (Hera and Pueyo 1986, cited in Roldan-Arjona *et al.* 1991). Bacteria were exposed in liquid for various time periods to VB at a concentration of 142 μmol/mL (corresponding to a minimum of 10 μL of VB). Under these test conditions, VB was mutagenic, and mutagenicity was enhanced by metabolic activation.

5.2 Lower eukaryotic systems

5.2.1 *Mutagenicity in* Drosophila melanogaster

The effect of VB in the *in vivo Drosophila melanogaster* w/w^+ eye mosaic assay was investigated by Vogel and Nivard (1993), who assessed damage to the somatic cells of *D. melanogaster* after exposure of larvae to concentrations of VB ranging from 0 to 64,000 ppm in air. VB was recombinagenic in the assay, as indicated in Table 5-1. A later study confirmed these results (Ballering *et al*. 1996).

Table 5-1. Mutagenicity of VB in the *Drosophila* w/w^+ eye mosaic assay

Conc. (ppm)	Eyes tested	Spots per 100 eyes tested			Average clone size	Clones per 10^4 cells	Activity
		S	L	Total			
0	700	2.86	0.57	3.4	2.4	2.1	—
4,000	250	5.20	1.60	6.8	3.6	6.1	weakly positive 0.085 ppm
8,000	500	3.80	1.20	5.0	2.8	3.5	inconclusive
32,000	500	6.40	1.60	8.0	3.6	7.2	weakly positive
64,000	166 [a]	11.45	4.22	15.7	5.3	20.8	positive

Source: Vogel and Nivard (1993).
Tested in C-4 strains: winscy, y, w females x w males; winscy, y, w/w females x y males.
[a] Reduced survival in relation to control.
L = large spots, clone size > 4 ommatidia.
S = small spots, clone size 1 –to 4 ommatidia.

VB at a concentration of 54,000 ppm in air induced sex-linked recessive mutations in the germ cells of male *D. melanogaster*. In addition to enhanced forward mutation rates (recessive lethal mutations), VB caused M_{exr-}/M_{exr+} hypermutability with *mus201* or *mei9* female genotypes (Ballering *et al.* 1996).

DNA sequence changes induced in the vermilion gene of *D. melanogaster,* following *in vivo* exposure of male flies to VB in air (27,000 ppm for 48 h) were investigated by Ballering *et al*. (1997). Because of low mutagenic activity of VB in nucleotide excision repair (NER^+) genotypes, vermilion mutants were isolated only from crosses of VB-treated males with NER^- females. A total of 14 mutants (5 from 391,039 F_1 females [mutation frequency (mf) = 0.13 x 10^4] and 9 from 125,000 F_2 offspring [mf = 0.72 x 10^{-4}]) were isolated, 3 of which carried large deletions (2 GC → AT transitions, 5 GC → TA transversions, 4 AT → TA transversions, and 3 intra-locus deletions).

In a 17 hour inhalation study, the genetic heterogenetic response of *D. melanogaster* to VB was investigated by Rodriguez-Arnaiz *et al.* (1993), in seven different strains (wild-type laboratory strains Leiden-S [LS], Oregon-K [OK], Berlin-K [BK], and 91-C and DDT-resistant strains 91-R, Hikone-R [HR], and Haag-79 [HG]) in combination with the w/w^+ eye mosaic assay for mitotic recombination. High exposure levels were required to see a significant number of spots in LS (2,000 to 64,000 ppm VB), OK (8,000 to 24,000 ppm VB), or BK (8,000 to 24,000 ppm). Mutation frequencies were highest in strains 91-C, HR, and HG, which were tested at 2,000 to 24,000 ppm VB. The highest concentration of VB (24,000 ppm) was toxic to strains HR and HG, and their mutagenic activity was lower at 24,000 ppm than at lower concentrations of VB at which they were tested. Induction rates were highest in 91-C and lowest in OK, with a 60-fold difference in response between these two strains. From highest to lowest induction rate, the strains responded in the following order: 91-C ≥ HG ~ HR > BK ~ LS ~ OK.

5.3 Mammalian systems *in vivo*

5.3.1 DNA damage

The alkaline single cell gel (SCG or Comet) assay was used to study the genotoxicity of VB in seven mouse organs: stomach, liver, kidney, bladder, lung, brain, and bone marrow (Sasaki *et al.* 1998). VB (2,000 mg/kg) dissolved in olive oil was administered orally to three groups of four male CD-1 mice. The animals were killed immediately (control) or 3 or 24 h after treatment, and necropsies of the seven organs were performed. DNA migration from the seven organs examined is presented in Table 5-2. VB induced DNA damage in all of the organs except bone marrow. The researchers observed no deaths, morbidity, distinctive clinical signs, or gross pathology. There were no microscopic signs of necrosis in the organs in which DNA damage was observed, implying that the DNA damage was not due to cytotoxicity (Sasaki *et al.* 1998).

Table 5-2. Migration of nuclear DNA from organs of mice orally administered 2,000 mg/kg of VB

Sampling time (h)		Migration (μm)						
		Stomach	Liver	Kidney	Bladder	Lung	Brain	Bone marrow
0	Mean	10.3	1.81	2.66	8.93	3.12	1.40	1.16
	SEM	0.88	0.50	0.29	0.75	0.17	0.48	0.76
3	Mean	28.2**	9.84***	8.62*	12.2	13.5***	2.66	0.70
	SEM	3.79	1.16	1.53	1.34	0.61	0.63	0.42
24	Mean	28.0**	8.36**	7.19	21.3**	6.73	11.8**	1.58
	SEM	3.51	1.05	1.48	3.02	1.54	2.12	0.24

Source: Sasaki *et al.* (1998)
SEM: Standard error of the mean. * $P < 0.05$. **$P < 0.01$. *** $P < 0.001$ (Dunnett test)

5.4 Summary

Like its structural analog VC, VB is mutagenic in *S. typhimurium* strains TA1530 and TA100 with or without metabolic activation. Exposure of *S. typhimurium* strains TA1530, TA1535, and G-46 to VB increased the number of histidine revertants/plate at rates 16, 12, or 5 times, respectively, the spontaneous mutation rate. The mutagenic response for strain TA1530 increased with metabolic activation by S9 liver fractions from rats or mice. Like VC, VB is clearly genotoxic (recombinagenic) to *D. melanogaster* in the *in vivo* w/w^+ eye mosaic assay. Both VC and VB are efficient clastogenic agents in *Drosophila* germ cells. VB and VC both can produce DNA and RNA adducts that are likely formed by their respective epoxide rearrangement products, bromoacetaldehyde and chloroacetaldehyde (as described in Section 6).

6 Other Relevant Data

6.1 Absorption and metabolism of VB

6.1.1 Absorption

VB is readily absorbed from the lungs of rats (Filser and Bolt 1979, 1981, Gargas and Andersen 1982, all cited in IARC 1986), and at equilibrium with inspired air, it exhibits an 11-fold bioaccumulation. Early evidence on VB metabolism indicated dose-related increases in plasma levels of nonvolatile bromide. Pretreatment of rats with phenobarbital accelerated the release of bromide from inhaled VB, suggesting a role for the cytochrome P-450 system (Van Stee *et al*. 1977, cited in IARC 1986).

6.1.2 Metabolism

The metabolic pathway in rats is saturable at inhalation exposure concentrations in excess of 250 mg/m^3 (55 ppm); however, the duration of exposure required for saturation was not reported (Van Stee *et al.* 1977, cited in IARC 1986). VB metabolism probably proceeds through epoxidation, with subsequent conjugation to macromolecules and other biologic compounds, similar to that seen for VC (Clayton and Clayton 1982).

The incidence of hepatic hemangiosarcoma in rats exposed to VB in air at a concentration of 10 ppm is 10%, compared with 1% in rats exposed to VC at 10 ppm (Maltoni *et al.* 1981). At a concentration of 50 ppm, the incidence of hemangiosarcoma is 36% in VB-exposed rats, compared with 7% in VC-exposed rats. The greater potency of VB in inducing hepatic hemangiosarcoma may be related to kinetic differences in the metabolism of the two compounds. The K_m for metabolism of VB is approximately an order of magnitude lower than that for VC (Bolt *et al.* 1982). Thus, VB may be metabolized to carcinogenic intermediates at a faster rate than VC.

The reactive metabolites of VB are produced in the hepatic microsomal fraction. When a mixture of VB and air was passed through a mouse liver microsomal system, a volatile alkylating metabolite was detected by trapping with 4-(4-nitrobenzyl)pyridine (Barbin *et al*. 1975; Bartsch *et al.* 1976, 1979, all cited in IARC 1986). VB incubated with liver microsomes from phenobarbital-treated rats alkylates the prosthetic group (heme) of cytochrome P-450. This alkylated moiety is the dimethyl ester of *N*-(2-oxoethyl)protoporphyrin IX (IARC 1986). A comparative study with isolated rat hepatocytes and hepatic sinusoidal cells revealed that metabolism of VB to reactive metabolites was confined primarily to hepatocytes (Ottenwalder and Bolt 1980).

The biotransformation of VB is similar to that observed for VC (IARC 1979; Guengerich *et al*. 1981, cited in Bolt 1988) and is summarized in Figure 6-1.

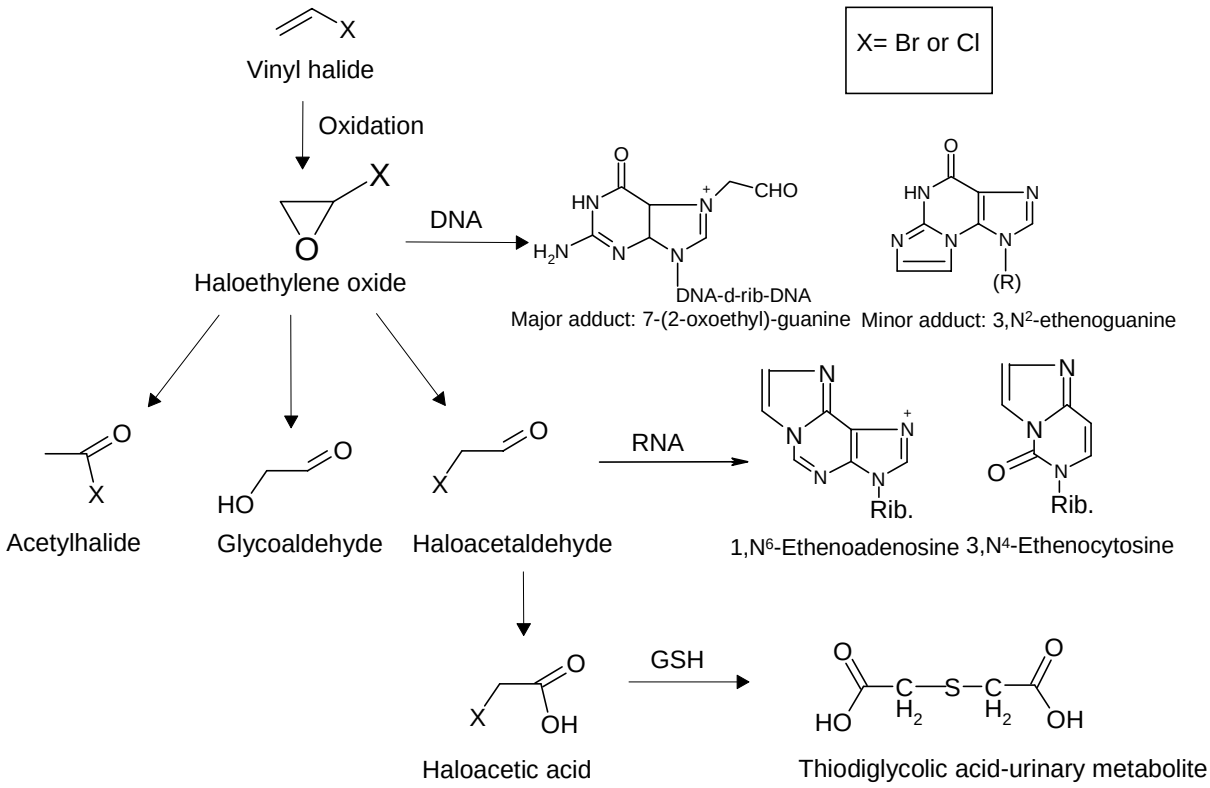

Figure 6-1. Proposed metabolic pathway of VB

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

VB is initially oxidized by microsomal monooxygenase(s) to bromoethylene oxide (bromooxirane). The resulting bromooxirane is highly reactive and probably can bind to nucleic acids (Amdur *et al*. 1991). Bromoethylene oxide can be deactivated by epoxide hydrolase or by gluthione (GSH) transferase. It also can rearrange to bromoacetaldehyde, which in turn is oxidized to bromoacetic acid, with a subsequent secondary metabolism after reaction with GSH.

6.2 Alkylating properties and DNA binding

After *in vitro* incubation of DNA with VB, alkylation following VB metabolism is caused largely by an epoxide intermediate similar to that observed for VC (Guengerich *et al*. 1981, cited by Bolt *et al.* 1986). This mechanism was confirmed for VC by the observation that alkylation of guanine in DNA (at 7-N) occurred after exposure of rats to VC, but not after exposure to 2,2'-dichlorodiethyl ether, a metabolic precursor of chloroacetaldehyde (Gwinner *et al.* 1983, cited in Bolt 1988). Thus, under conditions of *in vivo* exposure to VB (or VC), bromoethylene oxide (or chloroethylene oxide) appears to be the primary DNA-reactive intermediate.

The metabolism of ^{14}C-labeled VB in rat liver microsomes, reconstituted cytochrome P-450 systems, and isolated hepatocytes leads to products that bind irreversibly to DNA and protein. A role for cytochrome P-450 was confirmed in inhibition and reconstitution experiments. The major form of cytochrome P-450 involved in VB metabolism is not either of the major isozymes induced by phenobarbital or beta-napthoflavone. 2-Bromoethylene oxide and 2-

bromoacetaldehyde were found to be the substrates for rat liver epoxide hydrolase and equine liver alcohol dehydrogenase, respectively. Alcohol dehydrogenase was more effective than epoxide hydrolase in inhibiting the binding of VB metabolites to protein in microsomal incubations. Epoxide hydrolase was more effective than alcohol dehydrogenase in inhibiting the binding of VB metabolites to calf thymus DNA. Similar results were observed for VB metabolites binding to DNA in a reconstituted enzyme system. Reduced glutathione blocked nonenzymatic binding of 2-bromo(1,2- [^{14}C])acetaldehyde to protein, but not DNA. Studies with isolated rat hepatocytes suggest that a significant portion of the total reactive metabolites can be released by these cells. In these systems, binding of metabolites of VB to DNA outside the hepatocytes could be partially blocked by epoxide hydrolase or by alcohol dehydrogenase. This implies that as targets farther away from sources of reactive species are considered, the stabilities of these species become important for reaction with nucleophilic sites (Guengerich *et al*. 1981, cited in Bolt 1988; Guengerich *et al*. 1991).

The role of human cytochrome P-450 IIE1 in the oxidation of a number of suspect carcinogens was examined by Guengerich *et al*. (1991). The results indicated that the P-450 IIE1 is a major catalyst for the oxidation of both VB and VC

The metabolic activation and macromolecular binding of VB is similar to that of VC (Barbin *et al*. 1975; Ottenwalder *et al*. 1979; Guengerich *et al*. 1981, all cited in Bolt *et al*. 1986; Bonse and Henschler 1976; Bolt *et al.* 1978; Bartsch *et al*. 1979; Bolt *et al*. 1981). Chloroethylene and bromoethylene oxides bind mostly to the N-7 site of deoxyguanosine. Bromoacetaldehyde binds to RNA to form 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine metabolites. These metabolites also are capable of alkylating nonspecific proteins, preferably those containing free sulfhydryls (Swenberg *et al*. 1992).

When rat liver microsomes, a NADPH-regenerating system, DNA, and ^{14}C-labeled VC are incubated, 1,N^6-ethenodeoxyadenosine, 3,N^4-ethenodeoxycytidine, and 7-(2-oxoethyl)guanine, (the product of the hydrolysis of 7-(2-oxoethyl)deoxyguanosine), are formed. These cyclic DNA adducts, as well as N^2,3-ethenoguanine, were detected in liver, lung, and kidney of rats exposed to VC (Swenberg *et al.* 1992).

Laib and Bolt (1977, cited in Bolt 1988) described the formation of labeled 1,N^6-ethenoadenosine in hepatic RNA of rats dosed with ^{14}C-labeled VC. Similar results were seen in *in vitro* experiments wherein RNA and rat liver microsomes were incubated together with the substrate. Later, 3,N^4-ethenocytidine was identified in RNA hydrolysates under similar conditions (Laib and Bolt 1978). Ottenwalder *et al*. (1979) studied RNA alkylation after administration of ^{14}C-labeled VB to rats and observed results similar to those for VC. 1,N^6-Ethenoadenosine and 3,N^4-ethenocytidine were detected in hepatic RNA of exposed rats. Adducts formed by vinyl halide metabolites, as demonstrated in studies of RNA alkylation by VB metabolites and by analogy with VC, are illustrated in Figure 6-1.

Guengerich *et al*. (1981, cited in Bolt *et al.* 1986) advanced the hypothesis, based on *in vitro* experimentation, that the product of epoxidation of VB, bromoethylene oxide, is the major alkylating agent at the DNA level. In addition, bromoacetaldehyde (the rearrangement product of bromoethylene oxide) has the potential to bind covalently to proteins.

6.3 Structure-activity relationship

The metabolism of VB probably proceeds through the same pathway as that of VC (*known to be a human carcinogen*) and the *probable human carcinogen*, vinyl fluoride (VF). VB is less rapidly metabolized in rats and mice than VF and VC (Bolt *et al*. 1982). The metabolic process appears to be saturable, as observed for VC (Bolt *et al.* 1981).

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

Table 6-1. Summary of carcinogenicity, mutagenicity, and pharmacokinetics of VF, VB, and VC

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

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

—, Not reported; na, Not available.
[a]IARC 1986
[b]Bogdanffy *et al.* 1995, IARC 1995
[c]IARC 1979, NTP 1998
[d]IARC 1979
[e]Bogdanffy *et al.* 1995
[f]Bolt *et al.* 1979
[g]Bolt *et al.* 1981
[h]Laib *et al.* 1985
[i]IARC 1986, Roldan-Arjona *et al.* 1991
[j]Dupont de Nemours 1992a
[k]Vogel and Nivard 1993, Ballering *et al*. 1996.
[l]CMA 1988, IARC 1995
[m]Dupont de Nemours 1992b, IARC 1995
[n]Dupont de Nemours 1987, IARC 1995
[o]Richardson *et al.* 1983
[p]Cantonreggi and Keller 1997
[q]el Ghisassi *et al.* 1998
[r]Dilley *et al.* 1974
[s]Filser *et al.* 1982
[t]Bolt 1988
[u]Swenberg *et al.* 1995
[v]Swenberg *et al.* 1992

6.4 Summary

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

7 References

1. Amdur, M.O., J.Doull, and C.D. Klaasen (eds.). (1991). *Casarett and Doull's Toxicology*. Pergamon Press, 695P
2. Bales, R. E. (1978). *Vinyl Fluoride and Vinyl Bromide Industrial Hygiene Survey Report (DHEW) (NIOSH)*. Pub. No. 79-111; U.S. NTIS PS80-190150, 79-111 Cincinnati, OH, National Institute for Occupational Safety and Health.
3. Ballering, L.A., M.J. Nivard, and E.W. Vogel. (1996). Characterization by two-endpoint comparisons of the genetic toxicity profiles of vinyl chloride and related etheno-adduct forming carcinogens in Drosophila. *Carcinogenesis* 17:1083-1092.
4. Ballering, L.A.P., M.J.M. Nivard, and E.W. Vogel. (1997). Preferential formation of deletions following in vivo exposure of postmeiotic Drosophila germ cells to the DNA etheno-adduct- forming carcinogen vinyl carbamate. *Environ Mol Mutagen* 30:321-329.
5. Barbin, A., H. Bresil, A. Croisy, P. Jacquignon, C. Malaveille, R. Montesano, and H. Bartsch. (1975). Liver-microsome-mediated formation of alkylating agents from vinyl bromide and vinyl chloride. *Biochem Biophys Res Commun* 67:596-603.
6. Bartsch, H. (1976). Predictive Value of Mutagenicity Tests in Chemical Carcinogenesis. *Mutat Res* 3:177-190.
7. Bartsch, H., C. Malaveille, A. Barbin, and G. Planche. (1976). Alkylating and mutagenic metabolites of halogenated olefins produced by human and animal tissues (Abstract No. 67). *Proc Am Assoc Cancer Res* 17:17.
8. Bartsch, H., C. Malaveille, A. Barbin, and G. Planche. (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues. Evidence for oxirane formation by P450-linked microsomal mono-oxygenasesz. *Arch Toxicol* 41:249-277.
9. Benya, T.J., W.M. Busey, M.A. Dorato, and P.E. Berteau. (1982). Inhalation carcinogenicity bioassay of vinyl bromide in rats. *Toxicol Appl Pharmacol* 64:367-379.
10. Bogdanffy, M.S., G.T. Makovec, and S.R. Frame. (1995). Inhalation oncogenicity bioassay in rats and mice with vinyl fluoride. *Fundam Appl Toxicol* 26:223-238.
11. Bolt, H.M., J.G. Filser, and R.K. Hinderer. (1978). Rat liver microsomal uptake and irreversible protein binding of [1,2-^{14}C]-vinyl bromide. *Toxicol Appl Pharmacol* 44:481-489.
12. Bolt, H.M., R.J. Laib, and G. Stockle. (1979). Formation of pre-neoplastic hepatocellular foci by vinyl bromide in newborn rats. *Arch Toxicol* 43(1):83-84.
13. Bolt, H.M., J.G. Filser, and R.J. Laib. (1981). Covalent binding of haloethylenes. *Adv Exp Med Biol* 136:667-683.
14. Bolt, H.M., R.J. Laib, and J.G. Filser. (1982). Reactive metabolites and carcinogenicity of halogenated ethylenes. *Biochem Pharmacol* 31:1-4.

15. Bolt, H.M., R.J. Laib, H. Peter, and H. Ottenwalder. (1986). DNA adducts of halogenated hydrocarbons. *J Cancer Res Clin Oncol* 112:92-96.

16. Bolt, H.M. (1988). Roles of etheno-DNA adducts in tumorigenicity of olefins. *Crit Rev Toxicol* 18:299-309.

17. Bonse, G. and D. Henschler. (1976). Chemical reactivity, biotransformation, and toxicity of polychlorinated aliphatic compounds. *CRC Crit Rev Toxicol* 4:395-409.

18. Budavari, S., M.J. O'Neil, A. Smith, and P.E. Heckelman. (1996). *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*. Merck & Co., Inc., Whitehall, NJ.

19. Cantoreggi, S. and D.A. Keller. (1997). Pharmacokinetics and metabolism of vinyl fluoride in vivo and in vitro. *Toxicol Appl Pharmacol* 143:130-139.

20. Chemfinder. (1999). http://www.chemfinder.camsoft.com/ (Cas Registry Number 593-60-2). Cambridge Soft Corporation.

21. Clayton, G.D. and F.E. Clayton (eds.). (1982). *Patty's Industrial Hygiene and Toxicology*. In: *Toxicology* (Clayton, G. D. and F. E. Clayton, eds.) John Wiley Sons, 3542

22. CMA. (1988). Mutagenicity test on vinyl fluoride: Drosophila melanogaster sex-linked recessive lethal test (Final Report) with attachments and cover letter dated 8/15/88. NTIS/OTS0522809 Washington, D.C., U.S. EPA/ Office of Toxic Substances.

23. CRC. (1998). *CRC Handbook of Chemistry and Physics*. (Weast, R. C. and Astle, M. J., eds.) CRC Press, Inc., Boca Raton, FL.

24. Dilley, J.V., V.L.J. Carter, and E.S. Harris. (1974). Fluoride ion excretion by male rats after inhalation of one of several fluoroethylenes or hexafluoropropene. *Toxicol Appl Pharmacol* 27:582-590.

25. Dupont de Nemours and Co. (1987). *Mouse bone marrow micronucleus assay of vinyl fluoride*. U.S. EPA-OTS Document Id. No.87- 0515661 Washington, D.C., U.S. EPA/ Office of Toxic Substances.

26. Dupont de Nemours and Co. (1992a). Mutagenic activity of fluoroethylene in the Salmonella/Microsome Assay. U.S. EPA-OTS Document Id. No. 88-920002842 Washington, D.C., U.S. EPA/ Office of Toxic Substances.

27. Dupont de Nemours and Co. (1992b). Mutagenicity evaluation of vinyl fluoride in the CHO/prt assay. U.S. EPA-OTS Document Id. No. 88-920002841 Washington, D.C., U.S. EPA/ Office of Toxic Substances.

28. el Ghissassi, F., A. Barbin, and H. Bartsch. (1998). Metabolic activation of vinyl chloride by rat liver microsomes: low- dose kinetics and involvement of cytochrome P450 2E1. *Biochem Pharmacol* 55:1445-1452.

29. Filser, J.G. and H.M. Bolt. (1979). Pharmacokinetics of halogenated ethylenes in rats. *Arch Toxicol* 42:123-136.

30. Filser, J.G. and H.M. Bolt. (1980). Characteristics of haloethylene-induced acetonemia in rats. *Arch Toxicol* 45:109-116.

31. Filser, J.G. and H.M. Bolt. (1981). Inhalation pharmacokinetics based on gas uptake studies: 1. Improvement of kinetic models. *Arch Toxicol* 47:279-292.

32. Filser, J.G., P. Jung, and H.M. Bolt. (1982). Increased acetone exhalation induced by metabolites of halogenated C1 and C2 compounds. *Arch Toxicol* 49:107-116.

33. Gargas, M.L. and M.E. Andersen. (1982). Metabolism of inhaled brominated hydrocarbons: validation of gas uptake results by determination of a stable metabolite. *Toxicol Appl Pharmacol* 66:55-68.

34. Guengerich, F.P., P.S. Mason, W.T. Stott, T.R. Fox, and P.G. Watanabe. (1981). Roles of 2-haloethylene oxides and 2-haloacetaldehydes derived from vinyl bromide and vinyl chloride in irreversible binding to protein and DNA. *Cancer Res* 41:4391-4398.

35. Guengerich, F.P., D.H. Kim, and M. Iwasaki. (1991). Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* 4:168-179.

36. Gwinner, L.M., R.J. Laib, J.G. Filser, and H.M. Bolt. (1983). Evidence of chloroethylene oxide being the reactive metabolite of vinyl chloride towards DNA: comparative studies with 2,2'-dichlorodiethylether. *Carcinogenesis* 4:1483-1486.

37. Hera, C. and C. Pueyo. (1986). Conditions for the optimal use of the L-arabinose-resistance mutagenesis test with Salmonella typhimurium. *Mutagenesis* 1:267-273.

38. HSDB. (1996). Hazardous Substances Data Bank -- CAS# 593-60-2. MEDLARS Online Information Retrieval System, National Library of Medicine.

39. IARC. (1979). *Some Monomers, Plastics and Synthetic Elastomers, and Acrolein.* IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 19, pp. 367-755. Lyon, France, World Health Organization.

40. IARC. (1986). *Some Chemicals Used in Plastics and Elastomers*. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 39, pp. 133-145. Lyon, France, World Health Organization.

41. IARC. (1987). *Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42*. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Suppl 7, pp. 1-440. Lyon, France, World Health Organization.

42. IARC. (1995). *Dry cleaning, Some Chlorinated Solvents and Other Industrial Chemicals.* IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans. Vol. 63, pp. 467-475. Lyon, France, World Health Organization.

43. IARC. (1999). *Re-evaluation of Some Organic Chemicals, hydrazine, and Hydrogen Peroxide*. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans. Vol. 71, pp. 923-928. Lyon, France, World Health Organization.

44. Krost, K.J., E.D. Pellizzari, S.G. Walburn, and S.A. Hubbard. (1982). Collection and analysis of hazardous organic emissions. *Anal Chem* 54:810-817.

45. Laib, R.J. and H.M. Bolt. (1977). Alkylation of RNA by vinyl chloride metabolites in vitro and in vivo: formation of 1-N(6)-etheno-adenosine. *Toxicology* 8:185-195.

46. Laib, R.J. and H.M. Bolt. (1978). Formation of 3,N4-ethenocytidine moieties in RNA by vinyl chloride metabolities in vitro and in vivo. *Arch Toxicol* 39:235-240.

47. Laib, R.J., L.M. Gwinner, and H.M. Bolt. (1981). DNA alkylation by vinyl chloride metabolites: etheno-derivatives or 7-alkylation of guanine? *Chem-Biol Interact* 37:219.

48. Laib, R.J., K.P. Klein, and H.M. Bolt. (1985). The rat liver foci bioassay: I. Age-dependence of induction by vinyl chloride of ATPase-deficient foci. *Carcinogenesis* 6:65-68.

49. Lijinsky, W. and A.W. Andrews. (1980). Mutagenicity of vinyl compounds in Salmonella typhimurium. *Teratog Carcinog Mutagen* 1:259-267.

50. Maltoni, C., G. Lefemine, A. Ciliberti, G. Cotti, and D. Carretti. (1981). Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis. *Environ Health Perspect* 41:3-29:3-29.

51. NIOSH. (1978). Current 28. Joint NIOSH/OSHA. Vinyl Halides - Carcinogenicity. http://www.cdc.gov/niosh/79102_28.html .

52. NIOSH. (1990). National Occupational Exposure Survey (NOES) (1981-1983), unpublished provisional data as of July 1, 1990. Cincinnati, OH. National Institute for Occupational Safety and Health.

53. NIOSH. (1994). *NIOSH pocket guide to chemical hazards.* DHHS (NIOSH) publication # 94-116, U.S. Government Printing Office.

54. NTP. (1998). *Eighth Report on Carcinogens (Summary).* Vinyl Chloride (CAS No. 75-01-4). http://ntp-server.niehs.nih.gov/htdocs/8_RoC/KC/VinylChloride.html , National Toxicology Program.

55. Oser, J.L. (1980). Extent of industrial exposure to epichlorohydrin, vinyl fluoride, vinyl bromide and ethylene dibromide. *Am Ind Hyg Assoc J* 41:463-468.

56. Ottenwalder, H., R.J. Laib, and H.M. Bolt. (1979). Alkylation of RNA by vinyl bromide metabolites in vitro and in vivo. *Arch Toxicol* 41:279-286.

57. Ottenwalder, H. and H.M. Bolt. (1980). Metabolic activation of vinyl chloride and vinyl bromide by isolated hepatocytes and hepatic sinusoidal cells. *J Environ Pathol Toxicol* 4:411-417.

58. Pellizzari, E., Zweidinger, R. A., and Erickson, M. D. (1978). *Environmental Monitoring Near Industrial sites: Brominated Chemicals, Part II: Appendix*. Research Triangle Institute. EPA-560/6-78-002A; U.S. NTIS PB-286483 Research Triangle Park, NC, U.S. Environmental Protection Agency.

59. Physchem. (1999). http://www.Physchem.ox.ac.uk/msds/v/vinyl_bromide.html.

60. Richardson, C.R., J.A. Styles, and I.P. Bennett. (1983). Activity of vinyl chloride monomer in the mouse micronucleus assay. *Mutat Res* 122:139-142.

61. Rodriguez-Arnaiz, R., E.W. Vogel, and A. Szakmary. (1993). Strong intra-species variability in the metabolic conversion of six procarcinogens to somatic cell recombinagens in Drosophila. *Mutagenesis* 8(6):543-551.

62. Roldan-Arjona, T., P.M. Garcia, R.F. Luque, C. Hera, and C. Pueyo. (1991). An association between mutagenicity of the Ara test of Salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons. *Mutagenesis* 6:199-205.

63. Sasaki, Y.F., A. Saga, M. Akasaka, S. Ishibashi, K. Yoshida, Y.Q. Su, N. Matsusaka, and S. Tsuda. (1998). Detection of in vivo genotoxicity of haloalkanes and haloalkenes carcinogenic to rodents by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs. *Mutat Res* 419:13-20.

64. Spafford, R. B. and Dillon, H. K. (1981). *Analytical Methods Evaluation and Validation for Vinylidene Fluoride, Vinyl Bromide, Vinyl Fluoride, Benzenethiol, and n-Octanethiol: Research Report for Vinyl Bromide*. Southern Research Institute. U.S. NTIS PB83-133447 Birmingham, AL, National Institute of Occupational Safety and Health.

65. Swenberg, J.A., N. Fedtke, F. Ciroussel, A. Barbin, and H. Bartsch. (1992). Etheno-adducts formed in DNA of vinyl chloride-exposed rats are highly persistent in liver. *Carcinogenesis* 13(4):727-729.

66. Swenberg, J.A., D.K. La, N.A. Scheller, and K.-Y. Wu. (1995). Dose-Response Relationships for Carcinogens. *Toxicol Lett* 82/83:751-756.

67. Taylor, D.G. (1981). *NIOSH Manual of Analytical Methods* National Institute for Occupational Safety and Health, Cincinnati, OH.

68. TRI. (1996). *Vinyl Bromide (CAS# 593-60-2) Toxic Release Inventory Database*. http://toxnet.nlm.nih.gov/servlets/simple-search?1.15.0.2257 (& type CAS# 593-60-2) , U.S. EPA.

69. U.S. EPA. (1986). http:\\man.odsnet.com/TRIFACTS/166.html .

70. U.S. EPA. (1994). Vinyl bromide. http://www.epa.gov/opptintr/chemrtk/opptsrch.htm Washington, DC., U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics.

71. Van Duuren, B.L. (1977). Chemical structure, reactivity, and carcinogenicity of halohydrocarbons. *Environ Health Perspect* 21:17-23.

72. VanStee, E.W., J.M. Patel, B.N. Gupta, and R.T. Drew. (1977). Consequences of vinyl bromide debromination in the rat. *Toxicol Appl Pharmacol* (Abstract).

73. Vogel, E.W. and M.J. Nivard. (1993). Performance of 181 chemicals in a Drosophila assay predominantly monitoring interchromosomal mitotic recombination. *Mutagenesis* 8:57-81.

Appendix A: IARC. (1979). *Some Monomers, Plastics and Synthetic Elastomers, and Acrolein. (Vinyl Bromide).* Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 19. Lyon, France. World Health Organization. pp. 367-375.

Appendix B: IARC. (1986). *Some Chemicals Used in Plastics and Elastomers.* Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Vol 39. Lyon, France. World Health Organization. pp. 133-145.

Appendix C: IARC. (1987). *Overall Evaluation of Carcinogenicity: An Updating of IARC Monographs. Volumes 1 to 42.* Monographs on the Evaluation of the Carcinogenic Risk of Chemical to Humans. Suppl 7. Lyon, France. World Health Organization. p. 73.

Appendix D: IARC. (1999). *Re-evaluation of Some Organic Chemicals, Hydrazine, and Hydrogen Peroxide.* Monographs on the Evaluation of Carcinogenic Risk to Humans. Vol. 71. Lyon, France. World Health Organization. pp. D-1 – D-6.

Appendix E: IARC. (1979). *Some Monomers, Plastics and Synthetic Elastomers, and Acrolein. (Vinyl Chloride).* Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Vol. 19. Lyon, France. World Health Organization. pp 377-438.

Appendix F: IARC. (1987). *Overall Evaluations of carcinogenicity: An Updating of the IARC Monographs. Volumes 1 to 42.* Monographs on the Evaluation of the Carcinogenicity. Suppl. 7. Lyon, France. World Health Organization. pp. 373-376.