NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for TETRAFLUOROETHYLENE

FINAL
MARCH 1999

Prepared for

the October 30-31, 1997,
Meeting of the Report on Carcinogens Subcommittee
of the NTP Board of Scientific Counselors

Prepared by

Integrated Laboratory Systems
Post Office Box 13501
Research Triangle Park, North Carolina 27709
NIEHS Contract No. N01-ES-25346

TABLE OF CONTENTS

NTP Report on Carcinogens Listing for Tetrafluoroethylene 1
Listing Criteria from the Report on Carcinogens, Eighth Edition 2

1.0 CHEMICAL PROPERTIES 3
1.1 Chemical Identification 3
1.2 Physical-Chemical Properties 3

2.0 HUMAN EXPOSURE 4
2.1 Production 4
2.2 Use 4
2.3 Environmental Exposure 4
2.4 Occupational Exposure 4
Table 2-1 NIOSH National Occupational Exposure Survey (NOES, 1980-83): By Industry 4
2.5 Regulations 5

3.0 HUMAN STUDIES 8

4.0 EXPERIMENTAL CARCINOGENICITY 8
4.1 Mice 8
4.2 Rats 9

5.0 GENOTOXICITY 9
5.1 Prokaryotic Systems 10
5.2 *In Vitro* Mammalian Systems 10
5.3 *In Vivo* Mammalian Systems 10
Table 5-1 Summary of Tetrafluoroethylene Genotoxicity Studies 11

6.0 OTHER RELEVANT DATA 12
6.1 Absorption, Distribution, and Excretion 12
6.2 Metabolism 12
6.3 Pharmacokinetics 13
6.4 Structure-Activity Relationships 13
6.4.1 Cysteine *S*-Conjugates 13
6.4.2 Tetrachloroethylene 13
6.5 Tumor Mutations 14

7.0 MECHANISMS OF CARCINOGENESIS 14
Figure 7-1 Proposed Mechanism for the Metabolic Activation of TFE in the Male Rat 15

8.0 REFERENCES 15

APPENDIX A - Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Volume 19 (Some Monomers, Plastics and Synthetic Elastomers, and Acrolein), Tetrafluoroethylene and Polytetrafluoroethylene, pp. 285-301, 1979 ..... A-1

APPENDIX B - Excerpts from the NTP Technical Report, Toxicology and Carcinogenesis Studies of Tetrafluoroethylene in F344/N Rats and B6C3F1 Mice (Inhalation Studies), April 1997, pp. 3-32, 69-78 ..... B-1

APPENDIX C - Description of Online Searches for Tetrafluoroethylene C-1

APPENDIX D - Report on Carcinogens (RoC), 9th Edition Review Summary D-1

NTP Report on Carcinogens Listing for Tetrafluoroethylene

Carcinogenicity

Tetrafluoroethylene (TFE) is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of malignant tumor formation at multiple sites in multiple species of experimental animals (NTP, 1997). When administered by inhalation to F344 rats, TFE induced renal tubule neoplasms, hepatocellular neoplasms, liver hemangiosarcoma, and mononuclear cell leukemia. When administered by inhalation to B6C3F$_1$ mice, TFE induced liver hemangiomas and hemangiosarcomas, hepatocellular neoplasms, and histiocytic sarcomas.

There are no adequate data available to evaluate the carcinogenicity of TFE in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

In prokaryotic systems, TFE was negative for the induction of gene mutations in *Salmonella typhimurium* with and without S9 activation (Haskell Laboratory, 1986a; cited by HSDB, 1997). In mammalian systems *in vitro*, TFE was also negative for the induction of gene mutations in Chinese hamster ovary cells (Haskell Laboratory, 1986b; cited by HSDB, 1997). No increases in the frequency of micronucleated erythrocytes were observed in peripheral blood samples obtained from TFE-exposed mice (NTP, 1997).

The frequency of H *ras* codon 61 mutations observed in TFE-induced hepatocellular neoplasms (15%) was significantly less than the corresponding frequency (56-59%) in spontaneous liver neoplasms of B6C3F$_1$ mice, suggesting that TFE induces liver neoplasms via a *ras*-independent pathway (NTP, 1997).

The kidney-specific toxicity and carcinogenicity of TFE is most likely related to the selective uptake and subsequent processing of TFE-glutathione conjugates by renal β-lyase (Miller and Surh, 1994; Anders et al., 1988). In rats, a TFE cysteine conjugate is bioactivated in the kidney to a difluorothionacetyl fluoride, the putative reactive metabolite for TFE-induced nephrotoxicity (NTP, 1997).

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

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

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 A Human Carcinogen:

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

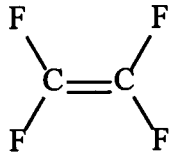
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to 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.

1.0 CHEMICAL PROPERTIES

Tetrafluoroethylene
[116-14-3]



1.1 Chemical Identification

Tetrafluoroethylene (C_2F_4, mol. wt. = 100.02) is also called:

Ethene, tetrafluoro- (9CI)
Ethylene, tetrafluoro- (8CI)
Ethylene, tetrafluoro- (inhibited)
Fluoroplast 4
Perfluoroethene
Perfluoroethylene
Tetrafluoroethene
Tetrafluoroethylene, inhibited (DOT)
1,1,2,2-Tetrafluoroethylene
TFE (VAN)

Tetrafluoroethylene has a UN shipping number of 1081.

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Colorless	Lewis (1992)
Physical State	Gas	Lewis (1992)
Melting Point, °C	-142.5	Lewis (1992)
Boiling Point, °C	-78.4	Lewis (1992)
Specific Gravity	1.519	Weast and Astle (1980)
Odor	Odorless to faint odor	Radian (1991)
Solubility:		
Water	Insoluble in water	Kennedy (1990)
Vapor pressure at 21.1 °C, mm Hg	22800	Radian (1991)

TFE is a highly flammable gas when exposed to heat or flame. When heated to decomposition, it emits highly toxic fluorocarbon fumes. Air and TFE will form a flammable mixture when the TFE molar percentage is between 6.5 and 46% (Fiumara, 1989) or the volume percentage is between 14 and 43% at ambient room temperature and pressure. It will explode at pressures above 2.7 bar (39.15 lb/in.^{2}, 27540 kg/m^3) if a terpene inhibitor is not added. Examples of terpene inhibitors used are *d*-limonene and terpene B (Gangal, 1980). TFE will also react violently with oxygen (Lewis, 1992). In the absence of oxygen, the violent thermal decomposition of TFE gives carbon and carbon tetrafluoride (Gangal, 1980).

2.0 HUMAN EXPOSURE

2.1 Production

TFE is mainly produced by the pyrolysis of chlorodifluoromethane or trifluoromethane (NTP, 1997). In 1996, two companies, ICI Americas, Inc., and PCR Inc., were listed by SRI International (1996) as producers of TFE.

2.2 Use

TFE is used primarily in the synthesis of polytetrafluoroethylene (Kennedy, 1990). It is also used to produce copolymers with monomers such as hexafluoropropylene and ethylene (Carson et al., 1986).

2.3 Environmental Exposure

No TFE was detected in the airspace of storage bags made from TFE-hexafluoropropylene copolymer. A gas chromatograph/mass spectrometer with a detection limit of 0.002 ppm was used (Kelly et al., 1985; cited by Kennedy, 1990). It has been reported to be present, along with several other low-molecular weight halogenated compounds, in volcanic emissions (Stoiber, 1971; cited by Gribble, 1994).

2.4 Occupational Exposure

According to NIOSH (National Institute of Occupational Safety and Health), the National Occupational Exposure Survey (NOES), conducted between 1980-1983, listed a total of 14,963 employees (including 325 female) potentially exposed to TFE in 870 plants (See Table 2-1) (NIOSH, 1990).

Table 2-1. NIOSH National Occupational Exposure Survey (NOES, 1980-83)[a]: By Industry

Industry	Number of Plants	Number of Employees	Number of Female Employees
Paper and Allied Products	60	510	
Printing and Publishing	807	14121	181
Transportation Equipment	3	332	145
Total	870	14963	326

[a] NIOSH (1990)

2.5 Regulations

The U. S. EPA regulates TFE under the Clean Air Act (CAA). It considers the compound a regulated flammable substance and designates a threshold quantity of 10,000 lb for accidental release prevention. FDA approves TFE polymers and copolymers for food-related uses. OSHA regulates the substance in the workplace. It has placed TFE in its list of toxic and reactive highly hazardous chemicals which have a potential for a catastrophic event at or above a designated threshold quantity (TQ); for TFE, the TQ is 5,000 lb.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 68—PART 68—CHEMICAL ACCIDENT PREVENTION PROVISIONS. Promulgated: 59 FR 4493, 01/31/94. U.S. Code: 42 U.S.C. 7412(r), 7601(a)(1), 7661-7661f.	This part sets forth the list of regulated substances and thresholds, the petition process for adding or deleting substances to the list of regulated substances, the requirements for owners or operators of stationary sources concerning the prevention of accidental releases, and the State accidental release prevention programs approved under section 112(r).
	40 CFR 68—Subpart F—Regulated Substances for Accidental Release Prevention.	
	40 CFR 68.130—Sec. 68.130 List of substances.	TFE is listed as a regulated flammable substance; its threshold quantity for accidental release prevention is 10,000 lb.
F D A	21 CFR 173—PART 173—SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION. Promulgated: 42 FR 14526, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, and 348.	
	21 CFR 173—Subpart A—Polymer Substances and Polymer Adjuvants for Food Treatment.	

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
F D A	21 CFR 173.21—Sec. 173.21 Perfluorinated ion exchange membranes. Promulgated: 59 FR 15623, 04/04/94.	Membrane, as copolymers of ethanesulfonyl fluoride, 2-[1-[difluoro-[(trifluoroethenyl)-oxy]methyl]-1,2,2,2-tetrafluoroethoxy]-1, 1,2,2,-tetrafluoro-, with TFE, may be safely used as ion exchange membranes intended for use in the treatment of bulk quantities of liquid food.
	21 CFR 177—PART 177—INDIRECT FOOD ADDITIVES: POLYMERS. Promulgated: 42 FR 14572, 03/15/77. U.S. Code: 21 U.S.C. 321, 342, 348, and 379e.	
	21 CFR 177—Subpart B—Substances for Use as Basic Components of Single and Repeated Use Food Contact Surfaces.	
	21 CFR 177.1380—Sec. 177.1380 Fluorocarbon resins. Promulgated: 42 FR 14572, 03/15/77, as amended at 57 FR 185, 01/03/92.	TFE may be used as a polymer to produce fluorocarbons resins that may be safely used as articles or components of articles intended for use in contact with food.
	21 CFR 177.1550—Sec. 177.1550 Perfluorocarbon resins. Promulgated: 43 FR 44834, 09/29/78 through 61 FR 14481, 04/02/96.	Perfluorocarbon resins produced using TFE as a polymer may be safely used as articles or components of articles intended for use in contact with food.
	21 CFR 177—Subpart C—Substances for Use Only as Components of Articles Intended for Repeated Use.	
	21 CFR 177.2400—Sec. 177.2400 Perfluorocarbon cured elastomers. Promulgated: 49 FR 43050, 10/26/84.	Perfluorocarbon cured elastomers may be safely used as articles or components of articles intended for repeated use in contact with nonacid food (pH >5.0), provided that the perfluorocarbon based polymer contains no less than 40 wt. % of polymer units derived from TFE.

REGULATIONS[a]

	Regulatory Action	Effect of Regulation/Other Comments
O S H A	29 CFR 1910—PART 1910—OCCUPATIONAL SAFETY AND HEALTH STANDARDS. Promulgated: 39 FR 23502, 06/27/74. U.S. Code: 29 U.S.C. 653, 655, and 657.	
	29 CFR 1910—Subpart H—Hazardous Materials.	
	29 CFR 1910.119—Sec. 1910.119 Process safety management of highly hazardous chemicals. Promulgated: 57 FR 6403, 02/24/92; 57 FR 7847, 03/04/92, as amended at 61 FR 9238, 03/07/96.	This section contains requirements for preventing or minimizing the consequences of catastrophic releases of toxic, reactive, flammable, or explosive chemicals. These releases may result in toxic, fire or explosion hazards. TFE is listed as a toxic and reactive highly hazardous chemical, which has a potential for a catastrophic event at or above the threshold quantity of 5,000 lb.
	29 CFR 1926—PART 1926—SAFETY AND HEALTH REGULATIONS FOR CONSTRUCTION. Promulgated: 44 FR 8577, 02/09/79; 44 FR 20940, 04/06/79.	
	29 CFR 1926—Subpart D—Occupational Health and Environmental Control.	
	29 CFR 1926.64—Sec. 1926.64 Process safety management of highly hazardous chemicals. Promulgated: 58 FR 35115, 06/30/93.	The requirements applicable to construction work under this section are the same as those specified in section 1910.119.

[a]The regulations in this table have been updated through the 1998 issues of Code of Federal Regulations titles 21, 29, and 40.

3.0 HUMAN STUDIES

IARC (1979, p. 288; see Appendix A) reported no human data for TFE. No additional information on human studies of TFE was located. [Literature searches from 1994 through July 1997 were conducted in TOXLINE, CANCERLIT, MEDLINE, EMBASE, BIOSIS, and Current Contents to update the literature search for the NTP bioassay (NTP, 1997) which was stated to be current as of 1995, the date of the publicly available draft.]

4.0 EXPERIMENTAL CARCINOGENICITY

No experimental carcinogenicity studies prior to 1979 were reported by IARC (1979, pp. 288; see Appendix A). In the NTP inhalation bioassay (NTP, 1997, pp. 5-11; see Appendix B), mice and rats chronically exposed to TFE via inhalation for 2 years had an increased incidence of neoplasms. Male and female mice ($B6C3F_1$) showed a significant increase in liver hemangiomas and hemangiosarcomas, hepatocellular neoplasms, and histiocytic sarcomas. Male and female rats (F344/N) exposed to similar levels for a similar duration showed an increased incidence of renal tubule and hepatocellular neoplasms, liver hemangiosarcomas, and mononuclear cell leukemia. Other experimental carcinogenicity studies were not found.

4.1 Mice

A 2-year mouse bioassay was conducted (NTP, 1997) in which groups of 58 male and female mice (B6C3F1) were exposed by inhalation to 312, 625, or 1250 ppm (1280, 2560, or 5110 mg/m^3) TFE for 6 hours per day, 5 days per week, for 95 weeks. A 15-month interim evaluation was done with 10 males and 10 females.

At this time point, a variety of liver neoplasms was observed in exposed males and females. Hemangiosarcoma was detected in males exposed to 1250 ppm and females exposed to 312 ppm. In males, adenoma and carcinoma were observed in all dose groups, and multiple adenomas were detected after exposure to 625 ppm. In females, adenoma and carcinoma were observed in all dose groups, and multiple adenoma were detected after exposure to 1250 ppm.

In the 2-year study, survival rates of all groups of exposed males and females were significantly lower than those of control animals. Because of reduced survival, the study was terminated at week 96. At study termination, neoplasms were detected in the liver and in the hematopoietic system.

In the liver, the incidence of hemangioma was significantly increased in males exposed to 312 and 625 ppm and in females exposed to 312 ppm TFE. Multiple hemangioma was significantly increased in the male 312-ppm dose group but not in the female dose group. Hemangiosarcoma was significantly increased at all doses in males and females. In addition, multiple hemangiosarcoma was significantly increased in dosed males and females.

The incidence of hepatocellular adenoma was significantly increased in females exposed to 625 ppm TFE, but not in males. Multiple hepatocellular adenoma were also significantly increased in females exposed to all doses, but not in males. Hepatocellular carcinoma and multiple hepatocellular carcinoma was significantly increased at all doses in males and females. The incidence of hepatocellular adenoma or carcinoma (combined) was also greater in all exposed males and females.

In the hematopoietic system, the incidence of histiocytic sarcoma (all organs) was significantly greater in all exposed males and females. The highest incidences of histiocytic sarcoma were seen in the liver and lung; this neoplasm was also found in the spleen, lymph nodes, bone marrow, and kidney.

4.2 Rats

Male F344/N rats in groups of 60 were exposed by inhalation to 156, 312, or 625 ppm (638, 1280, or 2560 mg/m^3) TFE. Groups of 60 F344/N female rats were exposed to 312, 625, or 1250 ppm (1280, 2560, or 5110 mg/m^3) TFE. The exposure duration was 6 hours per day, 5 days per week, for 103 weeks. An observation period of 11 days followed the final exposure. An interim evaluation at 15 months was done with 10 male and 10 female rats from each dose group (NTP, 1997).

At the 15-month interim evaluation, neoplasms were detected in the kidney and liver of males or females, but the incidence was not significantly greater than the incidence in controls. A renal tubule carcinoma was found in one high-dose male and a renal tubule adenoma was observed in two high-dose females. In addition, one high-dose female showed a malignant mixed tumor of the kidney. In the liver, hepatocellular adenoma was observed in one male exposed to 312 ppm TFE and hepatocellular carcinoma developed in one male exposed to 625 ppm TFE.

In the 2-year study, the survival rates were significantly decreased for males exposed to 625 ppm and for all exposed females. At study termination, kidney and liver neoplasms and mononuclear cell leukemia were seen in males and females.

The incidence of renal tubule adenoma in single sections was significantly increased in males exposed to 312 ppm TFE. An extended evaluation also showed a significant increase of renal tubule adenoma in males exposed to 625 ppm TFE. The incidence of renal tubule adenoma in single and step sections combined was significantly higher in males exposed to 312 and 625 ppm TFE. In females exposed to 1250 ppm TFE, the incidence of renal tubule adenoma or carcinoma was significantly increased in the standard evaluation, the extended evaluation, and when sections were combined.

Males exposed to 312 ppm TFE and females in all dose groups showed a significant increase in the incidence of hepatocellular adenoma or carcinoma (combined). The incidence of hepatocellular carcinoma was also increased in males exposed to 312 ppm and in females exposed to 312 and 625 ppm. In addition, females exposed to 625 ppm TFE showed a significant increase in the incidence of hemangiosarcoma. The incidence of mononuclear cell leukemia was significantly increased in males exposed to 156 and 625 ppm TFE, and in all exposed females.

5.0 GENOTOXICITY

No genotoxicity studies for TFE were reported by IARC (1979, p. 288; see Appendix A). Summaries of TFE genotoxicity studies published after IARC (1979) are summarized below and in **Table 5-1**. In prokaryotic systems, TFE was negative for the induction of gene mutations in *Salmonella typhimurium* and Chinese hamster ovary cells. *In vivo*, TFE did not induce *ras* mutations in mouse liver or micronuclei in mouse blood.

5.1 Prokaryotic Systems

TFE did not induce gene mutations in *S. typhimurium* strains TA1535, TA97, TA98, and TA100 both in the absence and presence of S9 metabolic activation (Haskell Laboratory, 1986a; cited by HSDB, 1997).

5.2 *In Vitro* Mammalian Systems

In Chinese hamster ovary (CHO) cells, TFE did not induce gene mutations at the *hprt* locus both with and without metabolic activation (Haskell Laboratory, 1986b; cited by HSDB, 1997).

5.3 *In Vivo* Mammalian Systems

NTP (1997, Appendix L; cited by Hong et al., 1996 abstr.; Hong et al., 1998) found no increase in *ras* mutations in the hepatocellular neoplasms induced in B6C3F1 mice by a 2-year inhalation exposure to TFE. Similarly, micronuclei were not induced in the peripheral blood normochromatic erythrocytes (NCE) of male or female B6C3F1 mice following 13 weeks of inhalation (NTP, 1997, p. 67; see Appendix B). The frequency of H *ras* codon 61 mutations observed in TFE-induced hepatocellular neoplasms (15%) was significantly less than the corresponding frequency (56-59%) in spontaneous liver neoplasms of B6C3F1 mice, suggesting that TFE induces liver neoplasms via a *ras*-independent pathway (NTP, 1997).

TABLE 5-1. SUMMARY OF TETRAFLUOROETHYLENE GENOTOXICITY STUDIES

System	Biological Endpoint	S9/Other Metabolic Activation	Form and Purity	Doses Used	Endpoint Response +/- Activation	Comments	Reference
5.1 Prokaryotic Systems							
Salmonella typhimurium strains TA1535, TA97, TA98, and TA100	*his* gene mutations (plate incorporation)	+/-	NG in source used	0.5, 3, 4, and 5% in air	negative/ negative	No positive response was observed in any tester strain.	Haskell Laboratory (1986a; cited by HSDB, 1997)
5.2 *In Vitro* Mammalian Systems							
Chinese hamster ovary (CHO) cells	*hprt* gene mutations	+/-	NG in source used	20, 40, 60, 80, and 100% in air (duration not reported in source used)	negative/ negative	No cultures produced mutant frequencies significantly greater than the solvent controls.	Haskell Laboratory (1986b; cited by HSDB, 1997)
5.3 *In Vivo* Mammalian Systems							
male and female B6C3F1 mice	*ras* oncogene mutations	n.a.	NG in source used	312, 625, and 1250 ppm (1290, 2580, and 5165 mg/m^3) via inhalation for 2 years	negative	*ras* mutations were not detected at codons 12, 13, and 61 in hepatocellular neoplasms induced by TFE.	Hong et al. (1996 abstr.); NTP (1997); Hong et al. (1998)
male and female B6C3F1 mice	micronuclei induction in peripheral blood erythrocytes	n.a.	NG in source used	1250, 2500, and 5000 ppm (5165, 10330, and 20660 mg/m^3) via inhalation for 13 weeks	negative	No significant increase was found in the number of micronucleated normochromatic erythrocytes (NCE) in either sex after 13 weeks.	NTP (1997)

n.a. = not applicable; NG = not given

6.0 OTHER RELEVANT DATA

Summary: Rabbits exposed by inhalation to TFE exhibited an alveolar absorption rate of 6.7%. The kidneys, bone, and lungs contained the highest fluoride levels following exposure. Rats and hamsters exposed to TFE by inhalation exhibited an increase in urinary fluoride.

The first step in the metabolism of TFE involves glutathione conjugation in the liver. The glutathione conjugates are then either excreted via the bile in the small intestine or are hydrolyzed to the cysteine-*S*-conjugate by renal peptidases. The cysteine-*S*-conjugate may be bioactivated by the renal enzyme cysteine-conjugate β-lyase to form reactive thiols; pyruvate and ammonia are also produced. No information on the metabolism of TFE in humans was found.

In the 2-year rodent bioassay conducted by the NTP, exposure to TFE by inhalation induced nonneoplastic changes in the liver and kidneys of mice and rats and in the spleen of mice.

Mutations in mouse hepatocellular neoplasms were not increased in the *ras* oncogene.

6.1 Absorption, Distribution, and Excretion

Rabbits exposed by inhalation to 1000 ppm (4090 mg/m^3) TFE exhibited an alveolar absorption rate of 6.7%. The kidneys, bone, and lungs contained the highest fluoride levels following exposure (Ding et al., 1980; cited by Kennedy, 1990).

Increased urinary fluoride was detected in rats exposed by inhalation to 3500 ppm (14,300 mg/m^3) TFE for 30 minutes (Dilley et al., 1974; cited by Kennedy, 1990) and in rats and hamsters exposed by inhalation to 101-2489 ppm (413-10,180 mg/m^3) TFE for 2 weeks (DuPont Co., 1981; cited by Kennedy, 1990) or to 200-2000 ppm (818-8182 mg/m^3) for 18 weeks (DuPont Co., 1982; cited by Kennedy, 1990). In the rats exposed to 3500 ppm, urinary fluoride levels returned to normal upon cessation of exposure (Kennedy, 1990).

6.2 Metabolism

Cytochrome P-450 oxidation is probably not involved in the metabolism of TFE (Lash and Anders, 1988; Wolf et al., 1984, and Odum and Green, 1984; cited by Lock and Berndt, 1988). Instead, a number of studies (reviewed by NTP, 1997; Miller and Surh, 1994; Kennedy, 1990) indicate that the first step in the metabolism of TFE involves conjugation with glutathione in the liver. The glutathione conjugates are then either excreted via the bile in the small intestine or are hydrolyzed to the cysteine-*S*-conjugate by renal peptidases. The cysteine-*S*-conjugate may be bioactivated by the renal enzyme cysteine-conjugate β-lyase to form a reactive thiol (NTP, 1997), also producing pyruvate and ammonia (Anders et al., 1988; Lash and Anders, 1988).

TFE was metabolized to *S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine, in *in vitro* preparations of rat liver fractions. The reaction was catalyzed by both microsomal and cytosolic glutathione *S*-transferases; the reaction rate catalyzed by the microsomal enzyme was four times higher than the cytosolic rate. Both cysteinylglycine and the cysteine conjugate of TFE have been detected in rat bile (Green and Odum, 1985; cited by Kennedy, 1990; Odum and Green, 1984; cited by Lock and Berndt, 1988).

Following i.p. injection of male F344/N rats with 30 mg TFE-cysteine conjugate per kilogram body weight, as many as five mitochondrial proteins were modified. Two of the proteins were identified as the heat shock proteins HSP60 (P1 protein) and HSP70-like protein (mortalin) (Bruschi et al., 1993; cited by NTP, 1997). In porcine renal epithelial cell cultures,

HSP70 and HSP70 mRNA were increased following addition of TFE-cysteine conjugate (Chen et al., 1992; cited by NTP, 1997). In rat renal mitochondrial and cytosolic preparations, addition of TFE-cysteine conjugate inhibited lipoyl dehydrogenase and glutathione reductase activity, respectively (Lock and Schnellmann, 1990; cited by NTP, 1997).

No information on the metabolism of TFE in humans was found.

6.3 Pharmacokinetics

No data were available.

6.4 Structure-Activity Relationships

6.4.1 Cysteine *S*-Conjugates

A number of halogenated hydrocarbons form glutathione *S*-conjugates that become nephrotoxic following conversion to the corresponding cysteine *S*-conjugate. For example, besides TFE, *vicinal*-dihaloalkanes, trichloroethylene, *S*-(1,2-dichlorovinyl)glutathione (DCVG), *S*-(1,2-dichlorovinyl)-*L*-cysteine (DCVC), chlorotrifluoroethylene, tetrachloroethylene, and hexachloro-1,3-butadiene are all metabolized to form nephrotoxic cysteine *S*-conjugates (Lash and Anders, 1988).

In general, cysteine *S*-conjugates of chloroalkenes are both nephrotoxic and genotoxic, whereas cysteine *S*-conjugates of fluoroalkenes are nephrotoxic, but not genotoxic. This difference may be the result of the higher stability of the carbon-fluorine bond, compared to the carbon-chloride bond (Green and Odum, 1985; cited by Lash and Anders, 1988).

6.4.2 Tetrachloroethylene

The NTP Report on Carcinogens has listed tetrachloroethylene as *reasonably anticipated to be a human carcinogen* since the fifth edition.

IARC (1995) has classified tetrachloroethylene as reasonably anticipated to be carcinogenic to humans, based on limited evidence in humans and sufficient evidence in experimental animals. In epidemiological studies, occupational exposure to tetrachloroethylene presented increased risk for esophageal cancer, non-Hodgkin's lymphoma, and cervical cancer. In experimental studies, mice given tetrachloroethylene by gavage showed an increased incidence of hepatocellular carcinomas (IARC, 1995). Mice exposed to high doses by inhalation showed exposure-related increases in hepatocellular adenomas and carcinomas (NTP, 1986). Rats exposed to high doses by inhalation showed a dose-related increase of mononuclear-cell leukemia; also observed but not statistically significant was an increase in the incidence of renal tubular cell adenomas and adenocarcinomas in male rats (NTP, 1986). In a study by Anna et al. (1994), the liver tumors induced in mice treated chronically with tetrachloroethylene for up to 76 weeks exhibited a decreased frequency of H-*ras* mutations and an increased frequency of K-*ras* mutations compared to liver tumors from concurrent and historical control animals.

Tetrachloroethylene is generally negative in most genetic toxicology assays. Radioactively labeled tetrachloroethylene binds to calf thymus DNA *in vitro* in the presence of metabolic activation, and to DNA and proteins of mouse and rat liver, kidney, and stomach *in vivo* (IARC, 1995). Tetrachloroethylene was not active in the SOS chromotest with *Escherichia coli* and was not mutagenic to bacteria in the absence of metabolic activation. Purified tetrachloroethylene was not mutagenic in *S. typhimurium* or *E. coli* in the presence of rat liver S9. However, purified tetrachloroethylene was mutagenic in *S. typhimurium* TA100 in the

presence of rat liver glutathione *S*-transferase, glutathione, and kidney microsomes (Vamvakas et al, 1989; cited in IARC, 1995). In stationary phase yeast, tetrachloroethylene did not induce gene conversion, mitotic recombination, or reverse mutations, while conflicting data were obtained for cells in logarithmic growth. Tetrachloroethylene did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*, unscheduled DNA synthesis in rat primary hepatocytes, chromosomal aberrations or sister chromatid exchanges in cultured Chinese hamster lung cells (with or without metabolic activation), or mutations in mouse lymphoma cells (with or without metabolic activation). It did induce cell transformation in Fischer rat embryo cells but not in mouse BALB/c-3T3 cells. In *in vivo* studies, the frequencies of gene conversion and reverse mutations were not increased in yeast recovered from the liver, lungs, and kidneys of mice treated with tetrachloroethylene. However, a significant increase in DNA damage (strand breaks/alkali-labile sites) in mouse liver and kidney, but not mouse lung, was detected after treatment.

IARC (1995) noted two genotoxicity studies of workers who smoked and were occupationally exposed to tetrachloroethylene. One study found small increases of peripheral lymphocytes showing numerical chromosome abnormalities (Ikeda et al., 1980; cited by IARC, 1995), and another study reported an increase in sister chromatid exchange frequency in subjects who smoked (Seiji et al., 1990; cited by IARC, 1995). In both studies, the possible confounding effects of smoking were not controlled.

6.5 Tumor Mutations

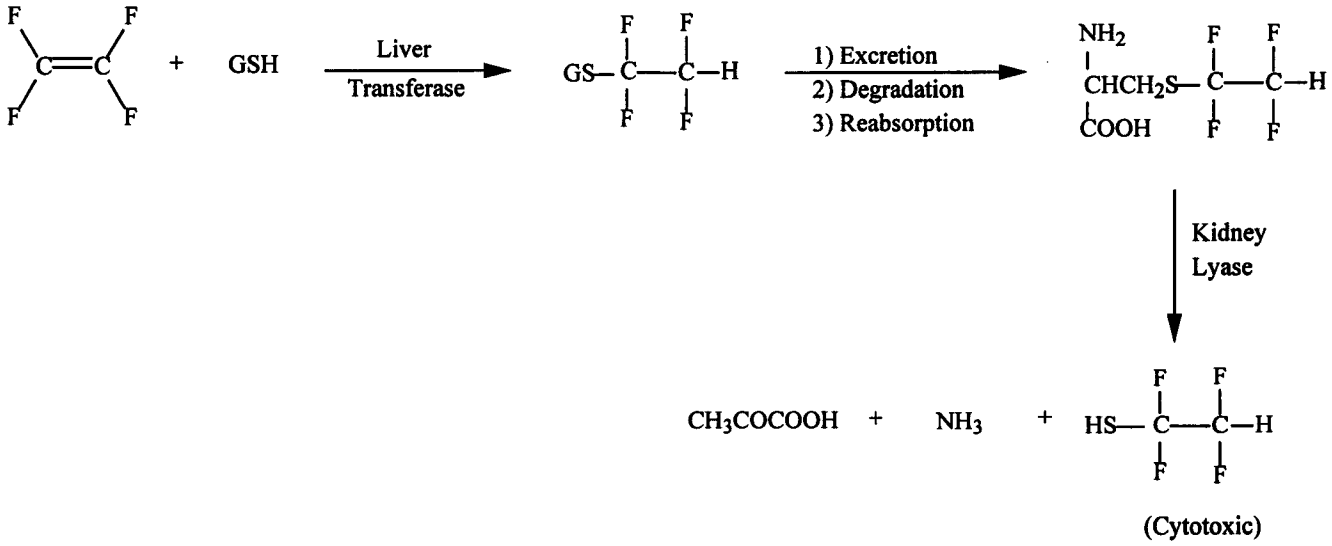
Hepatocellular neoplasms, induced in B6C3F1 mice by a 2-year inhalation exposure to TFE, showed no increase in *ras* mutations (NTP, 1997; Hong et al., 1998).

7.0 MECHANISMS OF CARCINOGENESIS

The kidney-specific toxicity and carcinogenicity of TFE is most likely related to the selective uptake and subsequent processing of TFE-glutathione conjugates by the renal enzyme β-lyase (Miller and Surh, 1994; Anders et al., 1988). TFE is conjugated in the liver with glutathione, released into general circulation, and hydrolyzed to cysteine-*S*-conjugates by renal peptidases. The TFE cysteine conjugate, *S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine, can cause renal damage *in vivo* identical to that caused by TFE and it can kill cultured human proximal tubule cells; it is bioactivated in rat kidney to a difluorothionacetyl fluoride, the putative reactive metabolite for TFE-induced nephrotoxicity (NTP, 1997).

Figure 7-1 presents a model for the metabolic activation of TFE to a nephrotoxin in the male rat. For a more detailed explanation refer to Section 6.2 (Metabolism). The mechanism by which it causes renal tumors has not been determined.

Figure 7-1. Proposed Mechanism for the Metabolic Activation of TFE in the Male Rat
Source: Kennedy (1990)



8.0 REFERENCES

Anders, M. W., L. Lash, W. Dekant, A. A. Elfarra, and D. R. Dohn. 1988. Biosynthesis and biotransformation of glutathione *S*-conjugates to toxic metabolites. Crit. Rev. Toxicol. 18:311-341.

Anna, C. H., R. R. Maronpot, M. A. Pereira, J. F. Foley, D. E. Malarkey, and M. W. Anderson. 1994. *ras* proto-oncogene activation in dichloroacetic-, trichloroethylene- and tetrachloroethylene-induced liver tumors in B6C3F1 mice. Carcinogenesis (London) 15:2255-2261. (Cited by IARC, 1995).

Bruschi, S. A., K. A. West, J. W. Crabb, R. S. Gupta, and J. L. Stevens. 1993. Mitochondrial HSP60 (P1 protein) and a HSP70-like protein (Mortalin) are major targets for modification during *S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine-induced nephrotoxicity. J. Biol. Chem. E285:23,157-23, 161. (Cited by NTP, 1997)

Carson, B. L., M.D. Erickson, and J. L. McCann. 1986. Literature Review of Pyrolysis and Combustion Products of Selected Utility Materials. Electric Power Research Institute (EPRI), Palo Alto, CA, pp. 3-6 - 3-10.

Chen, J. C., J. L. Stevens, A. L. Trifillis, and T. W. Jones. 1992. Renal cysteine conjugate β-lyase-mediated toxicity studied with primary cultures of human proximal tubular cells. Toxicol. Appl. Pharmacol. 103:463-473. (Cited by NTP, 1997)

Dilley, J. V.,. L. Carter, Jr., 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 ff. (Cited by Kennedy, 1990)

Ding, X., H. Yu, C. Liu, M. Hu, and F. Ko. 1980. Studies on the absorption, distribution and elimination of four organofluorine compounds in rabbits. Chung-hua Yu Fang I Hsueh Tsa Chih 14:39 ff. (Cited by Kennedy, 1990)

DuPont (E. I. DuPont de Nemours). 1981. Subacute inhalation toxicity of tetrafluoroethylene [unpublished data]. (Cited by Kennedy, 1990)

DuPont (E. I. DuPont de Nemours). 1982. 90-day inhalation toxicity study with tetrafluoroethylene (TFE) in rats and hamsters [unpublished data]. (Cited by Kennedy, 1990)

Fiumara, A. 1989. Poly(tetrafluoroethylene): Flammability problems in monomer-manufacturing plants. In: [Proceedings of] *Ber. Int. Kolloq. Verhütung Arbeitsunfällen Berufskrankh. Chem. Ind.*, 13th [13th International Symposium on the Prevention of Occupational Accidents and Diseases in the Chemical Industry]: 299-309; abstr. from STN, 112:124383.

Gangal, S. V. 1980. Fluorine compounds, organic. In: Kirk-Othmer Encyclopedia of Chemical Technology. M. Grayson, Ed. 3rd ed., vol. 11. John Wiley & Sons, New York, pp. 1-24.

Green, T., and J. Odum. 1985. Structure/activity studies of the nephrotoxic and mutagenic action of cysteine conjugates of chloro- and fluoro-alkenes. Chem. Biol. Interact. 54:15 ff. (Cited by Kennedy, 1990; cited by Lash and Anders, 1988)

Gribble, G. W. 1994. The natural production of chlorinated compounds. Environ. Sci. Technol. 28:310A-319A.

Haskell Laboratory (DuPont). 1986a. Mutagenicity evaluation of tetrafluoroethylene in *Salmonella typhimurium*. Final report. U. S. Environmental Protection Agency, report no. 86860000093. Available from NTIS, Springfield, VA; OTS-0510242. (Cited by HSDB, 1997).

Haskell Laboratory (DuPont). 1986b. Mutagenicity evaluation of tetrafluoroethylene in the CHO/HPRT assay. U. S. Environmental Protection Agency, report no. 86860000130. Available from NTIS, Springfield, VA; OTS-0510275. (Cited by HSDB, 1997)

Hong, H. L., T. R. Devereux, G. A. Boorman, and R. C. Sills. 1996. *ras* oncogene mutations in liver neoplasms of B6C3F1 mice exposed to tetrafluoroethylene for two years [abstract]. FASEB J. 10:A755.

Hong, H. L., T. R. Devereux, G. A. Boorman, and R. C. Sills. 1998. Frequency of *ras* mutations in liver neoplasms from B6C3F1 mice exposed to tetrafluoroethylene for two years. Toxicol. Pathol. 26(5):646-650.

HSDB (Hazardous Substances Data Bank). 1997. Tetrafluoroethylene profile. Online database produced by the National Library of Medicine. Profile last updated April 24, 1997.

IARC (International Agency for Research on Cancer). 1979. Tetrafluoroethylene and polytetrafluoroethylene. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 19(Some monomers, plastics and synthetic elastomers, and acrolein):285-301.

IARC (International Agency for Research on Cancer). 1995. Tetrachloroethylene. IARC Monogr. Eval. Carcinog. Risk Chem. Hum. 63(Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals):159-221.

Ikeda, M., A. Koizumi, T. Watanabe, A. Endo, and K. Sato. 1980. Cytogenetic and cytokinetic investigations on lymphocytes from workers occupationally exposed to tetrachloroethylene. Toxicol. Lett. 5:251-256. (Cited by IARC, 1995)

Kelly, N. A., K. L. Olson, and C. A. Wong. 1985. Tests for fluorocarbon and other organic vapor release by fluorocarbon film bags. Environ. Sci. Technol. 19:361 ff. (Cited by Kennedy, 1990)

Kennedy, G. L., Jr. 1990. Toxicology of fluorine-containing monomers. Crit. Rev. Toxicol. 21:149-170.

Lash, L. H., and M. W. Anders. 1988. Glutathione homeostasis and glutathione *S*-conjugate toxicity in the kidney. Rev. Biochem. Toxicol. 9:29-67.

Lewis, R. J. 1992. Sax's Dangerous Properties of Industrial Materials. 8th ed. Van Nostrand Reinhold, New York.

Lock, E. A., and W. O. Berndt. 1988. Studies on the mechanism of nephrotoxicity and nephrocarcinogenicity of halogenated alkenes. Crit. Rev. Toxicol. 19:23-42.

Lock, E. A., and R. G. Schnellmann. 1990. The effect of haloalkene cysteine conjugates on rat renal glutathione reductase and lipoyl dehydrogenase activities. Toxicol. Appl. Pharmacol. 104:180-190. (Cited by NTP, 1997)

Miller, J. A., and Y.-J. Surh. 1994. Historical perspectives on conjugation-dependent bioactivation of foreign compounds. Adv. Pharmacol. 27:1-16.

NIOSH (National Institute for Occupational Safety and Health). 1990. National Occupational Exposure Survey (1980-1983), unpublished provisional data as of July 1, 1990. U. S. Department

of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Surveillance, Hazard Evaluations and Field Studies, Surveillance Branch, Hazard Section.

NTP. 1986. Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) [CAS No. 127-18-4] in F344/N rats and B6C3F$_1$ mice (inhalation studies) NTP Technical Report No. 311; NIH Publication No. 86-2567. U. S. Department of Health and Human Services, Public Health Service, National Institutes of Health. (Cited by IARC, 1995)

NTP. 1997. NTP technical report on the toxicology and carcinogenesis studies of tetrafluoroethylene (CAS No. 116-14-3) in F344/N rats and B6C3F$_1$ mice (inhalation studies). NTP Technical Report No. 450; NIH Publication No. 97-3366. U. S. Department of Health and Human Services, NIEHS, National Toxicology Program, Research Triangle Park, NC.

Odum, J., and T. Green. 1984. The metabolism and nephrotoxicity of tetrafluoroethylene in the rat. Toxicol. Appl. Pharmacol. 76:306 ff. (Cited by Lock and Berndt, 1988)

Radian Corporation. 1991. Tetrafluoroethylene. In: NTP Chemical Repository Database. URL: http://ntp-db.niehs.nih.gov/NTP_Reports/NTP_chem_H&S/NTP_Chem1/Radian116-14-3.txt. Last accessed April 25, 1997.

Seiji, K., C. Jin, T. Watanabe, H. Nakatsuka, and M. Ikeda. 1990. Sister chromatid exchanges in peripheral lymphocytes of workers exposed to benzene, trichloroethylene, or tetrachloroethylene, with reference to smoking habits. Int. Arch. Occup. Environ. Health 62:171-176. (Cited by IARC, 1995)

SRI International. 1996. Directory of Chemical Producers, United States. SRI International, Menlo Park, pp. 291-292.

Stoiber, R. E., et al. 1971. Geol. Soc. Am. Bull. 82:2299 ff. (Cited by Gribble, 1994)

Vamvakas, S., M. Herkenhoff, W. Dekant, and D. Henschler. 1989. Mutagenicity of tetrachloroethylene in the Ames test—metabolic activation by conjugation with glutathione. J. Biochem. Toxicol. 4:21-27. (Cited by IARC, 1995)

Weast, R. C., and M. J. Astle, Eds. 1980. CRC Handbook of Chemistry and Physics. 61st ed. CRC Press, Boca Raton, FL.

Wolf, C. R., P. N. Berry, J. A. Nash, T. Green, and E. A. Lock. 1984. The role of microsomal and cytosolic glutathione *S*-transferases in the conjugation of hexachloro-1,3-butadiene and its possible relevance to toxicity. J. Pharmacol. Exp. Ther. 228:202 ff. (Cited by Lock and Berndt, 1988)

APPENDIX A

Excerpts from the IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans Volume 19 (Some Monomers, Plastics and Synthetic Elastomers, and Acrolein) Tetrafluoroethylene and Polytetrafluoroethylene pp. 285-301, 1979

IARC MONOGRAPHS
ON THE
EVALUATION OF THE CARCINOGENIC RISK OF CHEMICALS TO HUMANS

Some Monomers, Plastics and Synthetic Elastomers, and Acrolein

VOLUME 19

This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of the Carcinogenic Risk of Chemicals to Humans
which met in Lyon,
7-13 February 1978

February 1979

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

CONTENTS

LIST OF PARTICIPANTS 7

NOTE TO THE READER 11

PREAMBLE 13

BACKGROUND 13

OBJECTIVE AND SCOPE 13

SELECTION OF CHEMICALS FOR MONOGRAPHS 14

WORKING PROCEDURES 14

DATA FOR EVALUATIONS 15

THE WORKING GROUP 15

GENERAL PRINCIPLES FOR EVALUATING THE CARCINOGENIC RISK OF CHEMICALS 16

EXPLANATORY NOTES ON THE MONOGRAPH CONTENTS 21

GENERAL REMARKS ON THE SUBSTANCES CONSIDERED 35

THE MONOGRAPHS

Acrylic acid, methyl acrylate, ethyl acrylate and polyacrylic acid 47

Acrylonitrile, acrylic and modacrylic fibres, and acrylonitrile-butadiene-styrene and styrene-acrylonitrile copolymers 73

Caprolactam and nylon 6 115

Chloroprene and polychloroprene 131

Ethylene and polyethylene 157

Methyl methacrylate and polymethyl methacrylate 187

Propylene and polypropylene 213

Styrene, polystyrene and styrene-butadiene copolymers 231

Styrene oxide 275

Tetrafluoroethylene and polytetrafluoroethylene 285

2,4- and 2,6-Toluene diisocyanates, 1,5-naphthalene diisocyanate, 4,4'-methylenediphenyl diisocyanate, polymethylene polyphenyl isocyanate and flexible and rigid polyurethane foams 303

Vinyl acetate, polyvinyl acetate and polyvinyl alcohol 341

Vinyl bromide 367

Vinyl chloride, polyvinyl chloride and vinyl chloride-vinyl acetate copolymers 377

Vinylidene chloride and vinylidene chloride-vinyl chloride copolymers 439

N-Vinyl-2-pyrrolidone and polyvinyl pyrrolidone 461

Acrolein 479

SUPPLEMENTARY CORRIGENDA TO VOLUMES 1-18 495

CUMULATIVE INDEX 497

The following compounds were considered but no monograph was prepared because of lack of carcinogenicity data: adipic acid, hexamethylenediamine and nylon 6/6; dimethylterephthalate, terephthalic acid and polyethylene terephthalate; isoprene and polyisoprene; methacrylic acid; vinylidene fluoride and polyvinylidene fluoride.

TETRAFLUOROETHYLENE and POLYTETRAFLUOROETHYLENE

Tetrafluoroethylene

1. Chemical and Physical Data

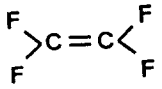
1.1 Synonyms and trade names

Chem. Abstr. Services Reg. No.: 116-14-3

Chem. Abstr. Name: Tetrafluoroethene

Perfluoroethene; perfluoroethylene

1.2 Structural and molecular formulae and molecular weight



C_2F_4 Mol. wt: 100

1.3 Chemical and physical properties of the pure substance

From Weast (1976), unless otherwise specified

(a) Description: Colourless gas (Hawley, 1971)

(b) Boiling-point: $-76.3^{\circ}C$

(c) Melting-point: $-142.5^{\circ}C$

(d) Density: $d^{-76.3}$ 1.519 (Grasselli & Ritchey, 1975); vapour density about 3 (air = 1) (Anon., 1972)

(e) Spectroscopy data: Infra-red and mass spectral data have been tabulated (Grasselli & Ritchey, 1975).

(f) Solubility: Insoluble in water

(g) Stability: Inflammable at 14-43% by volume in air at 760 mm (McCane, 1970)

(h) Reactivity: Polymerizes at high pressure (McCane, 1970)

(i) Conversion factor: 1 ppm in air = 4 mg/m^3

1.4 Technical products and impurities

A highly purified grade of tetrafluoroethylene is required for commercial production of polytetrafluoroethylene. Polymerization stabilizers such as dipentene, terpinolene and α-pinene are added if the tetrafluoroethylene is to be transported.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(a) Production

Tetrafluoroethylene was first prepared in 1933 by decomposition of tetrafluoromethane in an electric arc (McCane, 1970). It is produced commercially by the pyrolysis of chlorodifluoromethane at temperatures of 590-800°C.

Commercial production of tetrafluoroethylene in the US was first reported in 1960 (US Tariff Commission, 1961). In 1975, four companies reported production of 7.9 million kg (US International Trade Commission, 1977a), down from 11.2 million kg in 1974 (US International Trade Commission, 1976).

Six manufacturers in western Europe produced 11.5 million kg tetrafluoroethylene in 1977.

It was first produced commercially in Japan in 1953. In 1976, four companies produced a total of about 2-2.5 million kg.

Worldwide production of tetrafluoroethylene in 1977 is estimated to have been 15-20 million kg.

(b) Use

Virtually all tetrafluoroethylene produced in the US is used as a monomer for the production of homopolymers, copolymers and terpolymers. The major end-product is the homopolymer, polytetrafluoroethylene (PTFE); in 1974, about 7.3 million kg PTFE were used in the US. For a detailed description of the uses of PTFE, see p. 291.

Use of tetrafluoroethylene copolymers and terpolymer is the US amounted to 1.4-1.6 million kg in 1974. Three copolymer resins are made by one US company: fluorinated ethylenepropylene (FEP) resins, copolymers of tetrafluoroethylene and hexafluoropropylene; ethylene-tetrafluoroethylene (ETFE)

resins; and perfluoroalkoxy resins (PFA), copolymers of tetrafluoroethylene and perfluoropropyl vinyl ether. In general, the copolymers are a compromise of PTFE properties, resulting in lower temperature and specific chemical resistance. A terpolymer resin, vinylidene fluoride-hexafluoropropylene-tetrafluoroethylene, is used as a fluoroelastomer for: (1) applications requiring heat and fluid resistance or flame resistance for oxygen-enriched atmospheres; (2) critical automotive and aerospace components; (3) high vacuum equipment; and (4) equipment involving the use of cryogenic temperatures and radiation.

FEP resins are used primarily in electrical applications: 75% for wire and cable insulation and 25% for moulded electrical parts such as coil forms and tube sockets. Chemical applications of FEP resins include heat exchangers, laboratory ware, lined pipe and overbraided hose. FEP resins are also used in mechanical parts, e.g., chute liners, conveyor belt coatings, roll covers and seals.

ETFE resins are mainly used in wire and cable insulation but are also used in moulded parts for chemical processing equipment, electronic components, automotive parts, labware and liners for valves and fittings.

PFA resins are used mainly in injection-moulded articles for the chemical processing industry and as injection- or transfer-moulded liners for valves, pumps, pipes and fittings. They are also used for a variety of tubing, in film and roll covers, in wire insulation, and in extruded shapes and profiles.

Total use of tetrafluoroethylene in the European Economic Community in 1973 is estimated to have been 6.9 million kg. Essentially all of the monomer is used for production of fluorinated polymers, and more than two-thirds is used for the homopolymer.

In Japan, 70-80% of tetrafluoroethylene is used for the production of PTFE and 20-30% for FEP resins, ETFE resins and PFA resins.

2.2 Occurrence

Tetrafluoroethylene is not known to occur as a natural product.

No data were available concerning the content of tetrafluoroethylene in typical commercial grade PTFE; the very low boiling-point of tetrafluoroethylene suggests that the level of residues would be very small.

Tetrafluoroethylene has been detected as a decomposition product (1) when PTFE is heated in the absence of air (more than 90% of the decomposition product is tetrafluoroethylene) (Zapp, 1962); (2) during the high-temperature (400°C) processing of PTFE resins (Marchenko, 1966); and (3) as a result of pyrolysis of PTFE in air at 450°C (Waritz, 1975).

2.3 Analysis

Gas chromatography has been used to separate and detect tetrafluoroethylene in air, with a limit of detection of 7 mg/m^3 (2 ppm) (Dolgina *et al.*, 1966), and to detect tetrafluoroethylene in the pyrolysis products of PTFE (Waritz, 1975).

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

No data were available to the Working Group.

3.2 Other relevant biological data

(a) Experimental systems

The LC_{50} in rats is 160 g/m^3 (40 000 ppm) in air for a 4-hour exposure (Clayton, 1967). Male rats exposed to 14 g/m^3 (3500 ppm) tetrafluoroethylene in air for 30 minutes excreted small amounts of fluoride ion in the urine over a 14-day period, indicating that the compound can be metabolized. No gross pathology was noted in any of the organs (Dilley *et al.*, 1974).

No data on the embryotoxicity or mutagenicity of this compound were available to the Working Group.

(b) Humans

No data were available to the Working Group.

3.3 Case reports and epidemiological studies

No data were available to the Working Group.

Polytetrafluoroethylene

1. Chemical and Physical Data

1.1 Synonyms and trade names

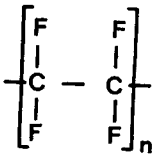
Chem. Abstr. Services Reg. No.: 9002-84-0

Chem. Abstr. Name: Tetrafluoroethene homopolymer

Poly(ethylene tetrafluoride); polytef; polytetrafluoroethene; PTFE; tetrafluoroethene polymer; tetrafluoroethylene homopolymer; tetrafluoroethylene polymers

Aflon; Algloflon; Algoflon SV; Alkathene RXDG33; AMIP 15M; Balfon 7000; BDH 29-801; Chromosorb T; Dixon 164; Duroid 5870; EK 1108GY-A; Ethicon PTFE; F 103; FBF 74D; F 4K20; Fluo-Kem; Fluon; Fluon CD 1; Fluon CD 023; Fluon CD 042; Fluon G4; Fluon G163; Fluon G201; Fluon GPI; Fluon L 169; Fluon L 170; Fluon L 171; Fluon L 169B; Fluoroflex; Fluorolon 4; Fluoropak 80; Fluoroplast 4B; Fluoroplast 4D; Fluoroplast 4M; Fluoropore FP 120; F 4MB; FN 3; FT-4; Ftorlon 4; Ftorlon 4M; Ftorlon F-4MB; Ftoroplast 4; Ftoroplast 4B; Ftoroplast 4D; Ftoroplast F-4; Ftoroplast F 4MB; Ftoroplast FBF 74D; Ftoroplast 4K20; Ftoroplast 4M; F 4Zh20; G 163; GORE-TEX; Halon G 80; Halon G 183; Halon G 700; Halon TFEG 180; Heydeflon; Hostaflon; Hostaflon TF; L 169; Molykote 522; Polifen; Politef; Polyfene; Polyflon; Polyflon D 1; Polyflon EK 4108GY; Polyflon EK 1108GY-A; Polyflon F 103; Polyflon M 12; Polyflon M 21; Polyflon M12A; PTFE-GM3; Soreflon 604; Soreflon 5A; T 8A; Tarflen; T 5B; TE 30; Teflon; Teflon 5; Teflon 6; Teflon 30; Teflon 110; Teflon 6C; Teflon K; Teflon T5; Teflon T 6; Teflon T 30; Tetran 30; Tetran PTFE; TL 102; TL 115; TL 125; TL 126; TL-R; TL-V; Unon P; Valflon; Zitex H 662-124; Zitex K 223-122

1.2 Structural and molecular formulae and molecular weight



$(C_2F_4)_n$ Mol. wt: 400 000-10 000 000

1.3 Chemical and physical properties of the polymer

From Windholz (1976), unless otherwise specified

(a) Description: Soft, waxy, milk-white solid with a low coefficient of friction (Hawley, 1971)

(b) Melting-point: Gels at 325°C

(c) Density: 2.25

(d) Solubility: No substance has been found which will dissolve the polymer.

(e) Stability: Upon heating, weight loss varies from 0.001%/hr at 290°C to 4%/hr at 450°C (Waritz, 1975). Polytetrafluoroethylene (PTFE) has been degraded at various temperatures, and the following compounds were detected: (1) at 450°C, tetrafluoroethylene (Waritz, 1975); (2) at 460°C, hexafluoropropylene (Waritz, 1975); (3) at 475°C, perfluoroisobutylene (Waritz, 1975); (4) between 500°-650°C, carbonyl fluoride (Coleman *et al.*, 1968); (5) above 650°C, carbon tetrafluoride and carbon dioxide (Coleman *et al.*, 1968). When PTFE was burned in air in an electric oven at 475-575°C, the pH of the exhaust gases was 2.5, and fluoride ion was detected at a level of 1580 ppm (vol/vol) (van Grimbergen *et al.*, 1975) [For studies of the toxic properties of thermal degradation products, see section 3.2 (a)].

(f) Reactivity: Ignites in fluorine-oxygen mixtures under extreme conditions.

1.4 Technical products and impurities

PTFE is available in three forms: (1) granular, for moulded parts and for extruding thick-walled tubing and rods; (2) coagulated dispersions (also referred to as fine powders), for extruding thin sections; and (3) aqueous dispersions, for coating, impregnation and preparation of fibres and films. Filled polymers are also available; these are generally made by mixing fillers such as glass fibre, graphite, molybdenum disulphide, metal oxides or ceramics and finely-divided granular PTFE. Reprocessed scrap and off-grade material is also used.

No detailed information on the possible presence of unreacted monomer in the polymer was available to the Working Group.

2. Production, Use, Occurrence and Analysis

2.1 Production and use

(a) Production

PTFE was first isolated in 1938 by Plunkett (McCane, 1970); it is manufactured commercially by the polymerization of tetrafluoroethylene at 7-70 atmospheres (5320-53 200 mm) in the presence of water and free-radical initiators (e.g., peroxydisulphates and organic peroxides).

Commercial production in the US was first reported in 1960 (US Tariff Commission, 1961). In 1976, three companies reported production of 7.1 million kg (US International Trade Commission, 1977b), a reduction from the 8.4 million kg reported in 1974 (US International Trade Commission, 1976). US imports of PTFE amounted to 600 thousand kg in 1976 and were from the following countries (per cent of total): the Federal Republic of Germany (43), France (40), Japan (11) and the UK (6) (US Department of Commerce, 1977). Data on exports of PTFE itself are not available; however, exports of all fluoropolymers (of which PTFE is the major component) are over 450 thousand kg per year.

Six companies in western Europe produce PTFE, and it is also believed to be produced in the USSR. Production in western Europe in 1977 was 7.5 million kg.

PTFE has been produced commercially in Japan since 1953; two companies currently manufacture it. Total production of all fluoropolymers based on tetrafluoroethylene in Japan amounted to 1.15 million kg in 1975.

Production facilities were reported to have been started in the People's Republic of China in 1965.

Worldwide production of PTFE in 1977 is estimated to have been 15-20 million kg.

(b) Use

Of the 7.3 million kg PTFE resins sold in the US in 1974, 69% was in granular resin form (including over 1 million kg of reprocessed scrap and off-grade material and over 1 million kg of filled PTFE, which contains 75-80% resins); 15% in coagulated dispersions (fine powders); and 16% in aqueous dispersions.

PTFE was used in the US in 1974 in chemical equipment (36%), and mechanical (25%), electrical (20%) and miscellaneous applications (19%).

The primary chemical equipment applications are in fluid handling parts (e.g., valve and pump linings, dip tubes, expansion bellows, nozzles, valve seats), packings, basic shapes (rods, sheets and tubes) and in over-braided hose. The major mechanical applications for PTFE are in seals and piston rings (e.g., fluid transmission systems, hydraulic cylinders and nonlubricated compressors) and in machine bearings and bearing pads, and, to a lesser extent, in mechanical tapes and impregnated glass fabrics. Wire and cable insulations account for over 85% of the PTFE used in electrical/electronic applications; it is also used in electrical components such as cable connectors, circuit breakers and stand-off insulators. Miscellaneous applications include anti-stick uses (coatings for consumer products, e.g., cooking utensils and tools, and coatings for industrial products, such as conveyor belts, chute liners, rolls and roller covers), fibres and lubricant powders.

In 1975, an estimated 5000 kg PTFE were used in Europe and the US in the manufacture of plastic materials for medical applications, such as (1) vascular grafts when fabricated into textiles; (2) knitted fabrics for the treatment of aneurysms; (3) heart valves and aorta implants; (4) shunts in haemodialysis equipment; (5) bone replacements (e.g., ossicles in the ear); and (6) injections near the vocal cords for the treatment of dysphonia (Halpern & Karo, 1977).

Use of PTFE in western Europe in 1977 was as follows: mechanical industry (30-45%), chemical industry (35%), electric/electronic industry (15-20%) and miscellaneous applications such as industrial coatings, housewares (10-15%).

In Japan, PTFE is used in chemical equipment (37%), appliances (31%), electrical/electronics (16%) and miscellaneous applications (16%).

Worldwide consumption of PTFE in 1974 is estimated to have been 13.6 million kg.

The US Food and Drug Administration permits the use of PTFE as a component of adhesives and in resinous and polymeric coatings (as release agents) intended for use in contact with food (US Food and Drug Administration, 1977).

2.2 Occurrence

PTFE is not known to occur as a natural product.

PTFE dust has been detected in workplace environments during the thermal processing of fluoroplastics (Okawa & Polakoff, 1973, 1974).

2.3 Analysis

PTFE elastomer has been identified by differential thermal analysis (Sircar & Lammond, 1972); and mass spectrometry (Okawa & Polakoff, 1973; Zeman, 1972) has been used in the identification and analysis of PTFE.

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) Subcutaneous and/or intramuscular administration

Mouse: A group of 89 random-bred female Swiss mice, 7-9-weeks old, received a s.c. implant in the left flank of a square sheet of polytetrafluoroethylene (PTFE) measuring 12 x 12 x 1.2 mm. The first local tumour developed 25 weeks after implantation; a total of 11 (12.5%) fibrosarcomas were found after an average latent period of 54.5 weeks (Tomatis & Shubik, 1963) [The Working Group noted that since the implant was not retained in 9 mice and since 70 mice were still alive at the appearance of the first tumour, the effective tumour incidence should be ≃16%].

Groups of 7-9-week old random-bred Swiss mice were given a s.c. implant of a 12 x 12 x 1.2 mm square of PTFE (89 females and 61 males) or of a 15 mm diameter PTFE disc (103 females), or of a Teflon fragment corresponding to one disc (size not specified) (53 females) or of a 20 mm diameter PTFE disc (54 females and 50 males). Tumours developed around the implant in 8/89 (10%) and 1/61 (2%), 23/103 (22.7%), 10/53 (21.2%) and 7/54 (15.2%) and 4/50 (8%) mice in the above groups, respectively. No similar tumours were seen in 200 female and 100 male untreated mice. Of 50 female mice implanted with 12 x 12 x 1.2 mm square glass coverslips, 6 developed sarcomas (13.6%); and of 48 females implanted with fragments of glass corresponding to one square, 2 developed sarcomas (4.3%). The average latent period for gross, palpable tumours was 55 weeks, with 2 tumours appearing as early as the 25th week and 9 at 65 weeks after implantation. All neoplasms were fibrosarcomas, and some had angiosarcomatous areas (Tomatis, 1963) [The Working Group noted that survival rates and the time at which the experiment was terminated were not reported. Mice at risk were considered to be those that retained the implant rather than those alive at the time of the appearance of the first tumour].

A group of 19 male and 27 female 7-9-week-old inbred C57BL mice received s.c. implants of 15 x 1.2 mm PTFE discs. At 50 weeks, 13 males and 13 females were still alive. Four local sarcomas (20%) developed in 20 females that retained the implant and were considered to be at risk at weeks 39, 47, 52 and 58, and 4 local sarcomas were found in the 15 males considered to be at risk (26%) at weeks 49, 51, 60 and 91. Mice were observed for 90 weeks, at which time only 3 males and 3 females were still alive. Tumours always occurred around the discs; 1 sarcoma tested was found to be transplantable in syngeneic mice. Tumours unrelated to the implant developed in 3 females and 1 male. In a control group of 30 male and 33 female non-implanted mice that were observed for 100 weeks, no s.c. sarcomas were found; 3 females and 2 males developed spontaneous tumours (Tomatis, 1966).

A group of 40 male and 40 female 8-week-old random-bred CTM albino mice received s.c. implants into the right flank of 15 x 1.2 mm PTFE discs and were observed for lifespan; 18 females and 9 males developed sarcomas around the disc, a total incidence of 38% of the 69 mice still alive at the time of the appearance of the first tumour. Average ages at death of tumour-bearing animals were 72 and 69 weeks for females and males, respectively. No s.c. fibrosarcomas were found in 99 male and 98 female control mice of the same strain observed for lifespan (Tomatis & Parmi, 1971).

Three groups of 38, 38 and 39, 6-7-week-old female BALB/c, C3Hf/Dp and C57BL/He mice received s.c. implants of PTFE discs (15 x 1.2 mm) in the dorsal area. Fibrosarcomas developed around the discs in 17/38 (44%) BALB/c, 36/38 (94%) C3Hf/Dp and 12/39 (30%) C57BL/He animals, with mean latent periods of 78, 61 and 82 weeks, respectively. All surviving mice were sacrificed at 120 weeks of age. Of the 56 tumours examined histologically, 2 were rhabdomyosarcomas and the rest were fibrosarcomas (Ménard & Della Porta, 1976) [The Working Group noted that the incidence of tumours was calculated on the basis of the number of mice treated initially].

Rat: In rats implanted subcutaneously with PTFE films, 4 sarcomas were produced after 2 years, at which time 15 animals were still alive (Oppenheimer *et al.*, 1953) [The Working Group noted the incomplete reporting of the experiment].

A group of 65 weanling Wistar rats of both sexes received single s.c. PTFE implants (4 x 5 x 0.16 mm) in the abdominal wall; 55 rats were still alive after 300 days and 45 at the time of appearance of the first tumour (659 days); all rats were killed within 800 days. Two s.c. sarcomas were induced; no tumours were observed in 20 control animals receiving glass implants, which survived 300 days and were observed for a similar period of time (Russell *et al.*, 1959).

Two groups of Wistar rats were implanted subcutaneously with discs of PTFE (15 x 0.02 mm) in the abdominal wall; in one group, the discs were perforated. The numbers of rats that survived the minimum latent period were 34 and 32 for the groups implanted with plain and perforated discs, respectively. Eight sarcomas (23.5%) were observed in the first group and 6 sarcomas (18.7%) in the second (Oppenheimer *et al.*, 1955) [The Working Group noted the incomplete reporting of the study].

A group of 39 male Evans rats received s.c. implants of squares (20 x 20 mm) of PTFE mesh surgical outflow patches. A further 40 rats were implanted with the shredded material, and 41 non-implanted rats served as controls. The experiment was terminated 19 months later, and no local tumours were observed; at that time, 28 controls and 24 and 23 PTFE-implanted rats were still alive (Bryson & Bischoff, 1969) [The Working Group noted the short period of observation].

(b) Intraperitoneal administration

Rat: Weanling Wistar rats of both sexes were implanted intraperitoneally with 10 x 2 x 2 mm PTFE rods (16 rats) or with equivalent amounts of PTFE powder (17 rats). After 365 days, 13/16 and 10/17 animals were still alive in the two groups, respectively, and after 800 days, 9/16 and 3/17 animals. Surviving animals were killed 27 months after implantation. No tumours were found in rod-implanted rats, whereas 2 sarcomas became palpable in the powder-treated animals at 354 and 476 days after implantation. Extraperitoneal tumours included 1 fibroadenoma in the inguinal region in rod-implanted rats and 1 liposarcoma in the upper part of the leg, 1 fibrosarcoma in the shoulder and 1 inguinal fibroadenoma in powder-treated rats. Among 25 untreated controls, 1 adenoma of the testis and a possible carcinoma in the inguinal region were observed (Simmers *et al.*, 1963).

3.2 Other relevant biological data

(a) Experimental systems

No toxicity was observed in male and female rats fed PTFE for 90 days, even with a level of 25% in the diet. The polymer has not been found to produce skin irritation or to act as an allergenic agent (Clayton, 1962; Zapp, 1962).

The toxicity of the pyrolysis products of PTFE, degraded at various temperatures, has been tested in several animal species.

PTFE heated to 300°C in air was lethal to rats (Zapp, 1962). Gases identified during pyrolysis of PTFE included tetrafluorethylene, hexafluorethylene, hexafluoropropylene, octafluorocyclobutane and octafluoroisobutylene (Lee *et al.*, 1976). Depending on the specific PTFE, generation of toxic gases does not occur until the temperature reaches 350°C (Clayton, 1967). When the polymer is heated to 375°C in air, toxic fumes are generated, as demonstrated in inhalation studies in several animal species (Treon *et al.*, 1955). One of the extremely toxic gases produced was reported to be octafluoroisobutylene, which has an approximate lethal concentration of 0.5 ppm in rats exposed for a few hours (Harris, 1959). At 400°C, no lethal products were produced from PTFE similar to that used in cooking utensils; tetrafluoroethylene was detected at 450°C, hexafluoropropylene at 460°C and perfluoroisobutylene (octafluoroisobutylene) at 475°C (Waritz, 1975).

At temperatures above 500°C, other toxic thermodegradation products are produced: when PTFE is heated in the temperature range of 500-650°C, the predominant product is carbonyl fluoride. If the temperature is increased above 650°C, the products formed are carbon tetrafluoride and carbon dioxide. The LC_{50} of carbonyl fluoride was found to be 350-450 ppm for 1 hour exposure; this toxicity was found to correlate with that of PTFE pyrolysed at 550°C (Coleman *et al.*, 1968). Exposure to pyrolysis products

equivalent to 50 ppm carbonyl fluoride for 1 hour daily increased the fluoride ion content of the urine of rats from 3 to 42 µg/ml in 5 days; the same exposure produced reversible fluoride toxicity in the lungs and liver of rats within 18 days (Scheel *et al.*, 1968a).

The toxicity of the pyrolysis products of PTFE is reduced and even suppressed by passing the pyrolysis stream through a 0.45 µ pore size filter (Clayton, 1967).

Rodents exposed to such pyrolysis products showed signs of pulmonary irritation and oedema and diffuse degeneration of the brain, liver and kidneys (Lee *et al.*, 1976; Scheel *et al.*, 1968b; Treon *et al.*, 1955).

No data on the embryotoxicity, teratogenicity, metabolism or mutagenicity of this compound were available to the Working Group.

(b) Humans

Thermodegradation products of PTFE produce influenza-like symptoms ('polymer-fume fever') in humans. These may include chills, headaches, rigour-like shaking of the limbs, mild respiratory discomfort and a high fever. These symptoms disappear within a 24- or 48-hour period if the worker is removed from the working environment and rests (Barnes & Jones, 1967; Brubaker, 1977; Harris, 1951; Kuntz & McCord, 1974; Lewis & Kerby, 1965; Okawa & Polakoff, 1974; Welti & Hipp, 1968; Williams *et al.*, 1974).

The chemical factor that causes the fever has not yet been identified (Evans, 1973). Polymer-fume fever generally occurs when the worker is exposed to polymer at temperatures between 300 and 500°C; a number of thermodegradation products have been identified in this temperature range (see section 1.3 (e)).

It has been suggested that smoking increases the risk of developing polymer-fume fever (Brubaker, 1977; Lewis & Kerby, 1965; Welti & Hipp, 1968).

Fluoride levels in urine are greater than normal in workers exposed to fumes of PTFE (Okawa & Polakoff, 1974).

Williams *et al.* (1974) reported a case of a female worker who had more than 40 attacks of polymer-fume fever without pulmonary oedema during a 9-month period. A few months after the last attack, she had no symptoms; however, 18 months later she complained of shortness of breath on exertion. Chest X-ray revealed no abnormalities, but pulmonary function studies demonstrated alveolar-capillary block. Robbins & Ware (1964) and Evans (1973) described 4 cases of pulmonary oedema that result from inhalation of fumes. Brubaker (1977) described a case of pulmonary oedema in a person who had smoked PTFE-contaminated cigarettes.

3.3 Case reports and epidemiological studies

Herrmann *et al.* (1971) and Burns *et al.* (1972) both report a case of a fibrosarcoma in a 31-year old man, which was diagnosed 10½ years after implantation of a 5 cm woven PTFE-dacron arterial prosthesis. The tumour (9 x 8 x 4 cm) constricted and encircled more than half of the length of the femoral artery, including the implant, but did not invade the vessel. There was no evidence of metastasis. The authors suggested that the fibrosarcoma arose due to implantation of the plastic vascular prosthesis.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

No data on the carcinogenicity or mutagenicity of tetrafluoroethylene were available to the Working Group. Polytetrafluoroethylene discs, squares, fragments or powder implanted subcutaneously or intraperitoneally in mice or rats induced local sarcomas.

4.2 Human data

No case reports or epidemiological studies relating to general or occupational exposure to tetrafluoroethylene or polytetrafluoroethylene were available to the Working Group. There is one case report of a patient who received a woven polytetrafluoroethylene/dacron prosthesis to repair a lacerated femoral artery; a fibrosarcoma was diagnosed at the site of the prosthesis 10½ years after the initial operation.

4.3 Evaluation

General and occupational exposure to tetrafluoroethylene and polytetrafluoroethylene and medical exposure to polytetrafluoroethylene are known to occur. The induction of sarcomas by polytetrafluoroethylene implants in mice and rats, together with a single case of a fibrosarcoma in a patient treated with a polytetrafluoroethylene/dacron implant, provide insufficient evidence to assess the carcinogenic risk of exposure to tetrafluoroethylene and polytetrafluoroethylene in humans. The long-term follow-up of patients with medical implants is recommended.

5. References

Anon. (1972) Fire Protection Guide on Hazardous Materials, 4th ed., Boston, Mass., National Fire Protection Association, pp. 49-212-49-213

Barnes, R. & Jones, A.T. (1967) Polymer-fume fever. Med. J. Aust., 54, 60-61

Brubaker, R.E. (1977) Pulmonary problems associated with the use of polytetrafluoroethylene. J. occup. Med., 19, 693-695

Bryson, G. & Bischoff, F. (1969) The limitations of safety testing. Progr. exp. Tumor Res., 11, 100-133

Burns, W.A., Kanhouwa, S., Tillman, L., Saini, N. & Herrmann, J.B. (1972) Fibrosarcoma occurring at the site of a plastic vascular graft. Cancer, 29, 66-72

Clayton, J.W., Jr (1962) The toxicity of fluorocarbons with special reference to chemical constitution. J. occup. Med., 4, 262-273

Clayton, J.W., Jr (1967) Fluorocarbon toxicity and biological action. Fluorine Chem. Rev., 1, 197-252

Coleman, W.E., Scheel, L.D., Kupel, R.E. & Larkin, R.L. (1968) The identification of toxic compounds in the pyrolysis products of polytetrafluoroethylene (PTFE). Am. ind. Hyg. Assoc. J., 29, 33-40

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

Dolgina, A.I., Alekseeva, A.D. & Meshcheryakova, A.N. (1966) Control of the content of difluorochloromethane and tetrafluoroethylene in air (Russ.). Gazov. Khromatogr., 4, 120-122 [Chem. Abstr., 67, 119996c]

Evans, E.A. (1973) Pulmonary edema after inhalation of fumes from polytetrafluoroethylene (PTFE). J. occup. Med., 15, 599-601

Grasselli, J.G. & Ritchey, W.M., eds (1975) CRC Atlas of Spectral Data and Physical Constants for Organic Compounds, 2nd ed., Vol. III, Cleveland, Ohio, Chemical Rubber Co., p. 283

van Grimbergen, M., Reybrouck, G. & van de Voorde, H. (1975) Air pollution due to the burning of thermoplastics. II. (Ger.). Zbl. Bakt. Hyg., I. Abt. Orig. B, 160, 139-147

Halpern, B.D. & Karo, W. (1977) Medical applications: In: Bikales, N.M., ed., Encyclopedia of Polymer Science and Technology, Plastics, Resins, Rubbers, Fibers, Suppl. Vol. 2, New York, Interscience, pp. 369, 383-384

Harris, D.K. (1951) Polymer-fume fever. Lancet, ii, 1008-1011

Harris, D.K. (1959) Some hazards in the manufacture and use of plastics. Br. J. ind. Med., 16, 221-229

Hawley, G.G., ed. (1971) The Condensed Chemical Dictionary, 8th ed., New York, Van Nostrand-Reinhold, pp. 712-713, 857

Herrmann, J.B., Kanhouwa, S., Kelley, R.J. & Burns, W.A. (1971) Fibrosarcoma of the thigh associated with a prosthetic vascular graft. New Engl. J. Med., 284, 91

Kuntz, W.D. & McCord, C.P. (1974) Polymer-fume fever. J. occup. Med., 16, 480-482

Lee, K.P., Zapp, J.A., Jr & Sarver, J.W. (1976) Ultrastructural alterations of rat lung exposed to pyrolysis products of polytetrafluoroethylene (PTFE, Teflon). Lab. Invest., 35, 152-160

Lewis, C.E. & Kerby, G.R. (1965) An epidemic of polymer-fume fever. J. Am. med. Assoc., 191, 103-106

Marchenko, E.N. (1966) Fundamental problems of industrial hygiene in processing of polyfluoroethylene resins (Russ.). Gig. Tr. Prof Zabol., 10, 12-18 [Chem. Abstr., 66, 68685v]

McCane, D.I. (1970) Tetrafluoroethylene polymers. In: Bikales, N.M., ed., Encyclopedia of Polymer Science and Technology, Plastics, Resins, Rubbers, Fibers, Vol. 13, New York, Interscience, pp. 623-624, 626-627, 654

Mênard, S. & Della Porta, G. (1976) Incidence, growth and antigenicity of fibrosarcomas induced by Teflon disc in mice. Tumori, 62, 565-573

Okawa, M.T. & Polakoff, P.L. (1973) Health Hazard Evaluation/Toxicity Determination, Modern Industrial Plastics Division, Duriron Co., Dayton, Ohio, available from US National Technical Information Service, PB Rep. No. 229167/2GA [Chem. Abstr., 81, 68004g]

Okawa, M.T. & Polakoff, P.L. (1974) Occupational health case reports - No. 7. J. occup. Med., 16, 350-355

Oppenheimer, B.S., Oppenheimer, E.T., Stout, A.P. & Danishefsky, I. (1953) Malignant tumors resulting from embedding plastics in rodents. Science, 118, 305-306

Oppenheimer, B.S., Oppenheimer, E.T., Danishefsky, I., Stout, A.P. & Eirich, F.R. (1955) Further studies of polymers as carcinogenic agents in animals. Cancer Res., 15, 333-340

Robbins, J.J. & Ware, R.L. (1964) Pulmonary edema from Teflon fumes. Report of a case. New Engl. J. Med., 271, 360-361

Russell, F.E., Simmers, M.H., Hirst, A.E. & Pudenz, R.H. (1959) Tumors associated with embedded polymers. J. natl Cancer Inst., 23, 305-315

Scheel, L.D., McMillan, L. & Phipps, F.C. (1968a) Biochemical changes associated with toxic exposures to polytetrafluoroethylene pyrolysis products. Am. ind. Hyg. Assoc. J., 29, 49-53

Scheel, L.D., Lane, W.C. & Coleman, W.E. (1968b) The toxicity of polytetrafluoroethylene pyrolysis products - including carbonyl fluoride and a reaction product, silicon tetrafluoride. Am. ind. Hyg. Assoc. J., 29, 41-48

Simmers, M.H., Agnew, W.F. & Pudenz, R.H. (1963) Effects of plastic polymers within the rat's peritoneal cavity. Bol. Inst. Estud. Med. Biol. Méx., 21, 1-13

Sircar, A.K. & Lamond, T.G. (1972) Identification of elastomers by thermal analysis. Rubber Chem. Technol., 45, 329-345

Tomatis, L. (1963) Studies in subcutaneous carcinogenesis with implants of glass and Teflon in mice. Acta unio int. contra cancrum, 19, 607-611

Tomatis, L. (1966) Subcutaneous carcinogenesis by implants and by 7,12-dimethylbenz[α]anthracene. Tumori, 52, 1-16

Tomatis, L. & Parmi, L. (1971) Effect of perinatal administration of 7,12-dimethylbenz[α]anthracene on the later response to a subcutaneous Teflon implant. Tumori, 57, 55-62

Tomatis, L. & Shubik, P. (1963) Influence of urethane on subcutaneous carcinogenesis by 'Teflon' implants. Nature (Lond.), 198, 600-601

Treon, J.F., Cappel, J.W., Cleveland, F.P., Larson, E.E., Atchley, R.W. & Denham, R.T. (1955) The toxicity of the products formed by the thermal decomposition of certain organic substances. Am. ind. Hyg. Assoc. Q., 16, 187-195

US Department of Commerce (1977) US Imports for Consumption and General Imports, TSUSA Commodity by Country of Origin, FT 246/Annual 1976, Bureau of the Census, Washington DC, US Government Printing Office, p. 234

US Food and Drug Administration (1977) Food and drugs. US Code Fed. Regul., Title 21, parts 175.105, 175.300, pp. 438, 446, 452, 457

US International Trade Commission (1976) Synthetic Organic Chemicals, US Production and Sales, 1974, USITC Publication 776, Washington DC, US Government Printing Office, pp. 128, 131, 203, 229

US International Trade Commission (1977a) Synthetic Organic Chemicals, US Production and Sales, 1975, USITC Publication 804, Washington DC, US Government Printing Office, pp. 198, 221

US International Trade Commission (1977b) Synthetic Organic Chemicals, US Production and Sales, 1976, USITC Publication 833, Washington DC, US Government Printing Office, pp. 182, 186

US Tariff Commission (1961) Synthetic Organic Chemicals, US Production and Sales, 1960, TC Publication 34, Washington DC, US Government Printing Office, p. 180

Waritz, R.S. (1975) An industrial approach to evaluation of pyrolysis and combustion hazards. Environ. Health Perspect., 11, 197-202

Weast, R.C., ed. (1976) CRC Handbook of Chemistry and Physics, 57th ed., Cleveland, Ohio, Chemical Rubber Co., p. C-298

Welti, D.W. & Hipp, M.J. (1968) Polymer fume fever. Possible relationship to smoking. J. occup. Med., 10, 667-671

Williams, N., Atkinson, W. & Patchefsky, A.S. (1974) Polymer-fume fever: not so benign. J. occup. Med., 16, 519-522

Windholz, M., ed. (1976) The Merck Index, 9th ed., Rahway, NJ, Merck & Co., pp. 985-986

Zapp, J.A., Jr (1962) Toxic and health effects of plastics and resins. Arch. environ. Health, 4, 335-346

Zeman, A. (1972) Identification of high polymers by thermal degradation in the mass spectrometer. In: Wiedemann, H.G., ed., Proceedings of the 3rd International Conference on Thermal Analysis, Basel, Vol. 3, Basel, Birkhaeuser, pp. 219-227 [Chem. Abstr., 78, 148551m]

APPENDIX B

Excerpts from the NTP Technical Report Toxicology and Carcinogenesis Studies of Tetrafluoroethylene in F344/N Rats and B6C3F1 Mice (Inhalation Studies) April 1997, pp. 3-32, 69-78

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF TETRAFLUOROETHYLENE

(CAS NO. 116-14-3)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

April 1997

NTP TR 450

NIH Publication No. 97-3366

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTENTS

ABSTRACT 5

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY 12

TECHNICAL REPORTS REVIEW SUBCOMMITTEE 13

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS 14

INTRODUCTION 15

MATERIALS AND METHODS 21

RESULTS 33

DISCUSSION AND CONCLUSIONS 69

REFERENCES 75

APPENDIX A Summary of Lesions in Male Rats in the 2-Year Inhalation Study of Tetrafluoroethylene 79

APPENDIX B Summary of Lesions in Female Rats in the 2-Year Inhalation Study of Tetrafluoroethylene 125

APPENDIX C Summary of Lesions in Male Mice in the 2-Year Inhalation Study of Tetrafluoroethylene 165

APPENDIX D Summary of Lesions in Female Mice in the 2-Year Inhalation Study of Tetrafluoroethylene 209

APPENDIX E Genetic Toxicology 253

APPENDIX F Organ Weights and Organ-Weight-to-Body-Weight Ratios 255

APPENDIX G Hematology, Clinical Chemistry, and Urinalysis Results 263

APPENDIX H Reproductive Tissue Evaluations and Estrous Cycle Characterization 273

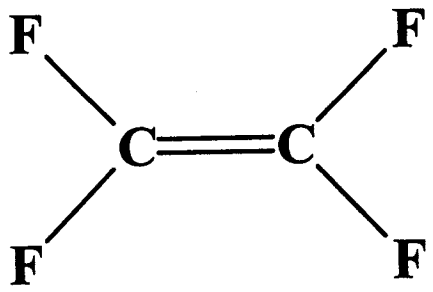
APPENDIX I Chemical Characterization and Generation of Chamber Concentrations 277

APPENDIX J Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration 299

APPENDIX K Sentinel Animal Program 303

APPENDIX L H-*ras* Codon 61 Mutation Spectra in Hepatocellular Neoplasms From B6C3F$_1$ Mice Exposed to Tetrafluoroethylene for 2 Years 307

ABSTRACT



TETRAFLUOROETHYLENE

CAS No. 116-14-3

Chemical Formula: C_2F_4 Molecular Weight: 100.02

Synonyms: Perfluoroethylene; tetrafluoroethene; 1,1,2,2-tetrafluoroethylene; TFE

Tetrafluoroethylene is used in the production of polytetrafluoroethylene (Teflon®) and other polymers. Tetrafluoroethylene was nominated by the National Cancer Institute for toxicity and carcinogenicity studies based on the potential for human exposure to the chemical due to the large production volume and on the lack of adequate data for tetrafluoroethylene in the literature. Male and female F344/N rats and B6C3F$_1$ mice were exposed to tetrafluoroethylene (98% to 99% pure) by whole body inhalation exposure for 16 days, 13 weeks, or 2 years. Genetic toxicity studies were conducted in mouse peripheral blood erythrocytes.

16-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were exposed to 0, 312, 625, 1,250, 2,500, or 5,000 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week for a total of 12 exposures during a 16-day period. All rats survived to the end of the study. The final mean body weights and body weight gains of males and females exposed to 5,000 ppm were significantly less than those of the controls. The mean body weight gain of females exposed to 2,500 ppm was also significantly less than that of the controls. There were no exposure-related clinical findings in male or female rats. There were no significant differences in hematology parameters that were considered to be related to tetrafluoroethylene exposure. Absolute and relative kidney weights of all exposed groups of males were significantly greater than those of the controls, as were those of females in the 2,500 and 5,000 ppm groups. The absolute kidney weight of females exposed to 1,250 ppm was also significantly greater than that of the controls. The relative liver weights of all exposed groups of males and the absolute liver weights of males in the 625 and 2,500 ppm groups were significantly greater than those of the controls. Increased incidences of renal tubule degeneration occurred in males and females exposed to 625 ppm or greater; this lesion was located predominantly at the corticomedullary junction. The severity of degeneration increased with increasing exposure concentration and was slightly greater in males than females.

16-DAY STUDY IN MICE

Groups of five male and five female B6C3F$_1$ mice were exposed to 0, 312, 625, 1,250, 2,500, or 5,000 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week for a total of 12 exposures during a 16-day period. All mice survived to the end of the study. Final mean body weights and body weight gains of all exposed groups of mice were similar to those of the controls. There were no exposure-related clinical findings in male or

female mice. There were no significant differences in hematology parameters that were considered to be related to tetrafluoroethylene exposure. The absolute and relative liver weights of females exposed to 5,000 ppm were significantly greater than those of the controls, as was the absolute kidney weight of females in that group and the absolute liver weight of females in the 2,500 ppm group. Renal tubule karyomegaly was observed in male and female mice in the 1,250, 2,500, and 5,000 ppm groups, and the severity of this lesion increased with increasing exposure concentration. Karyomegaly was located predominantly in the inner renal cortex.

13-Week Study in Rats

Groups of 10 male and 9 or 10 female F344/N rats were exposed to 0, 312, 625, 1,250, 2,500, or 5,000 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week, for 13 weeks. All rats survived to the end of the study. The final mean body weight and body weight gain of males exposed to 5,000 ppm were significantly less than those of the controls, as was the mean body weight gain of females in this exposure group. There were no clinical findings attributed to exposure to tetrafluoroethylene. Exposure of rats to tetrafluoroethylene resulted in a concentration-dependent normocytic, normochromic, nonresponsive anemia consistent with a secondary hypoproliferative anemia. An exposure concentration-dependent proteinuria also occurred, consistent with renal tubule degeneration observed histopathologically. The absolute and relative liver weights of all exposed groups of males and of females in the 5,000 ppm group were significantly greater than those of the controls. The absolute and relative right kidney weights of males and females exposed to 1,250 ppm or greater and of females in the 625 ppm group were also significantly greater than those of the controls. There were no differences in sperm morphology or vaginal cytology parameters between control and exposed groups of rats. Incidences of renal tubule degeneration in males exposed to 625 ppm or greater and in females exposed to 2,500 or 5,000 ppm were significantly greater than those in the controls. Renal lesions were similar to those observed in the 16-day study and were located predominantly at the corticomedullary junction.

13-Week Study in Mice

Groups of 10 male and 10 female B6C3F$_1$ mice were exposed to 0, 312, 625, 1,250, 2,500, or 5,000 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week, for 13 weeks. All mice survived to the end of the study. Final mean body weights and body weight gains of all exposed groups of male and female mice were generally similar to those of the controls. There were no clinical findings that were considered to be related to tetrafluoroethylene exposure. Exposure of mice to tetrafluoroethylene resulted in a concentration-dependent normocytic, normochromic, nonresponsive anemia, consistent with a secondary hypoproliferative anemia, and in polyuria. Differences in sperm morphology parameters and estrous cycle lengths were not considered to be exposure related. Incidences of karyomegaly of the renal tubule epithelial cells in male and female mice exposed to 1,250 ppm or greater were significantly greater than those in the controls. Karyomegaly was similar to that observed in the 16-day study and was observed primarily in the inner renal cortex.

2-Year Study in Rats

Groups of 60 male rats were exposed to 156, 312, or 625 ppm and groups of 60 female rats were exposed to 312, 625, or 1,250 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week, for 104 weeks, with an observation period of 11 days following the final exposure. Ten male and ten female rats from each exposure group were evaluated at 15 months for organ weights and clinical pathology.

Survival, Body Weights, and Clinical Findings

Survival rates of males in the 625 ppm group and of all exposed groups of females were significantly less than those of the controls. Mean body weights of males exposed to 625 ppm were lower than those of the controls from week 81 until the end of the study, and the mean body weight of 1,250 ppm females was slightly lower than that of the controls at the end of the study. The only clinical finding associated with exposure to tetrafluoroethylene was opacity of the eyes in exposed groups of female rats; this change was observed microscopically as cataracts.

Hematology, Clinical Chemistry, and Urinalysis

At the 15-month interim evaluation, there were no differences in hematology, clinical chemistry, or urinalysis parameters that were considered to be related to tetrafluoroethylene exposure.

Pathology Findings

The absolute and relative kidney weights of males exposed to 625 ppm and females exposed to 1,250 ppm and the absolute kidney weight of females exposed to 625 ppm were significantly greater than those of the controls at the 15-month interim evaluation. At 15 months, renal tubule hyperplasia was observed in one male exposed to 312 ppm and one male and one female exposed to 625 ppm; oncocytic hyperplasia was observed in one female exposed to 1,250 ppm. At the end of the study, incidences of renal tubule adenoma were greater in males and females exposed to 312 ppm or greater than those in the controls. This exposure-related increase was confirmed by examination of step sections (extended evaluations). At the end of the study, the incidences of renal tubule hyperplasia in males exposed to 625 ppm and females exposed to 1,250 ppm were significantly greater than those in the controls. The incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) in the extended evaluations and in the standard and extended evaluations (combined) in the 1,250 ppm female group and the 625 ppm male group were significantly greater than those in the controls, and the incidences occurred with significant positive trends. Oncocytic hyperplasia was observed at the end of the study in one male exposed to 312 ppm and in three females exposed to 1,250 ppm. At 15 months and at the end of the study, the incidences of renal tubule degeneration in all exposed groups of males and in females in the 625 and 1,250 ppm groups were greater than those in the controls. Renal tubule degeneration was similar to that observed in the 13-week study and was located predominantly at the corticomedullary junction. The severity of nephropathy generally increased with increasing exposure concentration in male rats at 15 months and 2 years.

The absolute and relative liver weights of females in the 1,250 ppm group and the absolute liver weight of females exposed to 625 ppm were significantly greater than those of the controls at the 15-month interim evaluation. At 2 years, the incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in males exposed to 312 ppm, the incidences of hepatocellular adenoma and adenoma or carcinoma (combined) in females in all exposed groups, and the incidences of hepatocellular carcinoma in females exposed to 312 or 625 ppm were significantly greater than those in the controls. Also at 2 years, the incidence of hemangiosarcoma in females exposed to 625 ppm was significantly greater than that in the controls. In all exposed groups of males, the incidences of clear cell foci at 15 months were greater than those in the controls; at 2 years, the incidences of eosinophilic foci in all exposed groups of males and the incidences of basophilic and mixed cell foci in males in the 312 and 625 ppm groups were greater than those in the controls. The incidences of mixed cell foci at 15 months in females exposed to 625 or 1,250 ppm and at 2 years in females exposed to 1,250 ppm were also significantly greater than those in the controls. At the end of the 2-year study, increased incidences of cystic degeneration occurred in the liver of all exposed groups of males, and increased incidences of hepatic angiectasis were observed in exposed groups of females.

Incidences of mononuclear cell leukemia in males exposed to 156 ppm and in all exposed groups of females were significantly greater than those in the controls.

Incidences of cataracts in females exposed to 1,250 ppm were greater than those in the controls at the end of the 2-year study.

At the end of the study, there were slight increases in the incidences of testicular interstitial cell adenoma in rats exposed to 312 or 625 ppm.

2-YEAR STUDY IN MICE

Groups of 58 male and 58 female B6C3F$_1$ mice were exposed to 0, 312, 625, or 1,250 ppm tetrafluoroethylene by inhalation for 6 hours per day, 5 days per week, for 95 to 96 weeks. Ten male and ten female mice from each exposure group were evaluated at 15 months for organ weights.

Survival, Body Weights, and Clinical Findings

The survival rates of all exposed groups of males and females were significantly less than those of the controls. Because of the reduced survival due to

exposure-related liver neoplasms, the study was terminated during week 96. Mean body weights of exposed groups of males and females were generally similar to those of the controls, except at the end of the study, when they were somewhat less than those of the controls. There were no clinical findings related to tetrafluoroethylene exposure.

Pathology Findings

At the 15-month interim evaluation, there were no differences in absolute or relative kidney, liver, or lung weights between exposed and control groups of mice. At the end of the study, the incidences of multifocal coagulative necrosis of the liver were increased in males in the 625 and 1,250 ppm groups. Also at the end of the study, females in all exposed groups had greater incidences of hematopoietic cell proliferation in the liver than the controls. Angiectasis occurred in all exposed groups of males and females at 15 months and at the end of the study. At the 15-month interim evaluation, hemangiosarcomas were observed in three males exposed to 1,250 ppm and in one female exposed to 312 ppm. The incidences of hemangiosarcoma in all exposed groups of males and females at the end of the study were significantly greater than those in the controls and exceeded the historical chamber control ranges. Also at the end of the study, the incidences of hemangioma in males and females exposed to 312 ppm and in males exposed to 625 ppm were also significantly greater than those in the controls and exceeded the range in historical chamber controls. At 15 months, hepatocellular adenomas and carcinomas occurred in control males and all exposed groups of males and females. Females exposed to 625 or 1,250 ppm had significantly greater incidences of eosinophilic foci than the controls at the 15-month interim evaluation. At the end of the study, the incidences of eosinophilic foci in males exposed to 625 or 1,250 ppm and in females exposed to 312 or 625 ppm were significantly greater than those in the controls. In male and female mice, increased incidences of a variety of hepatocellular neoplasms, including adenomas, multiple adenomas, carcinomas, and multiple carcinomas, were considered related to tetrafluoroethylene exposure.

At the end of the study, the incidences of histiocytic sarcoma (all organs) in all exposed groups of males and females were significantly greater than those in the controls and exceeded the historical control ranges for all organs. The greatest incidences of histiocytic sarcomas were observed in the liver and lung, but these neoplasms were also observed in the spleen, lymph nodes, bone marrow, and kidney.

Significantly increased incidences of renal tubule dilatation (males) and karyomegaly (males and females), located predominantly in the inner cortex, were observed in mice exposed to 625 or 1,250 ppm at 15 months. At the end of the study, the increased incidences of dilatation and karyomegaly in all exposed groups of males and of karyomegaly in 1,250 ppm females were generally significant.

Incidences of hematopoietic cell proliferation in the spleen of all exposed groups of males and females were significantly greater than those in the controls at the end of the study. Additionally, the severity of this lesion increased with increasing exposure concentration.

GENETIC TOXICOLOGY

No increases in the frequency of micronucleated erythrocytes were observed in peripheral blood samples obtained from male and female mice at the end of the 13-week inhalation study of tetrafluoroethylene.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of tetrafluoroethylene in male F344/N rats based on increased incidences of renal tubule neoplasms (mainly adenomas) and hepatocellular neoplasms. There was *clear evidence of carcinogenic activity* of tetrafluoroethylene in female F344/N rats based on increased incidences of renal tubule neoplasms, liver hemangiosarcomas, hepatocellular

neoplasms, and mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* of tetrafluoroethylene in male and female B6C3F$_1$ mice based on increased incidences of liver hemangiomas and hemangiosarcomas, hepatocellular neoplasms, and histiocytic sarcomas.

Slight increases in the incidences of mononuclear cell leukemia and testicular interstitial cell adenomas in male rats may have been related to exposure to tetrafluoroethylene.

Exposure of rats to tetrafluoroethylene resulted in increased incidences of renal tubule hyperplasia and degeneration in males and females, increased severity of kidney nephropathy in males, and increased incidences of liver angiectasis and cataracts in females. Exposure of mice to tetrafluoroethylene resulted in increased incidences of hematopoietic cell proliferation of the liver in females, liver angiectasis in males and females, renal tubule dilatation in males, renal tubule karyomegaly in males and females, and splenic hematopoietic cell proliferation in males and females.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Tetrafluoroethylene

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Exposure Concentrations	0, 156, 312, or 625 ppm	0, 312, 625, or 1,250 ppm	0, 312, 625, or 1,250 ppm	0, 312, 625, or 1,250 ppm
Body Weights	625 ppm group lower than controls	1,250 ppm group slightly lower than controls at end of study	Exposed groups lower than controls at end of study	Exposed groups lower than controls at end of study
Survival Rates	17/50, 12/50, 17/50, 1/50 (2 years)	28/50, 16/50, 15/50, 18/50 (2 years)	38/48, 11/48, 2/48, 1/48 (22 months)	36/48, 4/48, 6/48, 4/48 (22 months)
Nonneoplastic Effects	Kidney (renal tubule): hyperplasia (single sections - 1/50, 1/50, 1/50, 6/50; single and step sections - 7/50, 11/50, 7/50, 24/50); degeneration (2/50, 20/50, 50/50, 49/50); severity of nephropathy (2.3, 1.9, 2.7, 3.5)	Kidney (renal tubule): hyperplasia (single sections - 1/50, 3/50, 6/50, 12/50; single and step sections - 3/50, 6/50, 11/50, 25/50); degeneration (0/50, 0/50, 35/50, 46/50) Liver: angiectasis (0/50, 9/50, 9/50, 14/50) Eye: cataracts (15/50, 4/50, 10/50, 45/50)	Liver: angiectasis (0/48, 6/48, 10/48, 13/48) Kidney (renal tubule): dilatation (0/48, 4/48, 16/48, 36/48); karyomegaly (1/48, 2/48, 10/48, 28/48) Spleen: hematopoietic cell proliferation (14/48, 32/48, 41/46, 42/46)	Liver: hematopoietic cell proliferation (3/48, 19/48, 13/47, 15/47); angiectasis (1/48, 9/48, 6/47, 4/47) Kidney (renal tubule): karyomegaly (0/48, 0/48, 0/47, 38/48) Spleen: hematopoietic cell proliferation (18/48, 39/48, 41/46, 41/47)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Tetrafluoroethylene (continued)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic Effects	Kidney (renal tubule): adenoma (single sections - 0/50, 0/50, 6/50, 3/50; single and step sections - 2/50, 4/50, 9/50, 13/50); carcinoma (single sections - 1/50, 0/50, 2/50, 0/50; single and step sections - 1/50, 1/50, 2/50, 0/50); adenoma or carcinoma (single sections - 1/50, 0/50, 6/50, 3/50; single and step sections - 3/50, 5/50, 9/50, 13/50) Liver: hepatocellular carcinoma (1/50, 1/50, 10/50, 3/50); hepatocellular adenoma or carcinoma (4/50, 7/50, 15/50, 8/50)	Kidney (renal tubule): adenoma (single sections - 0/50, 3/50, 1/50, 3/50; single and step sections - 0/50, 3/50, 3/50, 8/50); carcinoma (single sections - 0/50, 0/50, 0/50, 2/50; single and step sections - 0/50, 0/50, 0/50, 3/50); adenoma or carcinoma (single sections - 0/50, 3/50, 1/50, 5/50; single and step sections - 0/50, 3/50, 3/50, 10/50) Liver: hemangiosarcoma (0/50, 0/50, 5/50, 1/50); hepatocellular adenoma (0/50, 4/50, 5/50, 6/50); hepatocellular carcinoma (0/50, 4/50, 9/50, 2/50); hepatocellular adenoma or carcinoma (0/50, 7/50, 12/50, 8/50) Mononuclear cell leukemia: (16/50, 31/50, 23/50, 36/50)	Liver: hemangioma (0/48, 10/48, 5/48, 2/48); hemangiosarcoma (0/48, 21/48, 27/48, 37/48); hemangioma or hemangiosarcoma (0/48, 26/48, 30/48, 38/48); hepatocellular carcinoma (11/48, 20/48, 33/48, 26/48); hepatocellular adenoma or carcinoma (26/48, 34/48, 39/48, 35/48) Hematopoietic system (all organs): histiocytic sarcoma (0/48, 12/48, 7/48, 7/48)	Liver: hemangioma (0/48, 5/48, 2/47, 1/47); hemangiosarcoma (0/48, 27/48, 27/47, 34/47); hemangioma or hemangiosarcoma (0/48, 31/48, 28/47, 35/47); hepatocellular carcinoma (4/48, 28/48, 22/47, 20/47); hepatocellular adenoma or carcinoma (17/48, 33/48, 29/47, 28/47) Hematopoietic system (all organs): histiocytic sarcoma (1/48, 21/48, 19/47, 18/48)
Uncertain Findings	Mononuclear cell leukemia: (34/50, 43/50, 38/50, 31/50) Testis: interstitial cell adenoma (39/50, 40/50, 48/50, 47/50)	None	None	None
Level of Evidence of Carcinogenic Activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence
Genetic Toxicology				
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:		Negative		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on tetrafluoroethylene on December 5, 1995, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Arnold L. Brown, M.D., Chairperson
University of Wisconsin Medical School
Madison, WI

Gary P. Carlson, Ph.D., Principal Reviewer
School of Health Sciences
Purdue University
West Lafayette, IN

Thomas L. Goldsworthy, Ph.D.
Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Robert LeBoeuf, Ph.D.
Corporate Professional and Regulatory Services
Human Safety Department
The Procter & Gamble Company
Cincinnati, OH

Janardan K. Reddy, M.D., Principal Reviewer*
Department of Pathology
Northwestern University Medical School
Chicago, IL

Irma Russo, M.D.
Fox Chase Cancer Center
Philadelphia, PA

Louise Ryan, Ph.D., Principal Reviewer
Division of Biostatistics
Dana-Farber Cancer Institute
Boston, MA

Robert E. Taylor, M.D., Ph.D.
Department of Pharmacology
Howard University College of Medicine
Washington, DC

Frederick L. Tyson, Ph.D.
St. Mary's Hospital and Medical Center
Cancer Research Institute
Grand Junction, CO

Jerrold M. Ward, D.V.M., Ph.D.*
National Cancer Institute
Frederick, MD

---

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 5 December 1995, the draft Technical Report on the toxicology and carcinogenesis studies of tetrafluoroethylene received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.H. Roycroft, NIEHS, introduced the toxicology and carcinogenesis studies of tetrafluoroethylene by describing the uses of the chemical, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplasms and nonneoplastic lesions in male and female rats and mice. Dr. R.C. Sills, NIEHS, presented data from ongoing molecular biology studies characterizing the H-*ras* codon 61 mutation spectra in hepatocellular neoplasms from control B6C3F$_1$ mice and mice exposed to tetrafluoroethylene for 2 years. Data were also presented comparing the mutation profiles of hepatocellular neoplasms from the current study with those from the NTP study of tetrachloroethylene. The proposed conclusions for the 2-year studies were *clear evidence of carcinogenic activity* in male and female F344/N rats and male and female B6C3F$_1$ mice.

Dr. Ryan, a principal reviewer, agreed with the proposed conclusions. She inquired as to whether short-burst, high exposures had been considered in the study design as being more similar to a typical occupational exposure. Dr. Roycroft replied that this regimen had not been considered; the 6-hour-per-day, 5-day-per-week design used was similar to continuous exposure in a workplace situation. Dr. Ryan asked whether there was information on the degree of human exposure encountered through spills or leaks. Dr. Roycroft noted that tetrafluoroethylene is produced and maintained in closed-capture systems, no occupational exposure limits have been established, and no information on spills or leaks has been found.

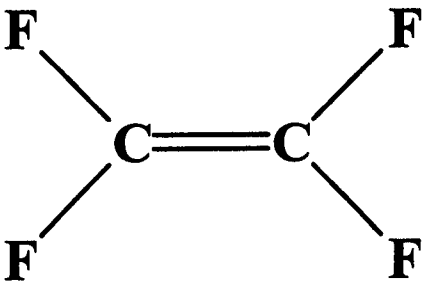
Dr. Carlson, the second principal reviewer, agreed with the proposed conclusions. He observed that the numbers of clinical pathology measurements appeared to be decreased from past reports and, in view of the types of lesions seen, wondered why. Dr. Roycroft responded that, in actuality, there were more measurements; in addition to standard time points and collections at 13 weeks, clinical pathology analyses were performed on animals killed at 15 months. Dr. Carlson said a sentence needed to be added to the Abstract regarding the reason for terminating the mouse study early. Dr. Roycroft agreed.

Dr. Reddy, the third principal reviewer, was unable to attend the meeting but had submitted his review, which Dr. L.G. Hart, NIEHS, read into the record. Dr. Reddy agreed with the proposed conclusions and found the report acceptable.

There was some discussion about the mixing of rats and mice in exposure chambers. Dr. Goldsworthy asked if hormonal alterations were seen. Dr. Roycroft said that hormone measurements were not made, but if a chemical to be studied was known to affect hormone levels, then separate housing might have to be considered. Dr. G.N. Rao, NIEHS, noted that rats and mice are produced in the same rooms so each species is accustomed to the other's presence; he pointed out that each inhalation chamber is by itself a room, and within the chamber, rats and mice are not side by side in cages, but rather in racks. This provides more efficient and economical use of available space. Dr. LeBoeuf commended the NTP for the molecular biology studies evaluating the H-*ras* activation profiles and noted the usefulness of such studies for risk assessment purposes.

Dr. Ryan moved that the Technical Report on tetrafluoroethylene be accepted with the revisions discussed and with the conclusions as written for male and female rats and male and female mice, *clear evidence of carcinogenic activity*. Dr. Carlson seconded the motion, which was accepted unanimously with seven votes.

INTRODUCTION



TETRAFLUOROETHYLENE

CAS No. 116-14-3

Chemical Formula: C_2F_4 Molecular Weight: 100.02

Synonyms: Perfluoroethylene; tetrafluoroethene; 1,1,2,2-tetrafluoroethylene; TFE

CHEMICAL AND PHYSICAL PROPERTIES

Tetrafluoroethylene is a colorless, odorless gas with a melting point of -142.5° C, a boiling point of -76.3° C, a specific gravity of 1.52 at -76° C, a vapor density of 3.87, and a vapor pressure of 428.4 mm Hg. It is insoluble in water. Tetrafluoroethylene is a highly flammable gas that reacts with oxidants and has a flash point of less than 0° C and an autoignition temperature of 188° C. The lower explosion limit of the chemical is 10% v/v and the upper explosion limit is 50% v/v. Tetrafluoroethylene can dimerize under appropriate temperature and pressure conditions to a stable perfluorocyclobutene and polymerizes easily in the absence of inhibitors or in the presence of heat or oxygen (IARC, 1979; Bretherick, 1985; Weast, 1985; NFPA, 1986; *Material Safety Data Sheet Collection*, 1993; HSDB, 1995).

PRODUCTION, USE, AND HUMAN EXPOSURE

Tetrafluoroethylene is generally produced by the pyrolysis of chlorodifluoromethane or trifluoromethane (IARC, 1979; Hawley, 1981; *Material Safety Data Sheet Collection*, 1993). Although tetrafluoroethylene was reported to the United States International Trade Commission for the year 1992, there was no estimate of the annual production (USITC, 1994). The most recent estimated United States annual production figure of 28 million pounds was reported in 1978. Virtually all of the tetrafluoroethylene produced in the United States is used in the synthesis of Teflon®, one of several polytetrafluoroethylene polymers (Kennedy, 1990). Because of its chemical properties, tetrafluoroethylene is produced and maintained in closed-capture systems. For this reason, human exposure would be primarily through leakage from these systems or through contact with one of several decomposition products of high-temperature processing or pyrolysis of Teflon® and other polymers (Kennedy, 1990). According to the National Occupational Exposure Survey, 14,963 male and 325 female workers were potentially exposed to tetrafluoroethylene from the years 1981 to 1983 (NIOSH, 1990). Currently, no occupational exposure limits have been established. There were no reports in the literature of the detection of tetrafluoroethylene in ambient air, drinking water, or wastewater, and the chemical is not known to occur naturally.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Dilley *et al.* (1974) reported the urinary excretion of fluoride ion following a 30-minute exposure of Sprague-Dawley rats to 3,500 ppm tetrafluoroethylene by inhalation. A significant increase in fluoride excretion occurred on days 6, 13, and 14 following exposure. Urinary creatinine, potassium, and volume were significantly increased following exposure to tetrafluoroethylene. Increased urinary fluoride was observed in male rats and hamsters exposed to 100 to 2,500 ppm tetrafluoroethylene by inhalation for 2 weeks; however, urinary fluoride concentrations returned to normal upon cessation of exposure (Kennedy, 1990). Male and female rats and hamsters exposed to 200 to 2,000 ppm tetrafluoroethylene by inhalation for 18 weeks displayed an exposure-related increase in urinary fluoride (Kennedy, 1990). Ding *et al.* (1980) exposed rabbits to 1,000 ppm tetrafluoroethylene for 60 minutes via a face mask and determined alveolar absorption to be 6.76%. Animals were evaluated during exposure and for a period of up to 75 minutes after exposure ended. The lung, bone, and kidney had the highest fluoride content of the sites examined.

As a part of an investigation of tetrafluoroethylene-induced nephrotoxicity, Odum and Green (1984) demonstrated that liver slices from Wistar rats metabolized tetrafluoroethylene to *S*-(1,1,2,2-tetrafluoroethyl)glutathione. The reaction was catalyzed by both microsomal and cytosolic glutathione *S*-transferases, with the rate of formation with microsomal fractions occurring at four times the rate with cytosol fractions. These same authors also identified the cysteinylglycine and cysteine conjugates of tetrafluoroethylene in bile from rats exposed to 6,000 ppm tetrafluoroethylene for 6 hours. Oral administration of the cysteine conjugate [*S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine] to rats caused renal damage identical to that caused by tetrafluoroethylene. Cytochrome P_{450} oxidation did not appear to be involved in tetrafluoroethylene metabolism.

Cysteine conjugates of the nephrotoxins hexachlorobutadiene, tetrafluoroethylene, and hexafluoropropane, together with those of trichloroethylene and perchloroethylene, have been chemically synthesized, and a relationship between their structures, nephrotoxicity, and mutagenicity *in vitro* has been determined (Green and Odum, 1985). All of the conjugates had a marked effect on the uptake of both the organic anion *p*-aminohippuric acid and the cation tetraethylammonium bromide into rat kidney slices, suggesting activation of the conjugates in the slices to a toxic species which interferes with ion transport. This observation is consistent with the known nephrotoxicity of hexachlorobutadiene, tetrafluoroethylene, and hexafluoropropene *in vivo*. Each of the conjugates was found to be metabolized by rat kidney slices and by semi-purified rat kidney β-lyase to pyruvate, ammonia, and an unidentified reactive metabolite (postulated at that time to be a thiol). When semi-purified β-lyase was used, stoichiometric amounts of pyruvate and ammonia were produced. Although all of the conjugates were activated by β-lyase and had a similar effect on ion transport, their mutagenicity differed markedly. The conjugates of hexachlorobutadiene, trichloroethylene, and tetrachloroethylene were mutagenic in the Ames bacterial mutation assay when activated by rat kidney S9. Metabolic cofactors were not required, suggesting that activation was due to the enzyme β-lyase. In the same assay, conjugates of tetrafluoroethylene and hexafluoropropene were not mutagenic either in the presence or absence of rat kidney S9 and cofactors. With a limited number of cysteine conjugates, a clear distinction was identified between the conjugates of fluoroalkenes that were similarly nephrotoxic but not mutagenic. The mutagenicity of the cysteine conjugate of hexachlorobutadiene is consistent with the known renal carcinogenicity of this chemical.

Since the initial observations by Odum and Green (1984), a number of laboratories have investigated the *in vitro* and *in vivo* metabolism of nephrotoxic haloalkenes (including tetrafluoroethylene) and their cysteine conjugates in order to determine the mechanism of haloalkene-induced nephrotoxicity. The bioactivation of the haloalkenes likely begins with glutathione conjugation in the liver. These conjugates are primarily excreted via the bile in the small intestine, where biliary and intestinal peptidases degrade them to cysteine-*S*-conjugates which may be reabsorbed from the small intestine. Glutathione conjugates released into the general circulation from the liver are hydrolyzed to the cysteine-*S*-conjugates by renal peptidases and are bioactivated by renal β-lyase to unstable, reactive thiols. Commandeur *et al.* (1988) administered tetrafluoroethylene-mercapturic acid intraperitoneally to Wistar rats at doses of 50 μmol/kg body weight or greater. After 48 hours,

nephrotoxicity, but not liver toxicity, ensued, as evidenced by increased blood urea nitrogen, urinary protein and glucose, and relative kidney weights. Excretion of difluoroacetic acid was dose related, represented 10% of the dose of 50 μmol/kg body weight, and occurred primarily during the first 24 hours. Difluoroacetic acid, pyruvate, hydrogen sulfide, and thiosulfate were formed during *in vitro* metabolism of the tetrafluoroethylene-cysteine conjugate by rat renal cytosol. Difluoroacetic acid formation was inhibited by aminooxyacetic acid, a β-lyase inhibitor, which points to a β-lyase dependency for its formation. This suggests that the tetrafluoroethylene-cysteine conjugate is bioactivated to a significant extent to difluorothionacyl fluoride and is subsequently metabolized to difluoroacetic acid (Commandeur *et al.*, 1988). Male Wistar rats were also injected with 50 μmol tetrafluoroethylene-cysteine conjugate/kg body weight. After 48 hours, 4% of the dose was excreted as the corresponding mercapturic acid. In order to evaluate the enzymatic activation or deactivation of various halogenated cysteine conjugates and mercapturic acids, rat liver and kidney cytosol (activation) and microsomes (deactivation) were incubated with the tetrafluoroethylene-cysteine conjugates and mercapturic acid or various chloro- and bromofluoro cysteine conjugates and mercapturic acids. Tetrafluoroethylene-cysteine conjugate incubated with β-lyase or tetrafluoroethylene-mercapturic acid with *N*-deacetylase demonstrated a greater activation activity with kidney cytosol than with liver. In addition, tetrafluoroethylene-cysteine conjugates and mercapturic acids demonstrated greater activity than chloro- or bromofluoro compounds. Deactivation as evidenced by *N*-acetyltransferase activity (microsomes) was greater with the chloro- and bromofluoro compounds than with tetrafluoroethylene conjugates or mercapturic acid. Deactivation activity was greatest with renal microsomes for both tetrafluoroethylene compounds (Commandeur *et al.*, 1991). The same laboratory also demonstrated that when isolated proximal tubule cells from Wistar rat kidneys were incubated with 100 μmol of tetrafluoroethylene-cysteine conjugates or mercapturic acid and other chloro- and bromofluoro compounds, tetrafluoroethylene compounds were more toxic, as evidenced by their ability to inhibit α-methylglucose uptake by the cells (Boogaard *et al.*, 1989). The cytotoxicity induced by both tetrafluoroethylene compounds was inhibited by aminooxyacetic acid; however, only the toxicity induced by tetrafluoroethylene-mercapturic acid could be inhibited by probenecid, suggesting that the two tetrafluoroethylene compounds are transported by a different carrier system. Tetrafluoroethylene cysteine accumulated in the proximal tubule cells on exposure to either tetrafluoroethylene-cysteine conjugate or tetrafluoroethylene-mercapturic acid (Boogaard *et al.*, 1989). Commandeur *et al.* (1989) demonstrated that the tetrafluoroethylene-cysteine conjugate may be bioactivated in the rat kidney and, to a lesser extent, in the rat liver to electrophilic thioacylating reactive intermediates, probably thionacetyl fluorides, which are the putative reactive intermediates for tetrafluoroethylene-induced nephrotoxicity.

A number of studies have demonstrated that the tetrafluoroethylene-cysteine conjugate and tetrafluoroethylene-derived thionacetyl fluorides affect rat and rabbit kidney macromolecules. Using isolated rat kidney mitochondria, Hayden and Stevens (1990) demonstrated that tetrafluoroethylene-cysteine conjugate was a good substrate for the mitochondrial β-lyase, inhibited ADP-stimulated respiration (primarily stage III), was covalently bound to macromolecules (blocked by aminooxyacetic acid), and was metabolized by mitochondria. Rabbit renal proximal tubules incubated with 25 μmol tetrafluoroethylene-cysteine conjugate produced similar results, including a sevenfold increase in lipid peroxidation (Groves *et al.*, 1993). Although the majority of binding in rat mitochondria is associated with the protein fraction, Hayden *et al.* (1992) have shown that significant binding occurs in the lipid fraction, and the major phospholipid adduct is the thioamide adduct of phosphatidylethanolamine.

Harris *et al.* (1992) administered 110 mmol tetrafluoroethylene-cysteine conjugate intraperitoneally to male Fischer rats. After 1 hour, kidneys were removed, and cytosolic, microsomal, and mitochondrial fractions were obtained. The single, stable amino acid adduct formed with renal protein was *N*-(difluorothioacetyl)lysine. Urine taken from bladders at necropsy was analyzed for metabolites. Mercapturic acids, dihaloacetic acids, and inorganic fluoride were present, as in previously reported studies. In a similar experiment, Hayden *et al.* (1991a) intraperitoneally administered 30 mg tetrafluoroethylene-cysteine conjugate/kg body weight to male Sprague-Dawley rats. The resulting kidney toxicity showed a good correlation with metabolite binding in that difluorothioamide adducts and toxicity were localized to the medullary ray, which contains

the S3C and S3M segments of the proximal tubule. Cell death occurred only in cells containing adducts.

Protein adducts such as *N*-acetyl-*N*-(difluorothionacetyl)lysine (Hayden *et al.*, 1991b) from rat renal subcellular fractions and *N*-(difluorothionoacetyl)-*S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine (Commandeur *et al.*, 1989) from rat renal and liver subcellular fractions have been isolated. Bruschi *et al.* (1993) have demonstrated that as many as five mitochondrial proteins are modified following intraperitoneal injection of male F344/N rats with 30 mg tetrafluoroethylene-cysteine conjugate/kg body weight; modification also occurs at the time of formation of the *N*-acetyl-*N*-(difluorothionacetyl) lysine adduct. Two of the proteins have been identified as the heat shock proteins HSP60 (P1 protein) and HSP70-like protein (mortalin). HSP70 and HSP70 mRNA are increased in porcine renal epithelial cell cultures when tetrafluoroethylene-cysteine conjugate is added (Chen *et al.*, 1992). Lock and Schnellmann (1990), using *in vitro* studies with rat renal mitochondrial or cytosolic preparations, have demonstrated that the addition of tetrafluoroethylene-cysteine conjugate inhibits lipoyl dehydrogenase and glutathione reductase activity, respectively.

Humans

No studies on the absorption, distribution, metabolism, or excretion of tetrafluoroethylene by humans were found in the available literature (National Library of Medicine, 1995).

TOXICITY

Experimental Animals

Sakharova and Tolgskaya (1977) reported LC_{50} values of 31,000 to 32,000 ppm for rats; 35,000 ppm for mice; and 28,000 ppm for guinea pigs following 4-hour inhalation exposures to tetrafluoroethylene. Kennedy (1990) reported a similar 4-hour LC_{50} of 28,500 ppm for hamsters. Zhemerdei (1958) reported that the lowest concentration of tetrafluoroethylene that caused mortality in rabbits was 40,000 ppm.

Odum and Green (1984) exposed male Wistar rats to tetrafluoroethylene for 6 hours at concentrations of 1,000, 2,000, 3,000, 4,000, and 6,000 ppm to evaluate the potential renal toxicity of the compound. Blood urea nitrogen values, urine volume and urinary excretion of glucose, and alkaline phosphatase and γ-glutamyltranspeptidase activities were significantly increased in rats exposed to 4,000 or 6,000 ppm. These changes are indicative of renal tubule damage. Histopathologic evaluations of the kidneys were conducted on rats in the control and 6,000 ppm groups. All exposed rats exhibited marked renal necrosis involving the pars recta of the proximal tubules. In addition, there were intertubule calcified deposits in the medulla. The no-observable-effect level for kidney toxicity was 2,000 ppm. As in previously discussed studies, oral exposure to tetrafluoroethylene-cysteine conjugate caused renal damage identical to that observed following exposure to tetrafluoroethylene via inhalation.

A number of industry-sponsored studies with tetrafluoroethylene have been conducted but not published. Kennedy (1990) has provided a brief review of some of these studies. Because few repeated-exposure studies with tetrafluoroethylene are presented in the available published literature, several of the studies are summarized here. Syrian hamsters and male Crl:CD rats were exposed to tetrafluoroethylene for 6 hours per day, 5 days per week, for 2 weeks at exposure concentrations of 0, 100, 500, 1,000, and 2,500 ppm. Some of the animals were evaluated at the end of the exposure period, and the remaining animals were evaluated following a 14-day recovery period. No clinical signs of toxicity were observed in either species. There were no significant differences in hamster absolute or relative organ weights (liver or kidney) at either evaluation period. After the tenth exposure, absolute and relative kidney and liver weights were significantly increased in rats exposed to 2,500 ppm tetrafluoroethylene; however, following the recovery period, kidney and liver weights were similar to those of the control group. Exposure-related kidney lesions in rats exposed to 2,500 ppm were characterized by swelling of the tubular epithelial cells and dilation of the tubular lumen. Sparse cellular degeneration was observed and localized to the juxtamedullary cortex. No significant lesions were apparent following the 14-day recovery period. No significant histopathologic changes were observed in tetrafluoroethylene-exposed hamsters after the tenth exposure; however, following the 14-day recovery period, testicular atrophy was observed in the 2,500 ppm group. This effect consisted of degeneration and sloughing of germinal epithelial cells, resulting in an absence or reduction in the number of mature sperm in both the testes and epididymides, and the presence of degenerative germ cells sloughed into

the lumen of the epididymal ducts. These effects were noted less frequently in animals exposed to lower concentrations.

Kennedy (1990) summarized the results of a study in which male and female rats and hamsters were exposed to 200, 600, or 2,000 ppm tetrafluoroethylene for 6 hours per day, 5 days per week, for 18 weeks. Hamsters appeared to be less affected than rats in that there was no reduction in the rate of weight gain in any exposed group of hamsters, while rats exposed to 2,000 ppm exhibited a reduction in the rate of weight gain. Additionally, toxic nephrosis associated with the straight portions of the proximal convoluted tubules was observed in rats exposed to 600 or 2,000 ppm tetrafluoroethylene. This was accompanied by increased urine volume and urine fluoride concentrations and decreased urine creatinine concentrations. The effects were more pronounced in female rats than in male rats. No effects on the kidneys of exposed groups of hamsters were observed; however, testicular atrophy similar to that reported in the 14-day study in male Syrian hamsters and Crl:CD rats was observed.

Although a number of animal studies have been performed with pyrolysis products of tetrafluoroethylene polymers, they are not reported here because these products are mixtures of a number of chemical vapors and particulates, and any observable effects cannot be solely attributed to the inhalation of tetrafluoroethylene monomer.

Humans

Epidemiological studies relative to exposure to tetrafluoroethylene alone were not found in the literature (National Library of Medicine, 1995). Although monomeric tetrafluoroethylene is generally considered an irritant to the eyes and respiratory tract, there is a paucity of firm toxicologic data. There are considerable data in the literature on human exposure to pyrolysis products of tetrafluoroethylene polymers. However, those studies are not considered here because toxic effects from exposure to the polymer fumes cannot be attributed solely to inhalation of tetrafluoroethylene monomer. Following exposure to pyrolysis products of tetrafluoroethylene, influenza-like symptoms commonly referred to as "polymer-fume fever" do occur. The symptoms include fever, chills, headaches, dizziness, nausea, weakness, cough, and rigor-like shaking of the limbs (IARC, 1979; Gosselin *et al.*, 1984; *Material Safety Data Sheet Collection*, 1993; *Patty's Industrial Hygiene and Toxicology*, 1994). The symptoms are transient if the oxygen supply to the subject is increased and the subject is removed from the contaminated environment.

S-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine and other cysteine and glutathione conjugates such as *S*-(1,2-dichlorovinyl)glutathione, *S*-(1,2-dichloro-vinyl)-*L*-cysteine, *S*-(1,2,3,4,4-pentachloro-butadienyl)-*L*-cysteine, and *S*-(2-chloro1,1,2-trifluoroethyl)-*L*-cysteine were found to be toxic to human proximal tubule cells in culture, as indicated by the release of lactate dehydrogenase (Chen *et al.*, 1990). Aminooxyacetic acid protected the cells from the toxicity of all the conjugates and inhibited the covalent binding of the sulfur-containing metabolites to cellular macromolecules and thus prevented cell death.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information on the reproductive or developmental toxicity of tetrafluoroethylene in experimental animals or in humans was found in a search of the available literature (National Library of Medicine, 1995).

CARCINOGENICITY

No information on the carcinogenicity of tetrafluoroethylene in experimental animals or in humans was found in a search of the available literature (National Library of Medicine, 1995). Tetrachloroethylene, which is structurally similar to tetrafluoroethylene, produced hepatocellular carcinomas in male and female B6C3F$_1$ mice (NCI, 1977). Male mice were administered 450 or 900 mg tetrachloroethylene/kg body weight in corn oil by gavage for 11 weeks, then 550 or 1,100 mg/kg for 67 weeks. Female mice were administered 300 or 600 mg/kg tetrachloroethylene in corn oil by gavage for 11 weeks, then 400 or 800 mg/kg for 67 weeks. Exposure of male and female F344/N rats to 200 or 400 ppm tetrachloroethylene by inhalation for 2 years resulted in increased incidences of renal tubule adenoma or adenocarcinoma in males and increased incidences of mononuclear cell leukemia in males and females. (NTP, 1986). In the same inhalation study, mice exposed to 100 or 200 ppm for 2 years developed increased incidences of hepatocellular adenoma (males) and carcinoma (males and females).

GENETIC TOXICOLOGY

Kennedy (1990) reported that tetrafluoroethylene was not mutagenic in *Salmonella typhimurium* with or without metabolic activation.

Cysteine conjugates of tetrafluoroethylene were not mutagenic in the *Salmonella* assay, with or without rat kidney S9 (Green and Odum, 1985).

The structurally similar tetrachloroethylene was not mutagenic in *Salmonella* (Haworth *et al.*, 1983), gave equivocal results in the mouse lymphoma mutagenicity assay with L5178Y mouse lymphoma cells (Myhr *et al.*, 1990), and did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells (Galloway *et al.*, 1987); all these tests were conducted with and without Aroclor-induced liver S9 enzymes.

STUDY RATIONALE

Tetrafluoroethylene was nominated to the NTP by the National Cancer Institute for testing following a review of related monomers and polymers. The nomination was based on the potential for human exposure to the chemical due to the large production volume and on the lack of adequate test data for tetrafluoroethylene. Inhalation was chosen as the route of exposure because human exposure occurs primarily via this route. Sixteen-day, 13-week, and 2-year whole body inhalation studies were performed in male and female F344/N rats and B6C3F$_1$ mice.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF TETRAFLUOROETHYLENE

Tetrafluoroethylene was obtained from SCM Specialty Chemicals (Gainesville, FL) in one lot (10271), which was used for the identity and purity analyses conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix I). Tetrafluoroethylene was also obtained from ICI Americas, Inc. (Bayonne, NJ), in five lots. The study laboratory assigned a lot number to each shipment of as many as 12 cylinders. Lot 12017-8 was used during the 16-day studies and during most of the 13-week studies; lot 12017-77 was used during the remainder of the 13-week studies; and lots 12438-6, 12438-79, and 12438-92 were used during the 2-year studies. Identity and purity analyses were also conducted by the study laboratory on each lot used during the studies. Reports on analyses performed in support of the tetrafluoroethylene studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

Each lot of the chemical, a colorless gas, was identified as tetrafluoroethylene by the analytical chemistry or study laboratory using infrared spectroscopy and the purity of each lot was determined by gas chromatography. Gas chromatography of lot 10271 indicated one major peak and one impurity with an area of 0.1% relative to the major peak. In addition, analysis for *d*-limonene, which is added to tetrafluoroethylene as a stabilizer, was conducted by the analytical chemistry laboratory using gas chromatography and indicated 0.03% ± 0.00% *d*-limonene. Gas chromatography conducted by the study laboratory for each cylinder of each lot used during the studies indicated that perfluorocyclobutane was present at concentrations less than or equal to 462 ppm (0.05%) for the 16-day studies and 2,604 ppm (0.26%) for the 13-week studies. During the 2-year studies, gas chromatography indicated peaks for perfluorocyclobutane with areas less than or equal to 1.21% relative to the major peak. Gas chromatography conducted by the study laboratory for each cylinder of each lot used during the 13-week studies indicated that *d*-limonene was present at concentrations less than or equal to 2,837 ppm (0.28%). During the 2-year studies, gas chromatography of lots 12438-6, 12438-79, and 12438-92 indicated peaks for *d*-limonene with areas less than or equal to 0.56% relative to the major peak. The manufacturer indicated that the following impurities were also present at concentrations less than or equal to 1.7 ppm: trifluoroethylene, methylene fluoride, vinyl fluoride, and vinylidene fluoride. The overall purity of lots 12017-8, 12017-77, and 12438-92 was determined to be greater than 99%. The overall purity of lots 12438-6 and 12438-79 was determined to be greater than 98%.

The manufacturer indicated that tetrafluoroethylene would be stable for up to 1 year when stored in the original containers at room temperature. To ensure stability, the bulk chemical was stored in the original metal cylinders at 10° to 22° C. Stability was monitored during the 13-week and 2-year studies using gas chromatography. The relative concentrations of perfluorocyclobutane were expected to increase due to the slow dimerization of tetrafluoroethylene; therefore, the gas cylinders were monitored for this dimer as well as for *d*-limonene during the 2-year studies. Two cylinders from lot 12438-6 contained higher concentrations of these compounds than canisters from the same lot that were analyzed earlier in the studies. One of these two cylinders, which contained 1.21% perfluorocyclobutane and 0.56% *d*-limonene, was replaced immediately after gas chromatographic analysis was performed; it is not known if the dimer formed in the canister due to degradation of tetrafluoroethylene or was present as an impurity at receipt. Stability was generally considered acceptable throughout the studies.

VAPOR GENERATION AND EXPOSURE SYSTEM

Because tetrafluoroethylene is a gas at room temperature, the generation and delivery system incorporated gas distribution under regulated pressure with individual adjustment and monitoring of the chemical flow rate to each chamber. Tetrafluoroethylene was taken

directly from the cylinder in which it was shipped and was metered to each exposure chamber. A sample line was included downstream from each gas cylinder. Stainless-steel chambers designed at Battelle Pacific Northwest Laboratories were used for all studies (Figure I3).

VAPOR CONCENTRATION MONITORING

The concentrations of tetrafluoroethylene were monitored using an on-line gas chromatograph, and samples were drawn and analyzed from each exposure chamber, the control chamber, the exposure suite, and an on-line standard. Summaries of the chamber concentrations during the studies are presented in Tables I1 through I3. The monthly mean exposure concentrations for the 2-year studies are presented in Figures I7 through I15; all were within 10% of the acceptable concentration range.

CHAMBER ATMOSPHERE CHARACTERIZATION

The time for the exposure concentration to build up to 90% of the final exposure concentration (T_{90}) used for all studies was 12 minutes. During the 16-day studies, T_{90} was approximately 12 minutes and T_{10} (the time for the exposure concentration to decay to 10% of the exposure concentration) was approximately 9 minutes with and without animals in the chambers. During the 13-week studies, T_{90} was 11 minutes with animals in the chambers and ranged from 11 to 14 minutes without animals; T_{10} ranged from 9 to 11 minutes with and without animals in the chambers. During the 2-year studies, T_{90} ranged from 8 to 10 minutes without animals in the chambers and from 10 to 16 minutes with animals present; T_{10} ranged from 7 to 9 minutes without animals and from 9 to 12 minutes with animals. The T_{90} value was not affected by the presence of animals.

Uniformity of tetrafluoroethylene concentration in the exposure chambers was measured using on-line gas chromatography before each of the studies, once during the 16-day and 13-week studies, and approximately every 3 months during the 2-year studies. Uniformity of exposure concentrations within each chamber was acceptable, and relative standard deviations were less than 5%.

The persistence of tetrafluoroethylene in the 1,250 ppm exposure chamber after shutting off the system was monitored without animals before the 2-year studies began and with animals present during the 2-year studies. The concentration of tetrafluoroethylene in the exposure chamber fell rapidly to less than 1% of the beginning concentration within 20 minutes. Tetrafluoroethylene concentrations in the building exhaust and room air were also monitored during all studies.

Information supplied by the manufacturer indicated that all cylinders contained *d*-limonene as a stabilizer. *d*-Limonene and perfluorocyclobutane are less volatile than tetrafluoroethylene and concentrate in the cylinder as the tetrafluoroethylene is removed. Cylinder usage was regulated to minimize concentrations of these chemicals in the exposure chambers; a maximum of 80% of the tetrafluoroethylene in each cylinder was used. Gas chromatography of chamber samples and cylinder headspace in used and unused cylinders was conducted for *d*-limonene and perfluorocyclobutane. The results indicated that tetrafluoroethylene was stable during generation and in the exposure chambers and that perfluorocyclobutane was not formed in significant quantities by the generation system.

16-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD). On receipt, the rats and mice were approximately 4 weeks old. Animals were quarantined for 11 days (rats) or 12 days (mice) and were approximately 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice were exposed to tetrafluoroethylene at concentrations of 0, 312, 625, 1,250, 2,500, and 5,000 ppm. The animals were exposed for 6 hours plus T_{90} (12 minutes) per day, 5 days per week for 12 exposure days during a 16-day period. Feed was available *ad libitum* except during exposure periods, and water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded two times during each exposure day for rats and mice. The animals were weighed on days 1, 8, and 15 and at necropsy. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 16-day studies, blood was collected from the retroorbital (rats) or supraorbital (mice) sinus

for hematology analyses. Rats and mice were anesthetized with CO_2. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. Analyses were performed with an Ortho ELT-8/ds hematology analyzer (Ortho Instruments; Westwood, MA). Leukocyte differential counts were determined by light microscopic examination of blood films stained with Wright-Giemsa. Reticulocyte counts were determined by light microscopy using smears stained supravitally with New Methylene Blue and a Miller disc for reticulocyte quantitation. Hematology parameters measured are listed in Table 1.

A necropsy was performed on all rats and mice. The brain, heart, left and right kidneys, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on all control and 5,000 ppm rats and mice. Selected organs were examined to a no-effect level in groups of animals exposed to lower concentrations of tetrafluoroethylene. Table 1 lists the tissues and organs examined.

13-WEEK STUDIES

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to tetrafluoroethylene and to determine the appropriate exposure levels to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, rats and mice were approximately 4 weeks old. Animals were quarantined for 12 days (rats) or 14 days (mice) and were approximately 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice were exposed to tetrafluoroethylene at concentrations of 0, 312, 625, 1,250, 2,500, or 5,000 ppm. (One female rat assigned to the 2,500 ppm group was missexed and discarded at week 6.) The animals were exposed for 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 13 weeks (excluding holidays). Feed was available *ad libitum* except during exposure periods, and water was available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded once weekly for rats and mice. The animals were weighed prior to the start of the studies and weekly thereafter. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 13-week studies, blood was collected from the retroorbital (rats) or supraorbital (mice) sinus for hematology analyses. Rats and mice were anesthetized with CO_2. Hematology determinations were analyzed as described for the 16-day study. Fourteen days (rats) or 10 days (mice) before the end of the studies, rats and mice were placed in metabolism cages (with access to water but not food) for a 16-hour urine collection. Four days before the end of the studies, rats were again placed in metabolism cages and were deprived of water for 16 hours to determine urine concentrating ability; urine was expressed from the bladders of all rats, and urine was collected for the following 4 hours while water deprivation continued. Specific gravity was determined on an A/O refractometer; urine glucose was determined using an Abbott VP glucose oxidase methodology (Abbott Laboratories, Abbott Park, IL); urine protein was determined by a Coomassie blue reaction; and fluoride was measured using an ion-specific electrode. Hematology and urinalysis parameters measured are listed in Table 1.

At the end of the 13-week studies, samples were collected for sperm morphology and vaginal cytology evaluations on rats and mice exposed to 0, 312, 1,250, and 5,000 ppm. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1984). For 7 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm morphology, count, and motility. The right testis and right epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis.

The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each right cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

A necropsy was performed on all animals. The brain, heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all control and 5,000 ppm rats and mice and on selected organs from rats and mice exposed to lower concentrations. Table 1 lists the tissues and organs examined.

2-YEAR STUDIES

Study Design

Groups of 60 male rats were exposed to tetrafluoroethylene at concentrations of 0, 156, 312, and 625 ppm for 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 104 weeks. Groups of 60 female rats and 58 male and female mice were exposed to tetrafluoroethylene at concentrations of 0, 312, 625, and 1,250 ppm for 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 104 weeks (female rats) or 95 to 96 weeks (mice). Following the last day of exposure, rats were observed for approximately 11 days before necropsy. Ten male and ten female rats and mice from each group were evaluated at 15 months for alterations in hematology, clinical chemistry, and urinalysis parameters (rats only) and organ weights (rats and mice).

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA) for use in the 2-year studies. Rats and mice were quarantined for 15 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats and mice were housed individually. Feed was available *ad libitum* except during exposure periods, and water was available *ad libitum*. Chambers and cages were rotated weekly. Further details of animal maintenance are provided in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded monthly until the last 13 weeks (rats) or 5 weeks (mice), when they were recorded twice monthly. Animals were weighed prior to the start of the studies, weekly for the first 13 weeks, and monthly thereafter, until the last 13 weeks (rats) or 5 weeks (mice), when they were weighed every 2 weeks.

Ten male and ten female rats and mice per exposure group were designated for interim evaluation at 15 months. Two weeks before the scheduled evaluation, designated rats were placed in metabolism cages for a 16-hour urine collection. One week later, male and female rats were again placed in metabolism cages for a urine concentrating study. Rats were deprived of water for 16 hours, and urine was expressed from the bladders of the animals; urine was then collected during the next 6 hours for a urine concentrating study. At the 15-month interim evaluation, blood was taken from the retroorbital plexus of rats for hematology and clinical chemistry analyses. The methods used were those described for the 13-week studies, and parameters measured are listed in Table 1.

A complete necropsy and microscopic examination were performed on all rats and mice. At the 15-month interim evaluation, the right kidney, liver, and lung were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, imbedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (i.e., adrenal gland, kidney, ovary), samples from each organ were examined. For extended evaluation of renal proliferative lesions, kidneys were step sectioned at 1 mm intervals, and six to ten additional sections were obtained from each kidney. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year rat and mouse studies, a quality assessment pathologist reviewed the following organs: adrenal medulla, kidney, liver, and spleen (male rats); eye, kidney, liver, and spleen (female rats); kidney, liver, and spleen (male mice); and harderian gland, liver, and spleen (female mice).

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists, the PWG chairperson, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, B5, C1, C5, D1, and D5 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences

For those neoplasms considered incidental in these studies, the primary statistical method used was logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if the fit of the model was not significantly enhanced. The neoplasm incidences of exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984).

In addition to logistic regression, other methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These methods include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal neoplasms, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test, a procedure based on the overall proportion of affected animals, was used.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoa data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973). Because the vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure levels.

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Consequently, neoplasm incidences from the NTP historical control database, which is updated yearly, are included in the NTP reports for neoplasms appearing to show compound-related effects.

QUALITY ASSURANCE METHODS

The 13-week and 2-year studies were conducted in compliance with Food and Drug Administration Good

Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of tetrafluoroethylene was assessed by testing the ability of the chemical to induce increases in the frequency of micronucleated erythrocytes in peripheral blood samples obtained from male and female mice at the end of the 13-week study. The protocol for this study and the results are given in Appendix E.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene

16-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
Battelle Pacific Northwest Laboratories (Richland, WA)	Battelle Pacific Northwest Laboratories (Richland, WA)	Battelle Pacific Northwest Laboratories (Richland, WA)
Strain and Species		
Rats: F344/N Mice: B6C3F$_1$	Rats: F344/N Mice: B6C3F$_1$	Rats: F344/N Mice: B6C3F$_1$
Animal Source		
Frederick Cancer Research Facility (Frederick, MD)	Taconic Farms, Inc. (Germantown, NY)	Simonsen Laboratories (Gilroy, CA)
Time Held Before Studies		
Rats: 11 days Mice: 12 days	Rats: 12 days Mice: 14 days	15 days
Average Age When Studies Began		
6 weeks	6 weeks	7 weeks
Date of First Exposure		
Rats: 30 September 1985 Mice: 1 October 1985	Rats: 25 March 1986 Mice: 27 March 1986	Rats: 23 June 1988 Mice: 9 June 1988
Duration of Exposure		
6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 16 days	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 13 weeks	6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 104 weeks (rats) (followed by an observation period of approximately 11 days), or 95 to 96 weeks (mice)
Date of Last Exposure		
Rats: 15 October 1985 Mice: 16 October 1985	Rats: 24 June 1986 (males) 25 June 1986 (females) Mice: 26 June 1986 (males) 25 June 1985 (females)	Rats: 15-month interim evaluation 17-18 September 1989 terminal sacrifice 14 June 1990 Mice: 15-month interim evaluation 6-7 September 1989 terminal sacrifice 4-5 April 1990
Necropsy Dates		
Rats: 16 October 1985 Mice: 17 October 1985	Rats: 24 June 1986 (males) 25 June 1986 (females) Mice: 27 June 1986 (males) 26 June 1986 (females)	Rats: 15-month interim sacrifice 18-19 September 1989 terminal sacrifice 25 June 1990 (males) or 26-27 June 1990 (females) Mice: 15-month interim sacrifice 7-8 September 1989 terminal sacrifice 5 April 1990 (males) or 6 April 1990 (females)

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene (continued)

16-Day Studies	13-Week Studies	2-Year Studies
Average Age at Necropsy 8 weeks	19 weeks	Rats: 15-month interim evaluation - 71 weeks terminal sacrifice - 111-112 weeks Mice: 15-month interim evaluation - 72 weeks terminal sacrifice - 102 weeks
Size of Study Groups 5 males and 5 females	10 males and 10 females (one rat assigned to the 2,500 ppm female group was determined to be missexed and was discarded during week 6)	15-Month interim evaluation: 10 males and 10 females Terminal sacrifice: 50 males and 50 females (rats) or 48 males and 48 females (mice)
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weight.	Same as 16-day studies	Same as 16-day studies
Animals per Cage 1	1	1
Method of Animal Identification Toe clips and placement within cage unit	Same as 16-day studies	Rats: Tail tattoos and placement within cage unit Mice: Toe clips and placement within cage unit
Diet NIH-07 pelleted feed (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum*, except during exposure periods	Same as 16-day studies	Same as 16-day studies
Maximum Storage Time for Feed 120 days post-milling	Same as 16-day studies	Same as 16-day studies
Water Tap water (Richland municipal supply) via automatic watering system (Edstrom Industries; Waterford, WI); available *ad libitum*	Same as 16-day studies	Same as 16-day studies
Cages Stainless steel (Hazleton Systems, Inc., Aberdeen, MD), changed weekly	Same as 16-day studies	Stainless steel (Lab Products, Inc., Harford Division, Aberdeen, Md), changed weekly
Bedding/Cage Board Techsorb®, Shepherd Specialty Papers, Inc. (Kalamazoo, MI), used during non-exposure periods, 7 days per week	Same as 16-day studies	Same as 16-day studies

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene (continued)

16-Day Studies	13-Week Studies	2-Year Studies
Chamber Air Supply Filters Duct pre-filter, dual and single HEPA (American Air Filter, Louisville, KY); checked biannually	Same as 16-day studies	Single HEPA (Flanders Filters, Inc., San Rafael, CA) and charcoal (RSE, Inc., New Baltimore, MI); changed before the studies began and once yearly thereafter
Chambers Stainless steel (Hazleton Systems, Inc., Aberdeen, MD); changed weekly	Stainless steel (Hazleton Systems, Inc., Aberdeen, MD); changed weekly	Stainless steel (Lab Products, Inc., Harford Division, Aberdeen, MD); changed weekly
Chamber Environment Temperature: 22.3° - 23.2° C Relative humidity: 52.7% - 55.4% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour	Temperature: 23.1° - 23.7° C Relative humidity: 56.1% - 60.5% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour	Temperature: 24.1° - 24.5° C Relative humidity: 53% - 58% Room fluorescent light: 12 hours/day Chamber air changes: 15/hour
Exposure Concentrations 0, 312, 625, 1,250, 2,500, or 5,000 ppm	0, 312, 625, 1,250, 2,500, or 5,000 ppm	Rats: males - 0, 156, 312, or 625 ppm females - 0, 312, 625, or 1,250 ppm Mice: 0, 312, 625, or 1,250 ppm
Type and Frequency of Observation All animals were observed twice daily. Clinical findings were recorded two times each exposure day. All animals were weighed on days 1, 8, 15, and at necropsy.	All animals were observed twice daily. Clinical findings were recorded once weekly for rats and mice. The animals were weighed before the studies began and weekly thereafter.	All animals were observed twice daily. Clinical findings were recorded monthly until the last 13 weeks (rats) or 5 weeks (mice), when they were recorded twice monthly. Animals were weighed before the studies began, weekly for the first 13 weeks, and monthly thereafter, until the last 13 weeks (rats) or 5 weeks (mice) when they were weighed every 2 weeks.
Method of Sacrifice Anesthetization with sodium pentobarbital followed by exsanguination	Anesthetization with CO_2 followed by exsanguination	Anesthetization with CO_2 followed by exsanguination
Necropsy Necropsy performed on all animals. Organs weighed were brain, heart, left and right kidneys, liver, lung, right testis, and thymus.	Necropsy performed on all animals. Organs weighed were brain, heart, right kidney, liver, lung, right testis, and thymus.	Necropsy performed on all animals. Organs weighed at the 15-month interim evaluation were the right kidney, liver, and lung.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene (continued)

16-Day Studies	13-Week Studies	2-Year Studies
Clinical Pathology		
Blood was collected from all animals from the retroorbital (rats) or supraorbital (mice) sinus for hematology analyses. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials	Blood was collected from all animals from the retroorbital (rats) or supraorbital (mice) sinus for hematology analyses. Fourteen days (rats) or 10 days (mice) before the end of the studies, animals were placed in metabolism cages (with access to water but not food) for a 16-hour urine collection. Four days before the end of the studies, rats were again placed in metabolism cages and were deprived of water for 16 hours; urine was expressed from the bladders of all rats, and urine was collected for the following 4 hours while water deprivation continued. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Urinalysis:*** fluoride, glucose, and protein concentrations; volume; and specific gravity	At the 15-month interim evaluation, blood was taken from the retroorbital plexus of rats for hematology and clinical chemistry analyses. Two weeks prior to the scheduled evaluation, designated rats were placed in metabolism cages for a 16-hour urine collection. One week prior to the evaluation, rats were again placed in metabolism cages and were deprived of water for 16 hours; urine was expressed from the bladders of all rats, and urine was collected for the following 6 hours while water deprivation continued. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, sodium, potassium, chloride, calcium, and phosphorus concentrations ***Urinalysis:*** glucose and protein concentrations; volume; and specific gravity
Histopathology		
Complete histopathology was performed on rats and mice exposed to 0 or 5,000 ppm. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland, esophagus, eye (if grossly abnormal), gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, sternebrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Additionally, the kidney of all other exposed groups of male and female rats and mice was examined, as was the pancreas of male and female rats exposed to 2,500 ppm.	Complete histopathology was performed on rats and mice exposed to 0 or 5,000 ppm. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland, esophagus, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, nose, ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, sternebrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Additionally, the following organs were examined in lower exposure groups: the kidney of all exposed groups of male and female rats and mice (except female rats exposed to 312 ppm); the cecum of 625 ppm male rats; the eye and mandibular lymph node of 2,500 ppm female rats; and the thymus and lung of 625 ppm male mice.	Complete histopathology was performed on all control and exposed rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, clitoral gland, esophagus, femur, gallbladder (mice only), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary gland, muscle (thigh), nose, oral cavity (larynx and pharynx), ovaries, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, sternebrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

TABLE 1
Experimental Design and Materials and Methods in the Inhalation Studies of Tetrafluoroethylene (continued)

16-Day Studies	13-Week Studies	2-Year Studies
Sperm Morphology and Vaginal Cytology		
None	At terminal sacrifice, sperm samples were collected from all male animals in the 0, 312, 1,250, and 5,000 ppm groups for sperm morphology evaluations. The parameters evaluated included sperm morphology, concentration, and motility. The right cauda, right epididymis, and right testis were weighed. Vaginal samples were collected for up to 7 consecutive days prior to the end of the studies from all females exposed to 0, 312, 1,250, or 5,000 ppm for vaginal cytology evaluations. The parameters evaluated included the relative frequency of estrous stages and estrous cycle length.	None

DISCUSSION AND CONCLUSIONS

Tetrafluoroethylene was evaluated for toxicity and carcinogenicity in 16-day, 13-week, and 2-year studies in male and female F344/N rats and B6C3F$_1$ mice, with whole body inhalation as the route of exposure.

In the 16-day studies, there were no exposure-related deaths, clinical findings, or effects on hematologic parameters. Mean body weights of male and female rats exposed to 5,000 ppm were lower than those of the controls. Absolute kidney weights of all exposed groups of male rats, female rats exposed to 1,250 ppm, and female mice exposed to 5,000 ppm were greater than those of controls. Relative liver weights of all exposed groups of male rats and of female mice exposed to 5,000 ppm were also greater than those of the controls; however, there were no histopathologic effects in the liver. There were exposure concentration-related effects on the kidneys of exposed rats and mice, although the effects were different between the species. In rats, the kidney lesion (renal tubule degeneration) was located predominantly in the inner cortical tubule epithelial cells at the junction of the cortex and medulla and was observed in male and female rats exposed to 625 ppm or greater. This lesion has been observed previously in rats exposed to tetrafluoroethylene and other halogenated compounds and is well documented in the literature. In mice, the lesion was characterized as renal tubule karyomegaly; however, it was also located primarily in the inner renal cortex.

In the 13-week studies, there were no exposure-related deaths or clinical findings. Mean body weight gains of male and female rats exposed to 5,000 ppm were marginally reduced. Although there were no treatment-related lesions in the livers of exposed rats or mice, absolute and relative liver weights of female rats exposed to 5,000 ppm and all exposed groups of male rats were significantly increased. Absolute and relative kidney weights of male rats exposed to 1,250 ppm or greater and females exposed to 625 ppm or greater were significantly increased. Absolute and relative kidney weights of exposed mice were not significantly different from those of the controls.

Exposure concentration-related renal lesions similar to those observed in the 16-day study were observed in male rats exposed to 625 ppm or greater and in female rats in the 2,500 and 5,000 ppm groups. These lesions were considered mild at most. As with exposed rats, mice exposed to 1,250 ppm or greater in the 13-week study also developed kidney lesions similar to those observed in mice at 16 days, and these lesions were slightly more severe in males than in females. A concentration-related proteinuria occurred in all exposed groups of rats with kidney lesions. Proteinuria could be related to either glomerular or proximal tubule renal injury and, in the present study, would be consistent with the renal tubule degeneration observed morphologically. Polyuria occurred in exposed mice and probably in rats and could be related to a variety of conditions, including overdrinking, osmotic diuresis, damage to the proximal and/or distal portions of the renal tubule, and alterations of metabolism or kidney response to antidiuretic hormone (Finco, 1989). Based on an appropriate response to a water concentration test (rats only), the ability of the kidney to concentrate the urine was not compromised, indicating that the pituitary-kidney ADH axis was intact and that there was no medullary washout (which could occur with conditions such as long-term overhydration or hyperadrenocorticism). Therefore, the renal damage observed morphologically, although different for rats and mice, would appear to involve primarily the proximal convoluted tubules.

An exposure concentration-dependent normocytic, normochromic, nonresponsive anemia occurred in exposed rats and mice in the 13-week studies. Normocytic, normochromic, nonresponsive anemias have been related to selective suppression of erythropoiesis in a variety of chronic disorders and may be related to decreased erythropoietin elaboration, bone marrow suppression, or defective iron metabolism (Jain, 1986). Because there was histopathologic evidence of exposure concentration-dependent renal lesions in male and female rats and mice, the anemia would be consistent with a secondary hypoproliferative anemia, possibly related to altered erythropoietin production/metabolism in the kidney.

In the 2-year study, survival was reduced in male rats exposed to 625 ppm and in all exposed female rats. Mean body weights of exposed rats were generally similar to those of the controls until the end of the study, at which time mean body weights were lower in males in the 625 ppm group and females in the 1,250 ppm group than in the controls. These reductions reflected the mean body weights of a very small number of surviving rats. The only exposure-related clinical finding was eye opacity in female rats, which upon histological examination was determined to be cataracts. At the 15-month interim evaluation, relative kidney weights of male rats exposed to 312 or 625 ppm and absolute kidney and liver weights of females exposed to 625 or 1,250 ppm were significantly greater than those of the controls.

The renal toxicity noted in male and female rats in the 13-week study was exacerbated in the 2-year study in that exposure to tetrafluoroethylene caused increased incidences of neoplasms and nonneoplastic lesions of the kidney in rats. As in the 13-week studies, renal tubule degeneration was associated with the proximal tubules at the corticomedullary junction, and the incidence was significantly increased at 15 months and 2 years. The only difference between the two time points was that the renal lesions were generally more severe and more numerous after 2 years.

Additional sections of the kidney were taken to further evaluate the renal proliferative effects. These sections did detect additional proliferative lesions and generally confirmed the findings of the single sections. Exposure to tetrafluoroethylene caused significantly increased incidences of hyperplasia at 15 months and 2 years in male and female rats. In the combined extended and standard evaluations, the incidences of renal tubule adenoma or carcinoma (combined) were significantly increased, with positive trends in exposed males and females. Because these neoplasms, which are considered uncommon, occurred consistently and because the incidences increased with increasing exposure concentration, the neoplasms are considered to have been caused by tetrafluoroethylene exposure. Similarly, increased incidences of renal tubule hyperplasia, adenoma, and carcinoma in male rats have been reported following inhalation exposure to 200 and 400 ppm tetrachloroethylene, a structurally related compound (NTP, 1986). In addition, incidences of karyomegaly were significantly increased in male and female rats exposed to tetrachloroethylene.

Considerable effort has been expended in the past 10 years investigating the mechanism of tetrafluoroethylene-induced renal proximal tubule toxicity, primarily in rats (Odum and Green, 1984; Boogaard *et al.*, 1989; Commandeur *et al.*, 1989; Hayden *et al.*, 1991a,b), and that induced by other halogenated compounds. Tetrafluoroethylene is conjugated in the liver with glutathione and subsequently excreted via the bile to the small intestine, where biliary and intestinal peptidases degrade them to cysteine-*S*-conjugates which may be reabsorbed from the small intestine. Glutathione conjugates released into the general circulation from the liver are also hydrolyzed to cysteine-*S*-conjugates by renal peptidases, where they are bioactivated by renal β-lyase to unstable reactive thiols. The tetrafluoroethylene cysteine conjugate, *S*-(1,1,2,2-tetrafluoroethyl)-*L*-cysteine, and tetrafluoroethylene mercapturic acid have been shown to cause renal damage *in vivo* identical to that caused by tetrafluoroethylene and to have greater enzyme activation activity than several chlorinated or brominated compounds; additionally, exposure to these compounds causes their accumulation in the proximal tubule cells (Commandeur *et al.*, 1989). Like tetrafluoroethylene, they are not mutagenic. The tetrafluoroethylene cysteine conjugate has also been shown to cause death to cultured human proximal tubule cells, as indicated by the release of lactate dehydrogenase. Toxicity and binding to macromolecules in human renal cells can be prevented with aminooxyacetic acid, a known β-lyase inhibitor; this process has also been demonstrated for rat renal cells (Chen *et al.*, 1990). In rats, the cysteine conjugate is further bioactivated in the kidney and, to a lesser extent, in the liver to a difluorothionacetyl fluoride, the putative reactive metabolite for tetrafluoroethylene-induced nephrotoxicity. The thionacetyl fluorides cause mitochondrial dysfunction by inhibiting adenosine diphosphate-stimulated respiration, increasing lipid peroxidation, modifying mitochondrial proteins (HSP60 and HSP70), and inhibiting lipoyl dehydrogenase; by binding to macromolecules; and by forming protein and phospholipid adducts. The difluorothioamide adduct has been shown to cause nephrotoxicity in the proximal tubule. Considering the similarity in effects and location of renal lesions in the present 2-year inhalation studies and the metabolism and toxicity studies reported in the literature, similar metabolites and common mechanisms may be involved in the carcinogenic response in the kidney of tetrafluoroethylene-exposed animals.

Although no liver lesions were observed in the 13-week studies, exposure to tetrafluoroethylene for 2 years caused increased incidences of nonneoplastic lesions and uncommon neoplasms of the liver in rats; these neoplasms include hemangiosarcomas, which have not been observed previously in chamber control rats from 2-year NTP inhalation studies. Hepatocellular foci (including clear cell, eosinophilic, and mixed cell) were observed in rats at the 15-month interim evaluation. At 2 years, the incidences of eosinophilic and mixed cell foci were significantly increased in exposed male rats and slightly increased in exposed female rats. At 15 months, one male exposed to 312 ppm had a hepatocellular adenoma and one male exposed to 625 ppm had a hepatocellular carcinoma. At the end of the 2-year study, male rats in the 312 ppm group had significantly increased incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined); in females, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) in all exposed groups and the incidences of hepatocellular carcinoma in the 312 and 625 ppm groups were significantly greater than those in the controls. There were no adenomas or carcinomas in control females. The incidences of hepatocellular adenoma or carcinoma (combined) occurred with positive trends in exposed males and females, even though the 8% incidence in the control male rats in the present study exceeds the previous highest incidence of 4% for inhalation chamber controls. The fact that fewer hepatocellular neoplasms occurred in male and female rats exposed to the highest concentrations (625 ppm for males, 1,250 ppm for females) than in rats exposed to lower concentrations may be a result of reduced survival. Also, the highest exposure concentration for males was half that for females.

Exposure to tetrafluoroethylene also caused hemangiosarcomas of the liver in female rats. These uncommon liver neoplasms have not been observed previously in male or female chamber controls from 2-year NTP inhalation studies or in untreated controls by any oral route of exposure. Hepatic angiectasis, characterized by multifocal, dilated, blood-filled sinusoids with prominent lining endothelial cells, was observed in exposed groups of female rats. This lesion is sometimes associated with hepatocellular neoplasms. Cystic degeneration of the liver occurs at a low incidence in aging rats; however, following exposure of rats to hepatocarcinogens, the incidence has been shown to be increased. In fact, the incidences of cystic degeneration in exposed groups of male rats were significantly greater than the control group incidence.

The incidence of mononuclear cell leukemia in male rats exposed to 156 ppm was significantly greater than in controls. In males, interpretation of the increased incidence of mononuclear cell leukemia is complicated by reduced survival in rats exposed to 625 ppm and the fact that the control incidence exceeded the previous historical chamber control range. The incidence of mononuclear cell leukemia in male rats exposed to 312 ppm, although not significantly greater than that in the control group, also exceeded the previous historical range. Whether this response in male rats was caused by tetrafluoroethylene exposure is uncertain. However, there was a significant increase in the incidence of mononuclear cell leukemia in all exposed groups of female rats. The incidence of mononuclear cell leukemia in female controls from the present study was within the historical control range. The increased incidence of mononuclear cell leukemia in female rats is considered to have been caused by exposure to tetrafluoroethylene. Exposure to tetrachloroethylene for 2 years (NTP, 1986) also caused an increase in the incidence of mononuclear cell leukemia in male and female F344/N rats exposed to 200 or 400 ppm.

At 15 months, there were no increases in the incidence of interstitial adenoma or interstitial cell hyperplasia. At the end of the 2-year study, there was a slight but statistically significant increase in the incidence of interstitial cell adenoma in the testes of rats exposed to 312 or 625 ppm. It is uncertain whether these increased incidences of adenomas were due to tetrafluoroethylene exposure; however, there was a similar slight but significant increase in the incidence of interstitial cell adenomas in F344/N rats exposed to 200 or 400 ppm tetrachloroethylene for 2 years (NTP, 1986).

Exposure to tetrafluoroethylene for 2 years caused a significant increase in the incidence of cataracts in female rats exposed to 1,250 ppm. The lesion was characterized by a disruption of the normal organization of lens fibers, with swelling, vacuolization, and mineralization.

In the 2-year mouse study, survival was reduced in all exposed male and female mice to the extent that the study was terminated at week 96. The reduction in

survival was considered to be the result of exposure-related neoplasms of the liver. Mean body weights of exposed mice were generally similar to those of controls except at the end of the study, where survival was significantly decreased.

As observed in the 2-year rat study, exposure of mice to tetrafluoroethylene caused significant increases in the incidences of nonneoplastic lesions and uncommon neoplasms of the liver in mice at 15 months and 2 years. Exposure to tetrafluoroethylene caused hemangiomas in the liver of male and female mice, and multiple hemangiomas were observed in males and females. These uncommon neoplasms were not observed in control mice. Hemangiosarcomas caused by exposure to tetrafluoroethylene were observed as early as 15 months in males in the 1,250 ppm group and females in the 312 ppm group. Hemangiosarcomas occurred with statistically significant incidences and with a positive trend in all groups of exposed males and females. The incidences of multiple hemangiosarcomas were significant in all exposed groups of males and females. In a few males and females, the hemangiosarcomas metastasized to the lung. Hemangiosarcomas were not observed in male or female control mice. The incidences of hemangioma or hemangiosarcoma (combined) were also highly significant and occurred with a positive trend for exposed groups of males and females. Hemangiomas and hemangiosarcomas are uncommon neoplasms in mice in studies using such routes as feed or dosed water, and the historical incidences of these neoplasms in untreated mice from 2-year NTP feed and dosed water studies are similar to those for inhalation chamber control mice. The rarity of these neoplasms further accentuates the striking results of the present study. These uncommon neoplasms probably had a significant effect on the survival of mice in the present study.

Although exposure to 100 or 200 ppm tetrachloroethylene for 2 years caused increased incidences of hepatocellular adenoma and carcinoma in male and female mice, there were no exposure-related increases in hemangioma or hemangiosarcoma incidences (NTP, 1986). However, chronic inhalation exposure to vinyl chloride (chloroethylene) has caused hemangiosarcomas and hepatocellular adenomas and carcinomas in several strains of rats, mice, and hamsters (IARC, 1979, 1987). More importantly, a number of epidemiologic studies have shown that workers exposed to vinyl chloride have higher mortality rates and a high incidence of neoplasms in the liver, brain, lung, and lymphatic and hematopoietic systems, all attributed to vinyl chloride exposure. Neoplasms observed in the liver of these workers include hemangiosarcomas and hepatocellular adenomas and carcinomas. Liver neoplasms caused by vinyl chloride exposure in laboratory animals and humans are similar to those observed in rats and mice exposed to tetrafluoroethylene.

Angiectasis, eosinophilic foci, and coagulative multifocal necrosis of the liver were observed at 15 months and were significantly increased in incidence and severity at 2 years. The incidences of hepatocellular adenoma or carcinoma (combined) were increased in all exposed groups of male mice and were significantly greater in all exposed groups of female mice than in the control group. Many animals had multiple adenomas or carcinomas. In all exposed groups, the incidences of multiple adenomas and multiple carcinomas in female mice and multiple carcinomas in male mice were significantly increased. A number of the carcinomas in exposed males and females, but only one carcinoma in each of the male and female control groups, metastasized to the lung. Incidences of hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) in control male and female mice were within the historical ranges in chamber control mice from 2-year NTP inhalation studies. The increased incidences of these neoplasms in exposed male and female mice were considered to be exposure related. A low frequency (15%) of H-*ras* codon 61 mutations was observed in tetrafluoroethylene-induced hepatocellular neoplasms when compared to the frequency in neoplasms in controls (59%) or in spontaneous liver neoplasms of $B6C3F_1$ mice (56%) (Appendix L). This frequency is similar to that detected in liver neoplasms in $B6C3F_1$ mice exposed to tetrachloroethylene (24%) (Anna *et al.*, 1994). Although a few neoplasms in the tetrachloroethylene study were found to have a K-*ras* mutation, none were found in neoplasms in the tetrafluoroethylene study. These data indicate that these two hepatocellular carcinogens induce liver neoplasms via a *ras*-independent pathway.

The incidence of histiocytic sarcoma was markedly increased in exposed male mice and significantly increased in all female mice, although not in an exposure concentration-related manner. Among mice with any histiocytic sarcomas, histiocytic sarcomas were present in the liver of all animals and in the lung

of many animals. The spleen, lymph nodes, bone marrow, and kidney were also affected. Combined with the hepatocellular adenomas and carcinomas, hemangiomas, and hemangiosarcomas, the presence of histiocytic sarcomas in exposed mice compromised liver function, ultimately leading to the death of the mice.

In the 13-week mouse study, renal tubule karyomegaly, accompanied by a polyuria, occurred in male and female mice exposed to 1,250 ppm or greater and was the only consideration for exposure concentration selection for the 2-year study. Although there were no kidney neoplasms in exposed groups of mice as were observed in the 2-year rat study, there were significantly increased incidences of renal tubule dilatation in exposed males and renal tubule karyomegaly, located in the tubules in the inner cortex, in males and females. These lesions were observed at 15 months as well as at the end of the study. In general, only the incidences of these lesions increased over the course of the study, while the severities remained essentially unchanged; in fact, the severity of karyomegaly was similar to that observed in the 13-week study at the same exposure concentration. Although these lesions were observed in all exposed groups of males at the end of the study, incidences of karyomegaly were observed only in 1,250 ppm females; however, this group had a high incidence. As in the 13-week study, males were affected more than females, and the increased incidences of both nonneoplastic lesions were considered to be related to exposure to tetrafluoroethylene. The effect of tetrafluoroethylene exposure on the kidney of mice is very similar to the effects of exposure to 100 or 200 ppm tetrachloroethylene for 2 years (NTP, 1986), in that tetrachloroethylene caused increased incidences of karyomegaly in male and female mice without causing increased incidences of kidney neoplasms.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity** of tetrafluoroethylene in male F344/N rats based on increased incidences of renal tubule neoplasms (mainly adenomas) and hepatocellular neoplasms. There was *clear evidence of carcinogenic activity* of tetrafluoroethylene in female F344/N rats based on increased incidences of renal tubule neoplasms, liver hemangiosarcomas, hepatocellular neoplasms, and mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* of tetrafluoroethylene in male and female B6C3F$_1$ mice based on increased incidences of liver hemangiomas and hemangiosarcomas, hepatocellular neoplasms, and histiocytic sarcomas.

Slight increases in the incidences of mononuclear cell leukemia and testicular interstitial cell adenomas in male rats may have been related to exposure to tetrafluoroethylene.

Exposure of rats to tetrafluoroethylene resulted in increased incidences of renal tubule hyperplasia and degeneration in males and females, increased severity of kidney nephropathy in males, and increased incidences of liver angiectasis and cataracts in females. Exposure of mice to tetrafluoroethylene resulted in increased incidences of hematopoietic cell proliferation of the liver in females, liver angiectasis in males and females, renal tubule dilatation in males, renal tubule karyomegaly in males and females, and splenic hematopoietic cell proliferation in males and females.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

REFERENCES

Anna, C.H., Maronpot, R.R., Pereira, M.A., Foley, J.F., Malarkey, D.E., and Anderson, M.W. (1994). *ras* Proto-oncogene activation in dichloroacetic acid-, trichloroethylene- and tetrachloroethylene-induced liver tumors in B6C3F1 mice. *Carcinogenesis* **15**, 2255-2261.

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.

Boogaard, P.J., Commandeur, J.N.M., Mulder, G.J., Vermeulen, N.P.E., and Nagelkerke, J.F. (1989). Toxicity of the cysteine *S*-conjugates and mercapturic acids of four structurally related difluoroethylenes in isolated proximal tubular cells from rat kidney. Uptake of the conjugates and activation to toxic metabolites. *Biochem. Pharmacol.* **38**, 3731-3741.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Bretherick, L. (1985). *Handbook of Reactive Chemical Hazards*, 3rd ed., p. 1348. Butterworths, Boston, MA.

Bruschi, S.A., West, K.A., Crabb, J.W., Gupta, R.S., and Stevens, J.L. (1993). Mitochondrial HSP60 (P1 protein) and a HSP70-like protein (Mortalin) are major targets for modification during *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine-induced nephrotoxicity. *J. Biol. Chem.* **268**, 23,157-23,161.

Chen, J.C., Stevens, J.L., Trifillis, A.L., and Jones, T.W. (1990). Renal cysteine conjugate β-lyase-mediated toxicity studied with primary cultures of human proximal tubular cells. *Toxicol. Appl. Pharmacol.* **103**, 463-473.

Chen, Q., Yu, K., and Stevens, J.L. (1992). Regulation of the cellular stress response by reactive electrophiles. The role of covalent binding and cellular thiols in transcriptional activation of the 70-kilodalton heat shock protein gene by nephrotoxic cysteine conjugates. *J. Biol. Chem.* **267**, 24,322-24,327.

Code of Federal Regulations (CFR) **21**, Part 58.

Commandeur, J.N.M., Brakenhoff, J.P.G., de Kanter, F.J.J., and Verneulen, N.P.E. (1988). Nephrotoxicity of mercapturic acids of three structurally related 2,2-difluoroethylenes in the rat. *Biochem. Pharmacol.* **37**, 4495-4504.

Commandeur, J.N.M., de Kanter, F.J.J., and Vermeulen, N.P.E. (1989). Bioactivation of the cysteine-*S*-conjugate and mercapturic acid of tetrafluoroethylene to acylating reactive intermediates in the rat: Dependence of activation and deactivation activities on acetyl coenzyme A availability. *Mol. Pharmacol.* **36**, 654-663.

Commandeur, J.N.M., Stijntjes, G.J., Wijngaard, J., and Vermeulen, N.P.E. (1991). Metabolism of L-cysteine *S*-conjugates and *N*-(trideuteroacetyl)-L-cysteine *S*-conjugates of four fluoroethylenes in the rat. Role of balance of deacetylation and acetylation in relation to the nephrotoxicity of mercapturic acids. *Biochem. Pharmacol.* **42**, 31-38.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

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

Ding, X.Z., Yu, H.T., Hu, M., Liu, C.F., and Ko, F.Z. (1980). Studies on the absorption, distribution, and elimination of four organofluorine compounds in rabbits. *Chung Hua Yu Fang I Hsueh Tsa Chih* **14**, 39-42.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* **6**, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumour prevalence data. *Appl. Statist.* **32**, 236-248.

Dixon, W.J., and Massey, F.J., Jr. (1951). *Introduction to Statistical Analysis*, 1st ed., pp. 145-147. McGraw-Hill Book Company, New York.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Finco, D.R. (1989). Kidney function. In *Clinical Biochemistry of Domestic Animals* (J.J. Kaneko, Ed.), 4th ed., pp. 496-542. Academic Press, Inc., San Diego, CA.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* **62**, 957-974.

Gosselin, R.E., Hodge, H.C., Smith, R.P., and Gleason, M.N. (1984). *Clinical Toxicology of Commercial Products: Acute Poisoning*, Vol. II, p. 412. Williams and Wilkins, Baltimore, MD.

Green, T., and Odum, J. (1985). Structure/activity studies of the nephrotoxic and mutagenic action of cysteine conjugates of chloro- and fluoro-alkenes. *Chem.-Biol. Interact.* **54**, 15-31.

Groves, C.E., Hayden, P.J., Lock, E.A., and Schnellmann, R.G. (1993). Differential cellular effects in the toxicity of haloalkene and haloalkane cysteine conjugates to rabbit renal proximal tubules. *J. Biochem. Toxicol.* **8**, 49-56.

Harris, J.W., Dekant, W., and Anders, M.W. (1992). *In vivo* detection and characterization of protein adducts resulting from bioactivation of haloethene cysteine *S*-conjugates by ^{19}F NMR: Chlorotrifluoroethene and tetrafluoroethene. *Chem. Res. Toxicol.* **5**, 34-41.

Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* **58**, 385-392.

Hawley, G.G., Ed. (1981). *The Condensed Chemical Dictionary*, 10th ed. Van Nostrand Reinhold, New York.

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., and Zeiger, E. (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ. Mutagen.* **5** (Suppl. 1), 3-142.

Hayden, P.J., and Stevens, J.L. (1990). Cysteine conjugate toxicity, metabolism, and binding to macromolecules in isolated rat kidney mitochondria. *Mol. Pharmacol.* **37**, 468-476.

Hayden, P.J., Ichimura, T., McCann, D.J., Pohl, L.R., and Stevens, J.L. (1991a). Detection of cysteine conjugate metabolite adduct formation with specific mitochondrial proteins using antibodies raised against halothane metabolite adducts. *J. Biol. Chem.* **266**, 18,415-18,418.

Hayden, P.J., Yang, Y., Ward, A.J.I., Dulik, D.M., McCann, D.J., and Stevens, J.L. (1991b). Formation of difluorothionacetyl-protein adducts by *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine metabolites: Nucleophilic catalysis of stable lysyl adduct formation by histidine and tyrosine. *Biochemistry* **30**, 5935-5943.

Hayden, P.J., Welsh, C.J., Yang, Y., Schaefer, W.H., Ward, A.J.L., and Stevens, J.L. (1992). Formation of mitochondrial phospholipid adducts by nephrotoxic cysteine conjugate metabolites. *Chem. Res. Toxicol.* **5**, 231-237.

Hazardous Substances Data Base (HSDB) (1995). Maintained, reviewed, and updated on the National Library of Medicine's Toxicology Data Network (TOXNET). Available through the Medlars System.

Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*, pp. 120-123. John Wiley and Sons, New York.

International Agency for Research on Cancer (IARC) (1979). Some Monomers, Plastics and Synthetic Elastomers, and Acrolein. In *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Vol. 19, pp. 285-301. Lyon, France.

International Agency for Research on Cancer (IARC) (1987). Overall Evaluations of Carcinogenicity: An Updating of *IARC Monographs*. In *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Vols. 1-42, Suppl. 7. Lyon, France.

Jain, N.C., Ed. (1986). Depression or hypoproliferative anemias. In *Schalm's Veterinary Hematology*, 4th ed., pp. 655-675. Lea and Febiger, Philadelphia, PA.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Kennedy, G.L. (1990). Toxicology of fluorine-containing monomers. In *Critical Reviews in Toxicology*, Vol. 21, pp. 149-170. CRC Press, Boca Raton, FL.

Lock, E.A., and Schnellmann, R.G. (1990). The effect of haloalkene cysteine conjugates on rat renal glutathione reductase and lipoyl dehydrogenase activities. *Toxicol. Appl. Pharmacol.* **104**, 180-190.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* **79**, 639-648.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Material Safety Data Sheet Collection (1993). Tetrafluoroethylene. Sheet No. 851. Genium Publishing Corp., Schenectady, NY.

Morrison, D.F. (1976). *Multivariate Statistical Methods*, 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.

Myhr, B., McGregor, D., Bowers, L., Riach, C., Brown, A.G., Edwards, I., McBride, D., Martin, R., and Caspary, W.J. (1990). L5178Y mouse lymphoma cell mutation assay results with 41 compounds. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 138-167.

National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1977). Bioassay of Tetrachloroethylene for Possible Carcinogenicity. (CAS No. 127-18-4). Technical Report Series No. 13. NIH Publication No. 77-813. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD.

National Fire Protection Association (NFPA) (1986). Fire Protection Guide on Hazardous Materials. 9th ed., Sect. 49, pp. 86-87. National Fire Protection Association, Quincy, MA.

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

National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). Specification NIH-11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Library of Medicine (1995). MEDLINE, CANCERLIT, TOXLINE. Toxicology Data Bank databases maintained by the National Library of Medicine. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD.

National Toxicology Program (NTP) (1984). Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice, 10/31/82 version (updated October 1984). Research Triangle Park, NC.

National Toxicology Program (NTP) (1986). NTP Technical Report on the Toxicology and Carcinogenesis of Tetrachloroethylene (Perchloroethylene) (CAS No. 127-18-4) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). Technical Report Series No. 311. NIH Publication No. 86-2567. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park.

Odum, J., and Green, T. (1984). The metabolism and nephrotoxicity of tetrafluoroethylene in the rat. *Toxicol. Appl. Pharmacol.* **76**, 306-318.

Patty's Industrial Hygiene and Toxicology (1994). 4th ed., Vol. II (G.D. Clayton and F.E. Clayton, Eds.), p. 1149. Wiley-Interscience, New York.

Sakharova, L.N., and Tolgskaya, M.S. (1977). Toxicity and the nature of action of some halogen derivatives of ethylene, such as difluorodichloroethylene, trifluorochloroethylene and tetrafluoroethylene. *Gig. Tr. Prof. Zabol.* **21**, Vol. 5, 36-42.

Schmid, W. (1976). The micronucleus test for cytogenetic analysis. In *Chemical Mutagens, Principles and Methods for Their Detection* (A. Hollaender, Ed.), Vol. 4, pp. 31-53. Plenum Press, New York.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

U.S. International Trade Commission (USITC) (1994). Synthetic Organic Chemicals, United States Production and Sales, 1992. USITC Publication No. 2720. USITC, Washington, DC.

Weast, R.C., Ed. (1985). *CRC Handbook of Chemistry and Physics*, 66th ed., p. C272. CRC Press, Boca Raton, FL.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Zhemerdei, A.I. (1958). Toxicology of tetrafluoroethylene. *Tr. Leningr. Sanit. Gig. Med. Inst.* **44**, 164.

APPENDIX C

Description of Online Searches for Tetrafluoroethylene

DESCRIPTION OF ONLINE SEARCHES FOR TETRAFLUOROETHYLENE

Searches were limited to 1994 (the year before the 1995 draft of the NTP bioassay, which has an extensive literature review) through September 1997.

Online searches for tetrafluoroethylene [CASRN 116-14-3] were performed in databases on the systems of the National Library of Medicine, STN International, DIALOG, and the Chemical Information System from 1994 to date. Toxicology information was sought in EMIC, EMICBACK, RTECS, TSCATS, BIOSIS, CANCERLIT, EMBASE, MEDLINE, and TOXLINE (using the MESH heading for all neoplasms in TOXLINE). Occupational safety and health information was obtained from NIOSHTIC. Chemical Abstracts file was searched by appropriate section codes (59, air pollution and industrial hygiene; 60, waste treatment and disposal; 61 water). Exposure information was also sought in NTIS. The Chemical Abstracts Service Registry file and SANSS provided chemical identification information.

Market information, including production, shipments, sales and consumption, labor use, and workers by type was sought in PROMT, *The Predicasts Overview of Markets and Technology*, and The Chemical Economics Handbook.

Regulatory information was obtained from the in-house FESA CD-ROM containing the latest *Code of Federal Regulations* and the *Federal Register* pertaining to CFR titles 21 (FDA), 29 (OSHA), and 40 (EPA).

Also, the review of 1200 life sciences journals was accomplished using Current Contents on Diskette® (and cumulative issues on CD-ROM).

APPENDIX D

Report on Carcinogens (RoC), 9th Edition
Review Summary

Report on Carcinogens (RoC), 9th Edition
Review Summary

Tetrafluoroethylene

NOMINATION
Review based on results of an NTP Bioassay of Tetrafluoroethylene (1995), reporting clear evidence of carcinogenicity in all experimental animal groups.

DISCUSSION
Tetrafluoroethylene is used in the production of polytetrafluoroethylene and other polymers. It has also been used as a propellant for food product aerosols. Experimental animal inhalation studies showed that Tetrafluoroethylene caused kidney and liver tumors and leukemia in rats and liver and reticuloendothelial tumors in mice. The recommendations from the three NTP reviews of this nomination are as follows:

Review Committee	Recommendation	Vote
NIEHS (RG1)	list as a reasonably anticipated human carcinogen	10 yes/0 no
NTP EC Working Group (RG2)	list as a reasonably anticipated human carcinogen	8 yes/0 no
NTP Board RoC Subcommittee	list as a reasonably anticipated human carcinogen	5 yes/0 no

Public Comments Received
One public comment was received.