Toxicology of trans-1,4-Dichloro-2-butene
Review of Literature

Prepared for

Errol Zeiger, Ph.D.
National Institute of Environmental Health Sciences
Post Office Box 12233
Research Triangle Park, North Carolina 27709
Contract No. N01-ES-65402
ILS Project No. L082

Submitted by

Raymond Tice, Ph.D.
Integrated Laboratory Systems
Post Office Box 13501
Research Triangle Park, North Carolina 27709

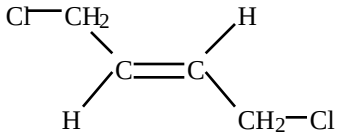
January 14, 1997

TABLE OF CONTENTS

1.0 INTRODUCTION

1.1 Chemical Identification

trans-1,4-Dichloro-2-butene



trans-1,4-Dichloro-2-butene ($C_4H_6Cl_2$, CASRN 110-57-6, mol. wt. = 125.00) is also called:

2-Butene, 1,4-dichloro-, (*E*)- (8CI9CI)

2-Butene, 1,4-dichloro-, *trans*-

2-Butylene dichloride

1,4-Dichloro-2-butene

1,4-Dichlorobutene-2, (*trans*)-

1,4-Dichloro-*trans*-2-butene

trans-1,4-Dichlorobutene

1.2 Physical-Chemical Properties

Property	Information	Reference
Melting Point, °C	1-3	Weast and Astle (1980)
Boiling Point, °C	155.5	Weast and Astle (1980)
Density at 20 °/4°C	1.183	Weast and Astle (1980)

Solubility:		
Organic Solvents	Soluble in: ethanol, diethyl ether, acetone, and benzene	Weast and Astle (1980)

2.0 PRODUCTION PROCESS ANALYSIS

Butadiene can be converted to *trans*-1,4-dichloro-2-butene (DCB) via a four-step process. In step one, a mixture of DCB and 3,4-dichloro-1-butene is produced as a result of vapor-phase chlorination of butadiene (SRI Int., 1996). An equilibrium results when the mixture is heated. The residue resulting from removal of 3,4-dichloro-1-butene, which has a lower boiling point, is normally used without further separation (IARC, 1977).

3.0 PRODUCTION AND IMPORT VOLUMES

Commercial production of DCB in the U.S. was not begun until 1963 (U.S. Tariff Commission, 1964; cited by IARC, 1977).

In the US, DCB has been used primarily as an intermediate in the manufacture of hexamethylenediamine and chloroprene. While U.S. production of hexamethylenediamine and polychloroprene rubber (neoprene) (the end-product from chloroprene) in 1975 was 340,000 and 143,900 metric tons (Mg), respectively, the percent originally derived from DCB was not known (Fishbein, 1979).

Two U.S. companies were reported by IARC (1977) as Acurrent@ manufacturers of DCB. However, for 1977, E.I. DuPont de Nemours & Co. was the only manufacturer of DCB which reported production levels (between 100 to 500 million pounds per year; 45,000 to 226,800 Mg/year) to the U.S. EPA (TSCAPP, 1983). More recent data are not available.

Import data were not located.

4.0 USES

In the U.S., DCB has been used as an intermediate in the manufacture of hexamethylenediamine and chloroprene since 1951 (U.S. Tariff Commission, 1964; cited by IARC, 1977). Hexamethylenediamine is used as a chemical intermediate in the production of nylon 66 and 612 polyamide resins; chloroprene is used in the production of polychloroprene (neoprene) rubber (Fishbein, 1979).

DCB is also used as a U.S. EPA RCRA Appendix VIII supplementary analytical standard (Tomkins et al., 1989).

5.0 ENVIRONMENTAL OCCURRENCE

5.1 Occurrence

DCB is not known to occur naturally (IARC, 1977).

5.2 Persistence

Hermens et al. (1985) calculated a hydrolysis rate constant (k) for DCB in a 1:1 acetone-water mixture using the equation

$$k = -1/t \ln(1-(H_3O^+)_t/(H_3O^+)_4)$$

where $(H_3O^+)_t$ is the concentration of liberated (H_3O^+) at time t, and $(H_3O^+)_4$ is the concentration of liberated (H_3O^+) after complete hydrolysis. With a reaction time t of 5 days, log k = - 5.09 (min^{-1}), while with a reaction time of 10 days, log k =

-5.01 (min^{-1}). Based on these rate constants, the half-life ($t_{1/2}$) of DCB in the acetone-water mixture was calculated to be 13 hours ($t_{1/2} = 1.155 \times 10^{-2}/k$).

6.0 HUMAN EXPOSURE

Occupational exposure to DCB may occur during its production or during its use as an intermediate in the manufacture of hexamethylenediamine and chloroprene (Fishbein, 1979). RTECS (1996) did not report on any occupational surveys conducted by the National Institute for Occupational Safety and Health (NIOSH).

No data on non-occupational exposures were found.

7.0 REGULATORY STATUS

REGULATIONS

EPA Regulatory Action	Effect of Regulation/Other Comments
40 CFR 60CStandards of Performance for New Stationary Sources; Volatile Organic Compound (VOC) Emissions From the Synthetic Organic Chemical Manufacturing Industry (SOCMI). Subpart NNNC Distillation Operations. Subpart RRRCReactor Processes.	These standards implement section 111 of the Clean Air Act (CAA), and requires all new, modified, and reconstructed SOCMI distillation and reactor process facilities to achieve an emission reduction that reflects the capabilities of the best demonstrated system of continuous emission reduction, considering costs, nonair quality health and environmental impacts and energy requirements. The chemicals (including DCB) affected for distillation operations are listed in 60.667 and those affected for reactor processes are listed in 60.707.
40 CFR 63CNational Emission Standards for Hazardous Air Pollutants for Source Categories.	Plants manufacturing DCB as a primary product whose emissions contain at least 10 tons per yr, or DCB in combination with another listed compound in amounts of at least 25 tons/yr must report their annual emissions to EPA. DCB is listed in Table 1 to Subpart FCSynthetic Organic Chemical Manufacturing Industry Chemicals.
40 CFR 258CCriteria for Municipal Solid Waste Landfills (MSLF)	Section 54 of CFR 258 requires detection monitoring at MSLF units for DCB.
40 CFR 264CRCRA. Appendix IXC Ground water monitoring list.	Lists DCB as one of the chemical substances for which suitable analytical methods are available for monitoring groundwater contamination at hazardous waste sites.
40 CFR 268CRCRA. Appendix III	Lists chemical substances, including DCB, regulated under 40 CFR 268.32 (land disposal prohibition; California list).

8.0 TOXICOLOGICAL DATA

Summary: The metabolic fate of DCB is not known. It may be metabolized to an epoxide which would structurally be a $-chloro ether. In rats, the oral LD_{50} is 89 mg/kg body weight (710 :mol/kg bw) while the 30-minute inhalation LC_{50} is 784 ppm (4000 mg/m^3; 32100 Fmol/m^3). In rats exposed to 62 ppm (320 mg/m^3; 2500 Fmol/m^3) DCB by inhalation for 4 hours, mortality occurred in 2/6 animals after 14 days. No short-term, chronic, or reproductive studies are available.

In a carcinogenicity study, DCB was evaluated for its potential to induce tumors in female ICR/Ha Swiss mice when administered by skin painting, or by intraperitoneal (i.p.) or subcutaneous (s.c.) injection. No tumors were observed when DCB was administered at 1.0 mg (8.0 :mol) in 0.1 mL acetone to the shaved dorsal skin, 3 times per week for 77 weeks. The tumor response was also not significantly increased in these mice when DCB was injected i.p. at 0.05 mg (0.4 :mol) in 0.05 mL tricaprylin once per week for 77 weeks. However, when administered by s.c. injection once per week for 77 weeks, this dose induced a significant increase in the incidence of sarcomas at the site of injection. In a 2-stage carcinogenicity bioassay, DCB was negative as an initiator when administered once at 1 mg (8 :mol) in 0.1 mL acetone to the shaved skin of 6- to 8-week-old female ICR/Ha Swiss mice skin, followed 14 days later with phorbol myristate acetate (2.5 :g/0.1 mL acetone) applied 3 times per week for 77 weeks.

DCB has been evaluated for genotoxicity in only a limited number of prokaryotic test systems. DCB was reported to induce gene mutations in *Salmonella typhimurium* in the absence or presence of active rat and human liver S9, and in *Escherichia coli* in the presence or absence of rat liver metabolic activation.

8.1 Human Data

No human data were found.

8.2 General Toxicology

8.2.1 Chemical Disposition, Metabolism, and Toxicokinetics

DCB may be metabolized to an epoxide which would structurally be a $-chloro ether (Van Duuren et al., 1975).

8.2.2 Acute Exposures

The studies described in this section are presented in **Table 8-1**.

8.2.2.1 Oral Administration

The oral LD_{50} of DCB in rats (age, strain, and sex not provided) was reported to be 89 mg/kg body weight (710 :mol/kg bw) (Smith et al., 1951; cited by IARC, 1977).

8.2.2.2 Inhalation Exposure

In rats (age, strain, and sex not specified) administration of 62 ppm (weight/vol)(320 mg/m^3; 2500 Fmol/m^3) DCB by inhalation for 4 hours caused mortality in 2/6 animals after 14 days (Smith et al., 1951; cited by IARC, 1977).

In an 8(e) submission by DuPont Chemical Co. (1974) for the Toxic Substances Control Act (TSCA), the acute inhalation toxicity of DCB in rats was evaluated. Young adult ChR-CD male rats (252-277 g)(6 per dose group) were exposed to DCB (1.43% *cis*-isomer; 97.17% *trans*-isomer) at 240, 370, 410, 440, 540, 760, or 3600 ppm (vol/vol) (1200 to 18000 mg/m^3; 9800 to 150000 Fmol/m^3) for 30 minutes. Gross and histopathologic examinations were performed on 2 rats per dose group at 7 days post-exposure (exposure information not provided) and on 1 rat exposed to 760 ppm and found dead at 13 days post-exposure. All other animals were observed for 14 days post-exposure.

The 30-minute LC_{50} was 784 ppm (4000 mg/m^3; 32100 Fmol/m^3). With the 760 ppm-dose (3900 mg/m^3; 31000 Fmol/m^3), destruction of the air passage,

kidney damage, testicular atrophy, and hypoplastic bone marrow occurred. The latter two changes were interpreted as a reflection of stress and emaciation and were considered not to be compound-related. Examination of other tissues (lymph nodes, stomach, duodenum, epididymis, thyroid, adrenal glands, brain, and eyes) revealed no compound-related effects. Exposure of rats to 410 ppm (2100 mg/m^3; 17000 Fmol/m^3) resulted in damage to the tracheobronchial epithelium.

8.2.3 Short-term and Subchronic Exposures

No data were found.

8.2.4 Chronic Exposures

No data were found.

8.2.5 Reproductive Effects

No data were found.

8.2.6 Carcinogenicity

The studies described in this section are presented in **Table 8-2**.

8.2.6.1 Dermal Application

No tumors were detected in female ICR/Ha Swiss mice administered 1.0 mg (8.0 :mol) DCB in 0.1 mL acetone on the shaved dorsal skin, 3 times per week for 77 weeks, beginning at 6 to 8 weeks of age (Van Duuren et al., 1975).

8.2.6.2 Subcutaneous Injection

There was a significant increase in the incidence of sarcomas at the site of injection when DCB at 0.05 mg (0.4 :mol) in 0.05 mL tricaprylin was administered once per week for 77 weeks into the left flank of female ICR/Ha Swiss mice beginning at 6 to 8 weeks of age (Van Duuren et al., 1975).

8.2.6.3 Intraperitoneal Injection

A significant increase in tumor incidence was not detected in female ICR/Ha Swiss mice administered DCB at 0.05 mg (0.4 :mol) in 0.05 mL tricaprylin by i.p. injection, once per week for 77 weeks, beginning at 6 to 8 weeks of age (Van Duuren et al., 1975). In a review of this study, IARC (1977) commented on the low dose used.

8.2.6.4 Initiation/Promotion Studies

In a 2-stage carcinogenicity study, DCB was negative as an initiator when applied as a single dose of 1 mg (8 :mol) DCB in 0.1 mL acetone to the skin of 6- to 8-week-old female ICR/Ha Swiss mice (Van Duuren et al., 1975). This application was followed 14 days later with 2.5 :g phorbol myristate acetate in 0.1 mL acetone, applied to the skin 3 times per week for 77 weeks.

8.3 Genetic Toxicology

Genotoxicity studies with DCB are summarized in **Table 8-3**.

Using the plate incorporation assay, DCB (76.8% trans/21.6% cis-isomer) at 10 to 1000 FM (7.7 to 768 FM *trans*-isomer) induced a significant increase in *his* gene mutations in *S. typhimurium* strain TA100 in the presence of mouse or

human liver S9 fractions with or without a NADPH-generating system (Bartsch et al., 1979). At 1000 FM, a 9-fold and a 5- to 6-fold increase in revertants over controls was observed with or without mouse S9 metabolic activation, respectively. DCB was 64% less mutagenic in the presence of active human liver S9 than when metabolically activated by mouse liver S9.

DCB is also reported to induce mutations in *E. coli* (Mukai and Hawryluk, 1973Abst.). Experimental details were not provided.

8.4 Immunotoxicity

No data were found.

ıble 8-1. Acute Toxicity of *trans*-1,4-Dichloro-2-butene

Age, Strain, Species	Exposed Animals	Control Animals	Chemical Form, Purity	Dose	Exposure Duration; Observation Period	Mortality	Results/Comments	Referenc e
.2.2.1 Oral Administration								
ıts (age nd strain ot given)	n.g.	n.g.	*trans*-DCB, purity not specified	n.g.	n.g.	LD_{50} = 89 mg/kg bw (710 :mol/kg bw)	No details were given.	Smith et al. (1951; cited by IARC, 1977)
.2.2.2 Inhalation Exposure								
ıts (age nd strain ot given)	6 (sex not specified)	n.g.	*trans*-DCB, purity not specified	62 ppm (w/v) (320 mg/m^3; 2500 Fmol/m^3)	4 h exposure period; 14 day observation period	14 days after exposure, 2/6 rats had died	No details were given.	Smith et al. (1951; cited by IARC, 1977)
oung dult hR-CD ıts (252-77 g)	6M per dose	none	*cis*-and *trans*-DCB (1.43% *cis*-isomer, 97.17% *trans*-isomer)	240, 370, 410, 440, 540, 760, or 3600 ppm (vol/vol)[1] (1200 to 18000 mg/m^3; 9800 to 150000 Fmol/m^3)	30 min exposure in an inhalation chamber; 14 days post-exposure observation period; 2 rats necropsied on day 7 post-exposure	6/6 (3600 ppm); 5/6 (760 ppm); 0/6 (540 ppm); 0/6 (440 ppm); 2/6 (410 ppm); 0/12 (370 ppm[2]); 0/6 (240 ppm) 30-min LC_{50} = 784 ppm (4000 mg/m^3; 32100 Fmol/m^3)	3600 ppm (18000 mg/m^3; 150000 Fmol/m^3): **Exposure Period:** inactive; closed eyes; salivation; hyperemia; gasping; spasms; flaccid paralysis; death by 2.5 h	DuPont Chem. (1974)
							760 ppm (3900 mg/m^3; 31000 Fmol/m^3): **Exposure Period:** inactive; pallor; irregular respiration; salivation. **Post-Exposure Period:** hypersensitive to touch; red nasal discharge; diarrhea; pilo-erection; death from 1-13 days (5/6); 1/6 found dead at 13 days and necropsied. Necropsied rats exhibited destruction of the air passage, kidney damage, testicular atrophy, and hypoplastic bone marrow. The latter 2 changes were interpreted as a reflection of stress and emaciation and were considered not to be compound-related.	
							540 ppm (2800 mg/m^3; 22000 Fmol/m^3): **Exposure Period:** inactive; closed eyes; shallow respiration; pale ears; salivation; lacrimation; wet fur. **Post-Exposure Period:** severe weight loss for 2 days followed by normal rate of weight gain.	
							440 ppm (2200 mg/m^3; 18000 Fmol/m^3): **Exposure Period:** see 540 ppm. **Post-Exposure Period:** severe weight loss for 1 day followed by normal rate of weight gain. Necropsied rats exhibited damage of the tracheobronchial epithelium.	
							410 ppm (2100 mg/m^3; 17000 Fmol/m^3): **Exposure Period:** closed eyes; pawing; pallor; irregular respiration. **Post-Exposure Period:** severe weight loss followed by moderate weight gain rate; death at 3-8 days (2/6)	
							370 ppm[2] (1900 mg/m^3; 15000 Fmol/m^3): **Exposure and Post-Exposure Periods:** see	

Age, Strain, Species	Exposed Animals	Control Animals	Chemical Form, Purity	Dose	Exposure Duration; Observation Period	Mortality	Results/Comments	Reference
							240 ppm (1200 mg/m^3; 9800 Fmol/m^3): **Exposure and Post-Exposure Periods:** see 540 ppm	

breviations: n.g. = not given; M = male.
ch exposure lasted 30 min unless all rats died sooner. Gross and histopathologic examinations were performed on 2 rats surviving exposure for 7 days, on 1 rat found dead after 13 rec
after 14 recovery days. [2] duplicate; [3]lymph nodes, stomach, duodenum, epididymis, thyroid, adrenal glands, brain, and eyes

ıble 8-2. Carcinogenicity of *trans*-1,4-Dichloro-2-butene

Age, Strain, Species	Exposed Animals	Control Animals	Chemical Form, Purity	Dose	Exposure Duration	Mortality	Comments	Reference
.2.6.1 Dermal Application								
- to 8-wk-ld ICR/Ha wiss mice	30F	30F (acetone) 85F (no treatment)	*trans*-DCB, purity not specified	1.0 mg /0.1 mL acetone (8.0 :mol/0.1 mL) applied to shaved dorsal skin, 3 times/ wk	77 weeks	Mean survival time in treated mice not decreased as compared to controls	Complete necropsies, except for cranial region, were performed on all mice. No tumors were detected in DCB-treated mice.	Van Duuren et al. (1975)
.2.6..2 Subcutaneous Injection								
- to 8-wk-ld ICR/Ha wiss mice	30F	50F (tricaprylin) 85F (no treatment)	*trans*-DCB, purity not specified	0.05 mg/ 0.05 mL tricaprylin (0.40 :mol/ 0.05 mL) injected s.c. into left flank, once/wk	77 weeks	Mean survival time in treated mice not decreased as compared to controls	Complete necropsies, except for cranial region, were performed on all mice. **Injection Site**: There was a significant increase in the incidence of sarcomas at the injection site in treated mice (3/30 vs. 0/50 tricaprylin controls and 0/85 untreated controls).	Van Duuren et al. (1975)
.2.6.3 Intraperitoneal Injection								
- to 8-wk-ld ICR/Ha wiss mice	30F	30F (tricaprylin) 85 F (no treatment)	*trans*-DCB, purity not specified	0.05 mg/0.05 mL tricaprylin (0.40 :mol/ 0.05 mL) injected i.p., once/wk	77 weeks	Mean survival time of treated mice was decreased as compared to tricaprylin controls (478 days vs. 513 days, respectively).	Complete necropsies, except for cranial region, were performed on all mice. There was no significant increase in tumor incidence in treated mice as compared to controls. In a review of this study, IARC (1977) noted the low dose used.	Van Duuren et al. (1975)
.2.6.4 Initiation/Promotion Studies								
- to 8-wk-ld ICR/Ha wiss mice	30F (*trans*-DCB + PMA)	30F (*trans*-DCB + acetone) 30F (PMA) 60F (no treatment)	*trans*-DCB, purity not specified	one application of 1 mg (8 :mol) *trans*-DCB in 0.1 mL acetone on skin; followed 14 days later with 2.5 :g PMA/ 0.1 mL acetone, applied to skin 3 times/wk	77 weeks	Mean survival time for groups 1, 2, 3, and 4 were 478, 526, 460, and 510 days, respectively.	Complete necropsies, except for cranial region, were performed on all mice. There was no significant increase in tumor incidence in mice treated with *trans*-DCB + PMA as compared to controls.	Van Duuren et al. (1975)

)breviations: PMA = phorbol myristate acetate; F = female; i.p. = intraperitoneal; s.c. = subcutaneous

ıble 8-3. Genotoxicity of *trans*-1,4-Dichloro-2-butene

Test System	Biological Endpoint	S9 Metabolic Activation	Purity	Doses Used	Endpoint Response	Comments	Reference
almonella yphimurium strain A100	*his* gene mutations (plate incorporation method)	+/- active mouse or human liver S9 (activity based on presence or absence of a NADP generating system)	76.8% *trans*/ 21.6% *cis*.	10 to 1000 FM (7.7 to 768 FM *trans*-isomer)	positive/positive	At 1000 FM, a 9-fold and a 5- to 6-fold increase in revertants over controls was observed with or without mouse S9 metabolic activation, respectively. DCB was 64% less mutagenic in the presence of active human liver S9 than when metabolically activated by mouse liver S9.	Bartsch et al. (1979)
Escherichia coli strain not provided)	gene mutations (locus not provided)	details not provided	n.p.	n.g.	positive	No experimental details were given.	Mukai and Hawryluk (1973Asbt.)

breviations: n.p. = not provided; n.g. = not given.

9.0 STRUCTURE-ACTIVITY RELATIONSHIPS

No data were found.

10.0 COMPLEX MIXTURES

Summary: The hepatotoxicity and nephrotoxicity of a complex waste mixture containing DCB (59 mg [470 Fmol]/g) were evaluated in rats. Centrilobular necrosis of the liver was detected in some rats treated with 1.0 or 5.0 mg waste mixture/kg (DCB = 0.059 or 0.30 mg/kg, respectively; 0.47 or 2.4 Fmols/kg, respectively). The relative liver weight, hepatic water content, and relative and absolute kidney weights for the treated groups were also increased. In addition, serum activities of alkaline phosphatase, lactate dehydrogenase, and ornithine carbamyl transferase, and the serum concentration of total bilirubin (all indicators of hepatic injury), as well as the serum concentration of urea nitrogen, were increased.

The same complex waste mixture containing DCB at 59 mg (470 Fmol)/g induced gene mutations in *S. typhimurium* TA100 at 0.1 to 2.0 Fg crude waste (DCB at 6-120 x 10^{-3}Fg; 0.05-0.9 x 10^{-3}Fmol)/plate in the presence and absence of rat liver S9 using the plate incorporation method. The same waste mixture induced lambda prophage in *E. coli.* The lowest effective dose (LED) for the complex mixture in the presence of S9 was 50 x 10^{-6} Fg/mL (DCB at 3 x 10^{-6} Fg/mL; 2 x 10^{-5} FM), while in the absence of S9 it was 2 x 10^{-7} Fg/mL (DCB at 1.2 x 10^{-8} Fg/mL; 9 x 10^{-8} FM). The maximal response was a 26-fold increase with metabolic activation versus a 5-fold increase without S9.

10.1 *In Vivo* Acute Toxicity

Studies described in this section are presented in **Table 10-1**. Simmons et al. (1988; see also Simmons and Berman, 1989) evaluated the hepatotoxicity of a waste sample containing DCB at 59 mg/g (470 :mol/g) (see **Table 10-1** for list of other chemicals detected in the sample). Male Fischer 344 rats (65 days of age)were administered a single dose of 1.0 or 5.0 mg waste sample/kg (0.06 or 0.3 mg DCB/kg; 0.5 or 2.4 Fmol DCB/kg) by gavage and were sacrificed 24 hours later. All high-dose rats died before the end of the study and could not be evaluated. AMarked@ centrilobular necrosis was detected in the livers of 4/5 low-dose rats (vs. 0/24 controls), while the liver of the remaining rat exhibited moderate (i.e., less severe than marked) centrilobular necrosis (vs. 0/24 controls). Relative liver weight (liver-to-body weight ratio) and hepatic water content (wet-to-dry-weight ratio) were significantly increased in low-dose rats. These rats also had significantly increased serum activities of several indicators of hepatic injury;

alkaline phosphatase, lactate dehydrogenase, ornithine carbamyl transferase, and total bilirubin.

In a further analysis of the study by Simmons et al. (1988), Simmons et al. (1995) evaluated the nephrotoxicity of the waste sample in the same rats. A description of renal histopathology was not given, but it was noted that relative and absolute kidney weights were significantly increased in the low-dose rats. The serum concentration of urea nitrogen (BUN), but not of creatinine (CREAT), was also significantly increased in these rats. The authors noted that serum BUN is Anot notably sensitive to low levels of renal damage@, suggesting that the renal damage must have been moderate to severe.

10.2 Genetic Toxicology: Prokaryotic Mutagenesis

Studies described in this section are presented in **Table 10-2**.

DeMarini et al. (1987) reported that a crude petrochemical waste containing DCB at 59 mg (470 Fmol)/g waste as well as a dichloromethane waste extract (concentration of DCB not determined) induced mutations in *S. typhimurium* strain TA100. Doses tested were 0.1 to 2.0 Fg crude waste/plate (DCB at 6 - 120 x 10^{-3}Fg/plate; 0.05 - 0.9 x 10^{-3} Fmol/plate), or waste extract in the presence and absence of metabolic activation using the plate incorporation method with strain TA100. The crude waste led to an approximate 3 fold higher response than the waste extract.

Houk and DeMarini (1988) reported that the same crude petrochemical waste induced lambda prophage in *E. coli* strain $WP2_S$(8) using the microscreen assay. The doses tested were 5 x 10^{-5} to 400 Fg crude waste/mL (DCB at 3 x 10^{-6} to 20 Fg/mL; 2.4 x 10^{-5} to 200 FM) in the presence of rat liver S9 and 2 to 15 x 10^{-7} Fg crude waste/mL (DCB at 1.2 - 9 x 10^{-8} Fg/mL; 9 - 71 x 10^{-8} FM) in the absence of S9. The lowest effective dose (LED) in the presence of S9 was 50 x 10^{-6} Fg crude waste/mL (DCB at 3 x 10^{-6} Fg/mL; 2 x 10^{-5} FM), while without

metabolic activation it was 2 x 10^{-7} Fg crude waste/mL (DCB at 1.2 x 10^{-8} Fg/mL; 9 x 10^{-8} FM). The maximal response was a 26-fold increase with S9 at 1.5 Fg crude waste/mL (DCB at 9 x 10^{-2} Fg/mL; 0.7 FM) vs. a 5-fold increase without S9 at 8 x 10^{-7} Fg crude waste/mL (DCB at 5 x 10^{-8} Fg/mL; 4 x 10^{-7} FM).

ıble 10-1. Toxicity of Complex Mixtures Containing *trans*-1,4-Dichloro-2-butene

Age, Strain, Species	Exposed Animals	Control Animals	Chemical Mixture Dose Exposure Duration Mortality Results/Comments Reference					
			10.1 In Vivo Acute Toxicity 65-day-old Fischer 344 rats 6M for both doses 16M (intubated with an empty gavage needle) ***trans*-DCB (59 mg/g [470 :mol/g])**, *cis*-1,4-DCB (18 mg/g), benzyl chloride (3 mg/g), carbon tetrachloride (68 mg/g), chloroform (2.9 mg/g), hexachloroethane (0.6 mg/g), methylene chloride (21 mg/g), naphthalene (< 0.1 mg/g[1]), tetrachloroethylene (11 mg/g), toluene (240 mg/g), trichloroethylene (4 mg/g), 1,1,1-trichloroethane (< 0.1 mg/g), water (1.8%), metals ([concentrations in :g/g] antimony [< 12], arsenic [< 24], barium [< 7], beryllium [< 2], cadmium [< 5], chromium [< 5], lead [< 19], mercury [< 22], nickel [68], selenium [< 470], silver [< 3], thallium [< 23]) Mixture was a collected waste sample. Not all components were identified.	1.0 or 5.0 mg waste sample/kg by gavage (0.06 or 0.30 mg DCB/kg; 0.5 or 2.4 Fmols DCB/kg)	single dose	1/6 LD and 6/6 HD rats died within 24 h of dosing; none of the controls died The observed mortality level did not match predicted values	Surviving rats were killed 24 h after dosing. The liver was the only organ evaluated in Simmons et al. (1988) and in Simmons and Berman (1989) . The kidneys was the only organ evaluated in Simmons et al. (1995). Due to early death, HD rats were not evaluated. **Histopathology:** AMarked@ centrilobular necrosis was detected in the livers of 4/5 LD rats (vs. 0/24 controls [the additional 8 controls were pooled from another group in the study]); with moderate (i.e., less severe than marked) centrilobular necrosis present in the liver of the remaining rat (vs. 0/24 controls). A description of renal histopathology was not given. **Organ Weight:** Relative liver weight (liver-to-body weight ratio x 100) was significantly increased in LD rats (4.65 " 0.08) vs. 3.35 " 0.11 in controls ; absolute weights not given). Relative and absolute kidney weights were significantly increased in LD rats (relative wt: 0.93 " 0.07 vs. 0.76 " 0.02 in controls; absolute wt: 2.00 " 0.22 vs. 1.67 " 0.03 in controls. **Organ Water Content:** Hepatic water content (wet-to-dry-weight ratio) was significantly increased in LD rats (4.02 " 0.34 vs. 3.49 " 0.07 in controls). Renal water content was not given. **Serum Indicators of Hepatic Injury**: The serum activities of alkaline phosphatase, lactate dehydrogenase, and ornithine carbamyl transferase, and the serum concentration of total bilirubin were significantly increased in LD rats as compared to controls. **Serum Indicators of Renal Injury:** The serum concentration of urea nitrogen (BUN) but not of creatinine (CREAT) was significantly increased in LD rats as compared to controls. Simmons et al. (1995) noted that serum BUN is Anot notably sensitive to low levels of renal damage.@, suggesting that the renal damage must have been moderate to severe.	Simmons et al. (1988; 1995); Simmons and Berman (1989)

)breviations: M = male; LD = low dose; HD = high dose.

able 10-2. Genotoxicity of Complex Mixtures Containing *trans*-1,4-Dichloro-2-butene

Test System	Biological Endpoint	S9 Metabolic Activation	Purity	Dose	Endpoint Response	Comments	Reference
. typhimurium train TA100	*his* reverse gene mutations (plate incorporation method)	-/+	59 mg DCB/g (470 Fmol/g) petrochemical waste.	0.1 to 2.0 Fg crude waste or waste extract/plate (6 - 120 x 10^{-3}Fg DCB/plate; 0.05 - 0.9 x 10^{-3} Fmol DCB/plate)	positive/positive	Both the crude waste and the dichloromethane extracted waste induced positive dose responses.	DeMarini et al. (1987)
. coli strain WP2$_s$(8)	lambda prophage induction (microscreen assay)	-/+	59 mg DCB/g (470 Fmol/g) petrochemical waste.	+S9 = 5 x 10^{-5} to 400 Fg waste/mL (3 x 10^{-6} to 20 Fg DCB/mL; 2.4 x 10^{-5} to 200.FM DCB) -S9 = 2 - 15 x10^{-7} Fg waste/mL (1.2 - 9 x 10^{-8} Fg DCB/mL; 9 - 71 x 10^{-8} FM DCB) for 20 h	positive/positive	S9 reduced the genotoxic potency of the waste; +S9 LED = 50x10^{-6} :g/mL (3 x 10^{-6} Fg DCB/mL; 2 x 10^{-5} FM DCB) vs. -S9 LED = 2x10^{-7} Fg/mL (1.2 x 10^{-8} Fg DCB/mL; 0.09 x 10^{-6} FM DCB while the maximal response was a 26-fold increase with S9 at 1.5 Fg/mL (9 x 10^{-2} Fg DCB/mL; 0.7 FM DCB) vs. a 5-fold increase without S9 at 8 x 10^{-7} Fg/mL (5 x 10^{-8} Fg DCB/mL; 4 x 10^{-7} FM DCB).	Houk and DeMarini (1988)

)breviations: LED = lowest effective dose.

11.0 ONLINE DATABASES AND SECONDARY REFERENCES SEARCHED

11.1 Online Databases

Chemical Information System Files

ISHOW (Information System for Hazardous Organics in Water)
SANSS (Structure and Nomenclature Search System)
TSCAPP (Toxic Substances Control Act Plant and Production)
TSCATS (Toxic Substances Control Act Test Submissions)

DIALOG Files

359 Chemical Economics Handbook

Internet Databases

Code of Federal Regulations full text. 1996 versions of various titles via GPO Gate, a gateway by the Libraries of the University of California to the GPO Access service of the Government Printing Office, Washington, DC. Internet URL http://www.gpo.ucop.edu/

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)

TRI (Toxics Release Inventory; compounds listed in the SARA 313 list [40 CFR 372])

STN International Files

BIOSIS (Biological Abstracts)
CA File (Chemical Abstracts)
CANCERLIT
CSNB (Chemical Safety News Base)
EMBASE (Excerpta Medica)
HSDB (Hazardous Substances Data Bank)
IPA (International Pharmaceutical Abstracts)
MEDLINE (Index Medicus)
RTECS (Registry of Toxic Effects of Chemical Substances)
TOXLINE
TOXLIT

TOXLINE includes the following subfiles, which often have only the toxicology information from the databases named:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicology Research Projects	CRISP
NIOSHTIC7	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

11.2 Secondary References Used

CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 1980. Listed in Section 12 as Weast and Astle (1980).

Fishbein, L. 1979. Potential Halogenated Industrial Carcinogenic and Mutagenic Chemicals. I. Halogenated Unsaturated Hydrocarbons. Sci. Total Environ. 11:111-161. Listed in Section 12.

IARC. 1977. *trans*-1,4-Dichlorobutene. IARC Monogr. Eval. Carcinog. Risks Hum. 15:149-154.

Listed in Section 12.

SRI Directory of Chemical Producers, SRI International, Menlo Park, CA, 1996. Listed in Section 12 as SRI International (1996).

12.0 REFERENCES

Bartsch, H., C. Malaveille, A. Barbin, and G. Planche 1979. Alkylating and Mutagenic Metabolites of Halo-Ethylenes, Chlorobutadienes, and Dichlorobutenes Produced by Rodent or Human Liver Tissues. Arch. Toxicol. 41:249-277.

DeMarini, D.M., J.P. Inmon, J.E. Simmons, E. Berman, T.C. Pasley, S.H. Warren, and R.W. Williams. 1987. Mutagenicity in Salmonella of Hazardous Wastes and Urine from Rats Fed These Wastes. Mutat. Res. 189(3):205-216.

DuPont Chemical Co. 1974. Initial Submission: Acute Inhalation Toxicity of *cis*- and *trans*-1,4-Dichlorobutene-2 (1.43% *cis*-1,4-DCB, 97.1% *trans*-1,4-DCB) in Rats, with Cover Letter Dated 10/15/92. U.S. EPA/OTS Public Files, Document Number 88-920009575. Fiche Number 0571232.

Fishbein, L. 1979. Potential Halogenated Industrial Carcinogenic and Mutagenic Chemicals, I. Halogenated Unsaturated Hydrocarbons. Sci. Total Environ. 11:111-161.

Hermens, J., F. Busser, P. Leeuwanch, and A. Musch. 1985. Quantitative Correlation Studies Between the Acute Lethal Toxicity of 15 Organic Halides to the Guppy (*Poecilia reticulata*) and Chemical Reactivity Towards 4-Nitrobenzylpyridine. Toxicol. Environ. Chem. 9:219-236.

Houk, V.S., and D.M. DeMarini. 1988. Use of the Microscreen Phage-Induction Assay to Assess the Genotoxicity of 14 Hazardous Industrial Wastes. Environ. Mol. Mutagen 11(1):13-30.

IARC (International Agency for Research on Cancer). 1977. *trans*-1,4-Dichlorobutene. IARC Monogr. Eval. Carcinog. Risks Hum. 15 (Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals):149-154.

Mukai, F.H., and I. Hawryluk. 1973. The Mutagenicity of Some Halo-Ethers and Halo-Ketones. Mutat. Res. 21:228. Abstract.

RTECS. 1996. Registry of Toxic Effects of Chemical Substances. Produced by NIOSH online version. File last reloaded on STN International, August 1996.

Simmons, J.E., D.M. DeMarini, and E. Berman. 1988. Lethality and Hepatotoxicity of Complex Waste Mixtures. Environ. Res. 46:74-85.

Simmons, J.E., and E. Berman. 1989. Toxicity of Complex Waste Mixtures: A Comparison of Observed and Predicted Lethality. J Toxicol. Environ. Health 27:275-286.

Simmons, J.E., R.S.H. Yang, and E. Berman. 1995. Evaluation of the Nephrotoxicity of Complex

Mixtures Containing Organics and Metals: Advantages and Disadvantages of the Use of Real-world Complex Mixtures. Environ. Health Perspect. 103(Suppl. 1):67-71.

SRI International. 1996. Chemical Economics Handbook. SRI International, Menlo Park, CA. Online version. DIALOG7 File 359.

Tomkins, B.A., J.E. Caton, Jr., M.D. Edwards, M.E. Garcia, R.L. Schenley, L.J. Wachter, and W.H. Griest. 1989. Determination of Regulatory Organic Compounds in Radioactive Waste Samples. Volatile Organics in Aqueous Liquids. Anal. Chem. 61:2751-2756.

TSCAPP (TSCA Plant and Production Search System). 1983. Online database available from the Chemical Information System, last update September 1983.

Van Duuren, B.L., B.M. Goldschmidt, and I. Seidman. 1975. Carcinogenic Activity of Di- and Trifunctional "-Chloro Ethers and of 1,4-Dichlorobutene-2 in ICR/HA Swiss Mice. Cancer Res. 35:2553-2557.

Weast, R.C., and M.J. Astle., Eds. 1980. CRC Handbook of Chemistry and Physics. CRC Press, Inc., Boca Raton, FL.