EVIDENCE ON THE CARCINOGENICITY OF

1,2-EPOXYBUTANE

FINAL

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**Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment
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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of carcinogenicity are identified as the members of the Carcinogen Identification Committee of the OEHHA Science Advisory Board (Title 22 Cal. Code of Regs. §12301).

1,2-Epoxybutane was assigned a final priority of 'high' carcinogenicity concern and placed on the Final Candidate list of chemicals for Committee review on March 12, 2004. A public request for information relevant to the assessment of the evidence on the carcinogenicity of this chemical was announced on March 12, 2004, in the *California Regulatory Notice Register*. No information was received as a result of this request. This document was developed to provide the Committee with the available scientific evidence on the carcinogenic potential of this chemical. It was released as a draft document in August 2004.

At their November 1, 2004 meeting the Committee, by a vote of two in favor and three against, did not find that 1,2-epoxybutane had been "clearly shown through scientifically valid testing according to generally accepted principles to cause cancer." Accordingly, 1,2-epoxybutane was not placed on the Proposition 65 list of chemicals known to the state to cause cancer.

The following is the final version of the document that was discussed by the Committee at their November 2004 meeting.

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EXECUTIVE SUMMARY

1,2-Epoxybutane is a short-chain epoxide used as a stabilizing agent primarily for chlorinated solvents. Its use in this capacity, as well as its high volume of production, suggests considerable potential for human exposure, principally in an occupational setting.

As an epoxide with alkylating activity, 1,2-epoxybutane was suspected to have and consistently showed genotoxic activity prior to its testing in long-term bioassays in rats and mice in 1988 by the National Toxicology Program (NTP). The observed genotoxicity was wide-ranging, with studies demonstrating the induction of base-pair substitutions in reverse mutation assays in *Salmonella*, mutagenicity in other bacteria and fungi, cell transformation and cytogenetic changes in mammalian cells *in vitro*, and cytogenetic changes in *Drosophila in vivo*. In the subsequent long-term inhalation studies in male rats conducted by the NTP, 1,2-epoxybutane treatment-related increases in lung carcinoma and nasal adenomas were observed, the latter of which occurs very rarely in untreated rats. 1,2-Epoxybutane-treated female rats developed a small number of nasal adenomas. Two structurally related carcinogens produce tumors at these sites: propylene oxide produces tumors of the nasal cavity in rats and mice and ethylene oxide produces lung tumors in mice. NTP reported no treatment-related increase in tumors following inhalation exposure of mice to 1,2-epoxybutane, although a single squamous cell papilloma of the nasal cavity developed in a treated male mouse and there was extensive toxicity to the nasal cavity, including squamous metaplasia. In oral gavage studies, a few forestomach tumors which are rare in control mice, were induced in mice treated with 1,2-epoxybutane-stabilized trichloro-ethylene but not in mice treated with trichloroethylene alone, or in control animals. The interpretation of this finding is complicated by the concomitant exposure to trichloroethylene.

2   INTRODUCTION

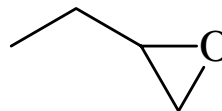## 2.1   Identity of 1,2-Epoxybutane

Molecular Formula: C_4H_8O

Molecular Weight: 72.1

CAS Registry No.:   106-88-7

Chemical Class: Short-chain epoxide

Synonym: 1-Butene oxide; 1,2-butene oxide; butylene oxide; 1,2-butylene oxide; 2-ethyloxirane; 2-ethyl oxirane; ethyl ethylene oxide

Boiling point: 63.3 ℃

2.2   Occurrence and Use

The primary use of 1,2-epoxybutane is as a stabilizer for chlorinated hydrocarbon solvents such as trichloroethylene (IARC, 1999). It is also used as a chemical intermediate in the production of other chemicals (butylene glycols and their derivatives: polybutylene glycols, mixed poly glycols and glycol ethers and esters; butanolamines; surface-active agents; gasoline additives) (IARC, 1999).

Ten to fifty million pounds of 1,2-epoxybutane are reported to be produced annually according to the 1998 Toxic Substance and Control Act update (U.S. EPA, 1998) and the chemical appears on U.S. Environmental Protection Agency's (U.S. EPA) list of high production volume chemicals (as 2-ethyl oxirane; U.S. EPA, 1990). Over 260,000 employees, including approximately 43,000 females, were estimated to be potentially exposed to 1,2-epoxybutane in the United States according to the National Institute for Occupational Safety and Health's (NIOSH) National Occupational Exposure Survey, which was conducted from 1981 to 1983 (NIOSH, 1990).

3   DATA ON 1,2-EPOXYBUTANE CARCINOGENICITY

1,2-Epoxybutane has been extensively tested for genotoxic potential in a variety of assays: *Salmonella* and *Escherichia coli* reverse mutation assays, *Klebsiella* forward mutation assays, mutagenicity assays in the fungi *Saccharomyces, Schizosaccharomyces*, and *Neurospora*, cell transformation assays in rat and hamster cells, the mouse lymphoma cell assay, assays for sister chromatid exchange and chromosomal aberrations in hamster cells, and sex-linked recessive lethal mutation and reciprocal translocation assays in *Drosophila.* Long-term carcinogenicity

studies of 1,2-epoxybutane have been conducted in both rats and mice. A skin-painting assay in mice was also reported.

3.1 Epidemiological Studies of Carcinogenicity in Humans

No data on long-term effects of human exposure to 1,2-epoxybutane were found in a recent search of the scientific literature by OEHHA.

3.2 Carcinogenicity Studies in Animals

A review of the scientific literature regarding carcinogenicity studies of 1,2-epoxybutane in experimental animals identified long-term inhalation studies in male and female rats and mice. A long-term skin painting study in mice was also identified.

3.2.1 Long-term Inhalation Studies in Rats

Male and female F344/N rats (50/sex/group) were exposed to 0, 200, or 400 ppm 1,2-epoxybutane for six hours per day, five days per week, for 103 weeks (NTP, 1988).[1] Among rats of both sexes, treatment resulted in slightly reduced body weights at all doses. Mean body weights of high dose male rats were four to eight percent lower than controls, but only after week 86. Mean body weights of high dose female rats were five to ten percent lower than control rats after week 22. Survival was lower among treated rats at all doses.

Among male rats, a significant increase in papillary adenomas of the nasal cavity, a rare tumor type in Fischer 344/N rats, was observed in the high dose group ($p = 0.0048$, by pairwise Poly-3 Test[2]), with a statistically significant positive trend across doses (see Table 1). An increase in alveolar/bronchiolar carcinomas of the lung in the high dose group relative to controls was statistically significant ($p = 0.025$, by pairwise Poly-3 Test) and a statistically significant positive trend across doses was observed ($p = 0.017$, by Poly-3 Trend Test). Combined alveolar/bronchiolar adenomas and carcinomas of the lung were significantly increased in the high dose group ($p = 0.0082$, by pairwise Poly-3 Test), also with a statistically significant positive trend across doses ($p = 0.0061$, by Poly-3 Trend Test). A marginally statistically significant increase in combined adenomas and carcinomas of the preputial gland in high dose male rats ($p = 0.072$, by pairwise Poly-3 Test) was observed, with a marginally statistically significant positive trend ($p = 0.060$, by Poly-3 Trend Test).

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[1] Estimates of the lifetime average daily doses of 1,2-epoxybutane in these studies are 63 and 126 mg/kg-day for male rats and 72 and 144 mg/kg-day for female rats, based on assumed breathing rates of 0.264 m^3/day for male rats and 0.201 m^3/day for female rats, respectively, average body weights of 450 g and 300 g for male and female rats, respectively (estimated from the 1988 NTP studies), and 100% fractional absorption. Inhalation rates for rats were estimated based on Anderson *et al.* (1983): Inhalation rate $= 0.105 \times$ (body weight$_{rats}$/0.113)$^{2/3}$.

[2] Poly-3 analysis is currently conducted routinely by NTP as a test of significance for pairwise comparison and overall exposure-related trend. The procedure takes survival into account, with animals without a given tumor dying prior to terminal sacrifice weighted by the fraction of total study time they survived raised to the third power. Since the NTP (1988) studies of 1,2-epoxybutane were conducted prior to the routine use of this procedure, Poly-3 analyses performed by OEHHA using the individual animal data are presented here.

NTP reported that, as of 1988, adenomas of the nasal cavity had not been observed in control male rats in inhalation studies conducted at the same laboratory at which 1,2-epoxybutane was tested (n = 249; Battelle Pacific Northwest Laboratories) (NTP, 1988). Using more recent historical control information from NTP (as of December 1999), adenomas of the nasal cavity had not been reported in any of 21 NTP inhalation bioassays in control male Fischer 344/N rats (n = 1048; NTP, 1999). Among 1977 control male F344/N rats at all NTP testing facilities by all routes, NTP observed two squamous cell papillomas of the nasal cavity (0.1% prevalence) (NTP, 1988).

Table 1. Tumors in Male F344/N Rats Exposed to 1,2-Epoxybutane Six Hours Per Day, Five Days Per Week for Two Years (NTP, 1988).

Tumor Site and Type		Exposure Level (ppm)			Trend Test[a]
		0	**200**	**400**	
Nasal cavity	Papillary adenomas	0/50	0/50		0.0010
Lung	Alveolar / bronchiolar adenomas	0/50	1/50	1/49	n.s.
	Alveolar / bronchiolar carcinomas	0/50	1/50		0.017
	Combined alveolar / bronchiolar adenomas and carcinomas	0/50	2/50		0.0061
Preputial gland	Adenomas (not otherwise specified)	2/50	0/50	5/50	0.091
	Carcinomas (not otherwise specified)	1/50	2/50	3/50	n.s.
	Squamous cell carcinomas	0/50	1/50	0/50	n.s.
	Combined adenomas, carcinomas, and squamous cell carcinomas	3/50	3/50	8/50[c]	0.060

[a] Poly-3 Trend Test (n.s. = not significant, p > 0.10).
[b] Statistically significant increase in incidence above controls by pairwise Poly-3 Test (p ≤ 0.05).
[c] Marginally significant increase in incidence above controls by pairwise Poly-3 Test (0.05 < p ≤ 0.1).

Among female rats, two rare papillary adenomas of the nasal cavity were observed in the high dose group compared to none in the control group (see Table 2). The incidence of nasal cavity papillary adenomas in high dose female rats was marginally statistically significant (p = 0.076, by pairwise Poly-3 Test), and the Poly-3 Trend Test was significant (p = 0.045). Follicular adenomas of the thyroid gland in the high dose group were also marginally statistically increased over controls (p = 0.074, by pairwise Poly-3 Test), with a statistically significant positive trend across doses (p = 0.044, by Poly-3 Trend Test). A marginally statistically significant increase in combined follicular cell adenomas and carcinomas was observed in the high dose group (p = 0.074, by pairwise Poly-3 Test), with a marginally statistically significant increase in positive trend across doses (p = 0.077, by Poly-3 Trend Test).

No nasal cavity tumors were reported in control animals in inhalation bioassays of female rats tested at Battelle Pacific Northwest Laboratories at the time of the 1988 NTP report on 1,2-epoxybutane (n = 247). Since that time, NTP's database of nasal tumors in control female rats in inhalation studies has expanded to 1044 female rats, still without a reported adenoma of the nasal cavity (NTP, 1999). Only a single nasal cavity papilloma (not otherwise specified) was reported among control female rats in NTP studies by all routes as of the 1988 report (n = 2021).

Table 2. Tumors in Female F344/N Rats Exposed to 1,2-Epoxybutane Six Hours Per Day, Five Days Per Week for Two Years (NTP, 1988).

Tumor Site and Type		Exposure Level (ppm)			Trend Test[a]
		0	200	400	
Nasal cavity	Papillary adenomas	0/50	0/50	2/50[b]	0.045
Thyroid gland	Follicular cell adenomas	0/45	0/48	2/48[b]	0.044
	Follicular cell carcinomas	0/45	1/48	1/48	n.s.
	Combined follicular cell adenomas or carcinomas	0/45	1/48	3/48[b]	0.077

[a] Poly-3 Trend Test p-value (n.s. = not significant, p > 0.10).
[b] Marginally significant increase in incidence above controls by pairwise Poly-3 Test (0.05 < p ≤ 0.1).

Non-cancer lesions of the nasal cavity and olfactory sensory epithelium were observed in both male and female rats treated with 1,2-epoxybutane. The incidence of squamous metaplasia and epithelial hyperplasia of the nasal cavity was significantly increased in both male and female rats in both exposure groups (p < 0.05).

NTP concluded that "under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity* of 1,2-epoxybutane for male F344/N rats, as shown by an increased incidence of papillary adenomas of the nasal cavity, alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined)" and that "[t]here was *equivocal evidence of carcinogenic activity* for female F344/N rats, as shown by the presence of papillary adenomas of the nasal cavity" (NTP, 1988).

3.2.2 Long-term Inhalation Studies in Mice

Male and female B6C3F$_1$ mice (50/sex/group) were exposed to 0, 50, or 100 ppm 1,2-epoxybutane six hours per day, five days per week for 102 weeks (NTP, 1988). Between weeks 47 and 69, mean body weights of high-dose male mice were comparable to those of controls, however, earlier in the study, their weights were generally higher, and after, were lower. Among low-dose male mice, mean body weights were higher than controls until week 86, after which they were lower. Survival among high-dose male mice was significantly lower than that of low-dose mice. After weeks 60 and 73, mean body weights of high- and low-dose female mice, respectively, were lower than controls. Survival of high-dose female mice was significantly lower than that of control and low-dose female mice. Other than inactivity and listlessness among high-dose female mice during the final two months of the study, NTP reported no other clinical signs of toxicity.

A single squamous cell papilloma in the incisive duct of the nasal cavity was observed in a male mouse exposed to 100 ppm 1,2-epoxybutane. This tumor was not considered to be treatment related by NTP. No statistically significant increases in tumor incidence were observed among 1,2-epoxybutane treated male or female mice relative to control mice. Statistically significant *decreases* in pituitary gland adenomas and combined adenomas and carcinomas were observed in 1,2-epoxybutane treated female mice at both levels relative to controls.

Extensive evidence of non-neoplastic lesions of the nasal cavity, nasal gland, nasolacrimal duct and olfactory sensory epithelium was observed in mice in response to exposure to 1,2-epoxybutane. These changes included statistically significant increases in the incidence of chronic inflammation, erosion, regeneration, epithelial hyperplasia, and squamous metaplasia of the nasal cavity at both exposure levels in male and female mice. Hyperplasia of the nasal gland, epithelial hyperplasia of the nasolacrimal duct, and atrophy of the olfactory sensory epithelium were also increased at both exposure levels in male and female mice.

NTP concluded "[t]here was *no evidence of carcinogenic activity* for male or female $B6C3F_1$ mice exposed at 50 or 100 ppm." NTP also indicated "1,2-epoxybutane exposure was associated with … inflammatory lesions of the nasal cavity in mice." (NTP, 1988).

3.2.3 Combined Exposures: Long-Term Gavage Studies in Mice

Five-week old male Swiss ICR/Ha mice (50/group) were treated by oral gavage with corn oil or 2400 mg/kg bodyweight trichloroethylene (amine base-stabilized) in corn oil, or trichloroethylene stabilized by 0.8% 1,2-epoxybutane (weight/weight) in corn oil five times per week for 18 months (Henschler *et al.*, 1984) and observed for an additional six months. Dosing was discontinued from weeks 35 to 40, week 65, and weeks 69 to 78 due to non-specific toxicity, and doses were reduced to half initial levels after week 40. Five-week old female mice (50/group) were treated similarly (including similar discontinuation of treatment), although the initial doses of trichloroethylene were 1800 mg/kg bodyweight up to week 35 prior to the reduction in dose by half from weeks 40 to 69.[3] Surviving animals were sacrificed at 106 weeks. Survival at 106 weeks was poor with six male and three female control mice, no trichloroethylene-treated mice, and one male and one female mouse treated with 1,2-epoxybutane-stabilized trichloroethylene surviving.

Squamous cell carcinomas of the forestomach, a rare tumor type, were reported in 3/49 male mice (age-adjusted p-value = 0.029, by χ^2-test, relative to controls) and 1/48 female mice treated with 1,2-epoxybutane-stabilized trichloroethylene, whereas no forestomach tumors were reported in trichloroethylene alone or corn oil control animals. Trichloroethylene is known to produce tumors of the liver, lung, and blood in mice, and tumors of the kidney and testes in rats, but not forestomach tumors.

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[3] An estimate of the average daily dose of 1,2-epoxybutane to the mice over the 18 months of treatment (taking into account the intermittent and reduced dosing) is approximately 8.4 mg/kg-day for male mice and 6.3 mg/kg-day for female mice, based on the 0.8% 1,2-epoxybutane contaminant level in the trichloroethylene used in the study.

3.2.4 Skin Painting Study in Mice

The shaved dorsal skin of 30 eight-week old female Swiss ICR/Ha mice was treated with 100 mg of a 10% solution of 1,2-epoxybutane in acetone three times per week for 77 weeks (Van Duuren *et al.*, 1967). One hundred untreated female mice and 40 female mice treated with 100% acetone for 85 weeks were included in the study as controls. All animals were sacrificed at 85 weeks. No evidence of tumorigenicity was observed in the study.

3.2.5 Discussion of Carcinogenicity Studies in Animals

The 1988 NTP studies of 1,2-epoxybutane provided evidence that rare tumors of the nasal cavity and alveolar / bronchiolar adenomas and carcinomas of the lung result from the exposure of male rats to the compound. The finding of two papillary adenomas in treated female rats was considered a treatment-related effect due to the rareness of this tumor type in untreated rats. The NTP studies in mice demonstrated extensive treatment-related toxicity to the nasal tissues, including metaplasia and hyperplasia in both sexes; however, no treatment related tumors were observed at any site. A report of a significant increase in rare forestomach tumors in male mice treated by oral gavage with 1,2-epoxybutane-stabilized trichloroethylene compared to amine base-stabilized trichloroethylene and control mice suggests a role for this epoxide in the tumor response, although the interpretation of this study is complicated by the established carcinogenicity of trichloroethylene itself (which did not manifest itself in this particular study) and the absence of a group treated with 1,2-epoxybutane alone. The exposure duration of this study was also less than lifetime.[4] A skin painting study in mice showed no evidence for the carcinogenicity of 1,2-epoxybutane, although the mice were neither exposed nor followed-up for a full lifetime.

3.3   Other Relevant Data

3.3.1 Genetic Toxicology

Extensive testing in bacterial mutagenesis assays clearly indicates the mutagenic potential of 1,2-epoxybutane. The *Salmonella* reverse mutation assay has been conducted in numerous strains showing that 1,2-epoxybutane induces base-pair mutations rather than frameshift mutations (see Table 3). This is consistent with the epoxide's properties as an alkylating agent. Many of the studies cited here included testing with and without metabolic activation systems in place. Metabolic activation generally did not influence the outcome of the reverse mutation assays, consistent with that of a compound that does not require metabolism to be active. Mutations have also been observed in several fungal assay systems, including *Saccharomyces*, *Schizosaccharomyces*, and *Neurospora* (Simmon, 1979a; Migliore *et al.*, 1982; Kolmark and Giles, 1955; Rossi *et al.*, 1983).

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[4] A direct comparison of 1,2-epoxybutane exposures/dose between the NTP (1988) 1,2-epoxybutane studies and the Henschler *et al.* (1984) trichloroethylene studies is uncertain because the exposures occurred by different routes and in different species. Using the estimates described earlier in the document ([1],[3]), on a mg/kg-day basis, it appears that the 1,2-epoxybutane doses in the NTP study were approximately 10- and 20-fold higher than that in the Henschler study.

In vitro tests with mammalian cells (see Table 4) have demonstrated that 1,2-epoxybutane induces cell transformation in rat embryo cells (Price and Mishra, 1980; Dunkel *et al.*, 1981), but does not induce unscheduled DNA synthesis in rat primary hepatocytes (Williams *et al.*, 1982). 1,2-Epoxybutane has tested positive for mutagenicity in the mouse lymphoma assay (Amacher *et al.*, 1980; McGregor *et al.*, 1987; Mitchell *et al.*, 1988; Myhr and Caspary, 1988; NTP, 1988; Knaap *et al.*, 1982) and has induced chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells (NTP, 1988; Anderson *et al.*, 1990). Clonal and focus formation assays in Syrian hamster embryo cells have also produced positive results for 1,2-epoxybutane (Pienta *et al.*, 1981; Dunkel *et al.*, 1981). *In vivo* assays in mice assessing dominant lethality and sperm head abnormalities have been negative (see Table 5; McGregor, 1981). *In vivo* tests in *Drosophila melanogaster* have produced evidence of sex-linked recessive lethal mutations and reciprocal translocations (Knaap *et al.*, 1982; NTP, 1988). 1,2-Epoxybutane did not exhibit covalent binding to DNA when tritiated compound was administered to the skin of ICR/Ha female mice (Paul and Pavelka, 1971).

Table 3. Non-Mammalian Species, Genotoxicity Tests *In Vitro*.

Species, Strain	Results	Reference
Gene Mutations: *Bacteria*		
Salmonella typhimurium, TA98, reverse mutation (frameshift)	Negative	Simmon, 1979b; De Flora, 1981; Gervasi *et al.*, 1985; Canter *et al.*, 1986; Katz *et al.*, 1980 NTP, 1988
Salmonella typhimurium, TA100, reverse mutation (base-pair substitution)	Positive	McCann *et al.*, 1975; Speck and Rosenkranz, 1976; Henschler *et al.*, 1977; De Flora, 1979; McMahon *et al.*, 1979; Katz *et al.*, 1980; De Flora, 1981; De Flora *et al.*, 1984; Gervasi *et al.*, 1985; Canter *et al.*, 1986; McGregor *et al.*, 1989; NTP, 1988
	Negative	Simmon, 1979b; Dunkel *et al.*, 1984; Rosman *et al.*, 1987
Salmonella typhimurium, TA100-FR1, reverse mutation	Positive	Rosenkranz and Speck, 1975
Salmonella typhimurium, TA1530, reverse mutation	Positive	Chen *et al.*, 1975

Species, Strain	Results	Reference
(base-pair substitution)		
Salmonella typhimurium, TA1535, reverse mutation (base-pair substitution)	Positive	McCann *et al.*, 1975; McMahon *et al.*, 1979; Rosenkranz and Poirier, 1979; Katz *et al.*, 1980; De Flora, 1981; Weinstein *et al.*, 1981; De Flora *et al.*, 1984; Canter *et al.*, 1986; Rosman *et al.*, 1987; NTP, 1988; McGregor *et al.*, 1989
	Negative	Simmon, 1979b; Dunkel *et al.*, 1984
Salmonella typhimurium, TA1535 / pSK1002, prophage, induction / SOS response / strand-break / or cross-links	Positive (induction of *umu* gene expression)	Nakamura *et al.*, 1987
Salmonella typhimurium, TA1536, reverse mutation (frameshift)	Negative	Simmon, 1979b
Salmonella typhimurium, TA1537, reverse mutation (frameshift)	Negative	Simmon, 1979b; Katz *et al.*, 1980; De Flora, 1981; Dunkel *et al.*, 1984; Canter *et al.*, 1986; NTP, 1988
Salmonella typhimurium, TA1538, reverse mutation (frameshift)	Negative	Rosenkranz and Poirier, 1979; Simmon, 1979a; Dunkel *et al.*, 1984; De Flora, 1981; NTP, 1988
Escherichia coli WP2 *uvrA*, reverse mutation	Positive	McMahon *et al.*, 1979
	Negative	Dunkel *et al.*, 1984
Escherichia coli pol A, differential toxicity	Positive	Rosenkranz and Poirier, 1979; McCarroll *et al.*, 1981
Escherichia coli rec, differential toxicity	Positive	McCarroll *et al.*, 1981

Species, Strain	Results	Reference
Escherichia coli PQ37, SOS-Chromotest	Negative	von der Hude *et al.*, 1990
Klebsiella pneumoniae, forward mutation	Positive	Voogd *et al.*, 1981; Knaap *et al.*, 1982
Gene Mutations: *Fungi*		
Saccharomyces cerevisiae D3, homozygosis	Positive	Simmon, 1979a
Schizosaccharomyces pombe	Positive	Migliore *et al.*, 1982; Rossi *et al.*, 1983
Neurospora crassa, reverse mutation	Positive	Kolmark and Giles, 1955

Table 4. Mammalian Species, Genotoxicity Tests *In Vitro*.

Species (Strain)	Cell Type	Results	Reference
Rat	Primary hepatocytes	Negative (unscheduled DNA synthesis)	Williams *et al.*, 1982
	RLV/Fischer rat embryo cells	Positive (cell transformation)	Price and Mishra, 1980; Dunkel *et al.*, 1981
Mouse	L5178Y lymphoma cells	Positive with and without S9 (*tk* locus)	Amacher *et al.*, 1980; McGregor *et al.*, 1987; Mitchell *et al.*, 1988; Myhr and Caspary, 1988; NTP, 1988
	L5178Y lymphoma cells	Positive with S9 (*hprt* locus)	Knaap *et al.*, 1982
	Balb/c 3T3 cells	Negative (cell transformation)	Dunkel *et al.*, 1981
Hamster	Chinese hamster V79	Positive (sister chromatid exchange)	von der Hude *et al.*, 1991
	Chinese hamster ovary cells	Positive (sister chromatid exchange)	NTP, 1988; Anderson *et al.*, 1990
	Chinese hamster ovary cells	Positive, although weak (chromosomal aberrations)	NTP, 1988; Anderson *et al.*, 1990
	Syrian hamster embryo cells	Positive (clonal assay)	Pienta *et al.*, 1981
	Syrian hamster embryo cells	Positive (focus assay)	Dunkel *et al.*, 1981

Table 5. Mammalian Species, Genotoxicity Tests *In Vivo*.

Species, Strain	Route	Cell Type	Results	Reference
Mouse	Inhalation	Sperm cells	Negative (dominant lethal)	McGregor, 1981
			Negative (sperm head abnormality)	McGregor, 1981

Table 6. Non-Mammalian Species, Genotoxicity Tests *In Vivo*.

Species	Test	Results	Reference
Drosophila melanogaster	Eye mosaic assay	Positive	Vogel and Nivard, 1993
	Sex-linked recessive lethal test	Positive	Knaap *et al.*, 1982; Yoon *et al.*, 1985; NTP, 1988
	Reciprocal translocations	Positive	Yoon *et al.*, 1985; NTP, 1988

3.3.2 Structure-Activity Comparisons

As a short-chain epoxide compound, 1,2-epoxybutane shares structural homology with other epoxide compounds including propylene oxide and ethylene oxide (see Figure 1). Both propylene oxide and ethylene oxide are on the Proposition 65 list of chemicals known to cause cancer. Like 1,2-epoxybutane, propylene oxide and ethylene oxide are direct-acting alkylating agents.

Figure 1. Chemicals Structurally Related to 1,2-Epoxybutane.

1,2-Epoxybutane   **Propylene Oxide**   **Ethylene Oxide**
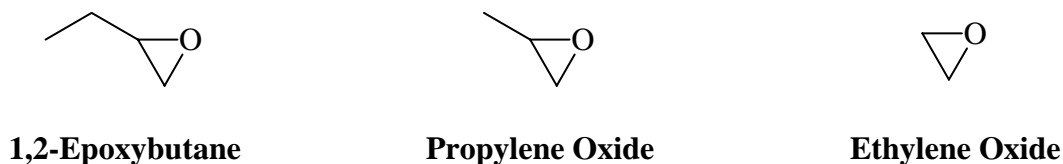
Propylene oxide is identified by NTP as "reasonably anticipated to be a human carcinogen" based upon evidence in experimental animals showing the development of tumors from inhalation exposure of mice (hemangiomas or hemangiocarcinomas of the nasal cavity) and rats (papillary adenomas of the nasal turbinates) (NTP, 2002). Inhalation exposure of weanling rats to propylene oxide led to increased incidence of adrenal pheochromocytomas and peritoneal mesotheliomas. Female rats administered propylene oxide by gavage developed an increased incidence of forestomach tumors.

Ethylene oxide is identified by NTP as "known to be a human carcinogen" based upon epidemiological evidence of lymphatic, hematopoietic, and breast cancer and evidence in experimental animals showing the development of tumors in mice (lung, hematopoietic system, harderian gland, mammary gland, uterus) and rats (hematopoietic system, brain, mesothelium) (NTP, 2002).

3.3.3 Pharmacokinetics and Metabolism

Information regarding the phamacokinetics and metabolism of 1,2-epoxybutane is limited. Female rats orally administered a single dose of 180 mg 1,2-epoxybutane per kg bodyweight

excreted 11% of the administered dose to the urine as 2-hydroxybutyl mercapturic acid (James *et al.*, 1968). In the same study, female rabbits orally administered 137 mg 1,2-epoxybutane per kg bodyweight excreted 4% as 2-hydroxylbutyl mercapturic acid.

3.3.4 Pathology

NTP described the adenomas of the nasal cavity appearing in the male and female rats as "exophytic papillary growths of a cuboidal to columnar nonciliated epithelium which were attached to the underlying mucosa by thin stalks or broad bases," with "no evidence of local invasive growth" (NTP, 1988). The lung tumors observed in rats were considered by NTP to meet standard criteria for alveolar / bronchiolar adenomas and carcinomas. Alveolar/bronchiolar adenomas and carcinomas are considered to be related in origin. Lung adenomas can progress to carcinomas, therefore, these tumor types are combined for purposes of carcinogen identification and risk assessment.

3.4   Mechanism

The demonstrated mutagenicity of 1,2-epoxybutane – specifically the induction of base-pair substitutions in bacterial DNA and cytogenetic damage in mammalian cells – strongly implicates DNA damage as a mode of action for carcinogenicity. Although very limited evidence has not shown the presence of alkyl adducts of 1,2-epoxybutane with DNA, the chemically reactive nature of the short-chain epoxides indicates a potential for this compound to interact directly with DNA. Two other alkylating agents with structural similarity to 1,2-epoxybutane produce tumors at the same sites: propylene oxide produces nasal tumors and ethylene oxide produces lung tumors in experimental animals. This provides some indirect evidence of a common mode of action.

In 1989, the International Agency for Research on Cancer (IARC) classified 1,2-epoxybutane as "not classifiable at to its carcinogenicity to humans (Group 3)" based on limited evidence in experimental animals and no data from studies in humans (IARC, 1989). In a 1999 reassessment, however, the IARC Working Group noted that "1,2-epoxybutane is a direct-acting alkylating agent which is mutagenic in a range of systems," and revised the classification to "possibly carcinogenic to humans (Group 2B)," based on limited evidence in experimental animals, no data in humans, and the genotoxicity findings (IARC, 1999).

4   SUMMARY AND CONCLUSIONS

4.1   Summary of Evidence

Extensive genotoxicity data from *in vitro* bacterial and mammalian cell assays of 1,2-epoxybutane suggest a potential to cause DNA damage as evidenced by increases in base-pair substitution mutations and cytogenetic changes. In male rats treated for two years by inhalation with 1,2-epoxybutane, lung carcinomas and rare nasal adenomas were significantly increased. Female rats exposed similarly developed a small number of these rare nasal adenomas. A gavage study with combined oral exposure of mice to 1,2-epoxybutane-stabilized

trichloroethylene suggested a carcinogenic effect on the forestomach of the mice when compared with mice treated with trichloroethylene alone; however, interpretation of this study is complicated by the carcinogenicity of trichloroethylene itself, although trichloroethylene is not known to cause forestomach tumors. 1,2-Epoxybutane is also structurally similar to both propylene oxide and ethylene oxide, both chemicals known to cause cancer.

In making a determination as to whether 1,2-epoxybutane should be established as "known to cause cancer," several issues must be considered. Of primary concern is whether the appearance of a small, but significant, increase in rare benign nasal adenomas in the 1,2-epoxybutane treated male rats with supporting, but less robust, evidence in female rats for nasal adenomas is indicative of the compound's potential to cause malignant disease. Male rats showed a significant increase in alveolar / bronchiolar carcinomas. The importance of the absence of a tumorigenic response in mice, which did show widespread evidence of toxicity to the nasal cavity, also needs to be considered. Important context for this determination is the known chemically reactive character of epoxide chemicals as alkylating agents coupled with extensive evidence of genotoxic potential including not only evidence of base-pair substitutions in bacteria, but also cytogenetic and transforming changes to mammalian cells. The degree to which the highly structurally similar carcinogens propylene oxide and ethylene oxide, which also produce nasal tumors and lung tumors, respectively, supports the findings with 1,2-epoxybutane must also be considered.

As noted by IARC, "in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents and mixtures for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans. The possibility that a given agent may cause cancer through a species-specific mechanism which does not operate in humans…should also be taken into consideration" (IARC, 1999). Extensive evidence supports genotoxicity as a plausible mode of action and no evidence of species-specificity has been identified for 1,2-epoxybutane. Therefore the data from studies in animals should be considered to be relevant to humans.

4.2   Conclusion

Evidence of mutagenicity and the broader genotoxicity of 1,2-epoxybutane, as well as its structural similarity to other carcinogens implicated 1,2-epoxybutane as a potential carcinogen prior to its testing in long-term inhalation bioassays. These bioassays subsequently revealed evidence for the carcinogenicity of 1,2-epoxybutane including the development of lung carcinomas and rare nasal adenomas in male rats and a small number of rare nasal adenomas in female rats treated for two years by inhalation.

5   REFERENCES

Amacher DE, Paillet SC, Turner GN, Ray VA, Salsburg DS (1980). Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation. *Mutat Res* **72**(3):447-74.

Anderson EL and the U.S. Environmental Protection Agency Carcinogen Assessment Group. (1983). Quantitative approaches in use to assess cancer risk. *Risk Anal* **3**:277-295.

Anderson BE, Zeiger E, Shelby MD, Resnick MA, Gulati DK, Ivett JL *et al*. (1990). Chromosome aberration and sister chromatid exchange test results with 42 chemicals. *Environ Mol Mutagen* **16 Suppl 18**:55-137.

Canter DA, Zeiger E, Haworth S, Lawlor T, Mortelmans K, Speck W (1986). Comparative mutagenicity of aliphatic epoxides in *Salmonella*. *Mutat Res* **172**(2):105-38.

Chen CC, Speck WT, Rosenkranz HS (1975). Mutagenicity testing with *Salmonella typhimurium* strains. II. The effect of unusual phenotypes on the mutagenic response. *Mutat Res* **28**(1):31-5.

De Flora S (1979). Metabolic activation and deactivation of mutagens and carcinogens. *Ital J Biochem* **28**(2):81-103.

De Flora S (1981). Study of 106 organic and inorganic compounds in the *Salmonella*/microsome test. *Carcinogenesis* **2**(4):283-98.

De Flora S, Zanacchi P, Camoirano A, Bennicelli C, Badolati GS (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. *Mutat Res* **133**(3):161-98.

Dunkel VC, Pienta RJ, Sivak A, Traul KA (1981). Comparative neoplastic transformation responses of Balb/3T3 cells, Syrian hamster embryo cells, and Rauscher murine leukemia virus-infected Fischer 344 rat embryo cells to chemical compounds. *J Natl Cancer Inst* **67**(6):1303-12.

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K *et al*. (1984). Reproducibility of microbial mutagenicity assays: I. Tests with *Salmonella typhimurium* and *Escherichia coli* using a standardized protocol. *Environ Mutagen* **6 Suppl 2**:1-251.

Gervasi PG, Citti L, Del Monte M, Longo V, Benetti D (1985). Mutagenicity and chemical reactivity of epoxidic intermediates of the isoprene metabolism and other structurally related compounds. *Mutat Res* **156**(1-2):77-82.

Henschler D, Eder E, Neudecker T, Metzler M (1977). Carcinogenicity of trichloroethylene: fact or artifact? *Arch Toxicol* **37**(3):233-6.

Henschler D, Elsasser H, Romen W, Eder E (1984). Carcinogenicity study of trichloroethylene, with and without epoxide stabilizers, in mice. *J Cancer Res Clin Oncol* **107**(3):149-56.

Huff JE (1994). Chemicals causally associated with cancers in humans and laboratory animals. A perfect concordance. In: Carcinogenesis. Waalkes MP, Ward JM, eds. New York: Raven Press.

pp. 25-37.

IARC (1989). International Agency for Research on Cancer. 1,2-Epoxybutane. *IARC Monogr Eval Carcinog Risks Hum* **47**:217-28.

IARC (1999). International Agency for Research on Cancer. 1,2-Epoxybutane. *IARC Monogr Eval Carcinog Risks Hum* **71 Pt 2**:629-40.

James SP, Jeffery DA, Waring RH, Wood PB (1968). Some metabolites of 1-bromobutane in the rabbit and the rat. *Biochem J* **109**(5):727-36.

Katz M, Kazmer S, Weinstein D (1980). An analysis of weak mutagens in the Ames assay. *J Environ Pathol Toxicol* **3**(5-6):171-87.

Knaap AG, Voogd CE, Kramers PG (1982). Comparison of the mutagenic potency of 2-chloroethanol, 2-bromoethanol, 1,2-epoxybutane, epichlorohydrin and glycidaldehyde in *Klebsiella pneumoniae*, *Drosophila melanogaster* and L5178Y mouse lymphoma cells. *Mutat Res* **101**(3):199-208.

Kolmark G, Giles NH (1955). Comparative studies of monoepoxides as inducers of reverse mutations in *Neurospora*. *Genetics* **40**:890-902.

McCann J, Choi E, Yamasaki E, Ames BN (1975). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. *Proc Natl Acad Sci U S A* **72**(12):5135-9.

McCarroll NE, Piper CE, Keech BH (1981). An *E coli* microsuspension assay for the detection of DNA damage induced by direct-acting agents and promutagens. *Environ Mutagen* **3**(4):429-44.

McGregor DB (1981). Tier II Mutagenic Screening of 13 NIOSH Priority Compounds: Individual Compound Report: Butylene Oxide. National Institute for Occupational Safety and Health, Cincinnati, OH. *NIOSH Report 28*.

McGregor DB, Martin R, Cattanach P, Edwards I, McBride D, Caspary WJ (1987). Responses of the L5178Y tk⁺/tk⁻ mouse lymphoma cell forward mutation assay to coded chemicals. I: Results for nine compounds. *Environ Mutagen* **9** (2):143-60.

McGregor DB, Reynolds DM, Zeiger E (1989). Conditions affecting the mutagenicity of trichloroethylene in *Salmonella*. *Environ Mol Mutagen* **13**(3):197-202.

McMahon RE, Cline JC, Thompson CZ (1979). Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. *Cancer Res* **39**(3):682-93.

Migliore L, Rossi AM, Loprieno N (1982). Mutagenic action of structurally related alkene oxides on *Schizosaccharomyces pombe*: the influence, 'in vitro', of mouse-liver metabolizing system. *Mutat Res* **102**(4):425-37.

Mitchell AD, Rudd CJ, Caspary WJ (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at SRI International. *Environ Mol Mutagen* **12 Suppl 13**:37-101.

Myhr BC, Caspary WJ (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at Litton Bionetics, Inc. *Environ Mol Mutagen* **12 Suppl 13**:103-94.

Nakamura SI, Oda Y, Shimada T, Oki I, Sugimoto K (1987). SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination with 151 chemicals. *Mutat Res* **192**(4):239-46.

National Institute for Occupational Safety and Health (NIOSH, 1990). National Occupational Exposure Survey (1981-1983). Last updated July 1, 1990. Estimated Numbers of Employees Potentially Exposed to Specific Agents by 2-Digit Standard Industrial Classification (SIC). Ethyl Oxirane, CAS No. 106-887. [cited 2004 May 4] Available from: URL: http://www.cdc.gov/noes/noes1/x9126sic.html.

National Toxicology Program (NTP, 1988). NTP Toxicology and Carcinogenesis Studies of 1,2-Epoxybutane (CAS No. 106-88-7) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). *Natl Toxicol Program Tech Rep Ser* **329**:1-176.

National Toxicology Program (NTP, 1999). NTP Historical Control Information for the NIH-07 Diet. Last updated Dec 1999. [cited 2004 May 3] Available from: URL: http://ehis.niehs.nih.gov/ntp/docs/ntp_hcrs.html.

National Toxicology Program (NTP, 2002). Report on Carcinogens, Tenth Edition. U.S. Department of Health and Human Services, Public Health Service. December 2002.

Paul JS, Pavelka MA (1971). Covalent binding of a carcinogenic epoxide to DNA *in vivo*. Abstract #448. *Fed Proc* **30**.

Pienta RJ, Lebherz WB III, Schuman RF (1981). The use of cryopreserved Syrian hamster embryo cells in a transformation test for detecting chemical carcinogens. In: Stich HF, San RHC, eds. *Short-Term Tests for Chemical Carcinogens*. Berlin: Springer-Verlag, 1981:323-37.

Price PJ, Mishra NK (1980). The use of Fischer rat embryo cells as a screen for chemical carcinogens and the role of the nontransforming type 'C' RNA tumor viruses in the assay. *Adv Mod Environ Toxicol* **1**:213-9.

Rosenkranz HS, Poirier LA (1979). Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. *J Natl Cancer Inst* **62**(4):873-92.

Rosenkranz HS, Speck WT (1975). Mutagenicity of metronidazole: activation by mammalian liver microsomes. *Biochem Biophys Res Commun* **66**(2):520-5.

Rosman LB, Gaddamidi V, Sinsheimer JE (1987). Mutagenicity of aryl propylene and butylene oxides with *Salmonella*. *Mutat Res* **189**(3):189-204.

Rossi AM, Migliore L, Barale R, Loprieno N (1983). *In vivo* and *in vitro* mutagenicity studies of a possible carcinogen, trichloroethylene, and its two stabilizers, epichlorohydrin and 1,2-epoxybutane. *Teratog Carcinog Mutagen* **3**(1):75-87.

Simmon VF (1979a). *In vitro* assays for recombinogenic activity of chemical carcinogens and related compounds with *Saccharomyces cerevisiae* D3. *J Natl Cancer Inst* **62**(4):901-9.

Simmon VF (1979b). *In vitro* mutagenicity assays of chemical carcinogens and related compounds with *Salmonella typhimurium*. *J Natl Cancer Inst* **62**(4):893-9.

Speck WT, Rosenkranz HS (1976). Mutagenicity of azathioprine. *Cancer Res* **36**(1):108-9.

Tomatis L, Aitio A, Wilbourn J, Shuker L (1989). Human carcinogens so far identified. *Jpn J Cancer Res* **80**(9):795-807.

U.S. Environmental Protection Agency (U.S. EPA, 1990). 1990 HPV Challenge Program Chemical List. Revised January 16, 2004. [cited 2004 May 21] Available from: URL: http://www.epa.gov/chemrtk/hpv_1990.pdf.

U.S. Environmental Protection Agency (U.S. EPA, 1998). Toxics Substances Control Act (TSCA) Inventory Update Rule Information Collection. Office of Pollution Prevention and Toxics.

Van Duuren BL, Langseth L, Goldschmidt BM, Orris L (1967). Carcinogenicity of epoxides, lactones, and peroxy compounds. VI. Structure and carcinogenic activity. *J Natl Cancer Inst* **39**(6):1217-28.

Vogel EW, Nivard MJ (1993). Performance of 181 chemicals in a *Drosophila* assay predominantly monitoring interchromosomal mitotic recombination. *Mutagenesis* **8**(1):57-81.

von der Hude W, Carstensen S, Obe G (1991). Structure-activity relationships of epoxides: induction of sister-chromatid exchanges in Chinese hamster V79 cells. *Mutat Res* **249**(1):55-70.

von der Hude W, Seelbach A, Basler A (1990). Epoxides: comparison of the induction of SOS

repair in *Escherichia coli* PQ37 and the bacterial mutagenicity in the Ames test. *Mutat Res* **231**(2):205-18.

Voogd CE, van der Stel JJ, Jacobs JJ (1981). The mutagenic action of aliphatic epoxides. *Mutat Res* **89**(4):269-82.

Weinstein D, Katz M, Kazmer S (1981). Use of rat/hamster S-9 mixture in the Ames mutagenicity assay. *Environ Mutagen* **3**(1):1-9.

Williams GM, Laspia MF, Dunkel VC (1982). Reliability of the hepatocyte primary culture/DNA repair test in testing of coded carcinogens and noncarcinogens. *Mutat Res* **97**(5):359-70.

Yoon JS, Mason JM, Valencia R, Woodruff RC, Zimmering S (1985). Chemical mutagenesis testing in *Drosophila*. IV. Results of 45 coded compounds tested for the National Toxicology Program. *Environ Mutagen* **7**(3):349-67.