FINAL

Report on Carcinogens Background Document for

Nitromethane

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FOREWORD

The Report on Carcinogens (RoC) is prepared in response to Section 301 of the Public Health Service Act as amended. The RoC contains a list of all substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, Department of Health and Human Services (DHHS) has delegated responsibility for preparation of the RoC to the National Toxicology Program (NTP) who prepares the Report with assistance from other Federal health and regulatory agencies and non-government institutions.

Nominations for listing in or delisting from the RoC are reviewed by a formal process that includes a multi-phased, scientific peer review and multiple opportunities for public comment. The review groups evaluate each nomination according to specific RoC listing criteria. This Background Document was prepared to assist in the review of the nomination of nitromethane. The scientific information in this document comes from publicly available, peer reviewed sources. Any interpretive conclusions, comments or statistical calculations, etc made by the authors of this document that are not contained in the original citation are identified in brackets [ ]. If any member(s) of the scientific peer review groups feel this Background Document does not adequately capture and present the relevant information they will be asked to write a commentary for this Background Document that will be included as an addendum to the document. In addition, a meeting summary that contains a brief discussion of the respective review group's review and recommendation for the nomination will be added to the Background Document, also as an addendum.

A detailed description of the RoC nomination review process and a list of all nominations under consideration for listing in or delisting from the RoC can be obtained by accessing the NTP Home Page at http://ntp-server.niehs.nih.gov. The most recent RoC, the 9th Edition, was published in May, 2000 and may be obtained by contacting the NIEHS Environmental Health Information Service (EHIS) at http://ehis.niehs.nih.gov (800-315-3010).

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

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

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

Reasonably Anticipated to be Human Carcinogens:

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

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

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

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

Executive Summary

Introduction

Nitromethane is a nitroalkane used as a synthesis intermediate for nitromethane derivatives, as a solvent, as an explosive, and as fuel or fuel additive. Nitromethane was nominated by the National Institute of Environmental Health Sciences for listing in the Report on Carcinogens based on the results of a National Toxicology Program (NTP) two-year inhalation study of nitromethane, which concluded that there was clear evidence of carcinogenicity in female F344/N rats and male and female B6C3F$_1$ mice. There was no evidence of carcinogenicity in male F344/N rats.

Human Exposure

Use. Nitromethane's primary use is in the synthesis of nitromethane derivatives used as pharmaceuticals, agricultural soil fumigants, and industrial antimicrobials. Nitromethane also is used as a fuel or fuel additive with methanol in racing cars, boats, and model engines, which accounts for less than 20% of the market for nitromethane. Past uses of nitromethane include its use as a chemical stabilizer to prevent the decomposition of various halogenated hydrocarbons, such as metal degreasers and aerosol propellants; as an additive for 1,1,1-trichloroethane; and in the explosive industry as a component in a binary explosive formulation with ammonium nitrate and in shaped charges.

Production. Nitromethane is produced commercially by high-temperature vapor-phase nitration of propane, a reaction that also yields nitroethane, 1-nitropropane, and 2-nitropropane. Nitromethane was produced commercially in the United States by Angus Chemical Co. (Buffalo Grove, IL) and W.R. Grace and Company (Columbia, MD); however, Angus Chemical Co. has reported that it is the only current commercial producer of nitromethane and produces 16 million pounds domestically per year.

Environmental exposure. Nitromethane has been detected in air, in surface water, and in drinking water. The general population can be exposed to nitromethane by inhalation from motor vehicle exhaust and cigarette smoke. Estimated nitromethane concentrations in motor vehicle exhaust in a simulated city driving study ranged from less than 0.8 to 5.0 ppm depending on the conditions. Nitromethane also may be released in air and wastewater during the manufacture of the munitions cyclotrimethylenetrinitramine and cyclotetramethylenetetranitramine. Maximum ground-level air concentrations of nitromethane at three plants on the boundary of an ammunition plant were 0.21, 2.0, and 2.0 $\mu g/m^3$. Nitromethane was identified, but not quantified, as a pollutant in drinking water in two of five cities (Philadelphia, PA, and Cincinnati, OH) tested in a 1975 United States Environmental Protection Agency survey. Human exposure also may occur through dermal contact or accidental ingestion of methanol-nitromethane fuel mixture; however, products containing nitromethane are not widely used by consumers.

Occupational exposure. Approximately 135,000 male and 46,500 female workers in the United States were potentially exposed to nitromethane from 1981 through 1983. Angus Chemical Co. reported that in its facility where nitromethane was produced, occupational exposure was in the 1.0-ppm range (8-h time-weighted average [TWA]). Occupational

exposure to nitromethane may have occurred in the past as a consequence of exposure to other chemicals, such as 1,1,1-trichloroethane, that may contain nitromethane as a contaminant.

Regulations. The Occupational Safety and Health Administration nitromethane exposure limit is 100 ppm, or 250 mg/m^3. The American Conference of Governmental Industrial Hygienists has set a TWA threshold limit value for nitromethane of 20 ppm, or 50 mg/m^3. Nitromethane is considered immediately dangerous to life or health at a concentration of 750 ppm (NIOSH 1997). Nitromethane also is regulated by the United States Environmental Protection Agency, with standards and record-keeping requirements for industrial facilities that produce nitromethane.

Human Cancer Studies

No studies have been reported on the relationship between human cancer and exposure to nitromethane.

Studies in Experimental Animals

The International Agency for Research on Cancer (IARC) concluded that there was sufficient evidence for the carcinogenicity of nitromethane in experimental animals, based on the NTP inhalation study in mice and rats. Increased incidences of harderian gland adenoma and adenoma or carcinoma (combined) occurred in male and female mice exposed to nitromethane by inhalation at a concentration of 375 or 750 ppm. Increased incidences of lung carcinoma occurred in males exposed at 750 ppm and females exposed at 375 ppm. Female mice exposed at 750 ppm had a significantly increased incidence of lung adenoma or carcinoma (combined). In addition, the incidences of hepatocellular adenoma and adenoma or carcinoma (combined) were significantly increased in female mice at 188 or 750 ppm. The NTP concluded that there was clear evidence for carcinogenicity of nitromethane in both male and female B6C3F$_1$ mice. In female F344/N rats exposed to nitromethane at 188 or 375 ppm for two years, the incidences of mammary gland fibroadenoma and fibroadenoma, adenoma, or carcinoma (combined) were significantly increased, and at 375 ppm, the incidence of mammary gland carcinoma was significantly increased. The NTP concluded that there was clear evidence that nitromethane was carcinogenic to female F344/N rats. There was no evidence that nitromethane was carcinogenic in male or female Long-Evans rats exposed to nitromethane by inhalation at 100 or 200 ppm for two years or in male F344/N rats exposed at 94, 188, or 375 ppm for two years. Evidence of only mild to moderate toxicity was observed in rabbits exposed to nitromethane at a concentration of 98 or 745 ppm by inhalation for 6 months.

Genotoxicity

Nitromethane was not mutagenic *in vitro* or *in vivo*. It did not induce reverse mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without induced rat liver S9; chromosomal aberrations or sister chromatid exchange in Chinese hamster ovary cells; or micronuclei in Syrian hamster embryo cells or mouse bone marrow cells or peripheral erythrocytes.

Other Relevant Data

Nitromethane has been shown to produce toxic effects in animals, including neurologic and reproductive effects. Relatively few reports have been published on the absorption, distribution, metabolism, and excretion of nitromethane. The available data suggest that absorption may occur by inhalation but that the amount absorbed after dermal exposure is negligible. Although nitromethane may be metabolized to formaldehyde by rat liver microsomes *in vitro*, no published reports have characterized the metabolism of nitromethane *in vivo*. Nitromethane is structurally related to other nitro compounds (i.e., 2-nitropropane and tetranitromethane) that have been evaluated by IARC and considered to be possibly carcinogenic to humans. The mechanism of carcinogenicity for nitromethane and these other nitro compounds is not known; however, it has been hypothesized that reactive radicals may play a key role in their carcinogenicity.

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1 Introduction

Nitromethane is a nitroalkane used as a synthesis intermediate for nitromethane derivatives, agricultural fumigants, biocides, and other products; as a solvent; and, in mixtures with ammonium nitrate, as an explosive in mining, oil-well drilling, and seismic exploration. Nitromethane also is used as a fuel or fuel additive to increase the power output of rockets, racing cars, boats, and model engines. Nitromethane has been found in air, surface and drinking water, and cigarette smoke and as a byproduct of hydrocarbon combustion and munitions manufacture. It is structurally related to two other nitroalkanes, 2-nitropropane and tetranitromethane, that are known animal carcinogens and are listed in the 9th Report on Carcinogens (NTP 2000) as *reasonably anticipated to be a human carcinogen*.

Nitromethane was nominated by the National Institute of Environmental Health Sciences for listing in the Report on Carcinogens based on the results of a National Toxicology Program (NTP) two-year inhalation study of nitromethane that concluded there was clear evidence of carcinogenicity in female F344/N rats (mammary gland fibroadenoma and carcinoma), female B6C3F$_1$ mice (liver neoplasms and harderian gland adenoma and carcinoma), and male B6C3F$_1$ mice (harderian gland adenoma and carcinoma) (NTP 1997). There was no evidence of carcinogenicity in male F344/N rats. Increased incidences of alveolar/bronchiolar adenoma and carcinoma in male and female mice also were observed and considered related to nitromethane exposure.

1.1 Chemical identification

Nitromethane (CH_3NO_2, mol wt 61.04, CASRN 75-52-5) is a colorless, oily liquid with a mild fruity or disagreeable odor (Budavari 1996). It is also known as nitrocarbol, nitrometan, NMT, and NM. Its RTECS number is PA9800000, and its Department of Transportation number and hazard class are UN 1261, flammable liquid. The structure of nitromethane is given in Figure 1-1.

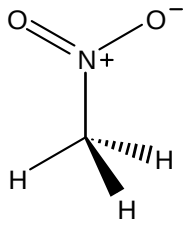


Figure 1-1. Structure of nitromethane

1.2 Physical-chemical properties

Nitromethane is a flammable liquid that may explosively decompose on shock, friction, concussion, or heating. Although nitromethane is relatively insensitive to detonation by shock at ordinary temperatures, it becomes more sensitive as the temperature increases. At 55°C to 60°C (130°F to 140°F), nitromethane has a 50% probability of detonation with a No. 8 blasting cap. Heating of nitromethane vapor results only in slow

decomposition even at temperatures above the critical point of 315°C (599°F). (The critical point is the temperature above which a liquefied gas will vaporize almost instantaneously if heat is added.) Heating nitromethane in the liquid phase, therefore, may be hazardous, as rapid expansion of confined volumes may result in ignition and explosion (Angus 2000).

Nitromethane reacts with alkalis and reacts violently with strong oxidants and strong reducing agents, causing fire and explosion hazards. The vapor is heavier than air and may travel along the ground; thus, distant ignition is possible. Nitromethane will slowly corrode steel and copper when wet (NIOSH 1998). The hazardous decomposition products of nitromethane are toxic fumes of nitrogen oxides (Ash and Ash 1996). The physical and chemical properties of nitromethane are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of nitromethane

Property	Information	Reference
Molecular weight	61.04	ChemFinder 2001, Budavari 1996
Color	colorless	ChemFinder 2001, Budavari 1996, NTP 2001
Odor	mild fruity or disagreeable	NTP 2001, ChemFinder 2001
Physical state	oily liquid	ChemFinder 2001, Budavari 1996
Melting point (^{o}C)	-29	ChemFinder 2001, Budavari 1996
Boiling point (^{o}C)	101.2	ChemFinder 2001, Budavari 1996
Evaporation rate	1.39	ChemFinder 2001, NTP 2001
Flash point (^{o}C)	35	ChemFinder 2001, NTP 2001
Density	1.1371	ChemFinder 2001
Vapor density	2.1	ChemFinder 2001, NTP 2001
Vapor pressure (mm Hg at 20^{o}C)	27.8	ChemFinder 2001, NTP 2001
Solubility (at 23^{o}C):		
water	9.50 g/100 mL	ChemFinder 2001
acetone	soluble	HSDB 2000
alcohol	soluble	Budavari 1996
ether	soluble	HSDB 2000
Henry's law constant (calc.) (atm-m^{3}/mol)	2.68 x 10^{-5}	(SRC 2001)

1.3 Other nitroparaffins

Four nitroparaffins, also called nitroalkanes or aliphatic nitro compounds (RNO_3), are available commercially as solvents and chemical intermediates in the synthesis of a variety of compounds (Kirk-Othmer 2001, Archer 1996, Markofsky 1991, Budavari 1996). These four compounds, nitromethane (Figure 1-1), nitroethane (Figure 1-2), 1-nitropropane (Figure 1-3), and 2-nitropropane (Figure 1-4), sometimes are referred to as the lower mononitroparaffins. Polynitro compounds include tetranitromethane (Figure 1-

5). The four mononitroparaffins and tetranitromethane all are liquids at room temperature, and all five compounds are slightly soluble in water and insoluble in alkanes but are soluble in most other organic solvents, including ethanol and ethyl ether. In addition, all five compounds are flammable, and some (nitromethane and tetranitromethane) are explosive. 2-Nitropropane and tetranitromethane have been reviewed by both an International Agency for Research on Cancer (IARC) Working Group and the NTP for potential carcinogenicity (see Section 6.4).

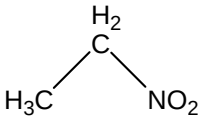


Figure 1-2. Structure of nitroethane

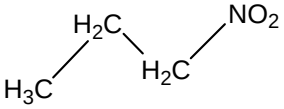


Figure 1-3. Structure of 1-nitropropane

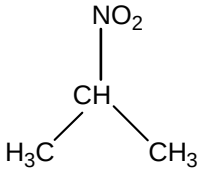


Figure 1-4. Structure of 2-nitropropane

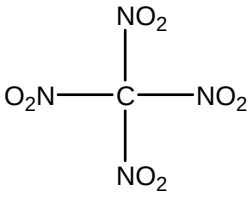


Figure 1-5. Structure of tetranitromethane

2 Human Exposure

2.1 Use

Nitromethane's primary use is in the synthesis of nitromethane derivatives used as pharmaceuticals, agricultural soil fumigants, and industrial antimicrobials. Table 2-1 summarizes the major nitromethane derivatives, their formation, and their uses (Markofsky 1991).

Table 2-1. Nitromethane derivatives

Derivative	Formation	Uses
Chloropicrin	reaction of nitromethane with sodium hypochlorite	fungicide and nematocidal fumigant
Tris(hydroxymethyl)nitromethane	reaction of formaldehyde and nitromethane (3:1 molar ratio) via the Henry reaction	biocide
Tris(hydroxymethyl) aminomethane	reduction of tris(hydroxymethyl) nitromethane	buffer and a component of resins and adhesives
Di(hydroxymethyl)nitromethane	reaction of formaldehyde and nitromethane (2:1 molar ratio) via the Henry reaction	chemical intermediate in the synthesis of the X-ray contrast agent iopamidol
2-Bromo-2-nitro-1,3-propanediol	bromination of di(hydroxymethyl)nitromethane	widely used biocide (bronopol)
β-nitrostyrene	reaction of benzaldehyde and nitromethane and dehydration	used as a chain transfer agent (lowers molecular weights of polymers in their free radical initiated states)
Bromonitrostyrene	treatment of β-nitrostyrene with bromine followed by dehydrobromination	slimicide
Nizatidine	commercial process	anti-ulcer drug
Ranitidine	commercial process	anti-ulcer drug
Sulpiride	commercial process	psychotropic agent

Source: Markofsky 1991.

In public comments in response to the nomination of nitromethane for listing in the Report on Carcinogens, Angus Chemical Company, a subsidiary of Dow Chemical Company, reported that 85% to 90% of the domestically produced nitromethane is used in the above industrial settings (Angus 2001). Angus Chemical Co. has estimated that use of nitromethane as a fuel or fuel additive with methanol in racing cars, boats, and model engines accounts for less than 20% of the market for nitromethane. Angus Chemical Co. also has reported that use of nitromethane as a chemical stabilizer to prevent the decomposition of various halogenated hydrocarbons, such as metal degreasers and aerosol propellants, has diminished to virtually zero. Nitromethane previously was used as an additive for 1,1,1-trichloroethane; however, the mandatory phase-out of 1,1,1-

trichloroethane in 1995 ended this use of nitromethane (Angus 2001). Nitromethane also was used in the explosive industry as a component in a binary explosive formulation with ammonium nitrate and in shaped charges (IARC 2000, NTP 1997). Angus Chemical Co. (2001) has reported that as of 2000, it no longer sells nitromethane for explosive-industry applications.

2.2 Production

Nitromethane is produced commercially by high-temperature vapor-phase nitration of propane, a reaction that also yields nitroethane, 1-nitropropane, and 2-nitropropane. Nitromethane was produced commercially in the United States by Angus Chemical Co. (Buffalo Grove, IL) and W.R. Grace and Company (Columbia, MD) (SRI 1992); however, Angus Chemical Co. has reported that it is the only current commercial producer of nitromethane (Angus 2001). Nitromethane also can be prepared by the reaction of sodium nitrite with sodium chloroacetate (Budavari 1996).

In its public comments, Angus Chemical Co. (2001) submitted a statement that approximately 16 million pounds of nitromethane are produced domestically per year. No information from the U.S. International Trade Commission on domestic nitromethane production was found.

2.3 Analysis

Nitromethane can be determined in workplace air by adsorption on Chromosorb, desorption with ethyl acetate, and analysis by gas chromotography with a nitrogen-specific detector. The applicable working range of this method (National Institute for Occupational Safety and Health [NIOSH] method 2527) is 60 to 360 ppm (150 to 900 mg/m^3) for a 2-L air sample (IARC 2000).

2.4 Environmental occurrence

Nitromethane has been detected in air, in surface water, and in drinking water. It also has been found in cigarette smoke and as a byproduct of hydrocarbon combustion and munitions manufacture (IARC 2000, NTP 1997).

2.5 Environmental fate

2.5.1 Atmospheric fate

Nitromethane does not persist in the environment, having a half-life of 4 to 9 hours in air; degradation is by photolysis. Reaction with photochemically produced hydroxyl radicals is not considered an important atmospheric fate, as this reaction is very slow, with a half-life of 100 days (HSDB 2000).

2.5.2 Aquatic fate

Nitromethane in water will be lost by volatilization. Nitromethane is slightly soluble in water (9.5 g/100 mL) and evaporates at about the same rate as water; thus, the aquatic half-life of nitromethane depends on the rate of evaporation (NTP 1997). The half-life of nitromethane was found to be 28.7 hours in a model river and 13 days in a model pond. The importance of biodegradation or photodegradation in the removal of nitromethane

from surface water has not been studied. Nitromethane is not expected to adsorb to sediment or particulate matter or to bioconcentrate in aquatic organisms (HSDB 2000).

2.5.3 Terrestrial fate

Nitromethane is expected to volatilize rapidly in soil because of its high vapor pressure, high calculated Henry's Law constant (which defines the equilibrium between the concentration of a solute gas in solution and the partial pressure of that gas above the solution), and low adsorptivity to soil. It may leach into soil, and degradation is expected to be low. In terrestrial studies, more nitromethane was lost as volatile products than through conversion to carbon dioxide (NTP 1997, HSDB 2000).

2.6 Environmental exposure

2.6.1 Atmospheric exposure

Vaporization of nitromethane in the atmosphere can lead to human exposure via inhalation. Nitromethane can be absorbed into the body by inhalation and by ingestion (NIOSH 1998). The general population will be exposed to nitromethane by inhalation from motor vehicle exhaust and cigarette smoke (HSDB 2000). Nitromethane concentrations in exhaust from automobiles using nine hydrocarbon test fuels were estimated under simulated city driving conditions. Estimated concentrations ranged from < 0.8 to 5.0 ppm. Environmental exposure to nitromethane also would occur in the use of specialty fuel blends for drag racing and hobby fuel (Angus 2001). Nitromethane also is a byproduct in the manufacture of the munitions cyclotrimethylenetrinitramine (RDX) and cyclotetramethylenetetranitramine (HMX) and may be released in air emissions and wastewaters during their manufacture (HSDB 2000, IARC 2000).

2.6.2 Drinking water

Nitromethane was identified, but not quantified, as a pollutant in drinking water in two of five cities tested (Philadelphia, PA, and Cincinnati, OH) in a 1975 U.S. Environmental Protection Agency (EPA) survey (HSDB 2000).

2.6.3 Other exposure

Human exposure may occur through dermal contact or accidental ingestion of methanol-nitromethane fuel mixtures (IARC 2000). Products containing nitromethane are not widely used by consumers; therefore, consumer exposure is presumed to be low (NTP 1997). In a study to evaluate the utility of human milk in specific pollutant studies, environmental pollutants in milk were identified by gas chromatography/mass spectrometry. In each of four urban areas selected based on the probability of emissions of various halogenated pollutants (Bridgeville, PA, Bayonne and Jersey City, NJ, and Baton Rouge, LA), up to 12 women were selected from various health clinics and hospitals. Nitromethane was detected by qualitative analysis in 1 of the 12 samples from one unspecified site. No quantitative analysis was done (Pellizzari *et al.* 1982).

In 1984, the munitions RDX and HMX (see Section 2.6.1) were manufactured only in one plant in Kingsport, TN (ATSDR 1995). Maximum ground-level air concentrations of

nitromethane at three sites on the boundary of this ammunition plant were 0.21, 2.0, and 2.0 $\mu g/m^3$ (HSDB 2000).

2.7 Occupational exposure

It was reported that approximately 135,000 male and 46,500 female workers in the United States were potentially exposed to nitromethane from 1981 through 1983 (NTP 1997). Angus Chemical Co. reported that in its facility where nitromethane was produced, occupational exposure was in the 1.0-ppm range (8-h time-weighted average [TWA]). No information on peak exposure to nitromethane was provided (Angus 2001). Exposure could have occurred in the past as a consequence of exposure to other chemicals, such as 1,1,1-trichloroethane, that may contain nitromethane as a contaminant (Henschler *et al.* 1980).

2.8 Biological indices of exposure

The acute symptoms of inhalation exposure to nitromethane are cough, drowsiness, headache, nausea, sore throat, unconsciousness, and vomiting (NIOSH 1998).

2.9 Regulations

The Occupational Safety and Health Administration (OSHA) nitromethane exposure limit is 100 ppm, or 250 mg/m^3 (OSHA 2001). The American Conference of Governmental Industrial Hygienists (ACGIH) has set a TWA threshold limit value for nitromethane of 20 ppm, or 50 mg/m^3 (ACGIH 1999). Nitromethane is considered immediately dangerous to life or health at a concentration of 750 ppm (NIOSH 1997).

Nitromethane is also regulated by the U.S. EPA requiring standards and record-keeping requirements for industrial facilities that produce nitromethane.

Table 2-2. EPA regulations

Regulatory action	Effect of regulation or other comments
40 CFR 60.480 – Subpart VV – Standards of Performance for Equipment Leaks of VOC in the Synthetic Organic Chemicals Manufacturing Industry. Promulgated: 48 FR 48335, 10/18/83.	The provisions of this subpart apply to affected facilities in the synthetic organic chemicals manufacturing industry that produce, as intermediates or final products, nitromethane. This subpart identifies standards, test methods, procedures, and record-keeping requirements for affected facilities.

Source: The regulations in this table have been updated through the 2001 Code of Federal Regulations 40 CFR, 1 July 2001.

Table 2-3. OSHA Regulations

Regulatory action	Effect of regulation or other comments
29 CFR 1910.119 – Sec. 1910.119. Process safety management of highly hazardous chemicals. U.S. Codes: 29 U.S.C. 653, 655, 657.	Nitromethane is listed as a toxic and reactive highly hazardous chemical that presents a potential for a catastrophic event at or above the threshold quantity of 2,500 lb.
29 CFR 1915.1000 – Subpart Z – Air contaminants. TABLE Z-1 Limits for Air Contaminants.	An employee's personal exposure level (PEL) for nitromethane shall be limited to 100 ppm (8-h TWA) or 250 mg/m^3 (8-h TWA).
29 CFR 1915.1000 – Subpart Z – Air contaminants. Toxic and Hazardous Substances.	An employee's PEL for nitromethane in shipyards shall be limited to 100 ppm (8-h TWA) or 250 mg/m^3 (8-h TWA).
29 CFR 1926.50ff – Subpart D – Occupational Health and Environmental Controls.	Exposure of employees to inhalation, ingestion, skin absorption, or contact with any material or substance at concentrations above those specified in the ACGIH "Threshold Limit Values of Airborne Contaminants for 1970" shall be avoided.

Source: The regulations in this table have been updated through the 2001 Code of Federal Regulations 29 CFR, 1 July 2001.

3 Human Cancer Studies

No studies have been reported on the relationship between human cancer and exposure to nitromethane.

4 Studies of Cancer in Experimental Animals

An IARC Working Group (2000) reviewed three animal cancer studies and several relevant metabolism and toxicity studies of nitromethane. Cancer studies were conducted with Long-Evans rats (Griffin *et al.* 1996), F344/N rats (NTP 1997), and B6C3F$_1$ mice (NTP 1997). The IARC Working Group concluded that there was sufficient evidence for the carcinogenicity of nitromethane in experimental animals. No carcinogenicity studies in experimental animals have been published since the IARC (2000) monograph. In addition, results from several subchronic toxicity studies in mice, rats, and rabbits are presented in Sections 4.1 through 4.3.

4.1 Rabbits

Male New Zealand white rabbits (15 per group) were exposed to nitromethane vapor at a concentration of 0, 98, or 745 ppm by inhalation, 7 hours/day, 5 days/week, for 24 weeks (Lewis *et al.* 1979). Five rabbits from each group were sacrificed after 1, 3, or 6 months of exposure. Evidence of only mild to moderate toxicity was observed in rabbits exposed to nitromethane at 98 or 745 ppm for 6 months. Growth rates and organ weights were not affected except for thyroid weight. Thyroid weight increased after 6 months of exposure at 745 ppm, and lower serum thyroxine levels were observed after exposure at 98 or 745 ppm after 1, 3, and 6 months. In addition, there was some evidence of pulmonary edema and other pulmonary abnormalities in rabbits exposed to either level of nitromethane for 1 month. However, no exposure-related gross or microscopic effects were seen in any of the tissues examined.

4.2 Mice

Nitromethane was selected for a two-year carcinogenicity bioassay in B6C3F$_1$ mice (NTP 1997) based on its potential for human exposure and its relationship to the known animal carcinogens 2-nitropropane and tetranitromethane. Inhalation studies first were conducted in the same strain of mice for 16 days (see Section 6) and 13 weeks. Results from the 13-week subchronic toxicity study and two-year carcinogenicity study are summarized here.

Three lots of nitromethane were obtained from W.R. Grace and Company (Lexington, MA) and analyzed for identity, purity, and stability by Midwest Research Laboratory (Kansas City, MO). Lot 1F-13-06 (used for the 16-day study, described in Section 6.1.3, and the beginning of the 13-week study) had a purity of approximately 99%, with 0.4% propionitrile and 0.017% 2-nitropropane. Lot 1-H-0501 (used for the remainder of the 13-week study and the beginning of the two-year study) had a purity of approximately 98%, with 1.71% total impurities. Lot 1-H-1004 (used for the remainder of the two-year study) had a purity of approximately 98%, with 1.5% total impurities (NTP 1997).

4.2.1 Subchronic toxicity

The 13-week study was conducted to evaluate the cumulative toxic effects of repeated exposure to nitromethane and to determine the exposure concentrations to be used in the two-year study (NTP 1997). Groups of B6C3F$_1$ mice (10 of each sex) were exposed to nitromethane vapor at a concentration of 0, 94, 188, 375, 750, or 1,500 ppm by inhalation, 6 hours/day, 5 days/week, for 13 weeks. Animals were housed individually;

water was available *ad libitum*; and feed was available *ad libitum* except during exposure periods. Clinical observations were recorded weekly, and animals were weighed initially, weekly, and at the end of the study. At the end of the study, samples were collected for sperm motility or vaginal cytology evaluations from all mice in all groups. Complete necropsies were performed on all animals and included histopathologic examination of all major organs and tissues. The heart, right kidney, liver, lungs, right testis, and thymus were weighed.

All mice survived to the end of the study. The final mean body weights and weight gains of exposed mice were generally similar to those of the controls. There were no treatment-related clinical findings. The absolute kidney weights of all groups of exposed male mice except the 1,500-ppm group and of all groups of female mice exposed at 188 ppm or more were significantly greater than those of the controls. The relative kidney weights also were significantly greater than those of controls in all males and females in the 750- and 1,500-ppm groups. The absolute liver weight of male mice in the 750-ppm group and the relative liver weights of males exposed at 375 ppm or more were significantly greater than those of the controls. Olfactory epithelial degeneration and respiratory epithelial hyaline droplets were observed microscopically in all male and female mice exposed at 375 ppm or more. Degeneration also occurred in females in the 188-ppm group, and hyaline droplets occurred in females in the 94- and 188-ppm groups. The average severity of the nasal lesions ranged from minimal to mild in males and from minimal to moderate in females. All males and 9 females in the 1,500-ppm groups also had minimal extramedullary hematopoiesis of the spleen.

4.2.2 Two-year carcinogenicity study

Exposure levels were based on the incidence and severity of nasal lesions and the presence of extramedullary hematopoiesis of the spleen in the 1,500-ppm groups in the subchronic toxicity study (see Section 4.2.1). Groups of seven-week-old B6C3F_1 mice (50 of each sex) were exposed to nitromethane vapor at a concentration of 0, 188, 375, or 750 ppm by inhalation, 6 hours/day, 5 days/week, for 103 weeks (NTP 1997). Water was available *ad libitum*, and feed was available *ad libitum* except during exposure periods. All animals were observed twice daily. Clinical findings were recorded monthly through week 91, then every two weeks until the end of the study. Animals were weighed initially, weekly through week 12, monthly from week 15 through week 91, every two weeks thereafter, and at the end of the study. Complete necropsies and microscopic examinations were performed on all tissues and organs of all animals.

Nitromethane exposure did not significantly affect survival, and the survival rate of females in the 750-ppm group was marginally greater than that of the controls (see Appendix B, pp. B-40 and B-41, Table 14 and Figure 3 in NTP 1997). The mean body weights of exposed females were generally slightly greater than the mean body weight of the controls during the study but generally similar to that of the controls at the end of the study. The mean body weights of exposed and control males were similar throughout the study (see Appendix B, pp. B-42 to B-44, Tables 15 and 16 and Figure 4 in NTP 1997). Clinical findings included swelling around the eyes and exophthalmos (abnormal protrusion of the eyeball) in exposed males and females. These findings were coincident with harderian gland neoplasms.

The incidences of harderian gland adenoma and adenoma or carcinoma (combined) in exposed mice increased with increasing exposure concentration and were significantly greater in males and females in the 375- and 750-ppm groups than in the controls (Table 4-1). The incidences of these neoplasms in all exposed groups of male mice were greater than the historical incidences for chamber-control mice in two-year NTP inhalation studies; however, the incidences in control males also exceeded the range of historical control incidences. Incidences of adenoma and adenoma or carcinoma (combined) in all exposed groups of female mice also exceeded the historical control incidences, except for adenoma in the low-dose group. The incidences of carcinoma in males and females in the 375- and 750-ppm groups also were slightly greater than the incidences in the controls. Although the differences were not statistically significant, the incidences of carcinoma in the 375- and 750-ppm groups were outside the historical control incidence range of 0% to 4%. The incidences of harderian gland hyperplasia in males and females in the 375-ppm groups were similar to those in the controls. A significant positive dose-related trend was observed for all harderian gland neoplasms except for carcinoma in female mice.

Table 4-1. Harderian gland tumor incidence in B6C3F$_1$ mice following inhalation exposure to nitromethane for up to two years

Sex	Exposure conc. (ppm)	Harderian gland tumor incidence[a] (%)		
		Adenoma	Carcinoma	Combined
Male	0	9/50 (18)	1/50 (2)	10/50 (20)
	188	10/50 (20)	1/50 (2)	11/50 (22)
	375	19/50 (38)*	6/50 (12)	25/50 (50)***
	750	32/50 (64)***	5/50 (10)	37/50 (74)***
	Hist. control	47/950 (0–14)	2/950 (0–4)	49/950 (0–14)
	Trend	$P < 0.001$	$P = 0.036$	$P < 0.001$
Female	0	5/50 (10)	1/50 (2)	6/50 (12)
	188	7/50 (14)	2/50 (4)	9/50 (18)
	375	16/50 (32)**	4/50 (8)	20/50 (40)**
	750	19/50 (38)**	3/50 (6)	21/50 (42)**
	Hist. control	26/941 (0–16)	6/941 (0–4)	32/941 (0–16)
	Trend	$P < 0.001$	$P = 0.305$	$P < 0.001$

Source: NTP 1997.

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ (logistic regression test).

[a]Number of mice with tumors/number of mice examined.

Female mice in the 188- and 750-ppm groups had significantly greater incidences of hepatocellular adenoma and adenoma or carcinoma (combined) than the controls (Table 4-2). The incidences of these neoplasms exceeded the historical control ranges of 0% to 40% for hepatocellular adenomas and 3% to 54% for hepatocellular adenomas or carcinomas (combined) for two-year NTP inhalation studies. Incidences of multiple hepatocellular adenomas also were higher in female mice in the 188-ppm (13/49, 27%)

and 750-ppm (13/50, 26%) groups than in the controls (3/50, 6%). The incidences of eosinophilic focus increased with increasing exposure concentration, and the incidences in the 375- and 750-ppm groups were significantly greater than the control incidence.

Table 4-2. Liver tumor incidence in B6C3F$_1$ mice following inhalation exposure to nitromethane for up to two years

Sex	Exposure conc. (ppm)	Liver tumor incidence[a] (%)		
		Adenoma	Carcinoma	Combined
Male	0	17/50 (34)	16/50 (32)	29/50 (58)
	188	14/50 (28)	14/50 (28)	24/50 (48)
	375	13/50 (26)	10/50 (20)	22/50 (44)
	750	17/50 (34)	9/50 (18)	26/50 (52)
	Hist. control	not reported	not reported	not reported
	Trend	$P = 0.484$	$P = 0.032$[b]	$P = 0.319$[b]
Female	0	14/50 (28)	10/50 (20)	19/50 (38)
	188	25/49 (51)*	14/49 (29)	34/49 (69)***
	375	17/49 (35)	8/49 (16)	22/49 (45)
	750	35/50 (70)***	12/50 (24)	40/50 (80)***
	Hist. control	114/937 (0–40)	103/937 (0–30)	200/937 (3–54)
	Trend	$P < 0.001$	$P = 0.329$	$P = 0.001$

Source: NTP 1997.

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ (logistic regression test).

[a]Number of mice with tumors/number of mice examined.

[b]Negative trend.

The incidences of alveolar/bronchiolar carcinoma in male and female mice in the 750-ppm groups were significantly greater than those in the controls and exceeded the historical control range of 0% to 16% for these neoplasms in two-year NTP inhalation studies. The incidence of alveolar/bronchiolar carcinoma in females in the 375-ppm group was significantly greater than in controls but was within the historical control range of 0% to 12%. The incidence of alveolar/bronchiolar adenomas in females in the 750-ppm group also exceeded the historical control range of 0% to 14%. Females in the 375-ppm group had a significantly greater incidence of cellular infiltration of histiocytes than the controls. The incidences of alveolar epithelial hyperplasia in exposed males and females were similar to those of the controls. The lung tumor incidence data are summarized in Table 4-3.

Table 4-3. Lung tumor incidence in B6C3F$_1$ mice following inhalation exposure to nitromethane for up to two years

Sex	Exposure conc. (ppm)	Alveolar/bronchiolar tumor incidence[a] (%)		
		Adenoma	Carcinoma	Combined
Male	0	11/50 (22)	2/50 (4)	13/50 (26)
	188	10/50 (20)	3/50 (6)	13/50 (26)
	375	9/50 (18)	3/50 (6)	12/50 (24)
	750	12/50 (24)	11/50 (22)**	20/50 (40)
	Hist. control	141/947 (6–36)	75/947 (0–16)	205/947 (10–42)
	Trend	P = 0.422	P = 0.001	P = 0.059
Female	0	3/50 (6)	0/50 (0)	3/50 (6)
	188	3/50 (6)	3/50 (6)	6/50 (12)
	375	2/49 (4)	5/49 (10)*	6/49 (12)
	750	9/50 (18)	3/50 (6)	12/50 (24)*
	Hist. control	61/939 (0–14)	38/939 (0–12)	97/939 (0–16)
	Trend	P= 0.022	P = 0.149	P = 0.007

Source: NTP 1997.

*$P \leq 0.05$, **$P \leq 0.01$ (logistic regression test).

[a]Number of mice with tumors/number of mice examined.

4.3 Rats

4.3.1 Subchronic toxicity

4.3.1.1 Sprague-Dawley rats

Male Sprague-Dawley rats (50 per group) were exposed to nitromethane vapor at a concentration of 0, 98, or 745 ppm (Lewis *et al.* 1979). The nitromethane had a reported purity of 96.5%. Animals were housed in inhalation chambers 24 hours/day but were exposed to nitromethane 7 hours/day, 5 days/week, for up to 24 weeks. Ten animals in each group were sacrificed and necropsied after 2 days, 10 days, 1 month, 3 months, and 6 months. The only effects of nitromethane exposure occurred at the high concentration and included decreased body-weight gain after 8 weeks, lowered hematocrit and hemoglobin level from 10 days through 6 months, and increased thyroid weight after 6 months. No compound-related macroscopic or microscopic lesions were observed.

4.3.1.2 F344/N rats

The NTP (1997) conducted a 13-week study to evaluate the cumulative toxic effects of repeated exposure to nitromethane and to determine the exposure concentrations to be used in the two-year study (see Section 4.2.2.1). Nitromethane was selected for study based on its potential for human exposure and its relationship to the known animal carcinogens 2-nitropropane and tetranitromethane.

Groups of F344/N rats (10 of each sex) were exposed to nitromethane vapor at a concentration of 0, 94, 188, 375, 750, or 1,500 ppm by inhalation, 6 hours/day, 5 days/week, for 13 weeks. Additional groups of rats (10 of each sex) designated for clinical pathology evaluations received the same exposure concentrations as the core study rats. Animals were housed individually; water was available *ad libitum,* and feed was available *ad libitum*; except during exposure periods. Clinical observations were recorded weekly. The core study animals were weighed initially, weekly, and at the end of the study. Neurobehavioral tests included forelimb and hindlimb grip strength measurements, response to stimulus (tail-flick latency), and startle response. Clinical pathology and clinical chemistry analyses were performed on rats designated for clinical pathology evaluation on days 3 and 23 and on core study rats at the end of the study. Samples also were collected for sperm motility and vaginal cytology evaluations from all rats in all groups at the end of the study. Complete necropsies were performed on all core study animals and included histopathologic examination of all major organs and tissues. The heart, right kidney, liver, lungs, right testis, thymus, and thyroid gland were weighed.

All rats survived to the end of the study. The final mean body weight and weight gain of male rats in the 1,500-ppm group were significantly less than those of the controls. Clinical findings included hindlimb paralysis in rats in the 750- and 1,500-ppm groups. Inhalation exposure of rats to nitromethane resulted in an exposure concentration–dependent, microcytic, responsive anemia; anemia was most pronounced in males and females exposed at 375 ppm or more. The presence of schistocytes, Heinz bodies, and spherocytes and increased mean cell hemoglobin and methemoglobin concentrations were evidence that a hemolytic process was occurring, which could have accounted, in part, for the anemia. Thrombocytosis accompanied the anemia and would be consistent with a reactive bone marrow or could have been due to the erroneous inclusion of small erythrocyte fragments in the platelet count. On day 23, transient decreases in serum triiodothyronine, thyroxine, and free thyroxine were observed in male rats exposed at 375 ppm or more and female rats in the 750- and 1,500-ppm groups. There was little or no pituitary response to the thyroid hormone decreases, as evidenced by the lack of significantly increased concentrations of thyroid-stimulating hormone in exposed rats. No biologically significant differences in organ weights were observed. The forelimb and hindlimb grip strengths of males in the 1,500-ppm group and the hindlimb grip strengths of females in the 750- and 1,500-ppm groups were significantly less than those of the controls. Minimal to mild hyperplasia of the bone marrow was observed microscopically in male rats in the 750- and 1,500-ppm groups and in females exposed at 188 ppm or more. Nasal lesions included olfactory epithelial degeneration in males and females exposed at 375 ppm or more and in one female in the 188-ppm group and respiratory epithelial hyaline droplets and goblet cell hyperplasia in males and females in the 750- and 1,500-ppm groups; the severity of nasal lesions in males and females was minimal to mild. Males and females exposed at 375 ppm or more had minimal to mild degeneration of the sciatic nerve and the lumbar spinal cord.

4.3.2 Chronic studies

4.3.2.1 F344/N rats

Although several effects were considered treatment related in the subchronic toxicity study (see Section 4.3.1.2), most were not severe or common enough for determination of exposure concentrations for the two-year study. Exposure concentrations for the two-year study were based on the neurotoxicologic findings of hindlimb paralysis at 750 and 1,500 ppm and sciatic nerve and spinal cord lesions at 375 ppm or more. Groups of seven-week-old F344/N rats (50 of each sex) were exposed to nitromethane at a concentration of 0, 94, 188, or 375 ppm by inhalation, 6 hours/day, 5 days/week, for 103 weeks. The animals were housed individually; water was available *ad libitum*, and feed was available *ad libitum* except during exposure periods. All animals were observed twice daily. Clinical findings were recorded monthly through week 91, then every two weeks until the end of the study. Animals were weighed initially, weekly through week 12, monthly from week 15 through week 91, every two weeks thereafter, and at the end of the study. Complete necropsies and microscopic examinations were performed on all tissues and organs.

Survival rates did not differ significantly between exposed and control male or female rats (see Appendix B, pp. B-27 and B-28, Table 6 and Figure 1 in NTP 1997). From week 23 to the end of the study, the mean body weight of females in the 375-ppm group was slightly greater than that of the control group. The mean body weights of exposed and control males were generally similar throughout the study (see Appendix B, pp. B-29 to B-31, Figure 2 and Tables 7 and 8 in NTP 1997).

Clinical findings (masses on shoulder and torso) consistent with mammary gland neoplasms were observed in females in the 188- and 375-ppm groups during the course of the study. There were no indications of hindlimb paralysis or other treatment-related clinical findings. The incidences of mammary gland fibroadenoma and fibroadenoma, adenoma, or carcinoma (combined) increased with increasing exposure concentration, and the incidences in the 188- and 375-ppm groups were significantly greater than those of the controls (Table 4-4). Additionally, the incidences of carcinoma and adenoma or carcinoma (combined) in the 375-ppm group were significantly greater than those of the controls. The incidences of fibroadenoma in the 188- and 375-ppm groups and carcinoma in the 94- and 375-ppm groups exceeded the historical ranges for these neoplasms (16% to 42% and 0% to 8%, respectively) in chamber-control female rats in two-year NTP inhalation studies. No treatment-related mammary gland neoplasms were observed in male rats.

Table 4-4. Mammary tumor incidence in female F344/N rats following inhalation exposure to nitromethane for up to two years

Exposure conc. (ppm)	Mammary tumor incidence[a]				
	Fibroadenoma	Adenoma	Carcinoma	Adenoma or carcinoma	All combined
0	19/50 (38)	2/50 (4)	2/50 (4)	4/50 (8)	21/50 (42)
94	21/50 (42)	0/50 (0)	7/50 (14)	7/50 (14)	25/50 (50)
188	33/50 (66)**	0/50 (0)	1/50 (2)	1/50 (2)	34/50 (68)**
375	36/50 (72)***	2/50 (4)	11/50 (22)*	13/50 (26)*	41/50 (82)***
Hist. control	180/653 (16–42)	8/653 (0–4)	25/653 (0–8)	NR[b]	202/653 (16–46)
Trend	$P < 0.001$	NS	$P = 0.009$	$P = 0.01$	$P < 0.001$

Source: NTP 1997.

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, logistic regression test. NS = not significant.

[a]Number of rats with tumor/number of rats examined.

[b]NR = not reported in NTP 1997.

Histopathologic evaluation of sections of spinal cords and sciatic nerves from approximately 15 rats of each sex from the control and 375-ppm groups revealed no significant differences between exposed and control rats.

4.3.2.2 Long-Evans rats

A chronic inhalation study of nitromethane in Long-Evans rats, conducted as one of a series of inhalation studies of nitroparaffins, was reported (Griffin *et al.* 1996). Previous investigations of carcinogenic potential were performed on similar rats exposed to 1-nitropropane, 2-nitropropane, or nitroethane. Of these three nitroparaffins, only 2-nitropropane exhibited carcinogenic potential, in male rats only. Griffin *et al.* (1996) exposed groups of Long-Evans rats (40 of each sex) to nitromethane vapor at a concentration of 0, 100, or 200 ppm. The low concentration corresponds to the occupational exposure limit established by OSHA. The nitromethane had a reported purity of 96.26%, with 2.79% nitroethane and 0.62% nitropropane. Animals were housed in the inhalation chambers and exposed to nitromethane 7 hours/day, 5 days/week, for two years. Animals were observed daily for signs of toxic effects. Moribund animals were sacrificed, and dead animals were necropsied and thoroughly examined for macroscopic and microscopic lesions. At the end of the study, all surviving animals were sacrificed and received thorough clinical and pathological examinations.

The proportion of male rats surviving to the end of the study was approximately the same in the control and the two exposed groups. Among female rats, survival was lower in the 200-ppm group; however, no statistical test was performed. Survival rates of controls were 62.5% for males and 75% for females, and survival rates in the 200-ppm groups were 62.5% for males and 60% for females. Body weight was not significantly affected in males, but in females it was slightly depressed after 9 months and significantly reduced during the last year of the study. There were no statistically significant differences in

hematology, serum chemistry, and organ weights between the exposed and the control groups. Further, the data suggest no exposure-related differences in non-neoplastic or neoplastic pathology.

4.4 Summary

IARC (2000) concluded that there was sufficient evidence for the carcinogenicity of nitromethane in experimental animals, based on the NTP (1997) study.

4.4.1 Rabbits

Evidence of only mild to moderate toxicity was observed in rabbits exposed to nitromethane at a concentration of 98 or 745 ppm by inhalation for 6 months.

4.4.2 Mice

Increased incidences of harderian gland adenoma and adenoma or carcinoma (combined) occurred in male and female mice exposed to nitromethane by inhalation at 375 or 750 ppm. Increased incidences of lung carcinoma occurred in males exposed at 750 ppm and females exposed at 375 ppm. Female mice exposed at 750 ppm had a significantly increased incidence of lung adenoma or carcinoma (combined). In addition, the incidences of hepatocellular adenoma and adenoma or carcinoma (combined) were significantly increased in female mice at 188 or 750 ppm. The NTP concluded that there was clear evidence for carcinogenicity of nitromethane in both male and female $B6C3F_1$ mice.

4.4.3 Rats

There was no evidence that nitromethane was carcinogenic in male or female Long-Evans rats exposed to nitromethane by inhalation at 100 or 200 ppm for two years or in male F344/N rats exposed at 94, 188, or 375 ppm for two years. In female F344/N rats exposed to nitromethane at 188 or 375 ppm for two years, the incidences of mammary gland fibroadenoma and fibroadenoma, adenoma, or carcinoma (combined) were significantly increased, and at 375 ppm, the incidence of mammary gland carcinoma was significantly increased. The NTP concluded that there was clear evidence that nitromethane was carcinogenic to female F344/N rats.

5 Genotoxicity

The genotoxicity of nitromethane was reviewed by the IARC Working Group (2000), which concluded that nitromethane was not genotoxic in any short-term tests except the Syrian hamster embryo (SHE) cell transformation assay (discussed in Section 6). No new studies on the genotoxicity of nitromethane have been published since the IARC (2000) monograph; the available literature is summarized below.

5.1 Prokaryotic systems: Reverse mutations in *Salmonella typhimurium*

In a study examining the mutagenicity of nitroaromatic and nitroheterocyclic compounds, Chiu *et al*. (1978) found that exposure to 0.1, 1, or 10 μmol of nitromethane did not induce reverse mutation in *Salmonella typhimurium* strains TA98 or TA100 in the absence of induced rat liver S9 metabolic activation. Similar results were obtained in studies by Löfroth *et al*. (1986) (20,000 to 50,000 μg [328 to 819 μmol), Mortelmans *et al*. (1986) (100 to 10,000 μg), Dayal *et al*. (1989) (50 to 200 μmol), Dellarco and Prival (1989) (0.3 to 100 μmol), and the NTP (1997) (100 to 10,000 μg[1.6-164 μmol). Nitromethane was not mutagenic in strains TA98 and TA100 in the presence of induced rat liver S9 (Mortelmans *et al.* 1986, Dellarco and Prival 1989, NTP 1997), strain TA1535 in the absence (Löfroth *et al.* 1986, Mortelmans *et al.* 1986, NTP 1997) or presence of induced rat liver S9 (Mortelmans *et al.* 1986, NTP 1997), or strain TA1537 in the presence or absence of induced rat liver S9 (Mortelmans *et al.* 1986, NTP 1997).

5.2 Non-mammalian eukaryotic systems: Sex-linked recessive lethal mutations in *Drosophila melanogaster*

Adult *Drosophila melanogaster* were given food spiked with nitromethane (125 mM), and sex-linked lethal mutations were scored in each of three successive broods (Gocke *et al.* 1981). Nitromethane did not induce sex-linked lethal mutations.

5.3 Mammalian systems

5.3.1 In vitro *assays*

The genotoxicity of nitromethane was assessed in tests that measure chromosomal and DNA damage.

5.3.1.1 Chromosomal aberrations

Chromosomal aberrations were not induced in Chinese hamster ovary (CHO) cells exposed to nitromethane at concentrations of 1,077 to 4,980 μg/mL in the presence or absence of induced rat liver S9 (NTP 1997).

5.3.1.2 Micronucleus test

Micronuclei were not induced in SHE cells exposed to nitromethane at concentrations of up to 5,000 μg/mL (Gibson *et al*. 1997).

5.3.1.3 Sister-chromatid exchange

The number of sister-chromatid exchanges (SCEs) per cell was not increased in CHO cells exposed to nitromethane at concentrations of 497 to 4,965 μg/mL in the presence or absence of induced rat liver S9 (NTP 1997).

5.3.2 In vivo *assays: Micronucleus test*

Micronucleus formation was not induced in bone-marrow erythrocytes of NMRI mice given nitromethane by intraperitoneal (i.p.) injection at a cumulative dose of 410, 1,830, or 3,660 mg/kg b.w. (Gocke *et al.* 1981) or in peripheral blood erythrocytes of $B6C3F_1$ mice exposed to nitromethane by inhalation at a concentration of 94 to 1,500 ppm for 13 weeks (NTP 1997).

5.4 Summary

Table 5-1 summarizes the data on nitromethane genotoxicity. Nitromethane was not mutagenic *in vitro* or *in vivo*. It did not induce reverse mutations in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without induced rat liver S9; chromosomal aberrations or SCEs in CHO cells; or micronuclei in SHE cells or mouse bone marrow cells or peripheral erythrocytes.

Table 5-1. Summary of genotoxicity studies of nitromethane

Test system	Endpoint	Results[a]		Reference
		without metabolic activation	with metabolic activation	
S. typhimurium TA98, TA100	reverse mutation	–	–	Mortelmans *et al.* 1986, NTP 1997
S. typhimurium TA98, TA100	reverse mutation	–	NR	Chiu *et al.* 1978, Löfroth *et al.* 1986, Dayal *et al.* 1989
S. typhimurium TA98, TA100	reverse mutation	NR	–	Dellarco and Prival 1989
S. typhimurium TA1535, TA1537	reverse mutation	–	–	Mortelmans *et al.* 1986, NTP 1997
S. typhimurium TA1535	reverse mutation	–	NR	Löfroth *et al.* 1986
D. melanogaster	sex-linked lethal mutations	–	NA	Gocke *et al.* 1981
CHO cells	chromosomal aberrations, SCEs	–	–	NTP 1997
SHE cells	micronuclei	–	NR	Gibson *et al.* 1997
Mouse bone marrow cells (i.p.)	micronuclei	–	NA	Gocke *et al.* 1981
Mouse peripheral erythrocytes (inhalation)	micronuclei	–	NA	NTP 1997

[a]NR = not reported, NA = not applicable.

6 Other Relevant Data

For the IARC (2000) review, no data were available on the toxicity, absorption, distribution, metabolism, or excretion of nitromethane in humans.

6.1 Toxicity

6.1.1 IARC review

IARC (2000) reviewed eight studies in which rats, mice, or rabbits were exposed to nitromethane by i.p. or subcutaneous injection or by inhalation. Toxic effects included histidinemia, neurologic effects, degeneration of the olfactory epithelia, and hyperplasia of the bone marrow. Reproductive toxicity was manifested in a dose-related decrease in sperm motility in rats and mice and a dose-related increase in the length of the estrus cycle in mice.

Injection of Wistar rats with nitromethane at lethal doses did not result in detectable methemoglobin in the blood; the heart, lungs, kidney, and spleen, but not the liver, contained low concentrations of nitrite. The only tissue containing detectable nitromethane after inhalation of a lethal concentration (33 g/m^3) for 6 hours was the liver.

6.1.2 Hepatotoxicity

The hepatotoxicity of nitroalkanes, including nitromethane, was studied in male and female BALB/c mice (Dayal *et al.* 1989). Nitromethane and nitroethane were administered by i.p. injection at 9 mmol/kg (549 and 675 mg/kg body weight [b.w.], respectively), and 2-nitropropane was administered at 4.5, 6.7, and 9 mmol/kg (400, 596, and 801 mg/kg b.w.). The animals were sacrificed after 24, 48, 72, or 96 hours, and blood samples were collected by cardiac puncture. The plasma activities of the hepatic enzymes sorbitol dehydrogenase, alanine aminotransferase, and aspartate aminotransferase were unchanged in male and female mice treated with either nitromethane or nitroethane. The activities of all three enzymes were significantly elevated in male mice at 48, 72, and 96 hours after administration of the high dose of 2-nitropropane, but not at 24 hours and not after administration of smaller doses. Hepatotoxicity was observed in female mice only at the middle dose of 2-nitropropane (6.7 mmol/kg, or 596 mg/kg b.w.).

The biochemical results were supported by histopathological evaluation of the livers of exposed mice. No significant abnormalities were observed in the livers of mice exposed to nitromethane or nitroethane. However, the livers of mice given 2-nitropropane at 9 mmol/kg (801 mg/kg b.w.) showed disruption of the normal parenchyma, together with extensive hemorrhagic necrosis. Apoptosis was a common feature of livers affected to varying degrees. The authors reported that 2-nitropropane was hepatotoxic at a dose of 9 mmol/kg, but that nitromethane and nitroethane were not hepatotoxic at this dose.

6.1.3 Miscellaneous toxicity studies

6.1.3.1 16-Day studies in rats and mice

The NTP (1997) carried out 16-day inhalation toxicity studies in male and female F344/N rats and $B6C3F_1$ mice. Groups of animals (5 of each sex) were exposed to nitromethane

at a concentration of 0, 94, 188, 375, 750, or 1,500 ppm, 6 hours/day, 5 days/week, for a total of 12 exposures. All rats and mice survived to the end of the study.

In rats of both sexes, clinical findings of toxicity at 1,500 ppm included increased preening, rapid breathing, hyperactivity at the beginning of the study, and hypoactivity and loss of coordination in the hindlimbs near the end of the study. Degeneration of the sciatic nerve, ranging in severity from minimal to moderate, was present in all rats exposed to nitromethane at 375 ppm or more. The nerve lesion increased in severity with increasing exposure concentration and was characterized by prominent, diffuse vacuolization and dilatation of the axonal sheaths and increased cellularity. In addition, significantly less myelin was present around the sciatic axons in rats exposed to nitromethane at 750 or 1,500 ppm than in control rats. Degeneration of the olfactory epithelium of the nasal turbinates (minimal to mild severity) was observed in all males exposed at 375 ppm or more, in all females in the 750- and 1500-ppm groups, and in four females in the 375-ppm group. No exposure-related lesions were found in the lungs of exposed rats of either sex. In all exposed groups of male rats, relative liver weights were significantly higher than those of controls, and absolute and relative liver weights were significantly increased in female rats at 375, 750, and 1,500 ppm. Also significantly increased were the relative kidney weights of male rats at 750 and 1,500 ppm and female rats at 1,500 ppm.

In mice of both sexes, clinical findings included hypoactivity and tachypnea at 1,500 ppm, near the end of the study. The absolute and relative liver weights of male mice at 750 and 1,500 ppm and female mice at all exposure levels and the relative liver weight of male mice at 375 ppm were significantly greater than in controls. Degeneration of the olfactory epithelium was observed microscopically in all males (minimal severity) and females (minimal to mild severity) exposed at 375 ppm or more.

A series of acute inhalation exposures of groups of 2 to 4 rats to nitromethane (2 hours) or simulated hair sprays containing nitromethane (15 minutes) was conducted (EPA 1989, 1992). When observed for up to 10 days after exposure, the rats showed signs of eye irritation, respiratory impairment, and central nervous system depression. The approximate lethal dose was 6,000 ppm, indicating low toxicity.

6.1.3.2 Bacterial luminescence toxicity test

Median effective concentrations (EC_{50}s) for nitroparaffins were determined with the Microtox test, a commercial system for toxicity testing with a luminescent bacterium, a strain of *Vibrio fischeri* (previously known as *Photobacterium phosphoreum*) (Thumm *et al.* 1992, AZUR Environmental 2001). Inhibition of cellular activity by a toxic substance results in decreased respiration and correspondingly decreased bioluminescence, which is a byproduct of cellular respiration. The test substances included nitromethane, nitroethane, 1-nitropropane, 2-nitropropane, 1-nitrobutane, tert-nitrobutane (2-nitro-2-methylpropane), 1-nitropentane, 1-nitrohexane, 2-nitrobutane, and iso-nitrobutane (1-nitro-2-methylpropane). From these data, quantitative structure-activity relationships were deduced suggesting that toxicity depends on the number of methyl and methylene groups in a molecule. Nitromethane was the least toxic material tested.

6.2 Mammalian absorption, metabolism, and excretion

6.2.1 Human studies

Gabrielli and Hammett-Stabler (1998) reported absorption of nitromethane by a patient as a result of accidental exposure to nitromethane fuel following a racing crash. The authors suggested that absorption was both dermal and by inhalation, but did not provide any quantitative data. Evidence for absorption was the apparent interference of nitromethane with a standard assay for serum creatinine; no evidence for a toxic effect was reported.

6.2.2 Animal studies

6.2.2.1 Dermal absorption in monkeys

A single dose of radiolabeled nitromethane (300 μL of an ether/ethanol solution containing 5.5% [^{14}C]nitromethane, for a total dose of 18.8 μg) was applied to the skin of two female adult rhesus monkeys (*Macaca fascicularis*) for 12 hours (EPA 1990). After 72 hours, blood, urine, feces, skin, and subcutaneous fat were collected and tested for radioactivity, and the skin samples were examined histologically.

There were no changes in general appearance, behavior, weight, or appetite or signs of toxicity or irritation throughout the study. After 72 hours, an average of 15.39 μg of nitromethane was excreted, of which 11.44 μg (0.062% of the total dose) was excreted in the urine. The plasma contained an average of 1.53 μg of nitromethane (0.008% of the total dose), and the skin contained an average of 3.49 μg (0.018% of the total dose). No nitromethane was found in subcutaneous fat, indicating that nitromethane and its metabolites are absorbed only in negligible amounts through the skin, with absorption to subcutaneous fat also negligible (EPA 1990).

6.2.2.2 Metabolism

No published reports of *in vivo* metabolism of nitromethane were available for this review.

Metabolism of nitromethane by liver microsomes from Fischer 344 rats resulted in formation of only trace amounts of formaldehyde (IARC 2000).

Wade *et al.* (1977) reported that nitromethane interacted with sodium dithionite-reduced rabbit liver microsomes and competed with carbon monoxide for binding to cytochrome P-450. These data suggested that the interaction of nitromethane with reduced hepatic microsomes was different from that of aromatic nitro compounds.

Formaldehyde released by metabolic reactions may be a factor in the irritancy of inhaled compounds, and a role for metabolically generated formaldehyde in the tumorigenicity of some compounds has been suggested. Dahl and Hadley (1983) tested 32 potential substrates for cytochrome P-450-dependent monooxygenases, including nitromethane, with rat nasal mucosal and liver microsomes. The formation of formaldehyde, which is a known nasal carcinogen in animal models, was detected after incubation of nitromethane with rat liver, but not nasal mucosal, microsomes.

6.3 Transformation potential in Syrian hamster embryo cells

The SHE cell transformation assay has been proposed as a model for testing chemical agents for their neoplastic transformation potential. SHE cells can metabolically activate many chemicals and follow a progressive, multistage pattern of neoplastic transformation that has been compared to *in vivo* carcinogenesis. Kerckaert *et al.* (1996) exposed SHE cell cultures to nitromethane (98% purity) at six concentrations (2,000, 2,500, 3,000, 3,500, 4,000, and 5,000 μg/mL) for 24 hours, followed by 6 to 7 days of growth. The two highest concentrations (4,000 and 5,000 μg/mL) produced significant increases in the morphological transformation frequency ($P = 0.0291$ and $P = 0.0027$, respectively; Fisher's exact test), and the trend test also was statistically significant ($P = 0.001$). Based on these results, the authors predicted that nitromethane is likely to be a carcinogen in rodents.

6.4 Carcinogenicity and mutagenicity of related compounds

Nitromethane belongs to the class of nitroparaffins, of which the four commercially important members are nitromethane, nitroethane, 1-nitropropane, and 2-nitropropane (Kirk-Othmer 2001). No evidence exists in the published literature for the carcinogenicity of either nitroethane or 1-nitropropane.

IARC (1999) lists 2-nitropropane as possibly carcinogenic to humans (Group 2B), based on occurrence of benign and malignant liver tumors in rats. 2-Nitropropane also is listed in the 9th Report on Carcinogens (NTP 2000) as *reasonably anticipated to be a human carcinogen,* based on sufficient evidence of carcinogenicity in experimental animals; when administered through inhalation, 2-nitropropane induced hepatocellular carcinoma in male rats and hepatocellular nodules in rats of both sexes. The IARC Working Group (1999) also reported that 2-nitropropane was mutagenic to bacteria (with and without exogenous metabolism) and was genotoxic to a wide range of organisms *in vitro* and *in vivo*.

Another nitroalkane, tetranitromethane, has been evaluated for potential carcinogenicity by both an IARC Working Group (1996) and the NTP (2000). The IARC Working Group (1996) concluded that tetranitromethane is possibly carcinogenic to humans (Group 2B) based on a marked increase in the incidence of alveolar/bronchiolar adenoma and carcinoma in mice and rats and of squamous-cell carcinoma of the lung in rats. The NTP (1990) concluded that there was clear evidence of carcinogenicity of tetranitromethane in male and female F344/N rats and male and female B6C3F_1 mice, based on increased incidences of alveolar/bronchiolar neoplasms in both species and squamous-cell carcinoma of the lung in rats. Tetranitromethane is listed in the Report on Carcinogens as *reasonably anticipated to be a human carcinogen*. The IARC Working Group (1996) also reported that tetranitromethane is genotoxic in bacteria and in cultured mammalian cells and that a GC:AT transition in the second base of codon 12 of the K-*ras* oncogene was identified in tumors from tetranitromethane-exposed rats and mice.

Dayal *et al.* (1989) investigated the mutagenicity of nitromethane (see Section 5), 2-nitropropane, and nitroethane and their nitronates (the nitronate, or anionic form, of nitromethane is ($H_2C{=}NO_2^-$) in *S. typhimurium* strains TA98, TA100, and TA102.

Nitromethane, nitroethane, and their nitronates were not mutagenic, but 2-nitropropane and its anionic form, propane-2-nitronate, were mutagenic, with the anionic form being the more potent mutagen.

There is no consensus on why tumor sites and mutagenicity test results have differed among the individual nitroparaffin compounds. The 2-nitroalkanes, including 2-nitropropane, produce electrochemically active species; therefore, it is possible that the metabolites, and not the compounds themselves, are responsible for the positive results in genotoxicity studies for some of the compounds. Other studies have shown that the enzymatic denitrification of 2-nitropropane to acetone results in the formation of free radicals, superoxide, and hydrogen peroxide. Chemicals with the aliphatic nitro group ($-C-NO_2$) are on an NTP list of DNA-reactive subgroups that should be considered for possible carcinogenic activity. It is not known whether these reactive radicals may be involved, either directly or indirectly, in the mechanism of carcinogenicity for nitromethane and other nitroparaffins (NTP 1997).

6.5 Summary

Nitromethane has been shown to produce toxic effects in animals, including neurologic and reproductive effects. Relatively few reports have been published on the absorption, distribution, metabolism, and excretion of nitromethane. The available data suggest that absorption may occur by inhalation, but that the amount absorbed after dermal exposure is negligible. Although nitromethane may be metabolized to formaldehyde by rat liver microsomes *in vitro*, no published reports have characterized the metabolism of nitromethane *in vivo*. Nitromethane is structurally related to other nitro compounds (i.e., 2-nitropropane and tetranitromethane) that have been evaluated by IARC and considered to be possibly carcinogenic to humans. The mechanism of carcinogenicity for nitromethane and these other nitro compounds is not known; however, it has been hypothesized that reactive radicals may play a key role in their carcinogenicity.

7 References

1. ACGIH. 1999. Guide to Occupational Exposure Values. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

2. Angus. 2000. Nitromethane (NM™). Dow Chemical Company, Buffalo Grove, IL.

3. Angus. 2001. Public comment to NTP. Dow Chemical Company, Buffalo Grove, IL.

4. Archer, W.L. 1996. Nitroparaffins. In Industrial Solvents Handbook. Marcel Dekker, New York. pp. 267-282.

5. Ash, M. and I. Ash. 1996. The Index of Solvents: An International Guide to 1700 Products by Trade Name, Chemical, Application, and Manufacturer. Gower, Brookfield, VT.

6. ATSDR. 1995. Toxicological Profile for RDX. Agency for Toxic Substances and Disease Registry, US Public Health Service. Available at http://www.atsdr.cdc.gov/toxprofiles/tp78.html.

7. AZUR Environmental. 2001. Microtox Rapid Toxicity Testing System. Available at: (http://azurenv.com/mtox.htm).

8. Budavari, S. 1996. The Merck Index. Budavari, S., M.J. O'Neil, A. Smith, P.E. Heckelman and J.F. Kinneary eds. Merck & Co., Inc., Whitehouse Station, NJ.

9. ChemFinder. 2001. Cambridge Soft Corporation. Nitromethane. Available at: (http://www.chemfinder.camsoft.com and search 75-52-5).

10. Chiu, C.W., L.H. Lee, C.Y. Wang, and G.T. Bryan. 1978. Mutagenicity of some commercially available nitro compounds for *Salmonella typhimurium*. *Mutat Res* 58:11-22.

11. Dahl, A.R. and W.M. Hadley. 1983. Formaldehyde production promoted by rat nasal cytochrome P-450-dependent monooxygenases with nasal decongestants, essences, solvents, air pollutants, nicotine, and cocaine as substrates. *Toxicol Appl Pharmacol* 67:200-205.

12. Dayal, R., A. Gescher, E.S. Harpur, I. Pratt, and J.K. Chipman. 1989. Comparison of the hepatotoxicity in mice and the mutagenicity of three nitroalkanes. *Fundam Appl Toxicol* 13:341-348.

13. Dellarco, V.L. and M.J. Prival. 1989. Mutagenicity of nitro compounds in *Salmonella typhimurium* in the presence of flavin mononucleotide in a preincubation assay. *Environ Mol Mutagen* 13:116-127.

14. EPA. 1989. Preliminary Toxicity Studies on Nitromethane and Simulated Hair Sprays Containing Nitromethane or Other Inhibitors with Cover Sheet Dated 06/12/89.

15. EPA. 1990. Skin Absorption and Metabolism/Toxicokinetic Study of 14C-Nitromethane in Female Rhesus Monkeys with Cover Letter Dated 4/23/90.

16. EPA. 1992. Initial Submission: Preliminary Toxicity Studies on Nitromethane and Simulated Hair Sprays Containing Nitromethane or Other Inhibitors with Cover Letter Dated 10/15/92.

17. Gabrielli, A. and C. Hammett-Stabler. 1998. False elevation of serum creatinine following skin absorption of nitromethane complicates the clinical diagnosis of rhabdomyolysis. *Chest* 113:1419-1422.

18. Gibson, D.P., R. Brauninger, H.S. Shaffi, G.A. Kerckaert, R.A. Leboeuf, R.J. Isfort, and M. Aardema. 1997. Induction of micronuclei in Syrian hamster embryo cells: Comparison of results in the SHE cell transformation assay for national toxicology program test chemicals. *Mutat Res* 392:61-70.

19. Gocke, E., M.T. King, K. Eckhardt, and D. Wild. 1981. Mutagenicity of cosmetics ingredients licensed by the European Communities. *Mutat Res* 90:91-109.

20. Griffin, T.B., F. Coulston, and A.A. Stein. 1996. Chronic inhalation exposure of rats to nitromethane. *Ecotoxicol Environ Saf* 34:109-117.

21. Henschler, D., D. Reichert, and M. Metzler. 1980. Identification of potential carcinogens in technical grade 1,1,1-trichloroethane. *Int Arch Occup Environ Health* 47:263-268.

22. HSDB. 2000. Hazardous Substance Data Bank, National Library of Medicine. Revised September 12, 2000. Nitromethane. Last reviewed February 28, 1992. Available at: (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB and search 75-52-5 and select “Nitromethane” in search results).

23. IARC. 1996. Printing Processes and Printing Inks, Carbon Black and Some Nitrocompounds. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. World Health Organization, International Agency for Research on Cancer, Lyon, France.

24. IARC. 1999. Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide, Vol. 71. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. World Health Organization, International Agency for Research on Cancer, Lyon, France.

25. IARC. 2000. Nitromethane. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. World Health Organization, International Agency for Research on Cancer, Lyon, France. pp. 487-501.

26. Kerckaert, G.A., R. Brauninger, R.A. Leboeuf, and R.J. Isfort. 1996. Use of the Syrian hamster embryo cell transformation assay for carcinogenicity prediction of chemicals currently being tested by the National Toxicology Program in rodent bioassays. *Environ Health Perspect* 104 Suppl 5:1075-1084.

27. Kirk-Othmer. 2001. Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, Inc. Nitroparaffins. Available at: (http://jws-edck.wiley.com:8095/articles/nitrboll.a02/sect1_3-fs.html, registered users only).

28. Lewis, T.R., C.E. Ulrich, and W.M. Busey. 1979. Subchronic inhalation toxicity of nitromethane and 2-nitropropane. *J Environ Pathol Toxicol* 2:233-249.

29. Löfroth, G., L. Nilsson, and J.R. Andersen. 1986. Structure-activity relationship of nitroalkane-induced mutagenicity in the Ames Salmonella assay. *Prog Clin Biol Res* 209B:149-155.

30. Markofsky, S.B. 1991. Nitro Compounds, Aliphatic. In Ullmann's Encyclopedia of Chemical Technology, 5th Rev Ed, Vol A17. Elvers, B., S. Hawkins and G. Schulz, eds. VCH Publishers, New York. pp. 401-409.

31. Mortelmans, K., S. Haworth, T. Lawlor, W. Speck, B. Tainer, and E. Zeiger. 1986. *Salmonella* mutagenicity tests. II. Results from the testing of 270 chemicals. *Environ Mutagen* 8:1-119.

32. NIOSH. 1997. NIOSH Pocket Guide to Chemical Hazards. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Washington, D.C.

33. NIOSH. 1998. National Institute for Occupational Safety and Health. International Chemical Safety CardsNitromethane. Available at: (http://www.cdc.gov/niosh/ipcsneng/neng0522.html).

34. NTP. 1997. Toxicology and Carcinogenesis Studies of Nitromethane in F344/N Rats and B6C3F_1 Mice (Inhalation Studies). National Toxicology Program Technical Report Series, TR 461.

35. NTP. 2000. National Toxicology Program. 9th Report on Carcinogens. Available at: (http://ehis.niehs.nih.gov/roc/).

36. NTP. 2001. National Toxicology Program. NTP Chemical Repository. Revised August 13, 2001. Nitromethane. Available at: (http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/ALL_SRCH/iH_ALL_SRCH_Frames.html and search 75-52-5 and select "H&S:Nitromethane 75-52-2" in search results.).

37. OSHA. 2001. Occupational Safety and Health Administration, U.S. Department of Labor. Regulations (Standards - 29 CFR), TABLE Z-1 Limits for Air

Contaminants. - 1910.1000TABLEZ-1. Available at: (http://www.osha-slc.gov/OshStd_data/1910_1000_TABLE_Z-1.html).

38. Pellizzari, E.D., T.D. Hartwell, B.S.d. Harris, R.D. Waddell, D.A. Whitaker, and M.D. Erickson. 1982. Purgeable organic compounds in mother's milk. *Bull Environ Contam Toxicol* 28:322-328.

39. SRC. 2001. Syracuse Research Corp. Available at: (http://esc.syrres.com/interkow/webprop.exe?CAS=75-52-5).

40. SRI. 1992. Directory of Chemical Producers-United States of America. SRI, International, Menlo Park, CA.

41. Thumm, W., R. Brueggeman, D. Freitag, and A. Kettrup. 1992. Toxicity tests with luminescent photobacteria and quantitative structure activity relationships for nitroparaffins. *Chemosphere* 24:1835-1843.

42. Wade, A.E., J.S. Evans, L.A. Sternson, and R.E. Gammans. 1977. Interaction of nitromethane with reduced hepatic microsomal cytochrome P-450. *Biochem Pharmacol* 26:963-967.

Appendix A: IARC (2000). Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Nitromethane. In Some Industrial Chemicals. V 77. PP A-1 – A-15.

NITROMETHANE

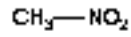
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 75-52-5
Chem. Abstr. Name: Nitromethane
IUPAC Systematic Name: Nitromethane
Synonyms: Nitrocarbol

1.1.2 *Structural and molecular formulae and relative molecular mass*



CH_3NO_2 Relative molecular mass: 61.04

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless, oily liquid with a moderately strong, somewhat disagreeable odour (Budavari, 1998)
(*b*) *Boiling-point*: 101.1 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: –28.5 °C (Lide & Milne, 1996)
(*d*) *Density*: 1.1371 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (grating [25]), Raman [296], ultraviolet [29], nuclear magnetic resonance (proton [9146], C-13[4002]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Slightly soluble in water (95 mL/L at 20 °C; Budavari, 1998) acetone, alkali (bases), carbon tetrachloride, diethyl ether and ethanol (Lide & Milne, 1996; Verschueren, 1996; American Conference of Governmental Industrial Hygienists, 1999)

(*g*) *Volatility*: Vapour pressure, 3.7 kPa at 20 °C; relative vapour density (air = 1), 2.11; flash-point, 35 °C (closed-cup) (Verschueren, 1996; American Conference of Governmental Industrial Hygienists, 1999)
(*h*) *Stability*: Lower explosive limit in air, 7.3% by volume; sensitive to adiabatic compression (Angus Chemical Co., 1998); forms an explosive sodium salt which bursts into flame on contact with water (Budavari, 1998)
(*i*) *Octanol/water partition coefficient (P)*: log P, –0.35 (Hansch *et al*., 1995)
(*j*) *Conversion factor*[1]: $mg/m^3 = 2.50 \times ppm$

1.1.4 *Technical products and impurities*

Nitromethane is commercially available with the following specifications (by weight): purity, 98.0% min.; total nitroparaffins, 99.0% min.; acidity (as acetic acid), 0.1% max.; and water, 0.1% max. Nitromethane can be made less sensitive to detonation by shock by the addition of compounds such as alcohols, hydrocarbons, esters and ketones. These desensitizers, with the minimum content by weight that must be present in the mixtures, are: cyclohexanone (25%), 1,4-dioxane (35%), 1,2-butylene oxide (40%), methanol (45%), 2-nitropropane (47%), 1-nitropropane (48%) or methyl chloroform (50%) (Angus Chemical Co., 1998).

1.1.5 *Analysis*

Nitromethane can be determined in workplace air by adsorbing the air sample on Chromosorb, desorbing with ethyl acetate and analysing by gas chromatography with nitrogen-specific detection (method 2527) (Eller, 1994).

1.2 Production

Nitromethane was first prepared in 1872 by Kolbe, and is produced commercially by high-temperature vapour-phase nitration of propane. The process, which uses nitric acid as the nitrating agent, is based on a free-radical reaction in which the active species is the NO_2 radical (Markofsky, 1991; Angus Chemical Co., 1998).

Information available in 1999 indicated that nitromethane was manufactured by four companies in China, two companies in India and one company each in Germany, Spain and the United States (Chemical Information Services, 1999).

[1] Calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming a temperature of 25 °C and a pressure of 101 kPa

1.3 Use

One of the most important direct uses for nitromethane is in the stabilization of halogenated hydrocarbons. For example, small amounts of nitromethane are widely used in industry to form stable non-corrosive mixtures with 1,1,1-trichloroethane for vapour degreasing, dry cleaning and for cleaning semiconductors and lenses. It is also used to stabilize the halogenated propellants for aerosols and to inhibit corrosion on the interiors of tin-plated steel cans containing water-based aerosol formulations (Markofsky, 1991; Angus Chemical Co., 1998).

Nitromethane is frequently used as a polar solvent for cellulose esters (Lundberg, 1989) and for cyanoacrylate adhesives and acrylic coatings. It is also used for cleaning electronic circuit boards. Nitromethane alone, and in mixtures with methanol and other nitroparaffins, is used as a fuel by professional drag racers and hobbyists. The explosives industry uses nitromethane in a binary explosive formulation and in shaped charges (Markofsky, 1991; Angus Chemical Co., 1998).

Nitromethane is used as a metal stabilizer for various chlorinated and fluorinated hydrocarbon solvents. The primary role of the nitromethane is to complex metal salts from the solvent–metal corrosion reaction (Archer, 1996).

1.4 Occurrence

1.4.1 *Natural occurrence*

Nitromethane is not known to occur as a natural product.

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999) as many as 135 000 workers in the United States were potentially exposed to nitromethane (see General Remarks).

1.4.3 *Environmental occurrence*

The production of nitromethane and its use as a solvent, fuel additive, stabilizer for halogenated alkanes, and intermediate may result in the release of nitromethane into the environment, principally into the atmosphere. Human exposure to nitromethane may additionally occur via dermal contact and accidental ingestion of methanol–nitromethane fuel mixtures (Kaiffer *et al*., 1972; Sandyk & Gillman, 1984; Dayal *et al*., 1989; De Leacy *et al*., 1989; Lundberg, 1989; National Toxicology Program, 1997; Mullins & Hammett-Stabler, 1998).

The concentration of nitromethane in automobile exhaust using nine hydrocarbon test fuels under simulated driving conditions ranged from < 0.8 to 5.0 ppm (Seizinger & Dimitriades, 1972).

Nitromethane has been found in one of twelve samples of mother's milk (Pellizzari *et al*., 1982).

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for nitromethane are presented in Table 1.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, seven weeks of age, were exposed by inhalation to 0, 188, 375 or 750 ppm [0, 470, 938 or 1875 mg/m^3] nitromethane (purity, 98%, with 0.25% nitroethane and 0.03% 2-nitropropane as contaminants) for 6 h plus T_{90} (12 min) per day on five days per week for 103 weeks [T_{90} is the time to achieve 90% of the target concentration]. The high dose was estimated to be the maximal tolerated dose. The average age of mice at necropsy was 111–112 weeks. The mean survival was 681, 700, 674 and 687 days among males and 662, 663, 673 and 695 days among females in the respective dose groups. As summarized in Table 2, statistically significant increases in the incidence of Harderian gland tumours and of alveolar/bronchiolar tumours in males and females and of hepatocellular adenomas in females were observed (National Toxicology Program, 1997).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, seven weeks of age, were exposed by inhalation to concentrations of 0, 94, 188 or 375 ppm [0, 135, 470 or 938 mg/m^3] nitromethane (purity, 98%, with 0.25% nitroethane and 0.03% 2-nitropropane as contaminants) for 6 h plus T_{90} (12 min) per day on five days per week for 103 weeks. The high dose was estimated to be the maximal tolerated dose. The average age of rats at necropsy was 111 weeks. The mean survival was 642, 631, 646 and 640 days among males and 683, 653, 679 and 670 days among females in the respective dose groups. The incidences of mammary gland fibroadenomas were increased in

Table 1. Occupational exposure limits and guidelines for nitromethane[a]

Country	Year	Concentration (mg/ m^3)	Interpretation[b]
Denmark	1993	250	TWA
Finland	1993	250	TWA
		375	STEL
France	1993	250	TWA
Germany	1998	250	TWA
Ireland	1997	250	TWA
		375	STEL
Netherlands	1997	50	TWA
Philippines	1993	250	TWA
Poland	1998	30	TWA
		240	STEL
Switzerland	1993	250	TWA
Turkey	1993	250	TWA
United Kingdom	1997	250	TWA
		375	STEL
United States			
ACGIH	1999	50	TWA
OSHA	1999	250	TWA

[a] From Finnish Ministry of Social Affairs and Health (1998); Occupational Safety and Health Administration (OSHA) (1999); American Conference of Governmental Industrial Hygienists (ACGIH) (1999); National Library of Medicine (1999)

[b] TWA, time-weighted average; STEL, short-term exposure limit

[c] These countries follow the recommendations of the ACGIH threshold limit values: Bulgaria, Colombia, Jordan, Republic of Korea, New Zealand, Singapore and Viet Nam

females (19/50, 21/50, 33/50 ($p < 0.001$, logistic regression test), and 36/50 ($p < 0.001$, logistic regression test)), as were those of mammary gland carcinomas (2/50, 7/50, 1/50 and 11/50 ($p < 0.05$, logistic regression test)) in the control, low-, mid- and high-dose groups, respectively (National Toxicology Program, 1997).

Groups of 40 male and 40 female BLU:(LE)BR Long-Evans rats [age unspecified] were exposed by inhalation to 0, 100 or 200 ppm [0, 250 or 500 mg/m^3] nitromethane (purity, 96.26%, with 2.79% nitroethane and 0.62% 2-nitropropane as contaminants) for 7 h per day on five days per week for two years. There was no difference in body weight gain in males, but body weight gain in females exposed to 100 or 200 ppm was slightly less than that of controls. The numbers of survivors at the end of the experiment were 25, 23 and 25 (males) and 30, 29 and 24 (females) in the control, low- and high-dose

Table 2. Incidence of tumours in B6C3F$_1$ mice exposed by inhalation to nitromethane

	Number of animals with tumours			
	0 ppm	188 ppm	375 ppm	750 ppm
Males				
Harderian gland adenoma	9/50	10/50	19/50*	32/50**
Harderian gland carcinoma	1/50	1/50	6/50	5/50
Harderian gland adenoma or carcinoma	10/50	11/50	25/50**	37/50**
Alveolar/bronchiolar adenoma	11/50	10/50	9/50	12/50
Alveolar/bronchiolar carcinoma	2/50	3/50	3/50	11/50**
Alveolar/bronchiolar adenoma or carcinoma	13/50	13/50	12/50	20/50
Females				
Harderian gland adenoma	5/50	7/50	16/50**	19/50**
Harderian gland carcinoma	1/50	2/50	4/50	3/50
Harderian gland adenoma or carcinoma	6/50	9/50	20/50**	21/50**
Hepatocellular adenoma	14/50	24/49**	17/49	35/50**
Hepatocellular carcinoma	10/50	14/49	8/49	12/50
Hepatocellular adenoma or carcinoma	19/50	34/49**	22/49	40/50**
Alveolar/bronchiolar adenoma	3/50	3/50	2/49	9/50
Alveolar/bronchiolar carcinoma	0/50	3/50	5/49*	3/50
Alveolar/bronchiolar adenoma or carcinoma	3/50	6/50	6/49	12/50*

From National Toxicology Program (1997)
* $p \leq 0.05$, logistic regression test
* $p \leq 0.01$, logistic regression test

groups, respectively. There was no significant increase in the incidence of tumours related to nitromethane (Griffin *et al.,* 1996).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Nitromethane was administered to Wistar rats [sex not specified] by a single lethal intraperitoneal injection of 1.5 g/kg body weight (bw), by eight injections of 0.11 g/kg

bw over two weeks, or by inhalation of a lethal concentration of 33 g/m^3 for about 6 h. In all cases no methaemoglobin was detected in the blood and low concentrations of nitrite were found in the heart, lungs, kidney and spleen, but not in the liver. After the inhalation study nitromethane was detected only in the liver (Dequidt *et al.*, 1973).

Formaldehyde generated from nitromethane was found only in trace amounts after incubation with microsomes from from Fischer 344 rat liver, but none was found after incubation with rat nasal microsomes (Dahl & Hadley, 1983). Nitromethane inhibited rabbit liver cytochrome P450 activity, apparently competing for the same ferrohaemochrome-binding sites as carbon monoxide (Wade *et al.*, 1977).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Nitromethane was administered intraperitoneally (200 mg/kg bw) to male Wistar rats (three months of age) as a 10% solution in olive oil. The effects of nitromethane in the liver were detected only 48 h after administration and included a decrease in NADPH-cytochrome c reductase activity with proliferation of the smooth endoplasmic reticulum. Nitromethane also caused an increase in brain acid proteinase (4 h after injection) and acetylcholine esterase activities (4, 24 and 48 h after injection) (Zitting *et al.*, 1982).

BALB/c male mice (19–25 g) received a single intraperitoneal injection of 4.5, 6.7 or 9.0 mmol/kg bw nitromethane in a volume of 0.2 mL saline. Control mice were injected with the same volume of 0.9% sodium chloride. Mice were killed 24, 48, 72 or 96 h after treatment. Blood was obtained by cardiac puncture and plasma was analysed for changes in sorbitol dehydrogenase, alanine aminotransferase and aspartate aminotransferase activity as measures of liver damage. Sections from three different liver lobes were processed and stained with haematoxylin and eosin for histopathological analysis. There were no significant changes in any of the enzymes measured or significant abnormalities in the livers of mice following nitromethane administration, demonstrating a lack of hepatotoxicity (Dayal *et al.*, 1989).

Nitromethane has been shown to produce histidinaemia in rats. Inbred weanling male Sprague-Dawley rats given subcutaneous injections of nitromethane (1.2 mol/L, 0.4 mL/100 g bw) every other day for one, three, six, 12 and 18 days. The histidine concentration in tissues increased gradually to reach a plateau after six days of treatment and after 18 days, levels were increased 4.7-fold in plasma, 2.7-fold in brain, 3.0-fold in liver and 1.7-fold in kidney (Lee & Wang, 1975). In the same strain of rats injected subcutaneously with nitromethane (1.8 mol/L, 0.8 mL/100 g bw) every day for six days, 61% of the rats had paralysis of the limbs and 15% had occasional

seizures. Liver weights and liver total protein did not change with treatment with nitromethane. Hepatic histidase activity decreased significantly in the nitromethane-treated rats compared with controls, with approximately a 3–3.5-fold corresponding increase in histidine concentration in plasma, liver and brain. No significant change in serotonin content of the various areas of the brain or in free amino acid concentration in plasma was detected. These results are consistent with nitromethane being a histidase inhibitor (Douay & Kamoun, 1980). In male Wistar rats (30 days of age), nitromethane (730 mg/kg bw) injected intraperitoneally three times over a 24-h period caused a 90% inhibition of histidase activity and higher serum histidine levels compared with controls. A consistently lower locomotor activity was observed in these histidinaemic rats compared with controls (Dutra-Filho *et al*., 1989).

Male and female Fischer 344/N rats and B6C3F$_1$ mice (seven weeks of age) were exposed to 0, 94, 188, 375, 750 or 1500 ppm [0, 235, 470, 938, 1875 or 3750 mg/m^3] nitromethane by inhalation for 6 h per day on five days per week over a 16-day period for a total of 12 exposure days. The mean body weight gain of male rats exposed to 1500 ppm [3750 mg/m^3] nitromethane only was slightly but significantly decreased. There was increased preening, rapid breathing, and hyperactivity early in the study and hypoactivity and loss of coordination in the hindlimbs near the end of the study in rats of both sexes. Exposure to nitromethane caused a concentration-related increase in the absolute and relative liver weights and minimal to mild degeneration of the olfactory epithelium in the nose of rats and mice. In nitromethane-exposed male and female rats, there was sciatic nerve degeneration. Concentrations of 750 or 1500 ppm [1875 or 3750 mg/m^3] nitromethane resulted in reduced myelin around sciatic axons in rats (National Toxicology Program, 1997).

Male and female Fischer 344/N rats and B6C3F$_1$ mice (six weeks of age) were exposed by inhalation to 0, 94, 188, 375, 750 or 1500 ppm [0, 235, 470, 938, 1875 or 3750 mg/m^3] nitromethane for 6 h per day on five days per week for 13 weeks to evaluate the cumulative toxic effects of repeated exposure to nitromethane and to determine the appropriate exposure concentrations to be used in a two-year study. Additional groups of rats were designated for clinical pathology evaluation on days 3 and 23. Neurobehavioural tests were carried out on all core study rats during week 11 of the study. Body weight and body weight gain were significantly less in male rats exposed to 1500 ppm [3750 mg/m^3] nitromethane than in the control group. Clinical findings included hindlimb paralysis in rats exposed to 750 and 1500 ppm [1875 and 3750 mg/m^3] nitromethane. Nitromethane caused exposure-related microcytic, responsive anaemia in male and female rats. Evidence that a haemolytic process occurred in exposed rats included the presence of schistocytes, Heinz bodies and spherocytes and increased mean cell haemoglobin and methaemoglobin concentration. On exposure day 23, there was a transient decrease in serum levels of triiodothyronine, and of total and free thyroxine in male and female rats exposed to nitromethane. Nitromethane exposure also caused minimal to mild hyperplasia of the bone marrow. Both rats and mice exposed to nitromethane had olfactory epithelial degeneration and

respiratory epithelial hyaline droplets. Goblet-cell hyperplasia occurred in male and female rats. Mild degeneration of the sciatic nerve and the lumbar spinal cord was also observed in male and female rats exposed to 375 ppm [938 mg/m^3] nitromethane. Forelimb and hindlimb grip strengths decreased in rats exposed to the highest concentration of nitromethane compared with controls. Both male and female mice in the 1500-ppm exposure group had minimal extramedullary haematopoiesis of the spleen (National Toxicology Program, 1997).

In a six-month inhalation study, New Zealand White rabbits and Sprague-Dawley rats were exposed by inhalation to 0, 98 or 745 ppm [0, 245 or 1860 mg/m^3] nitromethane for 7 h per day on five days per week for six months. Decreased body weight gain in rats was seen after eight weeks of exposure to 745 ppm. The most notable response in rabbits was an effect on the thyroid: increased thyroid weight and decreased serum thyroxine levels. There were no exposure-related gross or microscopic lesions in either rats or rabbits exposed to 98 or 745 ppm (Lewis *et al.*, 1979).

Male and female Long-Evans (BLU:(LE)BR) rats were exposed by inhalation to 0, 100 or 200 ppm [0, 250 or 500 mg/m^3] nitromethane for 7 h per day on five days per week for two years. Serum chemistry and haematology measurements were not found to be significantly different in nitromethane-exposed rats compared with rats exposed to room air. Body weights of exposed female rats were slightly lower than those of control rats. Tissues weights, however, were unaffected by chronic exposure to nitromethane. Non-neoplastic lesions were not related to nitromethane exposure but in most cases were similar to those found in populations of ageing laboratory rats (Griffin *et al.*, 1996).

In a two-year inhalation study, male and female Fischer 344/N rats and B6C3F$_1$ mice (six weeks of age) were exposed to 0, 94, 188 or 375 ppm [0, 235, 470 or 938 mg/m^3] and 0, 188, 375 or 750 ppm [0, 470, 938 or 1875 mg/m^3] nitromethane, respectively, for 6 h per day on five days per week for 103 weeks. Non-neoplastic lesions that developed with increased incidence included nasal lesions with degeneration and metaplasia of the olfactory epithelium and degeneration of the respiratory epithelium in male and female mice (National Toxicology Program, 1997).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

(*a*) *Developmental toxicity studies*

No data were available to the Working Group.

(*b*) *Reproductive toxicity studies*

In a 13-week inhalation study of nitromethane in male and female Fischer 344/N rats and B6C3F$_1$ mice exposed to 375, 750 or 1500 ppm [938, 1875 or 3750 mg/m^3] for 6 h per day on five days per week, a dose-related decrease in sperm motility was observed. The decrease was significant at doses of 750 and 1500 ppm in rats and at all dose levels in mice. In the 1500-ppm group, body weight as well as weight of cauda, epididymis and testis were decreased in rats. In female mice, estrous cycle length was dose-relatedly increased at all dose levels (National Toxicology Program, 1997).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

Nitromethane has given consistently negative results in bacterial mutagenicity assays. It also gave negative results in in-vitro mammalian tests for sister chromatid exchanges and chromosomal aberrations. It was not mutagenic in *Drosophila*. It did not induce micronuclei *in vitro* in Syrian hamster embryo cells or *in vivo* in mice. However, nitromethane did show a positive response at high concentration in a cell transformation assay in Syrian hamster embryo cells.

4.5 Mechanistic considerations

The results of short-term tests on nitromethane do not indicate that the compound has genotoxic activity.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Nitromethane is a volatile liquid that is added in small amounts to many halogenated solvents and aerosol propellants as a stabilizer. It is also used as a polar solvent for certain polymers and resins, in specialized fuels and in explosives. Exposures may occur from the use of solvents, propellants and fuels containing nitromethane.

5.2 Human carcinogenicity data

No data were available to the Working Group.

Table 3. Genetic and related effects of nitromethane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA98, reverse mutation	–	NT	610 µg/plate	Chiu *et al.* (1978)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	3600 µg/plate	Gocke *et al.* (1981)
Salmonella typhimurium TA100, TA1535, TA98, reverse mutation	–	–	20 000 or 50 000 µg/plate	Löfroth *et al.* (1986)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000 µg/plate	Mortelmans *et al.* (1986)
Salmonella typhimurium TA100, TA98, TA102, reverse mutation	–	NT	12 200 µg/plate	Dayal *et al.* (1989)
Salmonella typhimurium TA100, TA98, reverse mutation	NT	–	6100 µg/plate	Dellarco & Prival (1989)
Drosophila melanogaster, sex-linked recessive lethal mutations, Basc test	–		7625 in feed	Gocke *et al.* (1981)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	4965	National Toxicology Program (1997)
Micronucleus test, Syrian hamster embryo cells *in vitro*	–		5000	Gibson *et al.* (1997)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	4980	National Toxicology Program (1997)
Cell transformation, Syrian hamster embryo cells *in vitro*	+		4000	Kerckaert *et al.* (1996)
Micronucleus formation, male and female NMRI mouse bone marrow *in vivo*	–		1830 ip × 2	Gocke *et al.* (1981)
Micronucleus formation, male and female B6C3F$_1$ mouse peripheral blood erythrocytes *in vivo*	–		1500 ppm by inh × 13 w	National Toxicology Program (1997)

[a] +, positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inh, inhalation; w, week

5.3 Animal carcinogenicity data

Nitromethane was tested for carcinogenicity by inhalation in one experiment in mice and in two experiments in rats. In mice, it increased the incidence of Harderian gland and lung tumours in males and females as well as of hepatocellular adenomas in females. In one experiment in rats, nitromethane increased the incidence of benign and malignant mammary gland tumours in females, but produced no increase in the incidence of tumours in a second study in a different strain of rat.

5.4 Other relevant data

Nitromethane produces histidinaemia in rats by decreasing hepatic histidase activity, leading to increased tissue levels of histidine.

Neurological effects were observed in nitromethane-exposed rats.

Nitromethane caused mild degeneration of the olfactory epithelium of exposed rats and mice and microcytic anaemia with minimal to mild hyperplasia of the bone marrow in rats.

No data on reproductive or developmental effects in humans were available.

In rats and mice, dose-related decreases in sperm motility were found after inhalation of nitromethane. In females, estrous cycle length was increased in mice but not in similarly exposed rats.

Nitromethane gave negative results in all short-term tests for genetic effects, with the exception of a cell transformation assay in which it was positive at high concentration.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of nitromethane were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of nitromethane.

Overall evaluation

Nitromethane is *possibly carcinogenic to humans (Group 2B)*.

6. References

American Conference of Governmental Industrial Hygienists (1999) *TLVs and other Occupational Exposure Values—1999 CD-ROM*, Cincinnati, OH, ACGIH®

Angus Chemical Co. (1998) *Technical Data Sheet: Nitromethane (NM™)*, Buffalo Grove, IL

Archer, W.L. (1996) *Industrial Solvents Handbook*, Chap. 17: Nitroparaffins, NY, Marcel Dekker, pp. 267–282

Budavari, S., ed. (1998) *The Merck Index*, 12th Ed., Version 12:2 [CD-ROM], Whitehouse Station, NJ, Merck & Co.

Chiu, C.W., Lee, L.H., Wang, C.Y. & Bryan, G.T. (1978) Mutagenicity of some commercially available nitro compounds for *Salmonella typhimurium*. *Mutat. Res*., **58**, 11–22

Chemical Information Services, Inc. (1999) *Directory of World Chemical Producers*, Version 99.1.0 [CD-ROM], Dallas, TX

Dahl, A.R. & Hadley, W.M. (1983) Formaldehyde production promoted by rat nasal cytochrome P-450-dependent monooxygenases with nasal decongestants, essences, solvents, air pollutants, nicotine, and cocaine as substrates. *Toxicol. appl. Pharmacol.*, **67**, 200–205

Dayal, R., Gescher, A., Harpur, E.S., Pratt, I. & Chipman, J.K. (1989) Comparison of the hepatoxicity in mice and the mutagenicity of three nitroalkanes. *Fundam. appl. Toxicol*., **13**, 341–348

De Leacy, E.A., Brown, N.N. & Clague, A.E. (1989) Nitromethane interferes in assay of creatinine by the Jaffé reaction. *Clin. Chem*., **35**, 1772–1774

Dellarco, V.L. & Prival, M.J. (1989) Mutagenicity of nitro compounds in *Salmonella typhimurium* in the presence of flavin mononucleotide in a preincubation assay. *Environ. mol. Mutag*., **13**, 116–127

Dequidt, J., Vasseur, P. & Potencier, J. (1973) [Experimental toxicological study of some nitroparaffins. 4. Nitromethane.] *Bull. Soc. Pharm. Lille*, **1**, 29–35 (in French)

Deutsche Forschungsgemeinschaft (1999) *List of MAK and BAT Values 1999* (Report No. 35), Weinheim, Wiley-VCH, pp. 81, XII

Douay, O. & Kamoun, P.P. (1980) Serotonin in experimental histidinemia. *Neurochem. Res*., **5**, 897–903

Dutra-Filho, C.S., Wannmacher, C.M.D., Pires, R.F., Gus, G., Kalil, A.M. & Wajner, M. (1989) Reduced locomotor activity of rats made histidinemic by injection of histidine. *J. Nutr*., **119**, 1223–1227

Eller, P.M., ed. (1994) *NIOSH Manual of Analytical Methods (DHHS (NIOSH) Publ. No. 94-113)*, 4th ed., Cincinnati, OH, National Institute for Occupational Safety and Health [Method 2527]

Gibson, D.P., Brauninger, R., Shaffi, H.S., Kerckaert, G.A., LeBoeuf, R.A., Isfort, R.J. & Aardema, M.J. (1997) Induction of micronuclei in Syrian hamster embryo cells: comparison to results in the SHE cell transformation assay for National Toxicology Program test chemicals. *Mutat. Res*., **392**, 61–70

Gocke, E., King, M.-T., Eckhardt, K. & Wild, D. (1981) Mutagenicity of cosmetics ingredients licensed by the European Communities. *Mutat. Res*., **90**, 91–109

Griffin, T.B., Coulston, F. & Stein, A.A. (1996) Chronic inhalation exposure of rats to nitromethane. *Ecotoxicol. environ. Saf*., **34**, 109–117

Hansch, C., Leo, A. & Hoekman, D. (1995) *Exploring QSAR: Hydrophobic, Electronic, and Steric Constants*, Washington DC, American Chemical Society, p. 3

Kaiffer, M., Helmer, J., Jacob, F., Geneftet, B. & Larcan, A. (1972) [Acute toxic methaemoglobinaemia with anuria after use of nitrocellulose and nitromethane.] *Nouv. Presse méd*., **1**, 55–57 (in French)

Kerckaert, G.A., Brauninger, R., LeBoeuf, R.A. & Isfort, R.J. (1996) Use of the Syrian hamster embryo cell transformation for carcinogenicity prediction of chemicals currently being tested by the National Toxicology Program in rodent bioassays. *Environ. Health Perspect.*, **104** (Suppl. 5), 1075–1084

Lee, S.-C. & Wang, M-L. (1975) Histidinemia produced in the rat by treatment with nitromethane. *Nutr. Metab.*, **18**, 79–88

Lewis, T.R., Ulrich, C.E. & Busey, W.M. (1979) Subchronic inhalation toxicity of nitromethane and 2-nitropropane. *J. environ. Pathol. Toxicol.*, **2**, 233–249

Lide, D.R. & Milne, G.W.A. (1996) *Properties of Organic Compounds*, Version 5.0 [CD-ROM], Boca Raton, FL, CRC Press

Löfroth, G., Nilsson, L. & Andersen, J.R. (1986) Structure–activity relationship of nitroalkane-induced mutagenicity in the Ames Salmonella assay. Genetic toxicology of environmental chemicals, Part B: Genetic effects and applied mutagenesis. *Prog. clin. biol. Res.*, **209B**, 149–155

Lundberg, P. (1989) Consensus report for nitromethane. *Arbete och Halsa*, **32**, 86–91

Markofsky, S. (1991) Nitro compounds, aliphatic. In: Elvers, B., Hawkins, S. & Schulz, G., eds, *Ullmann's Encyclopedia of Chemical Technology*, 5th Rev. Ed., Vol. A17, New York, VCH Publishers, pp. 401–409

Ministry of Social Affairs and Health (1998) *Finnish Occupational Exposure Limits 1998. Concentrations Known to be Harmful*. [http://www.occuphealth.fi]oel_eng.htm

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B. & Zeiger, E. (1986) *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutag.*, **8**, 1–119

Mullins, M.E. & Hammett-Stabler, C.A. (1998) Intoxication with nitromethane-containing fuels: don't be 'fueled' by the creatinine. *Clin. Toxicol.*, **36**, 315–320

National Library of Medicine (1999) *Hazardous Substances Data Bank*, Bethesda, MD

National Toxicology Program (1997) *Toxicology and Carcinogenicity Studies of Nitromethane (CAS No. 75-52-5) in F344 Rats and B6C3F$_1$ Mice (Inhalation Studies)* (Technical Report Series No. 461; NIH Publication No. 97-3377), Research Triangle Park, NC

NOES (1999) *National Occupational Exposure Survey (1981 to 1983)*. Unpublished data as of July 1999. Cincinnati, OH, Department of Health and Human Services, Public Health Service, National Institute for Occupational Safety and Health

Occupational Safety and Health Administration (1999) Labor. *US Code Fed. Regul.*, **Title 29**, Part 1910, Subpart 1910.1000, pp. 7–19

Pellizzari, E.D., Hartwell, T.D., Harris, B.S.H., III, Waddell, R.D., Whitaker, D.A. & Erickson, M.D. (1982) Purgeable organic compounds in mother's milk. *Bull. environ. Contam. Toxicol.*, **28**, 322–328

Sadtler Research Laboratories (1980) *Sadtler Standard Spectra*, 1980 Cumulative Index (Molecular Formula Index), Philadelphia, PA, p. 2

Sandyk, R. & Gillman, M.A. (1984) Motor dysfunction following chronic exposure to a fluoroalkane solvent containing nitromethane. *Eur. Neurol.*, **23**, 479–481

Seizinger, D.E. & Dimitriades, B. (1972) Oxygenates in exhaust from simple hydrocarbon fuels. *J. Air Poll. Control Assoc.*, **22**, 47–51

Verschueren, K. (1996) *Handbook of Environmental Data on Organic Chemicals*, 3rd Ed., New York, Van Nostrand Reinhold, p. 1393

Wade, A.E., Evans, J.S., Sternson, L.A. & Gammans, R.E. (1977) Interaction of nitromethane with reduced hepatic microsomal cytochrome P 450. *Biochem. Pharmacol.*, **26**, 963–967

Zitting, A., Nickels, J. & Savolainen, H. (1982) Comparison of acute toxic effects of intraperitoneally injected nitromethane and nitroethane in rats. *Toxicol. Lett.*, **13**, 189–194

Appendix B: NTP (1997). Toxicology and Carcinogenesis Studies of Nitromethane (CAS No. 75-52-5) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). TR 461. PP B-1 – B-63.

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TOXICOLOGY AND CARCINOGENESIS STUDIES OF NITROMETHANE

(CAS NO. 75-52-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

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

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF NITROMETHANE

(CAS NO. 75-52-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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CONTENTS

ABSTRACT

CH_3NO_2

NITROMETHANE

CAS No. 75-52-5

Molecular Weight: 61.04

Synonym: Nitrocarbol

Nitromethane is used as a rocket and engine fuel; as a synthesis intermediate for agricultural fumigants, biocides, and other products; as a solvent; and as an explosive in mining, oil-well drilling, and seismic exploration. It has been detected in air, in surface and drinking water, and in cigarette smoke. Nitromethane was studied because of the potential for widespread human exposure and because it is structurally related to the carcinogens 2-nitropropane and tetranitromethane. Male and female F344/N rats and B6C3F$_1$ mice received nitromethane (purity 98% or greater) by inhalation for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and peripheral blood erythrocytes of mice.

16-Day Study in Rats

Groups of five male and five female rats were exposed to 0, 94, 188, 375, 750, or 1,500 ppm nitromethane by inhalation, 6 hours per day, 5 days per week, for 16 days. All rats survived until the end of the study. The mean body weight gain of male rats in the 1,500 ppm group was slightly but significantly less than that of the controls; the final mean body weights and mean body weight gains of exposed females were similar to those of the controls. Clinical findings in all male and female rats in the 1,500 ppm groups included increased preening, rapid breathing, hyperactivity early in the study, and hypoactivity and loss of coordination in the hindlimbs near the end of the study. The relative liver weights of all exposed groups of male rats and the absolute and relative liver weights of females exposed to 375 ppm or greater were significantly greater than those of the controls.

Minimal to mild degeneration of the olfactory epithelium was observed in the nose of males and females exposed to 375 ppm or greater. Sciatic nerve degeneration was present in all male and female rats exposed to 375 ppm or greater; rats exposed to 750 or 1,500 ppm also had reduced myelin around sciatic axons.

16-Day Study in Mice

Groups of five male and five female mice were exposed to 0, 94, 188, 375, 750, or 1,500 ppm nitromethane by inhalation, 6 hours per day, 5 days per week, for 16 days. All mice survived to the end of the study. The final mean body weights and weight gains of exposed males and females were similar to those of the controls. Clinical findings included hypoactivity and tachypnea in male and female mice in the 1,500 ppm groups. Absolute and relative liver weights of male mice in the 750 and 1,500 ppm groups and female mice in all exposed groups and the relative liver weight of males in the

375 ppm group were significantly greater than those of the controls. Degeneration of the olfactory epithelium of the nose was observed microscopically in all males and females exposed to 375 ppm or greater; this lesion was of minimal severity in males and minimal to mild severity in females.

13-Week Study in Rats

Groups of 10 male and 10 female rats were exposed to 0, 94, 188, 375, 750, or 1,500 ppm nitromethane by inhalation, 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 weight gain of male rats in the 1,500 ppm group were significantly less than those of the controls. Clinical findings included hindlimb paralysis in rats in the 750 and 1,500 ppm groups.

Inhalation exposure of rats to nitromethane resulted in an exposure concentration-dependent, microcytic, responsive anemia; anemia was most pronounced in males and females exposed to 375 ppm or greater. The presence of schistocytes, Heinz bodies, and spherocytes and increased mean cell hemoglobin concentration and methemoglobin concentration were evidence that a hemolytic process was occurring; this hemolytic process could have accounted, in part, for the anemia. Thrombocytosis accompanied the anemia and would be consistent with a reactive bone marrow or could have been due to the erroneous inclusion of small erythrocyte fragments as part of the platelet count. On day 23, transient decreases in serum triiodothyronine, thyroxine, and free thyroxine were observed in male rats exposed to 375 ppm or greater and female rats exposed to 750 or 1,500 ppm. There was little or no pituitary response to the thyroid hormone decreases, as evidenced by the lack of significantly increased concentrations of thyroid-stimulating hormone in exposed rats.

No biologically significant differences in organ weights were observed. The forelimb and hindlimb grip strengths of males in the 1,500 ppm group were significantly less than those of the controls. The hindlimb grip strengths of females in the 750 and 1,500 ppm groups were also significantly less than the control value.

Minimal to mild hyperplasia of the bone marrow was observed microscopically in male rats in the 750 and 1,500 ppm groups and in females exposed to 188 ppm or greater. Nasal lesions in exposed males and females included olfactory epithelial degeneration in males and females exposed to 375 ppm or greater and in one female exposed to 188 ppm and respiratory epithelial hyaline droplets and goblet cell hyperplasia in males and females in the 750 and 1,500 ppm groups; the severity of nasal lesions in males and females was minimal to mild. Males and females exposed to 375 ppm or greater had minimal to mild degeneration of the sciatic nerve and the lumbar spinal cord.

13-Week Study in Mice

Groups of 10 male and 10 female mice were exposed to 0, 94, 188, 375, 750, or 1,500 ppm nitromethane by inhalation, 6 hours per day, 5 days per week, for 13 weeks. All mice survived to the end of the study. The final mean body weights and weight gains of exposed mice were generally similar to those of the controls. There were no treatment-related clinical findings.

The absolute right kidney weights of all groups of exposed male mice except the 1,500 ppm group and of females exposed to 188 ppm or greater and the relative right kidney weights of all groups of exposed males and of females in the 750 and 1,500 ppm groups were significantly greater than those of the controls. The absolute liver weight of male mice in the 750 ppm group and the relative liver weights of males exposed to 375 ppm or greater were significantly greater than those of the controls.

Olfactory epithelial degeneration and respiratory epithelial hyaline droplets were observed microscopically in all male and female mice exposed to 375 ppm or greater. Degeneration also occurred in females in the 188 ppm group, and hyaline droplets occurred in females in the 94 and 188 ppm groups. The average severity of the nasal lesions ranged from minimal to mild in males. In females, the average severity of olfactory epithelial degeneration ranged from minimal to mild and the severity of respiratory epithelial hyaline droplets ranged from minimal to

moderate. All males and nine females in the 1,500 ppm groups also had minimal extramedullary hematopoiesis of the spleen.

2-Year Study in Rats

Groups of 50 male and 50 female rats were exposed to 0, 94, 188, or 375 ppm nitromethane by inhalation, 6 hours per day, 5 days per week, for 103 weeks.

Survival, Body Weights, and Clinical Findings

There were no significant differences in survival rates between exposed and control male or female rats. The mean body weight of females in the 375 ppm group was slightly greater than that of the control group; the mean body weights of exposed males were generally similar to the mean body weight of the controls throughout the study. Clinical findings were consistent with incidences of mammary gland neoplasms in females exposed to 188 or 375 ppm; no hindlimb paralysis, as occurred in rats in the 13-week study, was observed in male or female rats in the 2-year study.

Pathology Findings

The incidences of mammary gland fibroadenoma and fibroadenoma, adenoma, or carcinoma (combined) in female rats in the 188 and 375 ppm groups were significantly greater than those in the controls. Additionally, the incidence of mammary gland carcinoma in the 375 ppm group was significantly greater than in the controls.

2-Year Study in Mice

Groups of 50 male and 50 female mice were exposed to 0, 188, 375, or 750 ppm nitromethane by inhalation, 6 hours per day, 5 days per week, for 103 weeks.

Survival, Body Weights, and Clinical Findings

The survival rate of females in the 750 ppm group was marginally greater than that of the controls. The mean body weights of exposed females were generally slightly greater than the mean body weights of the controls during the study but were generally similar to the mean body weight of the controls at the end of the study. The mean body weights of exposed males were similar to those of the controls throughout the study. Clinical findings included swelling around the eyes and exophthalmos in exposed males and females; these findings were coincident with harderian gland neoplasms.

Pathology Findings

The incidences of harderian gland adenoma and adenoma or carcinoma (combined) in exposed mice increased with increasing exposure concentration and were significantly greater in males and females in the 375 and 750 ppm groups than those in the controls. The incidences of harderian gland carcinoma in males and females in the 375 and 750 ppm groups were also slightly greater than those in the controls.

Female mice in the 188 and 750 ppm groups had significantly greater incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) than the controls. The incidences of liver eosinophilic focus increased with increasing exposure concentration, and the incidences in the 375 and 750 ppm groups were significantly greater than the control incidence.

The incidences of alveolar/bronchiolar carcinoma in male mice in the 750 ppm group and female mice in the 375 ppm group were significantly greater than those in the controls. Females in the 750 ppm group also had a significantly greater incidence of alveolar/bronchiolar adenoma or carcinoma (combined) and a slightly greater incidence of alveolar/bronchiolar adenoma than the controls. Females in the 375 ppm group had a significantly greater incidence of cellular infiltration of histiocytes in the lung than the controls.

The incidences of degeneration and metaplasia of the olfactory epithelium and hyaline degeneration of the respiratory epithelium were significantly greater in exposed male and female mice than those in the controls. Additionally, males in the 375 and 750 ppm groups had significantly greater incidences of inflammation of the nasolacrimal duct than did the controls.

GENETIC TOXICOLOGY

Nitromethane was not mutagenic in any tests performed by the NTP. It did not induce mutations in *Salmonella typhimurium*, with or without S9 metabolic activation, and no induction of sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells exposed to nitromethane was noted with or without S9. No increase in the frequency of micronucleated erythrocytes was observed in peripheral blood samples of male and female mice at the end of the 13-week inhalation study of nitromethane.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity** of nitromethane in male F344/N rats exposed to 94, 188, or 375 ppm. There was *clear evidence of carcinogenic activity* of nitromethane in female F344/N rats based on increased incidences of mammary gland fibroadenomas and carcinomas. There was *clear evidence of carcinogenic activity* of nitromethane in male $B6C3F_1$ mice based on increased incidences of harderian gland adenomas and carcinomas. There was *clear evidence of carcinogenic activity* in female $B6C3F_1$ mice, based on increased incidences of liver neoplasms (primarily adenomas) and harderian gland adenomas and carcinomas. Increased incidences of alveolar/bronchiolar adenomas and carcinomas in male and female mice exposed to nitromethane were also considered to be related to chemical administration.

Exposure to nitromethane by inhalation for 2 years resulted in increased incidences of nasal lesions including degeneration and metaplasia of the olfactory epithelium and degeneration of the respiratory epithelium in male and female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

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

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Exposure concentrations	0, 94, 188, or 375 ppm	0, 94, 188, or 375 ppm	0, 188, 375, or 750 ppm	0, 188, 375, or 750 ppm
Body weights	Exposed groups similar to controls	375 ppm group slightly greater than controls	Exposed groups similar to controls	Exposed groups similar to controls
2-Year survival rates	13/50, 16/50, 14/50, 8/50	28/50, 19/50, 30/50, 23/50	31/50, 36/50, 30/50, 29/50	25/50, 28/50, 26/50, 36/50
Nonneoplastic effects	None	None	Nose: olfactory epithelium, degeneration (0/50, 10/49, 50/50, 50/50); olfactory epithelium, metaplasia (0/50, 1/49, 41/50, 49/50); respiratory epithelium, hyaline degeneration (5/50, 5/49, 50/50, 50/50)	Nose: olfactory epithelium, degeneration (0/50, 22/49, 50/50, 50/50); olfactory epithelium, metaplasia (0/50, 2/49, 46/50, 48/50); respiratory epithelium, hyaline degeneration (16/50, 39/49, 50/50, 50/50)
Neoplastic effects	None	Mammary gland: fibroadenoma (19/50, 21/50, 33/50, 36/50); carcinoma (2/50, 7/50, 1/50, 11/50); fibroadenoma, adenoma, or carcinoma (21/50, 25/50, 34/50, 41/50)	Harderian gland: adenoma (9/50, 10/50, 19/50, 32/50); carcinoma (1/50, 1/50, 6/50, 5/50); adenoma or carcinoma (10/50, 11/50, 25/50, 37/50) Lung: alveolar/ bronchiolar carcinoma (2/50, 3/50, 3/50, 11/50); alveolar/ bronchiolar adenoma or carcinoma (13/50, 13/50, 12/50, 20/50)	Harderian gland: adenoma (5/50, 7/50, 16/50, 19/50); adenoma or carcinoma (6/50, 9/50, 20/50, 21/50) Liver: hepatocellular adenoma (14/50, 25/49, 17/49, 35/50); hepatocellular adenoma or carcinoma (19/50, 34/49, 22/49, 40/50) Lung: alveolar/ bronchiolar adenoma (3/50, 3/50, 2/49, 9/50); alveolar/ bronchiolar carcinoma (0/50, 3/50, 5/49, 3/50); alveolar/ bronchiolar adenoma or carcinoma (3/50, 6/50, 6/49, 12/50)
Level of evidence of carcinogenic activity	No evidence	Clear evidence	Clear evidence	Clear evidence

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

Genetic toxicology	
Salmonella typhimurium gene mutations:	Negative in strains TA98, TA100, TA1535, and TA1537 with and without S9
Sister chromatid exchanges	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Chromosomal aberrations	
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
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 nitromethane 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.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On December 5, 1995, the draft Technical Report on the toxicity and carcinogenesis studies of nitromethane 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 toxicity and carcinogenesis studies of nitromethane by discussing the uses, describing the experimental design, reporting on the survival and body weight effects, and commenting on chemical-related neoplastic lesions in female rats and male and female mice and nonneoplastic lesions in male and female mice. The proposed conclusions for the 2-year studies in rats and mice were *no evidence of carcinogenic activity* in male F344/N rats and *clear evidence of carcinogenic activity* in female F344/N rats and male and female B6C3F$_1$ mice.

Dr. Russo, a principal reviewer, agreed with the proposed conclusions and found the report otherwise acceptable.

Dr. Ryan, the second principal reviewer, agreed with the proposed conclusions. She noted that 375 ppm female rats weighed more than the controls and wondered whether this could be related to chemical effects on the thyroid gland and, further, what impact the weight effect might have had on the increased incidence of mammary gland tumors. Dr. Roycroft responded that transient thyroid gland effects were seen early in the 13-week studies but not at the end and were not observed in the 2-year study, so he did not think the thyroid gland had an impact. Dr. J.K. Haseman, NIEHS, described a model developed by Dr. S. Seilkop using NTP historical control data to predict how certain tumors are affected by body weight and how body weights at certain ages are predictive of subsequent tumor development. Using the model, one would predict a 51% incidence of mammary gland tumors in 375 ppm female rats, while the actual incidence in the study was 82%. Dr. Haseman said in this case the increase in body weights could not account for the increase in tumors.

Dr. LeBoeuf, the third principal reviewer, agreed with the proposed conclusions in principle. He questioned whether the increases in hepatocellular adenomas alone in female mice were sufficient to support the conclusion of clear evidence. Dr. Roycroft said the incidences of 51% and 70% in the 188 and 750 ppm groups well exceeded the concurrent control incidence of 28% as well as the highest historical rate of 40% in any of the contemporary inhalation studies and justified their inclusion as support. Dr. LeBoeuf commented that since neurotoxicity was the prime determinant for dose setting for the 2-year rat study, there should have been histopathologic examination of sciatic nerve and spinal cord in animals from this study. Dr. Roycroft observed that the 13-week data indicated sciatic nerve degeneration was less severe than in the 16-day study, although there were obvious clinical observations in the longer study. He noted that the standard protocol calls for cutting sections of sciatic nerve, spinal cord, and other nervous system tissues when neurobehavioral effects are seen clinically, but such effects were not seen in the 2-year nitromethane study. However, Dr. Roycroft reported that subsequently, sections were taken from 375 ppm and control animals, and none of the lesions observed in prechronic studies were seen.

Dr. A. Bollmeier, Angus Chemical Company, said Angus was essentially the only manufacturer of nitroparaffins now in the country. He pointed out the variation among the three batches used for the studies and wondered if this might not play a role in differences in toxicology findings among the 16-day, 13-week, and 2-year studies. Dr. Bollmeier commented that the potential for human exposure estimates by NIOSH were done in 1981 to 1983, while current exposures would be much less, likely less than 10,000.

Dr. Russo moved that the Technical Report on nitromethane be accepted with the revisions discussed and with the conclusions as written. Dr. Ryan seconded the motion. Dr. J.R. Bucher, NIEHS, asked that the wording of the statement supporting the level of evidence for female mice be changed to add 'adenomas' after 'liver.' Dr. Brown said this would not be an amendment but should be kept in mind by the members when voting. Dr. Goldsworthy commented that one could argue for the same change with the harderian gland in female mice as the tumor response in this organ is primarily driven by the adenomas. Dr. Bucher proposed also using the less specific word 'neoplasm.' The revised sentence could read: "There was *clear evidence of carcinogenic activity* in female mice based on increased incidences of liver neoplasms (primarily adenomas) and harderian gland adenomas and carcinomas." The motion by Dr. Russo was then accepted unanimously with seven votes.

INTRODUCTION

CH_3NO_2

NITROMETHANE

CAS No. 75-52-5

Molecular Weight: 61.04

Synonym: Nitrocarbol

CHEMICAL AND PHYSICAL PROPERTIES

Nitromethane is a colorless, oily liquid with a moderately strong, disagreeable odor (*Merck Index*, 1989). Nitromethane has a melting point of −29° C, a boiling point of 101.2° C, a flash point of 112° F, and a lower explosive limit of 7.3%. Its density is 1.1322 at 25° C; the vapor density is 2.11 and the vapor pressure is 27.8 mm Hg at 20° C. Nitromethane is soluble in alcohol, ether, *N,N*-dimethylformamide, acetone, and alkali and is slightly soluble in water (9.5 g/L at 20° C). Nitromethane will explode when heated under confinement to near its critical temperature (315° C) or when rapidly compressed under adiabatic conditions. The sodium salt is also explosive and bursts into flames upon contact with water.

PRODUCTION, USE, AND HUMAN EXPOSURE

Nitromethane can be prepared by vapor phase nitration of propane or by the reaction of sodium nitrite with sodium chloroacetate (*Merck Index*, 1989). In the past, nitromethane was used extensively as a chemical stabilizer to prevent the decomposition of various halogenated hydrocarbons such as metal degreasers and aerosol propellants such as 1,1,1-trichloroethane. Nitromethane is used as a fuel or fuel additive to increase the power output of rockets, racing cars, boats, and model engines. Nitromethane is also used as a synthesis intermediate for a variety of chemicals, such as trichloronitromethane (chloropicrin), an agricultural soil and grain fumigant; the nitroalcohol, 2-hydroxymethyl-2-nitro-1,3-propanediol, which is used as a biocide for cutting fluids and as a source of formaldehyde for cross-linking amino resins; and the alkanolamine, 2-hydroxymethyl-2-amino-1,3-propanediol, which is used as a formaldehyde scavenger in resin curing and polyester resin modification and as a buffer. Nitromethane is used in a variety of solvent applications, such as solvent-extraction separation of aromatics from aliphatic compounds, in the crystallization of nitrofurantoin, as a reaction medium for aluminum chloride in Friedel-Crafts reactions, and as a solvent for resins such as α-cyanoacrylate. Nitromethane is used in mixtures with ammonium nitrate as an explosive in mining, oil-well drilling, and seismic exploration (*Biocides, U.S.A.*, 1974; *Remington's Pharmaceutical Sciences*, 1975; *Kirk-Othmer*, 1978, 1981; SRI, International, 1980).

Although nitromethane was reported to the U.S. International Trade Commission for the year 1992, the production volume was not published (USITC, 1994). According to the National Occupational Exposure Survey, approximately 135,000 male and 46,500 female workers in the U.S. were potentially exposed to nitromethane during the years 1981 to

1983 (NIOSH, 1990). The time-weighted average threshold limit value for nitromethane is 20 ppm, or 50 mg/m^3 (ACGIH, 1995). The exposure limit of nitromethane permitted by Occupational Safety and Health Administration is 100 ppm or 250 mg/m^3 (29 CFR, § 1910). Although an odor threshold of 3.5 ppm has been reported for nitromethane, the odor and sensory symptoms are not dependable warning properties (Davis, 1993). Products containing nitromethane are not widely used by consumers; therefore, consumer exposure to nitromethane from such products is presumed to be low.

Nitromethane has been detected in air and in surface and drinking water; its occurrence in the atmosphere results either from emissions from industrial processes or from its formation as a byproduct of certain chemical reactions. Nitromethane is found in cigarette smoke and is a byproduct of hydrocarbon combustion and munitions manufacture. It is possibly synthesized in the atmosphere by the photolytic reaction of nitrogen dioxide and ethylene.

Nitromethane is fairly reactive and therefore does not persist in the environment; the half-life ($t_{½}$) of nitromethane is from 4 to 9 hours in air and about 1 day in water (National Library of Medicine, 1995a). However, because nitromethane is slightly soluble in water and evaporates at about the same rate as water, the $t_{½}$ is somewhat dependent on the rate of evaporation. In the atmosphere and in water, nitromethane is degraded through its reaction with hydroxyl radicals; it may also undergo aerobic or anaerobic degradation. It may react with chlorine in water to form trichloronitromethane if the pH of the medium is high (Wade *et al.*, 1977).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Nitromethane is oxidized *in vitro* to formaldehyde, nitrite, and hydrogen peroxide by D-amino acid oxidase prepared from hog kidney (Porter *et al.*, 1972). However, when incubated with rat nasal and liver microsomes to determine if the cytochrome P_{450}-dependent demethylation produces formaldehyde, no formaldehyde was generated from nasal microsomes and only a trace was generated from liver microsomes (Dahl and Hadley, 1983). Flavoenzymes also oxidize nitromethane. Nitromethane inhibits rabbit liver cytochrome P_{450} activity, apparently competing for the same ferrohemochrome-binding sites as carbon monoxide (Wade *et al.*, 1977). Dequidt *et al.* (1973) administered nitromethane to Wistar rats by intraperitoneal injection (2,400 mg/kg) or by inhalation (13,000 ppm for 6 hours) and measured nitrite and methemoglobin concentrations in exposed animals. No methemoglobin was detected in rats treated with nitromethane by either route; nitrite was detected in low concentrations in the heart, lungs, kidneys, and spleen, but not the liver, of rats treated by each route. Following daily 6-hour inhalation exposures to 2,500 ppm nitromethane for 4 days, nitrite concentrations in heart, lung, kidney, and spleen tissues of Wistar rats were similar to those observed following a single exposure. No unchanged nitromethane was present in the organs of animals administered nitromethane intraperitoneally. In rats exposed by inhalation, nitromethane was detected only in the liver (0.27 g/100 g) and was not detected in rats exposed to less than 13,000 ppm.

Humans

No studies of absorption, distribution, metabolism, and excretion of nitromethane in humans were found in the literature (National Library of Medicine, 1995b).

TOXICITY

Experimental Animals

Oral LD_{50} values range from 940 to 1,210 mg/kg for rats (Subbotin, 1967; International Technical Information Institute, 1979) and from 950 to 1,440 mg/kg for mice (Machle *et al.*, 1940). In an early study of nitromethane toxicity performed by Gibbs and Reichert (1891), a minimum lethal dose of 565 to 1,130 mg/kg for dogs was reported. Injections of nitromethane caused lassitude, drowsiness, weakness, salivation, urination, defecation, and vomiting. Nitromethane first accelerated, then retarded, the pulse rate. Treated dogs exhibited progressive weakness, coma, paralysis, and terminal convulsions. Death by respiratory failure usually occurred within 24 hours of injection.

Dogs that were administered a single oral dose of nitromethane at a concentration of 200, 500, 1,000,

or 1,500 mg/kg died within 36 hours (Weatherby, 1955). Pathologic examination revealed hepatic edema, focal areas of necrosis, and cells with enlarged nuclei. Pathologic examinations performed on dogs that were administered a single, nonlethal oral dose of 125 mg/kg nitromethane revealed slight changes in the liver, including mild fatty change of the hepatic parenchyma and a few lymphocytes in the portal areas. Within 48 hours after dosing, regeneration of hepatic cells was observed. Liver damage was more severe as the dose was increased. Kidney damage (swollen glomeruli, swollen proximal and distal convoluted tubules, and hyaline casts in the tubules) was observed only in animals administered 1,500 mg/kg. All other tissues were normal.

Rabbits receiving gavage doses of 750 to 1,000 mg/kg nitromethane displayed progressive weakness and collapse, unsteadiness and incoordination ending in complete ataxia; their breathing was first slowed, then rapid (Machle *et al.*, 1940). There were no changes in blood chemistry variables and no methemoglobin formation. Liver damage (edema, cloudy swelling, and necrosis) was present in all animals that died from administration of the chemical. Inhalation experiments with rabbits, monkeys, and guinea pigs were also performed. The symptoms of nitromethane toxicity following inhalation were similar for each species, although guinea pigs seemed to be somewhat more susceptible than rabbits or monkeys (Machle *et al.*, 1940). Mortality was related to total dose (concentration of exposure multiplied by duration) at concentrations greater than 500 ppm. The LC_{L_0} for monkeys was determined to be 1,000 ppm. Rabbits and guinea pigs (two of each species) survived exposure to 30,000 ppm for 15 minutes or 10,000 ppm for 1 hour; however, all died when exposed to 30,000 ppm for 2 hours or 10,000 ppm for 6 hours (Machle *et al.*, 1940). A latency period was observed before the onset of symptoms of nitromethane toxicity and was inversely related to the concentration of nitromethane in the air. Central nervous system effects were observed within 30 minutes to 1 hour after exposure to 30,000 or 50,000 ppm; however, in animals exposed to 10,000 ppm, central nervous system effects were not observed until 5 hours after exposure began. Inhalation of nitromethane first caused restlessness and slight irritation of the respiratory tract. After the latency period, the animals began salivating, appeared ill, and showed signs of narcosis. As the exposure period progressed, the animals became weak, ataxic, and incoordinated and often exhibited circular movement, convulsions, and twitching. At necropsy, all exposed animals were found to have some liver damage (edema and necrosis). Animals that died from nitromethane inhalation exhibited general visceral and cerebral congestion and acute pulmonary congestion with edema. Application of nitromethane (dose not specified) to the clipped skin of rabbits caused neither skin irritation nor compound-related clinical findings (Machle *et al.*, 1940). In a study comparing hepatotoxicity, groups of three to five BALB/C mice were injected intraperitoneally with 275, 410, or 550 mg nitromethane, 2-nitropropane, or nitroethane per kilogram body weight and were killed 24, 48, 72, or 96 hours after dosing (Dayal *et al.*, 1989). Nitromethane and nitroethane were not hepatotoxic; treatment with 550 mg/kg 2-nitropropane caused increases in plasma sorbitol dehydrogenase, alanine aminotransferase, and aspartate aminotransferase activities and caused necrosis, degeneration, and cell proliferation in the liver.

Nitromethane has been used experimentally to cause histidinemia in inbred, weanling male Sprague-Dawley rats (Douay and Kamoun, 1980). The rats were injected subcutaneously with nitromethane at the rate of 0.8 mL of 110 g/L per 100 g body weight once daily for 6 days. Liver histidase in treated rats was 30% that of untreated control animals, while plasma, liver, and brain histidine increased threefold. Paralysis was observed in 61% of treated animals, while 15% displayed seizures. The rate of body weight gain was significantly reduced. No effect was observed on brain serotonin content or in plasma free amino acid concentrations. These findings indicate that animal histidinemia produced by nitromethane treatment is similar to human histidinemia.

In a study comparing the acute toxic effects of nitromethane and nitroethane, 3-month-old male Wistar rats were dosed intraperitoneally with 200 mg/kg of either compound (Zitting *et al.*, 1982). The rats were examined after 4, 24, or 48 hours. Both nitromethane and nitroethane caused an increase in brain acid proteinase and acetylcholine esterase activities. Nitromethane decreased NADPH-cytochrome C reductase activity in liver microsomes.

Nitroethane depressed 7-ethoxycoumarin *O*-deethylase and NADPH-cytochrome C reductase but increased epoxide hydrolase and UDP-glucuronosyltransferase activities in liver microsomes. In an earlier study, 2-nitropropane was shown to cause an increase in hepatic epoxide hydrolase and UDP-glucuronosyltransferase and brain acetylcholine esterase activities (Zitting *et al.*, 1982). Rats and rabbits provided drinking water containing 23, 47, or 94 mg nitromethane per kilogram body weight for 2 months had increased alanine transaminase and aspartate transaminase activities and α- and γ-globulin concentrations, liver impairment (decreased plasma prothrombin), and increased whole blood cholinesterase; there were no effects on blood cell morphology (Subbotin, 1967). However, in studies in which rats and rabbits were provided drinking water containing 0.05, 0.5, or 12.5 mg/kg nitromethane for 6 months, serum alanine and aspartate transaminase activities were increased only in animals administered 12.5 mg/kg (Subbotin, 1967).

In a 6-month inhalation study, Sprague-Dawley rats and New Zealand white rabbits were exposed to 98 or 745 ppm nitromethane or 27 or 207 ppm 2-nitropropane for 7 hours per day, 5 days per week, for 6 months (Lewis *et al.*, 1979). Interim evaluations were conducted on days 2 and 10 for rats and at 1 and 3 months for rats and rabbits. No deaths were attributed to nitromethane or 2-nitropropane administration in either species. Rats exposed to 745 ppm nitromethane did not gain weight as rapidly as the controls. There were no effects on hematologic parameters, prothrombin time, or alanine transaminase activity in either species, and no methemoglobin was produced in animals exposed to nitromethane. Rabbits in both groups exposed to nitromethane had depressed serum thyroxin concentrations throughout the study (statistically significant at 1 month for the 745 ppm group and at 6 months for both groups); this effect did not occur in animals exposed to 2-nitropropane. Rats and rabbits exposed to 745 ppm nitromethane also had greater thyroid gland weights than the controls. At all time points, lung weights of rats exposed to nitromethane were reduced. Lung and liver weights of rats exposed to 207 ppm 2-nitropropane were significantly increased at 3 and 6 months; 2-nitropropane did not cause organ weight effects in rabbits. There were no histopathologic changes related to nitromethane treatment in rats or rabbits at either concentration at any evaluation period. However, rats exposed to 207 ppm 2-nitropropane for 3 months had focal hepatocellular hypertrophy with large hepatocytes and basophilic foci containing small hyperplastic foci. Following 6 months of exposure to 207 ppm 2-nitropropane, all rats had multiple hepatocellular carcinomas (Lewis *et al.*, 1979).

In a study to investigate the potential hepatotoxicity of 1-nitropropane, Griffin *et al.* (1982) exposed male and female Long-Evans rats to 100 ppm 1-nitropropane by inhalation for 7 hours per day, 5 days per week, for up to 21½ months. No treatment-related mortality, weight-gain effects, clinical signs, or effects on hematologic indices were observed. 1-Nitropropane exposure did not cause changes in clinical chemistry indices indicative of hepatotoxicity. No neoplasms or nonneoplastic lesions were attributed to exposure; the liver effects observed in the 2-nitropropane study (Lewis *et al.*, 1979) did not occur in rats exposed to 1-nitropropane.

Male and female Long-Evans rats exposed to 100 or 200 ppm nitroethane by inhalation for 7 hours per day, 5 days per week, for 2 years had no treatment-related mortality or effects on body weight gains (Griffin *et al.*, 1988). Hematology and clinical chemistry evaluations performed at the end of the study indicated no treatment-related effects. Additionally, there were no neoplasms or nonneoplastic lesions associated with nitroethane exposure.

Humans

No epidemiological studies of nitromethane alone were found in the literature (National Library of Medicine, 1995b). The estimated oral LD_{50} for humans is 500 mg/kg (Gosselin *et al.*, 1984). Nitromethane and its decomposition products are toxic if ingested or inhaled. Eye and skin irritation has occurred from repeated exposure.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information on the reproductive or developmental toxicity of nitromethane in experimental animals or

in humans was found in the literature (National Library of Medicine, 1995b).

Carcinogenicity

No information on the carcinogenicity of nitromethane in experimental animals was found in the literature; additionally, no epidemiological studies or case reports examining the relationship between exposure to nitromethane and human cancer were found in the literature (National Library of Medicine, 1995b). As discussed previously, Sprague-Dawley rats exposed to 207 ppm 2-nitropropane by inhalation for 6 months had multiple hepatocellular carcinomas (Lewis *et al.*, 1979). Male and female Long-Evans rats exposed to 100 or 200 ppm nitroethane by inhalation for 2 years (Griffin *et al.*, 1988) or to 100 ppm 1-nitropropane by inhalation for 21½ months (Griffin *et al.*, 1982) had no neoplasms or nonneoplastic lesions associated with treatment.

Genetic Toxicity

Little information is available on the mutagenicity of nitromethane, but the results of published studies are uniformly negative. Results for induction of mutations in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 by nitromethane were negative with and without S9 metabolic activation in tests incorporating either the standard plate assay or preincubation (Chiu *et al.*, 1978; Mortelmans *et al.*, 1986; Dayal *et al.*, 1989; Dellarco and Prival, 1989). The nitronate form of nitromethane was negative for induction of mutations in *S. typhimurium* strains TA100 and TA102 (Dayal *et al.*, 1989). No significant increases in sex-linked recessive lethal mutations were noted in germ cells of male *Drosophila melanogaster* after administration of nitromethane by feeding (Gocke *et al.*, 1981). No induction of micronuclei was observed in bone marrow polychromatic erythrocytes of male NMRI mice administered two intraperitoneal injections of 205 to 1,830 mg nitromethane per kilogram body weight (Gocke *et al.*, 1981). In this test, bone marrow was sampled 6 hours after the second injection, so the effect of the second treatment is not likely to be reflected in these results; 24 hours is the preferred interval between treatment and observation of induced micronuclei. However, this negative result is in agreement with the results of the 13-week micronucleus study conducted by the NTP and presented in this report (Appendix E).

Study Rationale

Nitromethane was the sole chemical selected from the amines, amides, nitros, nitriles, ureas, and carbamates subclass of air pollutants and was subsequently nominated to the NTP for toxicity and carcinogenicity testing by the National Cancer Institute based on its high potential for human exposure and its structural relationship to 2-nitropropane and tetranitromethane, known animal carcinogens (NTP, 1990, 1994). Inhalation was chosen as the route of exposure because of the volatility of the chemical and because human exposure would likely occur by this route.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF NITROMETHANE

Nitromethane was obtained from W.R. Grace and Company (Lexington, MA) in three lots. Lot 1F 13 06 was used during the 16-day studies and the beginning of the 13-week studies; lot 1-H-0501 was used throughout the remainder of the 13-week studies and at the beginning of the 2-year studies. Lot 1-H-1004 was used throughout the remainder of the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Reports on the analyses performed in support of the nitromethane studies are on file at the National Institute of Environmental Health Sciences. The methods and results of these studies are detailed in Appendix J. The chemical, a clear, colorless liquid, was identified as nitromethane by infrared, ultraviolet/visible, and nuclear magnetic resonance spectrometry. The purity of each lot was determined by elemental analysis, Karl Fischer water analysis, functional group titration, and two gas chromatographic systems.

For lot 1F 13 06, elemental analyses of carbon and hydrogen agreed with the theoretical values for nitromethane, but results for nitrogen were low. Karl Fischer water analysis indicated 0.022% ± 0.004% water. Functional group titration indicated a purity of 100% ± 1%. Both gas chromatographic systems indicated one impurity with an area greater than 0.1% relative to the major peak. The area of the impurity peak was 0.62% relative to the major peak by one gas chromatographic system and 0.52% relative to the major peak by the second system. The overall purity of lot 1F 13 06 was determined to be approximately 99%. Gas chromatography/mass spectrometry used to identify the impurity indicated that the mass spectrum of the impurity was consistent with that of propionitrile; an additional impurity observed in the sample was identified as 2-nitropropane. The quantity of propionitrile was determined with gas chromatography to be 0.400% ± 0.001%; the quantity of 2-nitropropane was determined to be 0.017% ± 0.000%.

For lot 1-H-0501 (batch 2), the supplier indicated a purity of 99.3% for the bulk chemical, with 0.27% nitroethane present as a contaminant. Elemental analyses of carbon and hydrogen by the analytical chemistry laboratory agreed with the theoretical values for nitromethane, but results for nitrogen were low. Karl Fischer water analysis indicated 0.018% ± 0.003% water. Functional group titration indicated a purity of 98.9% ± 0.8%. Gas chromatography indicated three impurities with a combined area of 1.69% relative to the major peak by one system and two impurities with a combined area of 1.49% relative to the major peak by the second system. Batch 3 of lot 1-H-0501 was also analyzed with gas chromatographic system A; one major peak and three impurities with a total peak area 1.71% relative to the major peak were identified. Major peak comparisons of batch 2 with lot 1F 13 06 and of batch 3 with batch 2 were performed with gas chromatography; the results indicated a purity of 99.3% ± 0.3% for batch 2 of lot 1-H-0501 relative to lot 1F 13 06 and a purity of 99.5% ± 0.5% for batch 3 relative to batch 2. The overall purity of lot 1-H-0501 was determined to be approximately 98%.

For lot 1-H-1004, the supplier indicated a 99% purity of the bulk chemical, with nitroethane (0.25%) and 2-nitropropane (0.03%) present as contaminants. Elemental analyses of carbon and hydrogen by the analytical chemistry laboratory agreed with the theoretical values for nitromethane, but results for nitrogen were low. Karl Fischer water analysis indicated 0.086% ± 0.006% water. Functional group titration indicated a purity of 97.8% ± 0.5%. Gas chromatography indicated three impurities with a combined area of 1.5% relative to the major peak by one system and three impurities with a combined

area of 1.9% relative to the major peak by the second system. Major peak comparison of lot 1-H-1004 with lot 1F 13 06 by gas chromatography indicated a purity of 100.3% ± 0.9% for lot 1-H-1004 relative to lot 1F 13 06. The overall purity of lot 1-H-1004 was determined to be approximately 98%.

Accelerated stability studies of lots 1F 13 06 and 1-H-0501 of the bulk chemical were conducted with gas chromatography. Nitromethane was determined to be stable as a bulk chemical when stored in Teflon®-lined amber glass bottles, protected from light, for up to 2 weeks at temperatures up to 60° C. To ensure stability, the bulk chemical was stored in the original shipping containers (metal drums and amber glass bottles) at room temperature; lot 1F 13 06 was stored under a nitrogen headspace. Stability was monitored by the study laboratory throughout the studies with gas chromatography; no degradation of the bulk chemical was detected.

VAPOR GENERATION AND EXPOSURE SYSTEM

Nitromethane was held in a stainless-steel reservoir under a nitrogen blanket; a MasterFlex variable-speed peristaltic pump head (Cole-Parmer, Inc., Chicago, IL) was used to pump nitromethane through a liquid distribution manifold of stainless steel tubing to heated-wick vaporizers. During the 16-day studies, single vaporizers were used for each of the 750 and 1,500 ppm chambers, and a third vaporizer was located in the vapor distribution system that supplied the 94, 188, and 375 ppm chambers. During the 13-week and 2-year studies, one set of dual vaporizers supplied nitromethane vapor to all chambers. Detailed descriptions of the inhalation chambers and the vapor generation system are provided in Appendix J.

The vapor-laden air was transferred through the distribution line, where it was diluted with HEPA- and charcoal-filtered air, to the inhalation chambers; three-way valves mounted in the chamber inlet ducts allowed nitromethane vapors to be diverted to the exhaust until a stable concentration of nitromethane was built up in the distribution line. At each chamber, air moving through the chamber inlet duct was further diluted with HEPA- and charcoal-filtered air to the appropriate nitromethane concentration for the chamber with a metered three-way valve. The study laboratory designed the inhalation exposure chamber (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) so that uniform vapor concentrations could be maintained throughout the chamber with the catch pans in place. A small particle detector (Type CN, Gardner Associates, Schenectady, NY) was used with and without animals in the exposure chambers to ensure that nitromethane vapor, and not aerosol, was produced. No particle counts above the minimum resolvable level of approximately 200 particles/cm^3 were detected.

VAPOR CONCENTRATION MONITORING

Chamber concentrations were monitored with an on-line gas chromatograph. The monitor was coupled with the inhalation chambers by a computer-controlled 12-port stream select valve. The gas chromatograph was calibrated by a comparison of chamber concentration data to data from grab samples analyzed by an off-line gas chromatograph; the grab samples were collected in bubblers containing dimethylformamide. The off-line gas chromatograph was calibrated with gravimetrically prepared nitromethane standards. Chamber concentration uniformity was maintained throughout the 16-day, 13-week, and 2-year studies. Summaries of the chamber concentrations for the 16-day, 13-week, and 2-year studies are presented in Tables J1 through J3. The monthly mean exposure concentrations for the 2-year study chambers are presented in Figures J7 through J12.

CHAMBER ATMOSPHERE CHARACTERIZATION

Buildup and decay rates for chamber concentrations were determined with and without animals present in the chambers. The time to achieve 90% of the target concentration after the beginning of vapor generation (T_{90}) ranged from 10 to 13 minutes during the 16-day studies and from 6 to 13 minutes during the 13-week studies. The time for the concentration in the chamber to decay to 10% of the target concentration after vapor generation ended (T_{10}) ranged from 11 to 14 minutes during the 16-day studies and from 11 to

15 minutes during the 13-week studies. During the 2-year studies, T_{90} ranged from 11 to 14 minutes without animals and from 5 to 17 minutes with animals in the chambers; T_{10} ranged from 13 to 16 minutes without animals and from 13 to 19 minutes with animals. A T_{90} value of 12 minutes was selected for all studies.

Studies of nitromethane degradation and monitoring for impurities were conducted throughout the studies by comparing bubbler samples to a reference sample of nitromethane. No significant degradation of nitromethane was observed during the studies.

16-Day Studies

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). On receipt, the rats and mice were 5 weeks old. Animals were quarantined for 12 days (rats) or 13 days (mice) and were 7 weeks old on the first day of the studies. Before the studies began, two male and two female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease.

Groups of five male and five female rats and mice were exposed to 0, 94, 188, 375, 750, or 1,500 ppm nitromethane by inhalation, 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 16 days. Rats and mice received a total of 12 exposures, including two (rats) or three (mice) consecutive exposures before necropsy. Water was available *ad libitum*; feed was available *ad libitum* except during exposure periods. Rats and mice were housed individually. Clinical observations were recorded twice each day for rats and mice. The animals were weighed initially, on day 8, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 16-day studies, a necropsy was performed on all rats and mice. The heart, right kidney, liver, lungs, right testis, thymus, and thyroid glands (rats only) were weighed. Histopathologic examinations were performed on all rats and mice. Following the 13-week studies, sections of the sciatic nerve of rats from the 16-day study were stained with Sevier-Munger Luxol Fast Blue to allow for evaluation of myelin around sciatic nerve axons. The tissues and organs routinely examined are listed in Table 1.

13-Week Studies

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

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories (Gilroy, CA). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 13 or 14 days 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. Additionally, the kidneys of five male and five female mice were screened to ensure genetic integrity; the genetic profile of these mice was consistent with that of the B6C3F$_1$ strain. At the end of the study, serologic analyses were performed on five male and five female sentinel rats and control mice under the protocols of the NTP Sentinel Animal Program (Appendix L).

Groups of 10 male and 10 female rats and mice were exposed to 0, 94, 188, 375, 750, or 1,500 ppm nitromethane by inhalation, 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 13 weeks. Additional groups of 10 male and 10 female rats designated for clinical pathology evaluations received the same exposure concentrations as the core study rats. Water was available *ad libitum*; feed was available *ad libitum* except during exposure periods. Rats and mice were housed individually. Clinical observations were recorded weekly. The core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Neurobehavior tests including forelimb and hindlimb grip strength measurements, response to stimulus (tail flick latency), and startle response were performed on all male and female rats in the core study over a 2-day period during week 11. Rats were allowed to acclimate to the testing room for at least 2 hours. Forelimb and hindlimb grip strengths were measured

by allowing each rat to grip a triangular ring with its forepaws; the rat was pulled back along a channel until its forelimb grip was broken. While the backward motion continued, the rat was allowed to grasp a T-bar in the same channel with its hindpaws, then forced to release the bar by continued pulling. The strain required to break the forelimb and hindlimb grip was recorded with a calibrated push-pull strain gauge; for each animal, the means of three successive readings were determined for forelimb and hindlimb grip strength. Tail flick latency was measured with a Tail Flick Analgesiometer (Socrel, Varese, Italy), consisting of an infrared heat source (100 W) with radiant energy of adjustable intensity focused by a parabolic mirror on a photocell. Each rat was placed with its tail on the photocell window and a footpedal was depressed to activate the heat source and a timer; when the rat felt pain and flicked its tail, the photocell became energized, turning off the timer and the heat source. The reaction time was recorded as the time from heat onset to tail flick. Startle response to acoustic stimulation was measured with an SR Lab System (SRI, Scientific and Professional Support Group, La Jolla, CA); this system, which was located in an isolation chamber, measured the response of each rat to a series of six 40-millisecond bursts of 120 dB white noise, spaced 15 seconds apart, after the animal was acclimated to the system for 5 minutes. The maximum response amplitude and the time to reach the maximum response were measured.

Clinical pathology analyses were performed on rats designated for clinical pathology evaluation on days 3 and 23 and on core study rats at the end of the 13-week study. Rats were anesthetized and blood was withdrawn from the retroorbital plexus. Blood for hematology determinations was placed in collection tubes containing potassium EDTA as an anticoagulant. Blood for clinical chemistry evaluations was placed in tubes without anticoagulant and allowed to clot; these samples were then centrifuged and serum was removed. Erythrocyte and leukocyte counts, hematocrit, hemoglobin concentration, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and platelet counts were measured on an Ortho ELT-8/ds Hematology Counter (Ortho Instruments, Westwood, MA). Differential leukocyte counts, morphologic evaluation of blood cells, and nucleated erythrocyte counts were determined by light microscopic examination of blood films stained with Wright-Giemsa. Methemoglobin was measured within 30 minutes of blood collection with an IL co-oximeter (Instrumentation Laboratory, Inc., Lexington, MA). Clinical chemistry determinations were performed on an Abbott VP chemistry analyzer (Abbott Laboratories, Abbott Park, IL) with commercially available reagents. Serum triiodothyronine, thyroxine, free thyroxine, and thyroid-stimulating hormone concentrations were determined by ^{125}I radioimmunoassay techniques on a Packard Auto-Gamma counter (Packard Instrument Company, Downers Grove, IL). Reagents for triiodothyronine, thyroxine, and free thyroxine assays were obtained commercially; thyroid-stimulating hormone concentration measurements were performed with reference material obtained from the National Hormone and Pituitary Program. The hematology and clinical chemistry parameters measured are listed in Table 1.

At the end of the 13-week studies, samples were collected for sperm motility and vaginal cytology evaluations from all rats and mice in the 0, 375, 750, and 1,500 ppm groups. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm motility and vaginal cytology evaluations protocol (NTP, 1987). For 7 consecutive days before the 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 count and motility. The left epididymis and testis 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 left 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, the 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 core study animals. The heart, right kidney, liver, lungs, right testis, thymus, and thyroid gland (rats only) 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; additional sections of the spinal cord and sciatic nerve of rats were stained with Sevier-Munger Luxol Fast Blue to allow for a more complete evaluation of myelin around sciatic nerve axons. A complete histopathologic examination was performed on core study rats and mice in the 0 and 1,500 ppm groups. Additionally, all gross lesions and tissue masses and selected tissues of rats and mice in the lower exposure groups were examined. The tissues and organs examined are listed in Table 1.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were exposed to nitromethane by inhalation, 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 103 weeks. Rats were exposed to 0, 94, 188, or 375 ppm. Mice were exposed to 0, 188, 375, or 750 ppm.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA) for use in the 2-year studies. Rats and mice were quarantined for 14 days before the studies began. Five male and five female rats and mice were selected for parasite evaluation and gross observation of disease. Additionally, the kidneys of five male and five female mice were screened to ensure genetic integrity; the genetic profile of these mice was consistent with that of the B6C3F$_1$ strain. Rats and mice were approximately 6 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 L).

Animal Maintenance

Rats and mice were housed individually. Water was available *ad libitum*; feed was available *ad libitum* except during exposure periods. Cages were rotated within the inhalation chambers weekly. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded monthly through week 91, then every 2 weeks until the end of the studies. Animals were weighed at the beginning of the studies, weekly through week 12, monthly from week 15 through week 91, every 2 weeks thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. 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, embedded 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. Complete histopathologic examinations were performed on all animals. The sciatic nerves and spinal cords from approximately 15 male and 15 female rats in the 0 and 375 ppm groups were examined. For extended evaluation of renal tubule proliferative lesions in male rats, kidneys were step-sectioned at 1-mm intervals, and four additional sections were obtained from each kidney. The tissues and organs routinely examined 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 microscopic 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 studies, a quality assessment pathologist reviewed the lung, nasal cavity, and all neoplasms of all male and female rats; the kidney of all male rats; the mammary gland of all female rats; the sciatic nerve and spinal cord of approximately 15 male and 15 female rats in the 0 and 375 ppm groups; and the harderian gland, liver, lung, and nose and all neoplasms of all male and female mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed 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 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 quality assessment 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, A4, 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., skin, intestine, harderian gland, and mammary gland) 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

The majority of neoplasms in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, 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. For further discussion of these statistical methods, refer to Haseman (1984).

Using individual animal data from more than 3,000 rats and mice in the NTP historical control database, Seilkop (1995) demonstrated that certain site-specific neoplasms are strongly correlated with body weight. Seilkop also developed a logistic regression model that accurately predicted the control incidence of these neoplasms based on survival and 52-week body weights. The Seilkop model was used in these studies to evaluate the possible impact of survival and body weight differences on the incidence of mammary gland neoplasms in female F344/N rats and liver neoplasms in B6C3F$_1$ mice, the neoplasms having the strongest correlation with body weights.

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.

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 and neurobehavior data, which have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed with 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 with 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 nitromethane was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of nitromethane are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

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

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		
Simonsen Laboratories, Inc. (Gilroy, CA)	Simonsen Laboratories, Inc. (Gilroy, CA)	Simonsen Laboratories, Inc. (Gilroy, CA)
Time Held Before Studies		
Rats: 12 days Mice: 13 days	Rats: 13 (male) or 14 days (female) Mice: 14 days	14 days
Average Age When Studies Began		
7 weeks	6 weeks	7 weeks
Date of First Dose		
Rats: 14 March 1988 Mice: 15 March 1988	Rats: 5 (male) or 6 (female) July 1988 Mice: 6 July 1988	Rats: 7 September 1989 Mice: 31 August 1989
Duration of Dosing		
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 103 weeks
Date of Last Dose		
Rats: 29 March 1988 Mice: 30 March 1988	Rats: 3 (male) or 4 (female) October 1988 Mice: 5 (male) or 6 (female) October 1988	Rats: 28 August 1991 Mice: 21 August 1991
Necropsy Dates		
Rats: 30 March 1988 Mice: 31 March 1988	Rats: 4 (male) or 5 (female) October 1988 Mice: 6 (male) or 7 (female) October 1988	Rats: 9-11 September 1991 Mice: 3-6 September 1991
Average Age at Necropsy		
9 weeks	Rats: 19 weeks Mice: 19 (male) or 20 weeks (female)	Rats: 111 weeks Mice: 111-112 weeks
Size of Study Groups		
5 males and 5 females	10 males and 10 females	50 males and 50 females
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

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

16-Day Studies	13-Week Studies	2-Year Studies
Animals per Cage		
1	1	1
Method of Animal Identification		
Rats: tail tattoo Mice: toe clip	Tail tattoo	Tail tattoo
Diet		
NIH-07 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum* except during exposure periods, changed weekly	Same as 16-day studies	Same as 16-day studies
Water Distribution		
Softened tap water (City of Richland municipal supply) via automatic watering system (Systems Engineering, Napa, CA), available *ad libitum*	Softened tap water (City of Richland municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 16-day studies
Cages		
Stainless steel wire-bottom cages (Lab Products, Inc., Harford Systems, Aberdeen, MD); changed weekly and rotated in chamber daily	Same as 16-day studies, but rotated in chamber weekly	Stainless steel wire-bottom cages (Lab Products, Inc., Maywood, NJ); changed and rotated in chamber weekly
Chambers		
Stainless steel chambers (Lab Products Inc., Harford Systems, Aberdeen, MD), changed weekly	Same as 16-day studies	Same as 16-day studies
Chamber Filters		
Single HEPA (Flanders Filters, Inc., San Rafael, CA) and charcoal (RSE, Inc., New Baltimore, MI)	Same as 16-day studies	Same as 16-day studies
Chamber Environment		
Mean temperature: 22.5° to 24.2° C Mean relative humidity: 51% to 55% Room fluorescent light: 12 hours/day Chamber air: 14.5 to 15.9 ft^3/minute	Mean temperature: 23.2° to 24.1° C Mean relative humidity: 55% to 57% Room fluorescent light: 12 hours/day Chamber air: 14.4 to 14.6 ft^3/minute	Mean temperature: 23.9° to 24.3° C (rats), 23.5° to 23.9° C (mice) Mean relative humidity: 55% to 57% (rats), 54% to 56% (mice) Room fluorescent light: 12 hours/day Chamber air: 15.1 to 15.6 ft^3/minute (rats), 14.6 to 14.7 ft^3/minute (mice)
Exposure Concentrations		
0, 94, 188, 375, 750, or 1,500 ppm	0, 94, 188, 375, 750, or 1,500 ppm	Rats: 0, 94, 188, or 375 ppm Mice: 0, 188, 375, or 750 ppm

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

16-Day Studies	13-Week Studies	2-Year Studies
Type and Frequency of Observation		
Observed and clinical observations recorded twice daily; animals were weighed initially, on day 8, and at the end of the studies.	Observed twice daily; animals were weighed initially, weekly, and at the end of the studies; clinical observations were recorded weekly.	Observed twice daily; clinical observations were recorded monthly through week 91, then every 2 weeks until the end of the studies. Animals were weighed initially, weekly through week 12, monthly from week 15 through week 91, every 2 weeks thereafter, and at the end of the studies.
Method of Sacrifice		
Asphyxiation with 70% CO_2	Asphyxiation with 70% CO_2	Asphyxiation with 70% CO_2
Necropsy		
Necropsy performed on all animals. Organs weighed were heart, right kidney, liver, lungs, right testis, thymus, and thyroid glands (rats only).	Necropsy performed on all core study animals. Organs weighed were heart, right kidney, liver, lungs, right testis, thymus, and thyroid glands (rats only).	Necropsy performed on all animals.
Clinical Pathology		
None	Blood was collected from all clinical pathology group rats on days 3 and 23 and from all core study rats at the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital plexus of animals anesthetized with 70% CO_2 after 2 or 3 consecutive days of exposure. ***Hematology:*** hematocrit, hemoglobin concentration, erythrocyte counts, nucleated erythrocyte counts, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet counts, total leukocyte count and differentials, and methemoglobin concentration ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, and globulin concentrations; alanine aminotransferase, alkaline phosphatase, creatine kinase, and sorbitol dehydrogenase activities; bile acid, thyroid-stimulating hormone, triiodothyronine, and total and free thyroxine concentrations	None

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

16-Day Studies	13-Week Studies	2-Year Studies
Histopathology Histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: brain, larynx, lung and attached tracheobronchial lymph nodes, nose, sciatic nerve (rats), and trachea (longitudinal and transverse sections).	Complete histopathology was performed on core study rats and mice in the 0 and 1,500 ppm groups. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, eyes (if grossly abnormal), gallbladder (mice), heart, kidney, large intestine (cecum, colon, and rectum), larynx, liver, lung, lymph nodes (bronchial, mandibular, mediastinal, and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pharynx (if grossly abnormal), pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, and ileum), spinal cord and sciatic nerve (rats), spleen, stomach (forestomach and glandular stomach), testis, thigh muscle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). Additionally, the bone marrow, lung, and nose of male and female rats; cecum, larynx, and testis of male rats; and the nose and spleen of male and female mice in the 94, 188, 375, and 750 ppm groups were examined until a no-effect level was reached.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, bone and marrow, brain, clitoral gland, epididymis, esophagus, gallbladder (mice), harderian gland (mice), heart, kidney, large intestine (cecum, colon, and rectum), larynx, liver, lung, lymph nodes (bronchial, mandibular, mediastinal, and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, and ileum), spinal cord and sciatic nerve (a limited review of male and female rats in the 0 and 375 ppm groups), spleen, stomach (forestomach and glandular stomach), testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.
Sperm Motility and Vaginal Cytology Evaluations None	Rats and mice in the 0, 375, 750, and 1,500 ppm groups were evaluated. Sperm samples were collected at the end of the studies and evaluated for sperm count and motility. The left cauda, epididymis, and testis were weighed. Vaginal samples were collected for 7 consecutive days before the end of the studies and evaluated for the relative frequency of estrous stages and for estrous cycle length.	None
Neurobehavioral Evaluations None	Neurobehavior testing was performed on core study rats over a 2-day period during week 11 of the study. Parameters measured included forelimb and hindlimb grip strength, tail flick latency, and startle response.	None

RESULTS

RATS

16-DAY STUDY

All rats survived until the end of the study (Table 2). The mean body weight gain of male rats in the 1,500 ppm group was slightly but significantly less than that of the controls; the final mean body weights and mean body weight gains of exposed females were similar to those of the controls. Clinical findings of toxicity were observed in all male and female rats in the 1,500 ppm groups and included increased preening, rapid breathing, hyperactivity early in the study, and hypoactivity and loss of coordination in the hindlimbs near the end of the study.

The relative liver weights of all exposed groups of male rats and the absolute and relative liver weights of females exposed to 375 ppm or greater were significantly greater than those of the controls (Table F1). The relative kidney weights of male rats in the 750 and 1,500 ppm groups and female rats in the 1,500 ppm group were significantly greater than those of the controls; other differences in organ weights between exposed and control rats were secondary to body weight changes. Absolute and relative lung weights of exposed rats were similar to those of the controls.

TABLE 2
Survival and Body Weights of Rats in the 16-Day Inhalation Study of Nitromethane

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	145 ± 4	182 ± 4	38 ± 2	
94	5/5	147 ± 4	189 ± 5	43 ± 2	104
188	5/5	146 ± 4	187 ± 5	41 ± 2	103
375	5/5	145 ± 3	182 ± 6	37 ± 3	100
750	5/5	144 ± 3	177 ± 4	34 ± 1	97
1,500	5/5	146 ± 3	171 ± 4	25 ± 3**	94
Female					
0	5/5	116 ± 2	134 ± 3	17 ± 1	
94	5/5	116 ± 2	135 ± 3	18 ± 3	101
188	5/5	116 ± 2	133 ± 2	17 ± 1	99
375	5/5	116 ± 2	133 ± 2	18 ± 2	100
750	5/5	117 ± 2	132 ± 1	15 ± 2	99
1,500	5/5	117 ± 2	128 ± 2	11 ± 1	96

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

All males exposed to 375 ppm or greater, all females in the 750 and 1,500 ppm groups, and four females in the 375 ppm group had degeneration of the olfactory epithelium of the nasal turbinates; this lesion was of minimal to mild severity in exposed males and females (Table 3). There were no exposure-related lesions in the lungs of exposed male or female rats.

During the 16-day study, neurobehavioral effects were not sufficient to warrant evaluation of nervous system tissues. However, because of the hindlimb paralysis and histopathologic effects on the sciatic nerve in the 13-week study, the sciatic nerves from rats in the 16-day study were subsequently evaluated histopathologically. Sciatic nerve degeneration was present in all male and female rats exposed to 375 ppm or greater (Table 3). This lesion was characterized by prominent, diffuse vacuolization and dilatation of the axonal sheaths and increased cellularity, which was apparently due to Schwann cell hyperplasia. The severity of these lesions increased with increasing exposure concentration and ranged from minimal to moderate. Rats exposed to 750 or 1,500 ppm had significantly less myelin around the sciatic axons than did the controls.

Exposure Concentration Selection Rationale: Due to the lack of significant toxicologic or histopathologic effects, including the absence of histopathologic effects in the lung, nitromethane exposure concentrations selected for use in the 13-week study were the same as for the 16-day study. The sciatic nerve degeneration in rats in the 16-day study was not discovered until after the conclusion of the 13-week study.

TABLE 3
Incidences of Selected Nonneoplastic Lesions in Rats in the 16-Day Inhalation Study of Nitromethane

	0 ppm	94 ppm	188 ppm	375 ppm	750 ppm	1,500 ppm
Male						
Nose/Turbinates[a]	5	5	5	5	5	5
Degeneration, Olfactory Epithelium[b]	0	0	0	5** (1.0)[c]	5** (2.0)	5** (2.0)
Sciatic Nerve	4	5	5	5	5	5
Degeneration	0	0	0	5** (1.0)	5** (2.0)	5** (3.0)
Female						
Nose/Turbinates						
Degeneration, Olfactory Epithelium	5 0	5 0	5 0	5 4* (1.0)	5 5** (1.8)	5 5** (2.0)
Sciatic Nerve	5	5	5	5	5	5
Degeneration	0	0	0	5** (1.0)	5** (2.0)	5** (3.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected rats: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

13-Week Study

All rats survived to the end of the study (Table 4). The final mean body weight and weight gain of male rats in the 1,500 ppm group were significantly less than those of the controls. Clinical findings included hindlimb paralysis in all male and female rats in the 1,500 ppm groups, beginning on day 21, and one male and four females in the 750 ppm groups, beginning on day 63.

Hematology and clinical chemistry data are provided in Table G1. Exposure to nitromethane caused an exposure concentration-dependent, microcytic, responsive anemia in rats. The anemia was characterized by mild to moderate decreases in hematocrit values and hemoglobin concentrations, and the microcytosis was evidenced by minimal to moderate decreases in mean cell volume. Hematocrit values and hemoglobin concentrations were less than those of the controls for male and female rats in the 375, 750, and 1,500 ppm groups at all time points and in the 94 and 188 ppm groups at various time points. Additionally, erythrocyte counts on day 3 were minimally to mildly decreased in males exposed to 188 ppm or greater and females exposed to 750 or 1,500 ppm compared those of the controls; this finding is consistent with anemia. The decreases in mean cell volume occurred in all groups of exposed females at all time points and in all groups of exposed males at the end of the study; decreased mean cell volumes indicate increased numbers of smaller erythrocytes in the circulation. Review of erythrocyte size distribution information on day 23 revealed that two distinct populations of erythrocytes were present in rats in the higher exposure groups; one of these populations consisted of smaller

TABLE 4
Survival and Body Weights of Rats in the 13-Week Inhalation Study of Nitromethane

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	107 ± 3	334 ± 7	228 ± 6	
94	10/10	105 ± 2	323 ± 7	218 ± 7	97
188	10/10	113 ± 2	345 ± 4	232 ± 3	103
375	10/10	109 ± 3	336 ± 5	227 ± 4	101
750	10/10	106 ± 2	327 ± 4	221 ± 5	98
1,500	10/10	109 ± 2	295 ± 10**	185 ± 9**	88
Female					
0	10/10	95 ± 1	185 ± 5	90 ± 3	
94	10/10	96 ± 2	197 ± 3	101 ± 2	107
188	10/10	97 ± 2	197 ± 3	100 ± 2	106
375	10/10	95 ± 2	198 ± 5	103 ± 4**	107
750	10/10	96 ± 2	194 ± 4	97 ± 2	105
1,500	10/10	94 ± 2	177 ± 4	84 ± 3	96

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

erythrocytes. Additionally, microcytosis was observed in the blood smears of exposed rats; microcytosis is consistent with the decreased mean cell volume. At all time points, mean cell hemoglobin values of exposed rats were decreased compared to those of the controls; these decreases were attributed to the smaller erythrocyte sizes. The mean cell hemoglobin concentrations of males exposed to 750 or 1,500 ppm and females exposed to 1,500 ppm were minimally greater than those of the controls at all time points. Increases in mean cell hemoglobin concentration have been related to erythrocyte hemolysis (*in vivo* or *in vitro*). On day 23 and at week 13, a hematopoietic response was evidenced by increased numbers of nucleated erythrocytes in exposed animals compared to the controls.

Exposure concentration-dependent alterations in erythrocyte morphology occurred at all time points. On day 3, minimal numbers of Heinz bodies were observed in male rats in the 750 and 1,500 ppm groups; other red blood cell changes were present in males and females in the higher exposure groups on day 23 and at week 13. Morphologic alterations of red blood cells included anisocytosis (e.g., microcytes and spherocytes), poikilocytosis (e.g., schistocytes and acanthocytes), polychromasia, and target cells. The presence of Heinz bodies would be consistent with oxidative red blood cell damage. Schistocytes are irregular erythrocyte fragments that usually result from trauma to red blood cells; the presence of these would suggest a hemolytic process. Spherocytes have been observed in conjunction with immune-mediated and Heinz body anemias. Polychromasia would be consistent with the presence of young erythrocytes (reticulocytes) and would indicate a bone marrow response to the anemia.

Minimal, exposure concentration-dependent increases in methemoglobin concentration occurred in male and female rats, indicating oxidative red cell injury. Male rats exposed to 375 ppm or greater had minimally increased methemoglobin concentrations compared to the controls at all time points. Methemoglobin concentrations were also minimally increased in females in the 750 and 1,500 ppm groups compared to the controls on day 23 and at 13 weeks.

Platelet counts in all groups of exposed rats were mildly to markedly increased compared to the controls at all time points. A review of platelet size distribution information on day 23 revealed a wide size-distribution curve and indicated the presence of significant numbers of large platelets or particles counted as platelets. Increased platelet counts may be consistent with a reactive thrombocytosis, which can be caused by a variety of conditions, including bone marrow response to anemia. In light of the evidence suggestive of a hemolytic process (e.g., schistocytes, Heinz bodies, and increased mean cell hemoglobin concentration and methemoglobin concentration), the erroneous inclusion of small erythrocyte fragments in the platelet count could, in part, account for the platelet count increases.

On day 23, a hypothyroid state, evidenced by decreased serum triiodothyronine, thyroxine, and free thyroxine, occurred in males exposed to 375 ppm or greater and females exposed to 750 or 1,500 ppm; thyroxine concentrations of female rats in the 188 and 375 ppm groups were also decreased. There was little or no pituitary response to the thyroid hormone decreases, as evidenced by the lack of significantly increased thyroid-stimulating hormone concentrations in exposed rats. The change in thyroid hormone concentrations was transient, and at 13 weeks, hormone concentrations of exposed rats were similar to those of the controls. Differences in other hematology and clinical chemistry variables were not related to exposure and were not considered biologically significant.

No biologically significant differences in organ weights between exposed and control rats were observed (Table F2). The forelimb and hindlimb grip strengths of males in the 1,500 ppm group were significantly less than those of the controls (Table I1). The hindlimb grip strengths of females in the 750 and 1,500 ppm groups were also significantly less than the control value.

Male rats in the 750 and 1,500 ppm groups had significantly lower epididymal spermatozoal motility than the controls; the left cauda, epididymis, and testis weights of males in the 1,500 ppm group were also significantly less than those of the controls (Table H1). There were no biologically significant differences in the length of the estrous cycle or in the

relative amounts of time spent in the various estrous stages between exposed and control females.

At necropsy, no gross lesions were observed that were considered related to nitromethane exposure. Minimal to mild hyperplasia of the bone marrow was observed microscopically in male rats in the 750 and 1,500 ppm groups and in females exposed to 188 ppm or greater (Table 5). Olfactory epithelial degeneration was observed in males and females exposed to 375 ppm or greater and in one female in the 188 ppm group. Respiratory epithelial hyaline droplets and goblet cell hyperplasia were observed in males and females in the 750 and 1,500 ppm groups (Table 5); the severity of nasal lesions in exposed males and females ranged from minimal to mild.

Males and females exposed to 375 ppm or greater had minimal to mild degeneration of the spinal cord and sciatic nerve (Table 5). Sciatic nerve degeneration, as observed in the 16-day study, was observed in most rats exposed to 375 ppm and in all rats exposed to 750 or 1,500 ppm; however, the degeneration in exposed rats in the 13-week study was less severe than that observed in the 16-day study. In rats exposed to 1,500 ppm, the lesion was considered mild and was characterized by focal dilatation, with foci containing eosinophilic debris, and vacuolization of the axonal sheaths. Increased cellularity, presumably due to Schwann cell hyperplasia, was apparent, primarily in rats exposed to 1,500 ppm. The presence of inflammatory cells and myelin debris was less prevalent than in the 16-day study. Minimal to mild degeneration of the lumbar spinal cord was present in some rats exposed to 375 ppm and in all rats exposed to 750 or 1,500 ppm. This lesion was characterized by focal vacuolization in the white matter of the lumbar region of the cord and, to a greater extent, in the spinal nerves. The foci contained eosinophilic, granular debris.

Exposure Concentration Selection Rationale: Due to the increased incidences and severity of degeneration of the sciatic nerve and spinal cord in rats exposed to 750 or 1,500 ppm and to the rather minimal changes in the 375 ppm groups, nitromethane exposure concentrations selected for the 2-year study in rats were 94, 188, and 375 ppm.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 13-Week Inhalation Study of Nitromethane

	0 ppm	94 ppm	188 ppm	375 ppm	750 ppm	1,500 ppm
Male						
Bone Marrow[a]	10	10	10	10	10	10
Hyperplasia[b]	0	0	0	0	9** (1.1)[c]	10** (2.0)
Nose/Turbinates	10	—[d]	10	10	10	10
Degeneration, Olfactory Epithelium	0		0	9** (1.0)	10** (1.0)	10** (1.0)
Hyaline Droplets, Respiratory Epithelium	0		0	0	1 (1.0)	8** (1.0)
Hyperplasia, Goblet Cell	0		0	0	1 (1.0)	10** (2.0)
Sciatic Nerve	10	—	10	10	10	10
Degeneration	0		0	5* (1.0)	10** (1.2)	10** (1.5)
Spinal Cord	10	—	10	10	10	10
Degeneration	0		0	9** (1.0)	10** (1.4)	10** (2.0)
Female						
Bone Marrow	10	10	10	10	10	10
Hyperplasia	0	0	1 (2.0)	6** (1.0)	7** (1.1)	10** (1.7)
Nose/Turbinates	10	10	10	10	10	10
Degeneration, Olfactory Epithelium	0	0	1 (1.0)	10** (1.0)	10** (1.2)	10** (1.8)
Hyaline Droplets, Respiratory Epithelium	0	0	0	0	4* (1.0)	10** (1.0)
Hyperplasia, Goblet Cell	0	0	0	0	2 (1.5)	10** (1.7)
Sciatic Nerve	10	—	10	10	10	10
Degeneration	0		0	8** (1.0)	10** (1.1)	10** (1.8)
Spinal Cord	10	—	10	10	10	10
Degeneration	0		0	2 (1.0)	10** (1.4)	10** (1.9)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected rats: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[d] Not examined at this exposure concentration

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 1). There were no significant differences in survival rates between exposed and control male or female rats.

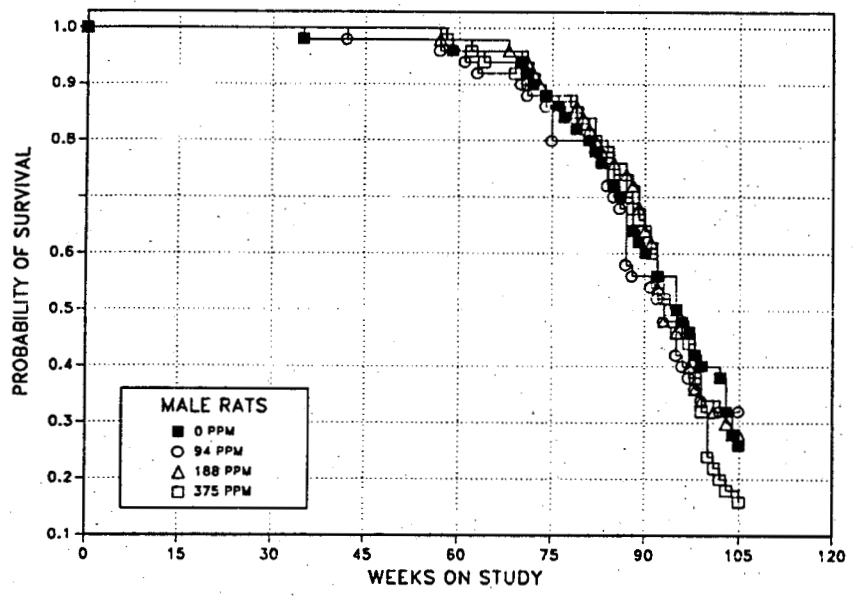
Body Weights and Clinical Findings

Mean body weights are given in Figure 2 and Tables 7 and 8. From week 23 to the end of the study, the mean body weight of females in the 375 ppm group was slightly greater than that of the control group. The mean body weights of exposed males were generally similar to the mean body weight of the controls throughout the study. Clinical findings (masses on shoulder and torso) consistent with mammary gland neoplasms were observed in females in the 188 and 375 ppm groups during the course of the study; there were no indications of hindlimb paralysis, as observed in the 13-week study, or other treatment-related clinical findings during the study.

TABLE 6
Survival of Rats in the 2-Year Inhalation Study of Nitromethane

	0 ppm	94 ppm	188 ppm	375 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	33	31	34	39
Natural deaths	4	3	2	3
Animals surviving to study termination	13	16	14	8
Percent probability of survival at end of study[a]	26	32	28	16
Mean survival (days)[b]	642	631	646	640
Survival analysis[c]	P=0.378	P=1.000N	P=1.000N	P=0.361
Female				
Animals initially in study	50	50	50	50
Moribund	17	26	18	25
Natural deaths	5	5	2	2
Animals surviving to study termination	28	19	30	23
Percent probability of survival at end of study	56	38	60	46
Mean survival (days)	683	653	679	670
Survival analysis	P=0.780	P=0.083	P=0.900N	P=0.404

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A lower mortality in an exposed group is indicated by N.



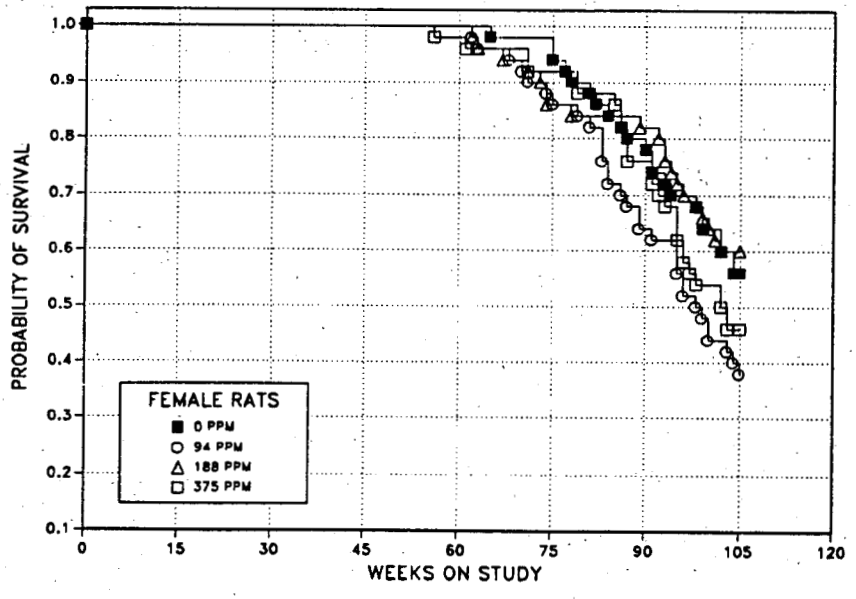


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Nitromethane by Inhalation for 2 Years

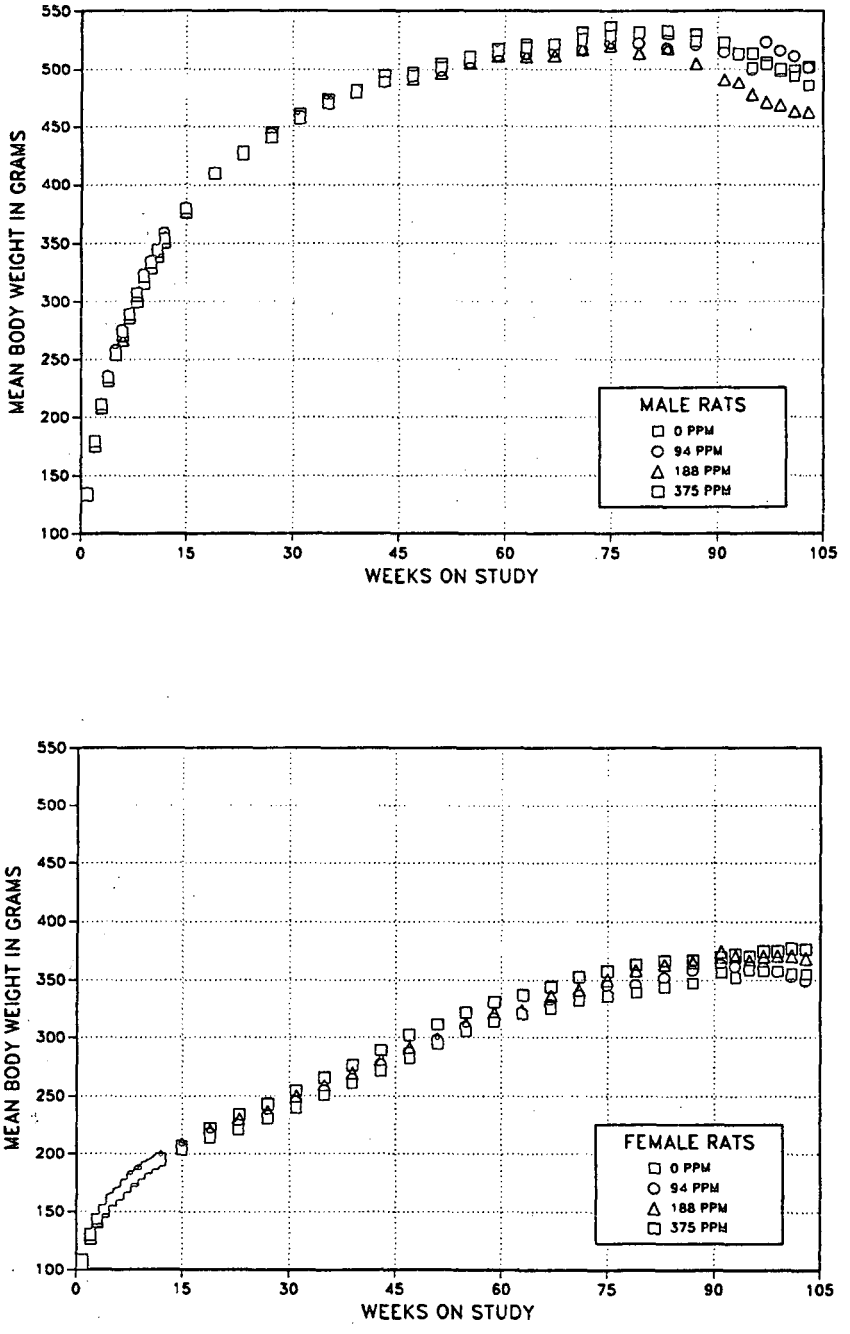


FIGURE 2
Growth Curves for Male and Female Rats Exposed to Nitromethane by Inhalation for 2 Years

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Inhalation Study of Nitromethane

Weeks on Study	0 ppm		94 ppm			188 ppm			375 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	135	50	135	100	50	135	100	50	133	98	50
2	180	50	179	100	50	179	100	50	175	98	50
3	211	50	211	100	50	211	100	50	208	99	50
4	234	50	236	101	50	235	100	50	231	99	50
5	255	50	258	101	50	255	100	50	253	99	50
6	274	50	276	101	50	267	98	50	271	99	50
7	289	50	290	100	50	289	100	50	286	99	50
8	307	50	308	100	50	306	99	50	300	98	50
9	322	50	324	101	50	322	100	50	316	98	50
10	333	50	335	101	50	334	100	50	329	99	50
11	345	50	344	100	50	343	100	50	338	98	50
12	354	50	359	102	50	355	100	50	351	99	50
15	379	50	380	100	50	380	100	50	376	99	50
19	409	50	410	100	50	410	100	50	410	100	50
23	425	50	428	101	50	426	100	50	429	101	50
27	441	50	444	101	50	441	100	50	445	101	50
31	456	50	460	101	50	458	100	50	462	101	50
35	470	49	473	101	50	471	100	50	474	101	50
39	480	49	481	100	50	480	100	50	482	101	50
43	489	49	488	100	49	490	100	50	495	101	50
47	495	49	494	100	49	492	99	50	497	101	50
51	502	49	498	99	49	496	99	50	505	101	50
55	510	49	505	99	49	506	99	50	511	100	50
59	516	48	512	99	48	511	99	49	518	100	49
63	518	48	513	99	46	511	99	49	521	101	48
67	521	48	515	99	46	512	98	49	522	100	47
71	526	46	517	98	44	517	98	47	532	101	45
75	529	44	523	99	40	521	98	44	537	102	44
79	531	41	523	98	40	514	97	43	532	100	42
83	533	38	518	97	39	518	97	39	531	100	40
87	524	35	521	100	30	505	96	37	530	101	35
91	522	30	514	99	28	491	94	31	522	100	31
93	512	28	513	100	25	489	95	27	513	100	28
95	501	27	499	100	24	478	95	24	514	103	24
97	503	23	523	104	19	471	94	22	506	100	22
99	500	21	516	103	18	469	94	18	499	100	18
101	493	20	511	104	17	463	94	17	499	101	11
103	486	17	501	103	16	462	95	16	502	103	9
Mean for weeks											
1-13	270		271	100		269	100		266	99	
14-52	455		456	100		454	100		458	101	
53-103	514		514	100		496	96		518	101	

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Inhalation Study of Nitromethane

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	94 ppm Av. Wt. (g)	94 ppm Wt. (% of controls)	94 ppm No. of Survivors	188 ppm Av. Wt. (g)	188 ppm Wt. (% of controls)	188 ppm No. of Survivors	375 ppm Av. Wt. (g)	375 ppm Wt. (% of controls)	375 ppm No. of Survivors
1	108	50	108	100	50	108	100	50	107	99	50
2	130	50	130	100	50	130	99	50	126	97	50
3	143	50	143	100	50	142	99	50	140	98	50
4	151	50	152	100	50	150	99	50	149	99	50
5	159	50	161	101	50	160	101	50	159	100	50
6	164	50	166	101	50	167	101	50	165	100	50
7	172	50	174	101	50	173	101	50	171	100	50
8	178	50	181	102	50	180	101	50	177	99	50
9	182	50	186	102	50	186	102	50	182	100	50
10	187	50	189	101	50	191	102	50	188	101	50
11	191	50	193	101	50	194	102	50	191	100	50
12	194	50	196	101	50	198	102	50	196	101	50
15	203	50	206	102	50	209	103	50	207	102	50
19	214	50	218	102	50	221	103	50	222	104	50
23	220	50	227	103	50	230	105	50	234	107	50
27	230	50	234	102	50	237	103	50	243	106	50
31	239	50	246	103	50	250	104	50	255	107	50
35	250	50	255	102	50	260	104	50	266	106	50
39	261	50	265	102	50	270	104	50	276	106	50
43	272	50	277	102	50	281	103	50	289	107	50
47	282	50	287	102	50	291	103	50	303	107	50
51	294	50	298	101	50	299	102	50	311	106	50
55	306	50	310	101	50	312	102	50	322	106	50
59	314	50	317	101	50	321	103	50	332	106	49
63	320	50	323	101	48	324	101	49	337	105	48
67	325	49	329	101	48	337	104	47	344	106	48
71	332	49	337	101	45	342	103	46	353	106	46
75	336	47	346	103	43	350	104	43	358	107	46
79	340	45	346	102	42	358	106	42	364	107	44
83	344	43	352	102	38	364	106	42	367	107	44
87	347	40	359	103	34	365	105	42	367	106	38
91	356	37	364	102	32	375	105	41	370	104	37
93	352	37	362	103	31	370	105	39	372	106	35
95	358	35	359	100	30	367	103	37	371	104	33
97	358	35	361	101	26	370	104	35	375	105	28
99	358	32	357	100	24	371	104	33	375	105	27
101	355	32	353	99	22	371	104	31	377	106	27
103	355	30	350	99	22	369	104	30	376	106	25
Mean for weeks											
1-13	163		165	101		165	101		163	100	
14-52	247		251	102		255	103		261	106	
53-103	341		345	101		354	104		360	106	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the mammary gland and kidney and in the incidences of mononuclear cell leukemia. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Mammary Gland: The incidences of fibroadenoma, fibroadenoma or adenoma (combined), and fibroadenoma, adenoma, or carcinoma (combined) in female rats increased with increasing exposure concentration, and the incidences in the 188 and 375 ppm groups were significantly greater than those in the controls (Tables 9 and B3). Additionally, the incidences of carcinoma and adenoma or carcinoma (combined) in the 375 ppm group were significantly greater than those in the controls. The incidences of fibroadenoma and carcinoma exceeded the ranges of historical incidences for these neoplasms in untreated (chamber control) female rats in 2-year NTP inhalation studies (Table B4). No treatment-related mammary gland neoplasms were observed in male rats (Table A1).

The morphology of the mammary gland fibroadenomas was typical, with the lesions characterized by dense, fibrous tissue surrounding scattered glands (Plate 1). Adenomas were composed of glands with scant fibrous tissue. The carcinomas were very cellular lesions (Plate 2) that often contained a well-differentiated glandular formation (Plate 3); other mammary gland carcinomas exhibited a papillary pattern or showed more solid areas of growth. Mammary gland carcinomas contained occasional areas of necrosis and hemorrhage, and in some cases mitotic activity was quite high. The two carcinomas that metastasized to the lung (Plate 4) did not vary appreciably in morphology from the carcinomas that did not metastasize.

Hematopoietic System: The incidences of mononuclear cell leukemia in exposed female rats were lower than the control incidence (0 ppm, 22/50; 94 ppm, 13/50; 188 ppm, 14/50; 375 ppm, 9/50), and the difference was significant in the 375 ppm group (Table B3). The incidences in all exposed groups fell below the historical range (30% to 54%) for leukemias (all types) in female chamber control rats in 2-year NTP inhalation studies. In addition, the incidences of mononuclear cell leukemia in exposed males were slightly, although not significantly, less than the control incidence (35/50, 27/50, 33/50, 25/50; Table A3); however, these decreases was not related to exposure concentration. The biological significance of these decreases is uncertain. However, the incidences in exposed females were well within the range of historical incidences for leukemias in untreated female control rats in NTP noninhalation (feed) studies (14%-52%), and the incidences in exposed males were within or slightly above the range of historical incidences in untreated males (32%-64%).

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Mammary Gland in Female Rats in the 2-Year Inhalation Study of Nitromethane

	0 ppm	94 ppm	188 ppm	375 ppm
Mammary Gland[a]	50	50	50	50
Hyperplasia[b]	0	0	1 (3.0)[c]	2 (2.5)
Hyperplasia, Atypical	12 (1.7)	17 (1.2)	14 (1.4)	15 (1.7)
Fibroadenoma				
Overall rate[d]	19/50 (38%)	21/50 (42%)	33/50 (66%)	36/50 (72%)
Adjusted rate[e]	58.2%	68.5%	80.0%	92.1%
Terminal rate[f]	15/28 (54%)	10/19 (53%)	22/30 (73%)	20/23 (87%)
First incidence (days)	454	435	468	552
Logistic regression test[g]	P<0.001	P=0.219	P=0.003	P<0.001
Adenoma				
Overall rate	2/50 (4%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Carcinoma[h]				
Overall rate	2/50 (4%)	7/50 (14%)	1/50 (2%)	11/50 (22%)
Adjusted rate	6.0%	29.3%	2.0%	33.0%
Terminal rate	1/28 (4%)	4/19 (21%)	0/30 (0%)	5/23 (22%)
First incidence (days)	631	588	440	425
Logistic regression test	P=0.009	P=0.052	P=0.447N	P=0.011
Fibroadenoma, Adenoma, or Carcinoma[i]				
Overall rate	21/50 (42%)	25/50 (50%)	34/50 (68%)	41/50 (82%)
Adjusted rate	62.4%	74.9%	80.4%	95.2%
Terminal rate	16/28 (57%)	11/19 (58%)	22/30 (73%)	21/23 (91%)
First incidence (days)	454	435	440	425
Logistic regression test	P<0.001	P=0.112	P=0.006	P<0.001

[a] Number of animals necropsied
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Number of animals with neoplasm per number of animals necropsied
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by N.
[h] Historical incidence for all 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 25/653 (3.8% ± 2.7%); range, 0%-8%. Historical incidence (Battelle Pacific Northwest Laboratories): 14/348 (4.0% ± 2.6%); range, 0%-8%.
[i] Historical incidence (all laboratories): 202/653 (30.9% ± 9.1%); range, 16%-46%. Historical incidence (Battelle Pacific Northwest Laboratories): 108/348 (31.0% ± 8.1%); range, 22%-46%.

Kidney: At the end of the study, renal tubule hyperplasia and adenoma were observed in a few exposed males (Tables 10, A3, and A5); the incidences were not related to exposure concentration. The incidences of renal tubule adenoma in exposed males were within or only slightly above the historical incidence range of 0% to 4% for these neoplasms in male chamber control rats in NTP inhalation studies; however, because no hyperplasia or adenomas were observed in the control group, additional step sections of the kidneys of control and exposed males were prepared. Adenomas were observed in step sections of the kidneys of two males in each of the control and 94 ppm groups and four males in the 375 ppm group (Tables 10 and A3), including multiple adenomas in one male each in the 94 and 375 ppm groups. However, the combined incidences of renal tubule adenoma (from the single and step sections) were not significantly different from the control incidence. Renal tubule hyperplasia was also identified in step sections of kidneys from exposed and control males; the incidence in the 375 ppm group (from step sections and combined single and step sections) was slightly, but not significantly, greater than the control incidence.

Renal tubule hyperplasia consisted of tubules that were dilated approximately two to four times the normal diameter with lumens filled by clusters of renal tubule epithelial cells; these cells were somewhat pleomorphic, often with large nuclei with prominent nucleoli, and with cytoplasm varying from eosinophilic to slightly basophilic. Renal tubule adenomas consisted of cells that resembled those in the hyperplasia. However, the adenomas were larger (five or more tubule diameters) and generally had a more complex structure, often consisting of multiple, variably sized tubule-like structures or multiple solid clusters of cells separated by fine bands of stroma.

Nervous System: Histopathologic evaluation of hematoxylin- and eosin-stained sections of spinal cords and sciatic nerves from approximately 15 male and 15 female rats per group from the 0 and 375 ppm groups revealed no significant differences between exposed and control rats.

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Male Rats in the 2-Year Inhalation Study of Nitromethane

	0 ppm	94 ppm	188 ppm	375 ppm
Single Sections (Standard Evaluation)				
Kidney[a]	50	50	50	50
Nephropathy[b]	50 (2.8)[c]	50 (2.9)	50 (3.1)	50 (3.2)
Renal Tubule, Hyperplasia	0	3 (2.7)	2 (2.5)	1 (2.0)
Renal Tubule, Adenoma[d]				
Overall rate[e]	0/50 (0%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate[f]	0.0%	14.9%	14.3%	12.5%
Terminal rate[g]	0/13 (0%)	1/16 (6%)	2/14 (14%)	1/8 (13%)
First incidence (days)	—[i]	636	733 (T)	733 (T)
Logistic regression test[h]	P=0.487	P=0.107	P=0.252	P=0.403
Step Sections (Extended Evaluation)				
Kidney	50	50	50	50
Renal Tubule, Hyperplasia	6 (2.5)	7 (2.3)	5 (1.4)	12 (2.0)
Renal Tubule, Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	0/50 (0%)	4/50 (6%)
Adjusted rate	15.4%	12.5%	0.0%	22.7%
Terminal rate	2/13 (15%)	2/16 (13%)	0/14 (0%)	1/8 (13%)
First incidence (days)	733 (T)	733 (T)	—	650
Logistic regression test	P=0.184	P=0.622N	P=0.219N	P=0.283
Single Sections and Step Sections (Combined)				
Kidney	50	50	50	50
Renal Tubule, Hyperplasia	6 (2.5)	8 (2.5)	6 (1.7)	12 (2.0)
Renal Tubule, Adenoma				
Overall rate	2/50 (4%)	5/50 (10%)	2/50 (4%)	5/50 (10%)
Adjusted rate	15.4%	26.3%	14.3%	33.7%
Terminal rate	2/13 (15%)	3/16 (19%)	2/14 (14%)	2/8 (25%)
First incidence (days)	733 (T)	636	733 (T)	650
Logistic regression test	P=0.181	P=0.173	P=0.675N	P=0.158

(T)Terminal sacrifice
[a] Number of animals with kidney examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected rats: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Historical incidence for all 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 6/652 (0.9% ± 1.3%); range, 0%-4%. Historical incidence (Battelle Pacific Northwest Laboratories): 5/347 (1.4% ± 1.5%); range, 0%-4%.
[e] Number of animals with neoplasm per number of animals with kidney examined microscopically
[f] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[g] Observed incidence in animals surviving until the end of the study
[h] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by N.
[i] Not applicable; no neoplasms in animal group

MICE

16-DAY STUDY

All mice survived to the end of the study (Table 11). The final mean body weights and mean body weight gains of exposed males and females were similar to those of the controls. Clinical findings included hypoactivity and tachypnea in male and female mice in the 1,500 ppm groups near the end of the study.

The absolute and relative liver weights of male mice in the 750 and 1,500 ppm groups and female mice in all exposed groups were significantly greater than those of the controls (Table F3). The relative liver weight of males in the 375 ppm group was also significantly greater than that of the controls.

At necropsy, no lesions were observed grossly that were attributed to nitromethane exposure. Degeneration of the olfactory epithelium of the nose was observed microscopically in all males and females exposed to 375 ppm or greater; this lesion was of minimal severity in males and minimal to mild severity in females.

Exposure Concentration Selection Rationale: Due to the lack of significant toxicologic or histopathologic effects (including the absence of histopathologic effects in the lung), nitromethane exposure concentrations selected for use in the 13-week study were the same as for the 16-day study.

TABLE 11
Survival and Body Weights of Mice in the 16-Day Inhalation Study of Nitromethane

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	24.4 ± 0.3	26.7 ± 0.4	2.2 ± 0.3	
94	5/5	24.5 ± 0.3	27.5 ± 0.6	3.0 ± 0.6	103
188	5/5	24.5 ± 0.2	27.2 ± 0.5	2.8 ± 0.6	102
375	5/5	24.5 ± 0.2	26.5 ± 0.4	2.0 ± 0.4	99
750	5/5	24.6 ± 0.2	27.2 ± 0.5	2.5 ± 0.3	102
1,500	5/5	24.5 ± 0.2	27.1 ± 0.4	2.6 ± 0.3	102
Female					
0	5/5	18.8 ± 0.3	21.9 ± 0.4	3.1 ± 0.5	
94	5/5	18.8 ± 0.2	22.2 ± 0.3	3.4 ± 0.2	102
188	5/5	18.8 ± 0.3	22.0 ± 0.5	3.3 ± 0.5	101
375	5/5	18.8 ± 0.3	21.8 ± 0.5	3.0 ± 0.5	100
750	5/5	18.8 ± 0.3	22.4 ± 0.3	3.6 ± 0.4	102
1,500	5/5	18.9 ± 0.3	22.3 ± 0.3	3.4 ± 0.4	102

[a] Number of animals surviving at 16 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the control group were not significant by Dunnett's test.

13-Week Study

All mice survived to the end of the study (Table 12). The final mean body weights and mean body weight gains of exposed males were similar to those of the controls. The final mean body weights and mean body weight gains of exposed females were similar to or slightly greater than those of the controls. There were no treatment-related clinical findings.

The absolute right kidney weights of all groups of exposed male mice except the 1,500 ppm group and the relative right kidney weights of all groups of exposed males were significantly greater than those of the controls (Table F4); the absolute right kidney weights of females exposed to 188 ppm or greater and the relative right kidney weights of females in the 750 and 1,500 ppm groups were also significantly greater than those of the controls. The absolute liver weight of male mice in the 750 ppm group and the relative liver weights of males exposed to 375 ppm or greater were significantly greater than those of the controls.

Exposed male mice had significantly less epididymal spermatozoal motility than the controls (Table H2). The estrous cycle lengths of exposed females were significantly longer than the cycle length of the controls; exposed females spent more time in metestrus and proestrus and less time in estrus than the controls.

TABLE 12
Survival and Body Weights of Mice in the 13-Week Inhalation Study of Nitromethane

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	24.5 ± 0.2	34.3 ± 0.4	9.8 ± 0.4	
94	10/10	24.4 ± 0.2	34.1 ± 0.5	9.7 ± 0.6	100
188	10/10	24.0 ± 0.3	34.3 ± 0.8	10.3 ± 0.7	100
375	10/10	24.1 ± 0.4	34.1 ± 0.7	10.0 ± 0.5	100
750	10/10	24.3 ± 0.3	33.6 ± 0.3	9.3 ± 0.3	98
1,500	10/10	24.4 ± 0.3	34.1 ± 0.6	9.7 ± 0.4	99
Female					
0	10/10	19.8 ± 0.3	28.9 ± 0.7	9.1 ± 0.5	
94	10/10	19.9 ± 0.3	29.7 ± 0.7	9.8 ± 0.8	103
188	10/10	20.0 ± 0.3	30.9 ± 0.7	10.8 ± 0.6	107
375	10/10	20.4 ± 0.2	31.7 ± 0.8*	11.3 ± 0.8	110
750	10/10	20.4 ± 0.1	29.8 ± 0.6	9.4 ± 0.6	103
1,500	10/10	20.1 ± 0.3	29.1 ± 0.5	9.1 ± 0.3	101

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

At necropsy, no lesions were observed grossly that were attributed to nitromethane exposure. Olfactory epithelial degeneration and respiratory epithelial hyaline droplets (not observed in the 16-day study) were observed microscopically in all male and female mice exposed to 375 ppm or greater (Table 13). Seven females in the 188 ppm group also had epithelial degeneration; one male and nine females in the 188 ppm groups and two females in the 94 ppm group had hyaline droplets. Olfactory epithelial degeneration was characterized by loss of orderly arrangement of the olfactory epithelium and thinning of the epithelium due to a loss of bipolar sensory neurons. Some of the remaining epithelial (sustentacular) cells contained hyaline droplets. Inflammation associated with these changes was minimal. Olfactory epithelial degeneration was more prominent along the dorsal meatus but was occasionally present along the medial aspects of the ethmoid turbinates close to the septum. Hyaline droplet formation of the respiratory epithelium, similar to that observed in the olfactory epithelium, was striking and was most frequently observed in the epithelium of the nasopharyngeal duct, nasal septum, and medial aspects of the nasal turbinates. The respiratory epithelium near the olfactory epithelium seemed particularly vulnerable.

In males, the average severity of the nasal lesions was minimal in the 188, 375, and 750 ppm groups and mild in the 1,500 ppm group. In females, the severity of olfactory epithelial degeneration was minimal in the 188 and 375 ppm groups and mild in the 750 and 1,500 ppm groups; the severity of respiratory epithelial hyaline droplets was minimal in the 94 and 188 ppm groups, mild in the 375 and 750 ppm groups, and moderate in the 1,500 ppm group.

All males and nine females in the 1,500 ppm groups had minimal extramedullary hematopoiesis of the spleen (Table 13); although this lesion was also observed in a few males and females exposed to 375 or 750 ppm, the incidences were low and the change was very subtle in these groups. No kidney, liver, or lung lesions were observed in exposed mice.

Exposure Concentration Selection Rationale: Due to the increased severity and extent of nasal lesions in mice in the 13-week study compared to those in the 16-day study and to the presence of splenic hematopoiesis in mice in the 1,500 ppm groups, the nitromethane exposure concentrations selected for the 2-year study in mice were 188, 375, and 750 ppm.

TABLE 13
Incidences of Selected Nonneoplastic Lesions in Mice in the 13-Week Inhalation Study of Nitromethane

	0 ppm	94 ppm	188 ppm	375 ppm	750 ppm	1,500 ppm
Male						
Nose/Turbinates[a]	10	10	10	10	10	10
Degeneration, Olfactory Epithelium[b]	0	0	0	10** (1.0)[c]	10** (1.3)	10** (2.0)
Hyaline Droplets, Respiratory Epithelium	0	0	1 (1.0)	10** (1.0)	10** (1.0)	10** (2.0)
Spleen	10	10	10	10	10	10
Extramedullary Hematopoiesis	0	1 (1.0)	0	1 (1.0)	2 (1.0)	10** (1.0)
Female						
Nose/Turbinates	10	10	10	10	10	10
Degeneration, Olfactory Epithelium	0	0	7** (1.0)	10** (1.0)	10** (2.0)	10** (3.0)
Hyaline Droplets, Respiratory Epithelium	0	2 (1.0)	9** (1.0)	10** (2.0)	10** (2.0)	10** (3.0)
Spleen	10	10	10	10	10	10
Extramedullary Hematopoiesis	0	0	0	2 (1.0)	3 (1.0)	9** (1.0)

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected mice: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 14 and in the Kaplan-Meier survival curves (Figure 3). The survival rate of females in the 750 ppm group was marginally greater than that of the controls.

Body Weights and Clinical Findings

Mean body weights are given in Tables 15 and 16 and Figure 4. The mean body weights of exposed females were generally slightly greater than the mean body weights of the controls during the study but were generally similar to the mean body weight of the control females at the end of the study. The mean body weights of exposed and control males were similar throughout the study.

Clinical findings included swelling around the eyes and exophthalmos in exposed males and females. These findings were coincident with harderian gland neoplasms.

TABLE 14
Survival of Mice in the 2-Year Inhalation Study of Nitromethane

	0 ppm	188 ppm	375 ppm	750 ppm
Male				
Animals initially in study	50	50	50	50
Moribund	14	11	16	16
Natural deaths	5	3	4	5
Animals surviving to study termination	31	36	30	29
Percent probability of survival at end of study[a]	62	72	60	58
Mean survival (days)[b]	681	700	674	687
Survival analysis[c]	P=0.519	P=0.321N	P=0.960	P=0.949
Female				
Animals initially in study	50	50	50	50
Accidental deaths[d]	2	0	1	0
Moribund	16	17	20	12
Natural deaths	7	5	3	2
Animals surviving to study termination	25	28[e]	26	36
Percent probability of survival at end of study	52	56	53	72
Mean survival (days)	662	663	673	695
Survival analysis	P=0.046N	P=1.000N	P=0.993N	P=0.056N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or a lower mortality in an exposed group is indicated by N.
[d] Censored from survival analyses
[e] Includes one animal that died during the last week of the study

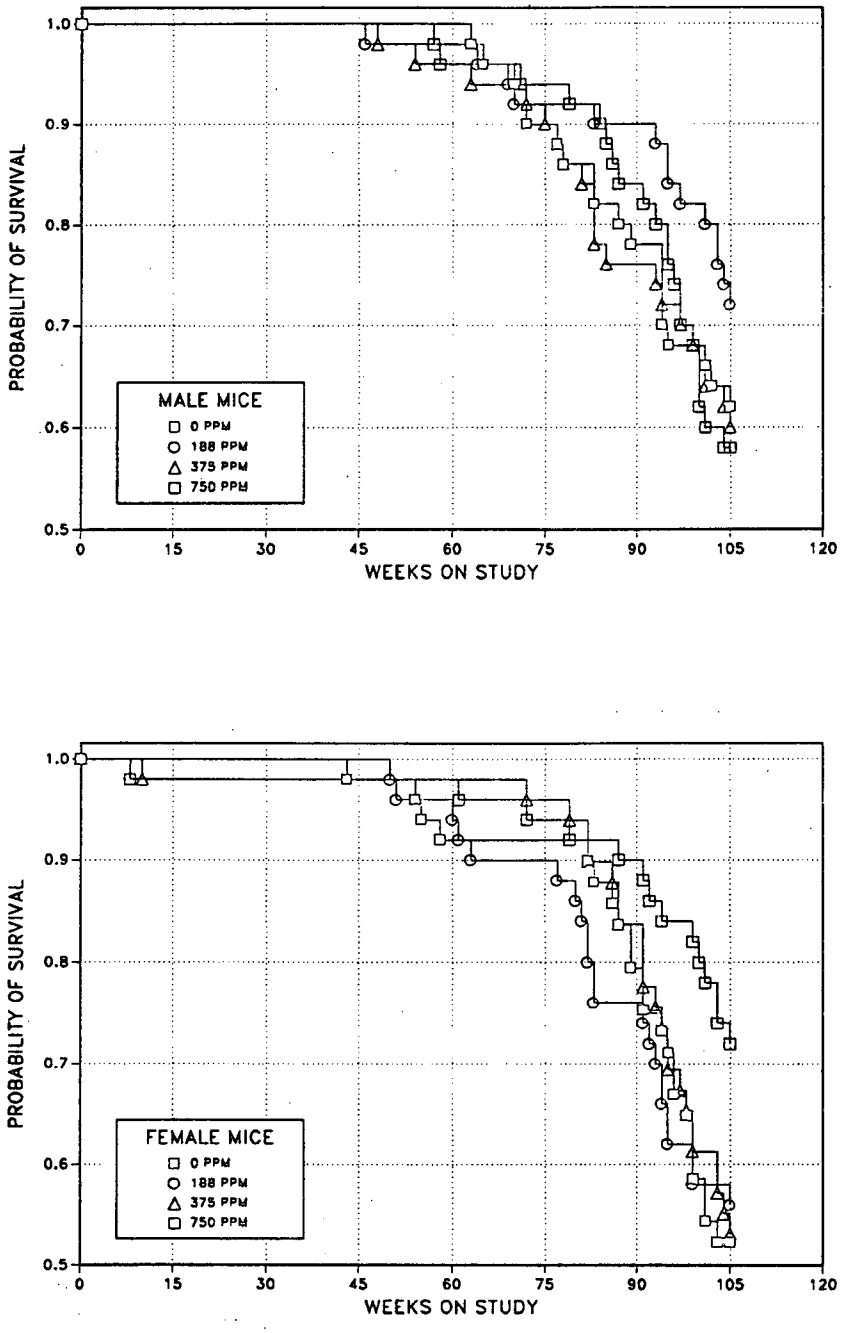


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Nitromethane by Inhalation for 2 Years

TABLE 15
Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of Nitromethane

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	188 ppm Av. Wt. (g)	188 ppm Wt. (% of controls)	188 ppm No. of Survivors	375 ppm Av. Wt. (g)	375 ppm Wt. (% of controls)	375 ppm No. of Survivors	750 ppm Av. Wt. (g)	750 ppm Wt. (% of controls)	750 ppm No. of Survivors
1	25.4	50	25.0	98	50	25.3	100	50	25.4	100	50
2	27.7	50	27.0	98	50	27.7	100	50	28.0	101	50
3	28.9	50	28.2	98	50	29.0	100	50	29.2	101	50
4	29.7	50	29.1	98	50	30.2	102	50	30.4	102	50
5	30.6	50	30.0	98	50	30.9	101	50	31.3	102	50
6	31.2	50	30.0	96	50	31.5	101	50	31.9	102	50
7	31.7	50	30.7	97	50	32.2	102	50	32.0	101	50
8	32.3	50	31.6	98	50	32.7	101	50	32.7	101	50
9	33.1	50	32.2	97	50	33.5	101	50	33.8	102	50
10	33.9	50	33.4	99	50	34.2	101	50	34.5	102	50
11	34.3	50	33.3	97	50	34.2	100	50	34.9	102	50
12	35.1	50	34.0	97	50	34.8	99	50	35.5	101	50
15	36.9	50	35.7	97	50	36.2	98	50	37.7	102	50
19	39.6	50	38.7	98	50	38.6	98	50	40.1	101	50
23	41.3	50	39.5	96	50	39.8	96	50	41.2	100	50
27	43.2	50	42.0	97	50	42.2	98	50	43.3	100	50
31	45.6	50	43.9	96	50	44.5	98	50	45.8	100	50
35	46.6	50	44.7	96	50	45.6	98	50	46.7	100	50
39	47.9	50	46.6	97	50	46.9	98	50	48.0	100	50
43	48.5	50	47.4	98	50	48.1	99	50	49.5	102	50
47	48.7	50	47.8	98	49	47.5	98	50	49.6	102	50
51	48.9	50	48.4	99	49	48.4	99	49	50.2	103	50
55	49.4	50	48.7	99	49	49.5	100	48	51.0	103	50
59	49.9	50	49.9	100	49	50.1	100	48	51.3	103	48
63	50.4	50	49.9	99	49	50.2	100	47	51.7	103	48
67	50.8	48	50.3	99	48	50.9	100	47	51.8	102	48
71	50.8	47	50.6	100	46	50.2	99	47	51.5	101	47
75	51.5	45	50.8	99	46	50.8	99	45	51.7	100	47
79	51.9	43	50.6	98	46	51.2	99	43	51.8	100	46
83	52.1	41	50.7	97	45	52.2	100	39	51.3	99	46
87	51.3	40	50.2	98	45	51.6	101	38	51.5	100	42
91	51.4	39	50.5	98	45	51.9	101	38	51.7	101	41
93	50.5	39	50.2	99	44	51.5	102	37	51.4	102	40
95	50.9	35	49.9	98	43	51.3	101	36	50.9	100	39
97	50.5	34	49.1	97	42	50.1	99	36	50.6	100	37
99	50.0	34	49.1	98	41	49.4	99	35	50.6	101	34
101	49.1	33	48.0	98	41	48.4	99	33	50.4	103	30
103	49.2	32	47.7	97	40	48.3	98	32	50.2	102	30
Mean for weeks											
1-13	31.2		30.4	97		31.4	101		31.6	101	
14-52	44.7		43.5	97		43.8	98		45.2	101	
53-103	50.6		49.8	98		50.5	100		51.2	101	

TABLE 16
Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of Nitromethane

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	188 ppm Av. Wt. (g)	188 ppm Wt. (% of controls)	188 ppm No. of Survivors	375 ppm Av. Wt. (g)	375 ppm Wt. (% of controls)	375 ppm No. of Survivors	750 ppm Av. Wt. (g)	750 ppm Wt. (% of controls)	750 ppm No. of Survivors
1	19.9	50	20.2	102	50	20.0	101	50	20.1	101	50
2	22.2	50	22.3	101	50	22.4	101	50	22.6	102	50
3	23.0	50	23.5	102	50	23.9	104	50	23.8	104	50
4	23.7	50	24.3	103	50	24.7	104	50	24.9	105	50
5	25.0	50	25.5	102	50	25.7	103	50	25.9	104	50
6	25.5	50	25.7	101	50	26.5	104	50	26.5	104	50
7	25.6	50	26.3	103	50	27.1	106	50	26.5	104	50
8	26.5	50	27.2	103	50	28.1	106	50	28.0	106	49
9	26.9	50	27.5	102	50	28.4	106	50	28.3	105	49
10	27.8	50	28.7	103	50	29.3	105	49	29.2	105	49
11	27.4	50	28.6	104	50	29.6	108	49	29.6	108	49
12	27.9	50	29.0	104	50	29.5	106	49	29.8	107	49
15	29.1	50	31.0	107	50	31.2	107	49	31.6	109	49
19	32.0	50	33.9	106	50	33.6	105	49	34.6	108	49
23	33.1	50	35.6	108	50	35.8	108	49	36.1	109	49
27	35.0	50	37.7	108	50	37.6	107	49	38.2	109	49
31	38.0	50	40.0	105	50	40.6	107	49	40.6	107	49
35	39.6	50	42.0	106	50	42.2	107	49	42.7	108	49
39	42.2	50	44.3	105	50	43.6	103	49	44.2	105	49
43	43.1	49	45.8	106	50	45.7	106	48	45.8	106	49
47	44.2	49	47.1	107	50	45.3	103	48	46.3	105	49
51	45.5	49	47.6	105	49	47.5	104	48	48.0	106	49
55	47.3	47	49.9	106	48	49.1	104	48	49.8	105	49
59	49.1	46	51.5	105	48	50.7	103	48	51.1	104	49
63	49.7	44	52.6	106	46	51.3	103	48	51.7	104	48
67	50.9	44	53.8	106	45	52.7	104	48	52.9	104	48
71	51.6	44	53.8	104	45	53.0	103	48	53.8	104	48
75	51.5	44	53.7	104	45	52.9	103	47	54.3	105	47
79	52.1	44	53.7	103	44	53.7	103	46	54.7	105	46
83	53.3	42	54.6	102	38	53.0	99	44	53.8	101	46
87	52.8	40	53.7	102	38	52.4	99	41	53.7	102	45
91	52.8	36	53.7	102	37	52.1	99	40	54.2	103	44
93	52.0	36	52.7	101	35	52.1	100	37	53.1	102	43
95	52.4	34	52.8	101	32	51.2	98	36	52.9	101	42
97	51.3	32	51.6	101	31	51.2	100	34	52.0	101	42
99	51.2	31	50.6	99	31	49.9	98	32	51.2	100	41
101	50.7	27	50.4	99	29	48.1	95	30	50.1	99	39
103	51.4	25	49.9	97	29	47.0	91	29	49.3	96	38
Mean for weeks											
1-13	25.1		25.7	102		26.3	105		26.3	105	
14-52	38.2		40.5	106		40.3	105		40.8	107	
53-103	51.3		52.4	102		51.3	100		52.4	102	

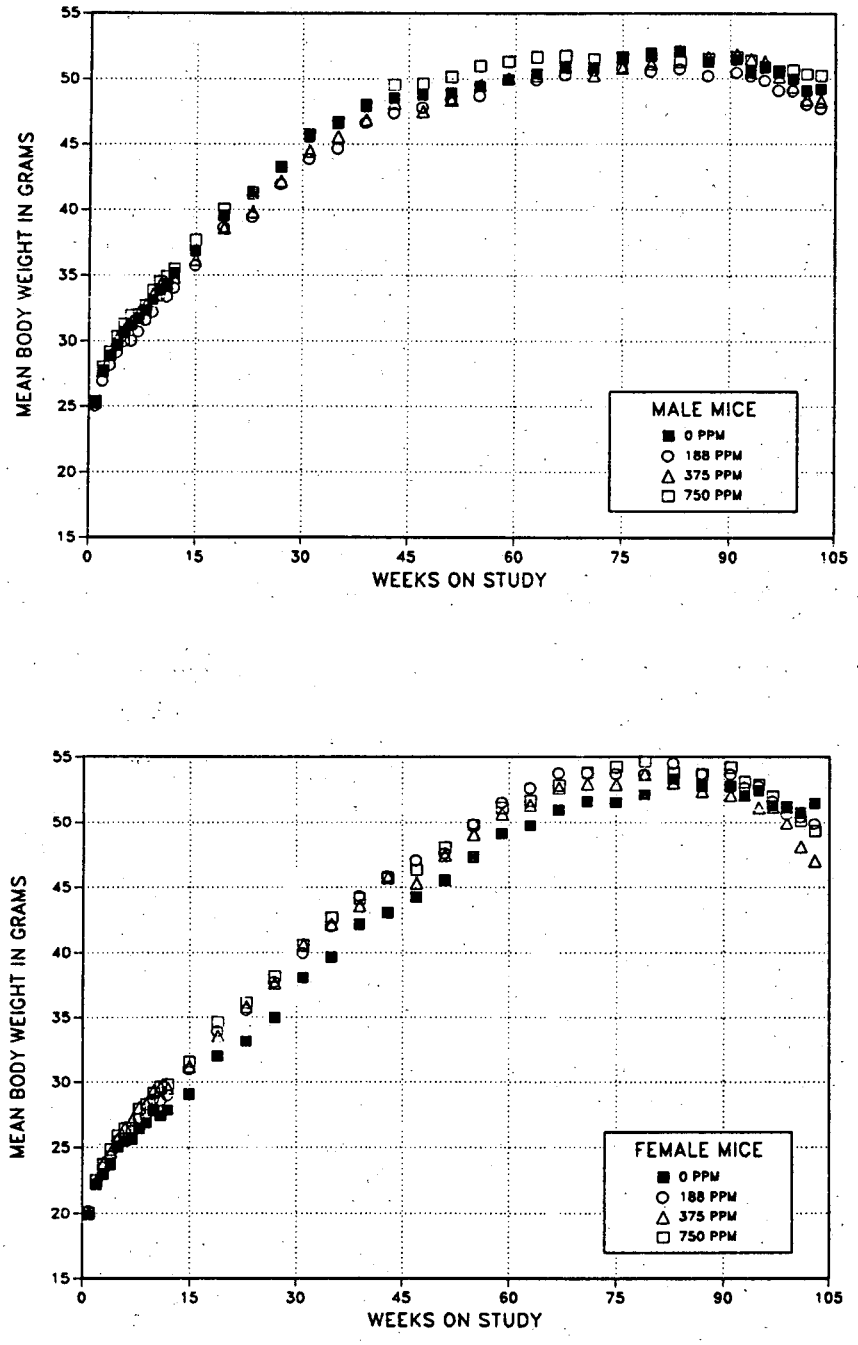


FIGURE 4
Growth Curves for Male and Female Mice Exposed to Nitromethane by Inhalation for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the harderian gland, liver, lung, and nose. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Harderian Gland: The incidences of harderian gland adenoma and adenoma or carcinoma (combined) in exposed mice increased with increasing exposure concentration and were significantly greater in males and females in the 375 and 750 ppm groups than in the controls (Tables 17, C3, and D3). The incidences of these neoplasms in all exposed groups of males and females were greater than the historical control incidences for chamber control mice in 2-year NTP inhalation studies; however, the incidences of adenoma and adenoma or carcinoma (combined) in control males also exceeded the range of historical control incidences (Tables C4a and D4a). The incidences of carcinoma in males and females in the 375 and 750 ppm groups were also slightly greater than the incidences in the controls; although the differences were not statistically significant, the incidences of carcinoma in the 375 and 750 ppm groups were outside the historical incidence range of 0% to 4% for these neoplasms in male and female chamber control mice in 2-year NTP inhalation studies. The incidences of harderian gland hyperplasia in males and females in the 375 ppm groups

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Harderian Gland in Mice in the 2-Year Inhalation Study of Nitromethane

	0 ppm	188 ppm	375 ppm	750 ppm
Male				
Harderian Gland[a]	50	50	50	50
Hyperplasia[b]	2 (3.0)[c]	2 (1.5)	6 (1.7)	2 (3.5)
Adenoma				
Overall rate[d]	9/50 (18%)	10/50 (20%)	19/50 (38%)	32/50 (64%)
Adjusted rate[e]	26.6%	22.8%	51.9%	75.5%
Terminal rate[f]	7/31 (23%)	4/36 (11%)	13/30 (43%)	19/29 (66%)
First incidence (days)	545	448	520	497
Logistic regression test[g]	P<0.001	P=0.505	P=0.019	P<0.001
Carcinoma[h]				
Overall rate	1/50 (2%)	1/50 (2%)	6/50 (12%)	5/50 (10%)
Adjusted rate	2.6%	2.8%	16.5%	14.7%
Terminal rate	0/31 (0%)	1/36 (3%)	3/30 (10%)	3/29 (10%)
First incidence (days)	653	734 (T)	436	595
Logistic regression test	P=0.036	P=0.762N	P=0.062	P=0.104
Adenoma or Carcinoma[i]				
Overall rate	10/50 (20%)	11/50 (22%)	25/50 (50%)	37/50 (74%)
Adjusted rate	28.4%	25.3%	63.2%	83.7%
Terminal rate	7/31 (23%)	5/36 (14%)	16/30 (53%)	22/29 (76%)
First incidence (days)	545	448	436	497
Logistic regression test	P<0.001	P=0.506	P=0.001	P<0.001

(continued)

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Harderian Gland in Mice in the 2-Year Inhalation Study of Nitromethane (continued)

	0 ppm	188 ppm	375 ppm	750 ppm
Female				
Harderian Gland	50	50	50	50
Hyperplasia	3 (1.3)	2 (3.5)	5 (3.0)	1 (1.0)
Adenoma				
Overall rate	5/50 (10%)	7/50 (14%)	16/50 (32%)	19/50 (38%)
Adjusted rate	16.0%	21.4%	43.1%	45.9%
Terminal rate	2/25 (8%)	4/28 (14%)	7/26 (27%)	14/36 (39%)
First incidence (days)	609	639	498	503
Logistic regression test	P<0.001	P=0.380	P=0.008	P=0.003
Carcinoma[j]				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	3/50 (6%)
Adjusted rate	2.9%	6.7%	14.1%	8.3%
Terminal rate	0/25 (0%)	1/28 (4%)	3/26 (12%)	3/36 (8%)
First incidence (days)	663	693	679	734 (T)
Logistic regression test	P=0.305	P=0.501	P=0.194	P=0.365
Adenoma or Carcinoma[k]				
Overall rate	6/50 (12%)	9/50 (18%)	20/50 (40%)	21/50 (42%)
Adjusted rate	18.4%	27.1%	53.5%	50.8%
Terminal rate	2/25 (8%)	5/28 (18%)	10/26 (38%)	16/36 (44%)
First incidence (days)	609	639	498	503
Logistic regression test	P<0.001	P=0.175	P=0.002	P=0.002

(T)Terminal sacrifice
[a] Number of animals necropsied
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Number of animals with neoplasm per number of animals necropsied
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by N.
[h] Historical incidence for all 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 2/950 (0.2% ± 0.9%); range, 0%-4%. Historical incidence (Battelle Pacific Northwest Laboratories): 2/450 (0.4% ± 1.3%); range, 0%-4%.
[i] Historical incidence (all laboratories): 49/950 (5.2% ± 4.5%); range, 0%-14%. Historical incidence (Battelle Pacific Northwest Laboratories): 38/450 (8.4% ± 4.0%); range, 2%-14%.
[j] Historical incidence (all laboratories): 6/941 (0.6% ± 1.4%); range, 0%-4%. Historical incidence (Battelle Pacific Northwest Laboratories): 6/447 (1.3% ± 1.7%); range, 0%-4%.
[k] Historical incidence (all laboratories): 32/941 (3.4% ± 4.4%); range, 0%-16%. Historical incidence (Battelle Pacific Northwest Laboratories): 27/447 (6.0% ± 5.0%); range, 0%-16%.

were similar to those in the controls (Tables 17 and C5). Hyperplasias were small focal lesions with increased numbers of secretory cells causing little if any compression of the adjacent parenchyma. Adenomas were generally larger and more expansive and caused compression of the adjacent parenchyma (Plate 5); some cellular atypia was noted, and the cells formed papillary, cystic, and glandular patterns. Harderian gland carcinomas were large neoplasms, often seen at necropsy, that involved the entire gland (Plate 6); many of these neoplasms showed encapsulation or local invasion.

Liver: Female mice in the 188 and 750 ppm groups had significantly greater incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) than the controls (Tables 18 and D3); the incidences of these neoplasms exceeded the historical control ranges of 0% to 40% for hepatocellular adenomas and 3% to 54% for hepatocellular adenomas or carcinomas (combined) for 2-year NTP inhalation studies (Table D4b). Females in the 188 and 750 ppm groups also had greater incidences of multiple hepatocellular adenomas than the controls. The incidences of eosinophilic focus increased with increasing exposure concentration, and the incidences in the 375 and 750 ppm groups were significantly greater than the control incidence (Tables 18 and D5).

Eosinophilic foci consisted of isolated foci of increased numbers of hepatocytes and/or enlarged hepatocytes, usually of similar tinctorial nature as the rest of the liver; these lesions, which sometimes caused slight tissue compression, blended into the normal parenchyma at short angles so that a demarcation line was not always easily seen. Hepatocellular adenomas were usually larger than eosinophilic foci and compressed the surrounding tissue; these neoplasms contained basophilic cells and were sharply demarcated from the surrounding parenchyma due to the sharp angle of abutment and contrasting tinctorial quality. Hepatocellular carcinomas were large and irregular, with cellular atypia and pleomorphism, and contained trabecular patterns of cell growth.

TABLE 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Female Mice in the 2-Year Inhalation Study of Nitromethane

	0 ppm	188 ppm	375 ppm	750 ppm
Liver[a]	50	49	49	50
Eosinophilic Focus[b]	4	7	11*	15*
Hepatocellular Adenoma				
Overall rate[c]	14/50 (28%)	25/49 (51%)	17/49 (35%)	35/50 (70%)
Adjusted rate[d]	45.5%	68.6%	49.0%	83.1%
Terminal rate[e]	9/25 (36%)	17/28 (61%)	10/26 (38%)	29/36 (81%)
First incidence (days)	597	534	498	426
Logistic regression test[f]	P<0.001	P=0.013	P=0.364	P<0.001
Hepatocellular Adenoma, Multiple				
Overall rate	3/50 (6%)	13/49 (27%)**	4/49 (8%)	13/50 (26%)**
Hepatocellular Carcinoma[g]				
Overall rate	10/50 (20%)	14/49 (29%)	8/49 (16%)	12/50 (24%)
Adjusted rate	29.7%	35.7%	26.6%	25.6%
Terminal rate	3/25 (12%)	6/28 (21%)	6/26 (23%)	2/36 (6%)
First incidence (days)	576	534	548	426
Logistic regression test	P=0.329	P=0.195	P=0.383N	P=0.200
Hepatocellular Adenoma or Carcinoma[h]				
Overall rate	19/50 (38%)	34/49 (69%)	22/49 (45%)	40/50 (80%)
Adjusted rate	54.6%	82.4%	62.6%	86.9%
Terminal rate	10/25 (40%)	21/28 (75%)	14/26 (54%)	30/36 (83%)
First incidence (days)	576	534	498	426
Logistic regression test	P=0.001	P<0.001	P=0.368	P<0.001

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
[a] Number of animals with liver examined microscopically
[b] Number of animals with lesion
[c] Number of animals with neoplasm per number of animals with liver examined microscopically
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by N.
[g] Historical incidence for all 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 103/937 (11.0% ± 6.7%); range, 0%-30%. Historical incidence (Battelle Pacific Northwest Laboratories): 54/446 (12.1% ± 8.1%); range, 2%-30%.
[h] Historical incidence (all laboratories): 200/937 (21.3% ± 11.9%); range, 3%-54%. Historical incidence (Battelle Pacific Northwest Laboratories): 95-446 (21.3% ± 14.8%); range, 6%-54%.

Lung: The incidence of alveolar/bronchiolar carcinoma in male mice in the 750 ppm group was significantly greater than that in the controls (Tables 19 and C3) and exceeded the historical control range for these neoplasms in 2-year NTP inhalation studies (Table C4b). The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in females in the 750 ppm group was also significantly greater than in the controls and exceeded the historical control range (Tables 19, D3, and D4c). The incidence of alveolar/bronchiolar carcinoma in the female 375 ppm group was significantly greater than in controls but was within the historical control range. The incidence of alveolar/bronchiolar adenomas in females in the 750 ppm group also exceeded the historical control range. Females in the 375 ppm group had a significantly greater incidence of cellular infiltration of histiocytes than the controls (Tables 19 and D5); the incidences of alveolar epithelial hyperplasia in exposed males and females were similar to those of the controls.

Alveolar/bronchiolar adenomas consisted of focal proliferations of cuboidal or columnar cells in alveolar areas; adenomas usually caused compression of surrounding tissue and loss of the basic alveolar structure. Alveolar/bronchiolar carcinomas had cellular anaplasia and compression; there was evidence of tissue invasion. Carcinomas had a higher nucleus:cytoplasm ratio than alveolar/bronchiolar adenomas.

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Inhalation Study of Nitromethane

	0 ppm	188 ppm	375 ppm	750 ppm
Male				
Lung[a]	50	50	50	50
Infiltration Cellular, Histiocyte[b]	7 (2.4)[c]	2 (3.0)	3 (2.7)	6 (2.7)
Alveolar Epithelium, Hyperplasia	1 (1.0)	1 (1.0)	3 (2.7)	1 (3.0)
Alveolar/bronchiolar Adenoma				
Overall rate[d]	11/50 (22%)	10/50 (20%)	9/50 (18%)	12/50 (24%)
Adjusted rate[e]	30.8%	26.0%	30.0%	35.1%
Terminal rate[f]	8/31 (26%)	8/36 (22%)	9/30 (30%)	8/29 (28%)
First incidence (days)	449	646	734 (T)	497
Logistic regression test[g]	P=0.422	P=0.456N	P=0.412N	P=0.511
Alveolar/bronchiolar Carcinoma[h]				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	11/50 (22%)
Adjusted rate	6.5%	8.3%	10.0%	30.4%
Terminal rate	2/31 (6%)	3/36 (8%)	3/30 (10%)	6/29 (21%)
First incidence (days)	734 (T)	734 (T)	734 (T)	586
Logistic regression test	P=0.001	P=0.569	P=0.485	P=0.009
Alveolar/bronchiolar Adenoma or Carcinoma[i]				
Overall rate	13/50 (26%)	13/50 (26%)	12/50 (24%)	20/50 (40%)
Adjusted rate	36.8%	33.9%	40.0%	51.2%
Terminal rate	10/31 (32%)	11/36 (31%)	12/30 (40%)	11/29 (38%)
First incidence (days)	449	646	734 (T)	497
Logistic regression test	P=0.059	P=0.517N	P=0.515N	P=0.105

(continued)

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Inhalation Study of Nitromethane (continued)

	0 ppm	188 ppm	375 ppm	750 ppm
Female				
Lung	50	50	49	50
Infiltration Cellular, Histiocyte	0	1 (1.0)	6* (2.5)	4 (2.8)
Alveolar Epithelium, Hyperplasia	3 (2.3)	1 (2.0)	5 (2.0)	1 (3.0)
Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	3/50 (6%)	2/49 (4%)	9/50 (18%)
Adjusted rate	11.5%	10.7%	4.3%	22.7%
Terminal rate	2/25 (8%)	3/28 (11%)	0/26 (0%)	6/36 (17%)
First incidence (days)	716	734 (T)	498	426
Logistic regression test	P=0.022	P=0.632N	P=0.514N	P=0.083
Alveolar/bronchiolar Carcinoma[j]				
Overall rate	0/50 (0%)	3/50 (6%)	5/49 (10%)	3/50 (6%)
Adjusted rate	0.0%	8.3%	15.0%	7.2%
Terminal rate	0/25 (0%)	1/28 (4%)	2/26 (8%)	1/36 (3%)
First incidence (days)	—[k]	534	602	503
Logistic regression test	P=0.149	P=0.119	P=0.033	P=0.110
Alveolar/bronchiolar Adenoma or Carcinoma[l]				
Overall rate	3/50 (6%)	6/50 (12%)	6/49 (12%)	12/50 (24%)
Adjusted rate	11.5%	18.5%	16.8%	28.7%
Terminal rate	2/25 (8%)	4/28 (14%)	2/26 (8%)	7/36 (19%)
First incidence (days)	716	534	498	426
Logistic regression test	P=0.007	P=0.243	P=0.238	P=0.015

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
(T)Terminal sacrifice
[a] Number of animals with lung examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Number of animals with neoplasm per number of animals with lung examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposed group is indicated by N.
[h] Historical incidence for all 2-year NTP inhalation studies with chamber control groups (mean ± standard deviation): 75/947 (7.9% ± 5.7%); range, 0%-16%. Historical incidence (Battelle Pacific Northwest Laboratories): 37/448 (8.3% ± 5.8%); range, 0%-16%.
[i] Historical incidence (all laboratories): 205/947 (21.7% ± 8.0%); range, 10%-42%. Historical incidence (Battelle Pacific Northwest Laboratories): 108/448 (24.1% ± 9.5%); range, 10%-42%.
[j] Historical incidence (all laboratories): 38/939 (4.1% ± 3.2%); range, 0%-12%. Historical incidence (Battelle Pacific Northwest Laboratories): 15/446 (3.4% ± 2.4%); range, 0%-6%.
[k] Not applicable; no neoplasms in animal group
[l] Historical incidence (all laboratories): 97/939 (10.3% ± 3.7%); range, 0%-16%. Historical incidence (Battelle Pacific Northwest Laboratories): 46/446 (10.3% ± 4.6%); range, 0%-16%.

Nose: The incidences of several nonneoplastic nasal lesions, similar to but more severe than those observed in the 13-week study, were generally significantly greater in exposed male and female mice than those in the controls (Tables 20, C5, and D5). These lesions included degeneration and metaplasia of the olfactory epithelium and hyaline degeneration of the respiratory epithelium (not observed in the 13-week study). The minimal to moderate olfactory degeneration was most prominent along the middle and posterior sections of the dorsal meatus and at the tips of the ethmoid turbinates; this lesion consisted of degeneration and loss of sensory neurons, nerve atrophy, and dilation of Bowman's glands (Plates 7 and 8). The metaplastic lesions, which represented a sequelae to degeneration, were characterized by replacement of the damaged olfactory epithelium with ciliated respiratory epithelium. Hyaline degeneration of the respiratory epithelium was characterized by accumulation of eosinophilic hyaline droplets in cells lining the nasopharyngeal duct, nasal septum, medial surface of the middle nasoturbinates, and some of the glandular epithelium beneath the respiratory epithelium of the middle nasal section. In addition, there was minimal suppurative inflammation of the nasolacrimal duct in males in the 375 and 750 ppm groups. The association of this marginally increased incidence with exposure to nitromethane is uncertain.

TABLE 20
Incidences of Nonneoplastic Lesions of the Nose in Mice in the 2-Year Inhalation Study of Nitromethane

	0 ppm	188 ppm	375 ppm	750 ppm
Male				
Nose[a]	50	49	50	50
Nasolacrimal Duct, Inflammation[b]	2 (1.5)[c]	3 (1.3)	10* (1.9)	10* (1.9)
Olfactory Epithelium, Atrophy, Focal	3 (1.0)	8* (1.1)	0	0
Olfactory Epithelium, Degeneration	0	10** (1.1)	50** (2.5)	50** (3.1)
Olfactory Epithelium, Metaplasia	0	1 (2.0)	41** (1.8)	49** (2.0)
Respiratory Epithelium, Degeneration, Hyaline	5 (1.0)	5 (1.2)	50** (1.9)	50** (2.0)
Female				
Nose	50	49	50	50
Nasolacrimal Duct, Inflammation	1 (2.0)	0	3 (1.7)	3 (2.0)
Olfactory Epithelium, Atrophy, Focal	2 (1.0)	6 (1.0)	0	0
Olfactory Epithelium, Degeneration	0	22** (1.1)	50** (2.7)	50** (3.2)
Olfactory Epithelium, Metaplasia	0	2 (1.0)	46** (1.9)	48** (2.2)
Respiratory Epithelium, Degeneration, Hyaline	16 (1.1)	39** (1.5)	50** (2.0)	50** (2.5)

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
[a] Number of animals with nose examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

GENETIC TOXICOLOGY

Nitromethane was not mutagenic *in vitro* or *in vivo*. Results of tests for induction of mutations by nitromethane (100 to 10,000 μg/plate) in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 were negative with and without induced S9 enzymes (Table E1; Mortelmans *et al.*, 1986). No induction of sister chromatid exchanges (Table E2) or chromosomal aberrations (Table E3) was observed in cultured Chinese hamster ovary cells treated with up to 5,000 μg/mL nitromethane. Nitromethane administered by inhalation for 13 weeks at concentrations up to 1,500 ppm did not induce increased frequencies of micronucleated erythrocytes in the peripheral blood of male or female mice (Table E4).

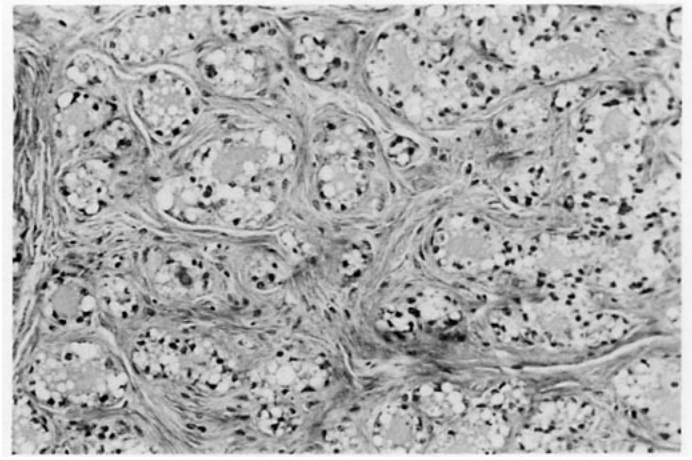


PLATE 1
Mammary gland fibroadenoma from a female F344/N rat exposed to 375 ppm nitromethane by inhalation for 2 years. The neoplasm contains nests of glands separated by prominent bands of collagen. H&E; 150×

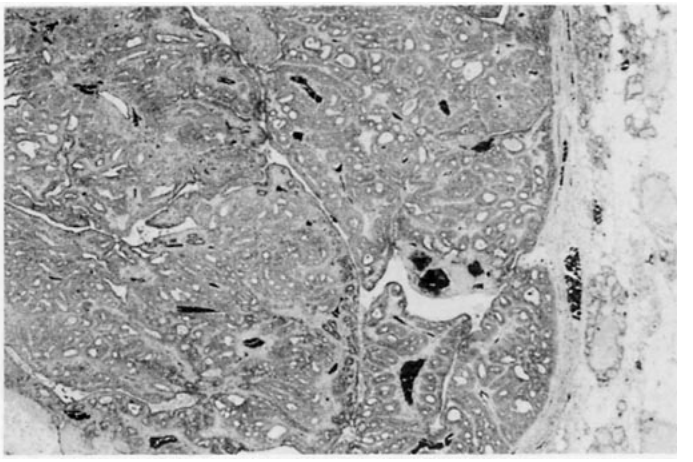


PLATE 2
Mammary gland carcinoma from a female F344/N rat exposed to 375 ppm nitromethane by inhalation for 2 years. The neoplasm has a glandular pattern. H&E; 35×

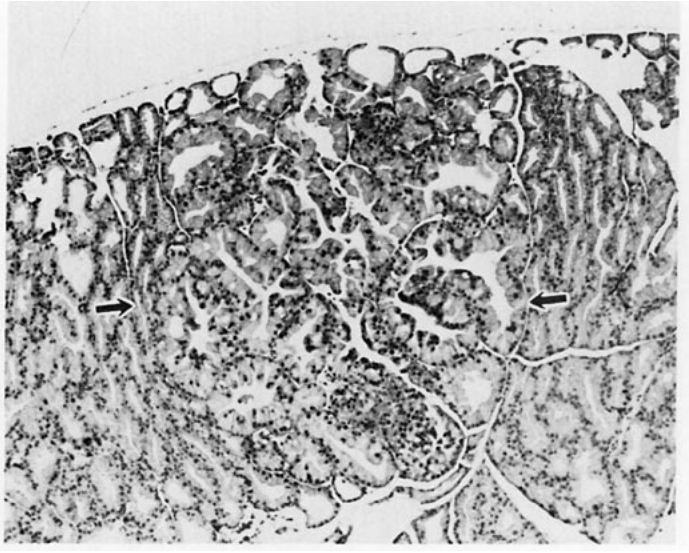


PLATE 3
Detail of a mammary gland carcinoma from a female F344/N rat exposed to 375 ppm nitromethane by inhalation for 2 years. Mitotic figures are common (arrows). H&E; 240×

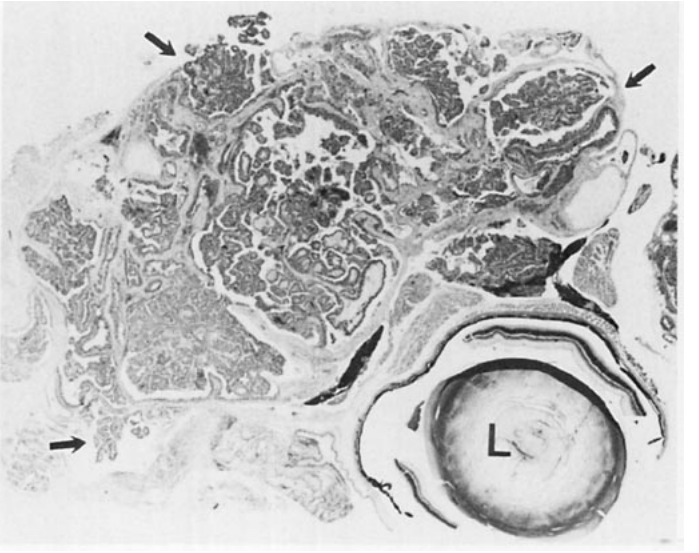


PLATE 4
Metastatic mammary gland carcinoma in the lung of a female F344/N rat exposed to 375 ppm nitromethane by inhalation for 2 years. The neoplasm obliterates a major pulmonary vessel (arrows). Bronchus (B). H&E; 35×

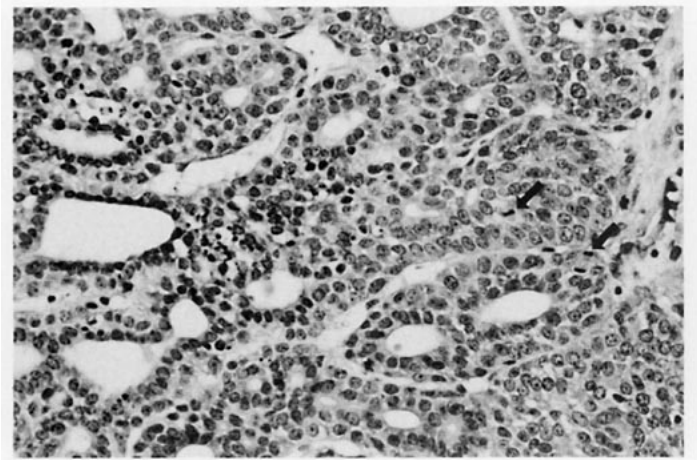


PLATE 5
Small harderian gland adenoma (between arrows) from a male B6C3F$_1$ mouse exposed to 750 ppm nitromethane by inhalation for 2 years. H&E; 11×

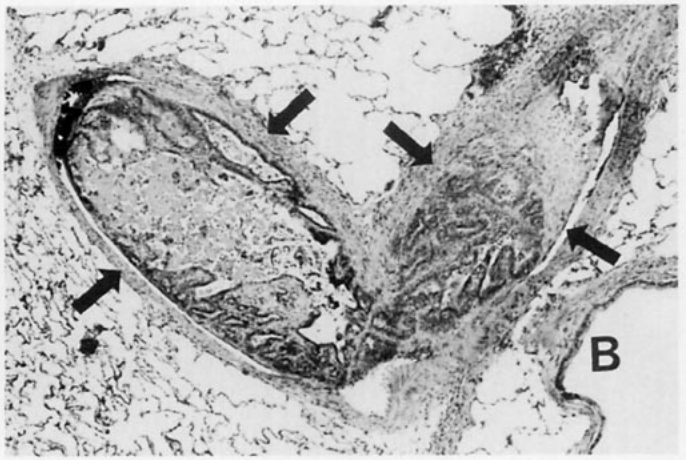


PLATE 6
Large harderian gland carcinoma (between arrows) from a male B6C3F$_1$ mouse exposed to 750 ppm nitromethane by inhalation for 2 years. The neoplasm nearly surrounds the eye of the mouse. Note the lens (L) of the eye. H&E; 55×

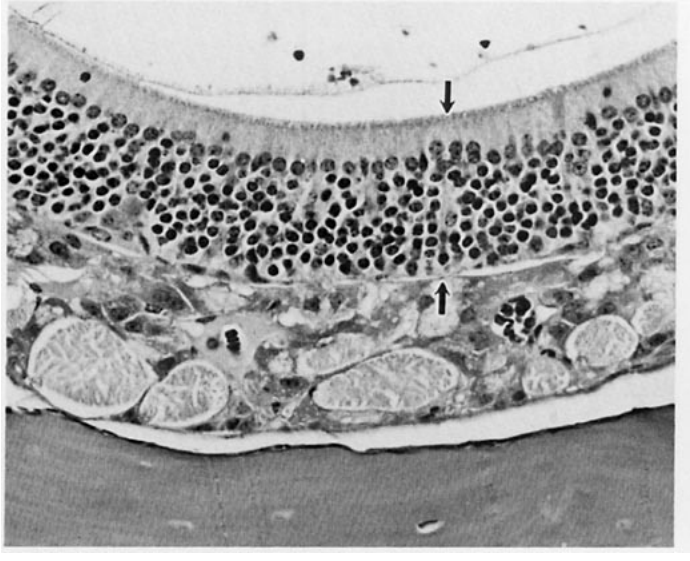


PLATE 7
Normal olfactory epithelium at level II of the nasal cavity of a control male B6C3F$_1$ mouse. Note the plump olfactory epithelium between the arrows. H&E; 340×

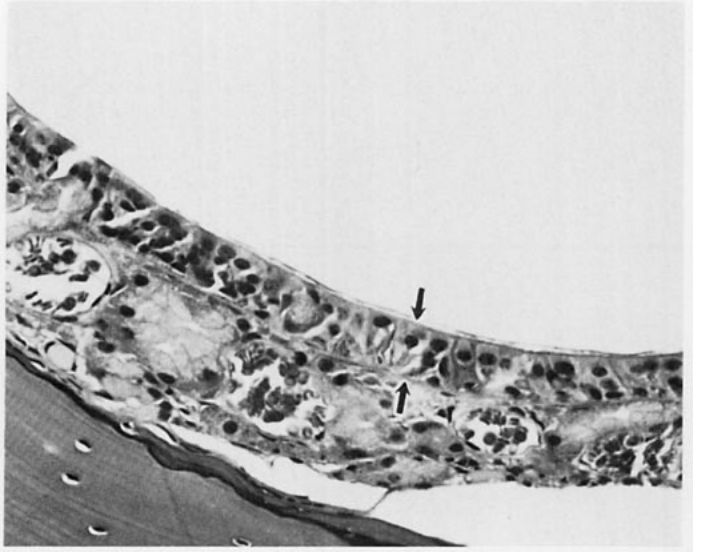


PLATE 8
Degeneration of the olfactory epithelium at level II of the nasal cavity in a male B6C3F$_1$ mouse exposed to 750 ppm nitromethane by inhalation for 2 years. Note the attenuated olfactory epithelium between the arrows. H&E; 340×

DISCUSSION AND CONCLUSIONS

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

Although there were a number of effects that were considered treatment related in rats in the 13-week study, most were not of great enough severity or incidence to determine exposure concentrations for the 2-year study. The deciding factor for the selection of exposure concentrations for the 2-year study was the neurotoxicologic findings in the 13-week study: loss of grip strength in males exposed to 1,500 ppm, hindlimb paralysis in rats in the 750 and 1,500 ppm groups, and sciatic nerve and spinal cord lesions in rats exposed to 375 ppm or greater. The effects in rats exposed to 750 ppm or greater were considered too severe, while the lesions observed at 375 ppm were very subtle and were less severe than those observed in the 16-day study. In the absence of significant short-term exposure-related effects, 375 ppm was selected as the highest exposure concentration for the 2-year rat study.

The primary factors influencing the selection of exposure concentrations for the 2-year mouse study were the incidence and severity of nasal lesions in male and female mice exposed to 1,500 ppm in the 13-week study. Also considered in the selection was the presence of extramedullary hematopoiesis of the spleen in male and female mice exposed to 1,500 ppm. Because the nasal lesions were considered too severe, 750 ppm was selected as the highest exposure concentration for the 2-year study.

There was a marked microcytic, regenerative anemia in exposed rats in the 13-week study, accompanied by red cell fragmentation, Heinz body formation, and increased methemoglobin production, although not extensive. Bone marrow hyperplasia, consistent with the anemia, was present in exposed rats. However, as one might expect with such hematotoxic effects, there were no treatment-related findings in the spleen of exposed rats. Incidences of extramedullary hematopoiesis of the spleen were significantly increased in male and female mice exposed to 1,500 ppm in the 13-week study. No hematologic evaluation was performed for mice in this study. Reductions in serum thyroxine concentration and increased thyroid gland weights have been observed in New Zealand white rabbits exposed for 6 months to 745 ppm nitromethane (Lewis *et al.*, 1979). On day 23 of the 13-week study, a hypothyroid state, as evidenced by reduced serum triiodothyronine, thyroxine, and free thyroxine concentrations, was observed in exposed rats. However, this effect was transient in that thyroid gland hormone concentrations and thyroid gland weights were similar to those of the controls at the end of the study.

In the 2-year rat study, neither survival rates nor weight gains were significantly affected by nitromethane exposure. The lack of neurobehavioral clinical signs and neuropathological changes in the 2-year study suggests that the exposure concentrations were sufficiently low to prevent cumulative neurotoxic effects and that the rats had adapted to any effects that might have occurred early in the study; evaluation of spinal cords and sciatic nerves from male and female rats in the 0 and 375 ppm groups revealed no significant differences between exposed and control rats. There were no treatment-related clinical findings other than the gross observation of mammary gland swellings in female rats. Nitromethane exposure caused increased incidences of mammary gland neoplasms in female rats, as evidenced by exposure concentration-related increases in fibroadenoma; carcinoma; and fibroadenoma, adenoma, or carcinoma (combined). While the incidence of mammary gland neoplasms in control female rats (42%) was near the upper bound of the historical control range (16%-46%), the observed control neoplasm incidence was similar to the 46% rate predicted by the Seilkop logistic regression model (Seilkop, 1995) for control animals with an equivalent survival rate and 52-week mean body weight. Moreover, the slightly elevated body weights of females in the 188 and 375 ppm groups

could not account for the markedly increased incidences of mammary gland neoplasms observed in these groups. There were no treatment-related increases in the incidences of neoplasms or nonneoplastic lesions at any site in male rats. The presence of renal tubule adenomas only in exposed animals (0/50, 3/50, 2/50, 1/50) was investigated by preparing kidney step sections for control and exposed male rats. Additional adenomas were observed in step sections from exposed and control groups (2/50, 2/50, 0/50, 4/50); however, the combined incidences of renal tubule adenoma for step sections and original kidney sections (2/50, 5/50, 2/50, 5/50) did not indicate a significant treatment-related increase in the incidence of this neoplasm.

During the 2-year mouse study, the survival rate for females in the 750 ppm group was marginally greater than that of controls. Exposed female mice generally weighed slightly more than the controls; however, body weights of control and exposed females were similar at the end of the study. Survival rates and body weights of treated male mice were similar to those of the controls. The only clinical findings observed during the 2-year study were swelling around the eyes and exophthalmos. These findings were consistent with increased incidences of harderian gland adenomas and carcinomas in exposed mice. The incidences of harderian gland neoplasms in control mice were somewhat greater than the historical control mean; however, the increased incidences of harderian adenomas or carcinomas (combined) were highly significant in males and females exposed to 375 or 750 ppm and were considered to be caused by exposure to nitromethane.

Nitromethane exposure caused a significant increase in the incidences of hepatocellular adenomas and adenomas or carcinomas (combined) in female mice in the 188 and 750 ppm groups. The incidences of multiple adenomas were increased in these two groups as well. The incidences of eosinophilic foci, considered to be part of the continuum of hepatic neoplasms, were marginally increased with increasing exposure concentration. As stated previously, exposed female mice had slightly greater mean body weights and lived slightly longer than control females; moreover, liver neoplasm incidences did correlate with body weight. However, application of the Seilkop logistic regression model (Seilkop, 1995) suggests that marginal body weight differences and/or increased survival rates could not account for the increased incidences of neoplasms observed in the 188 and 750 ppm groups. There is a somewhat unusual inversion in the treatment response in that the 375 ppm group incidence is consistent with the control incidence. The control incidence (38%) is slightly greater than the mean historical control incidence of 21.3%, but is still well within the historical control range (3%-54%). The increased incidences of these hepatocellular neoplasms were considered to be caused by nitromethane exposure.

The secondary nitroalkane 2-nitropropane has been shown to cause increased incidences of hepatocellular neoplasms in rats administered the compound by inhalation (Lewis *et al.*, 1979) or gavage (Fiala *et al.*, 1987). The primary alkanes nitroethane (Griffin *et al.*, 1988), 1-nitropropane (Griffin *et al.*, 1982), and tetranitromethane (NTP, 1990) have not been shown to cause increased incidences of such neoplasms in rats. In addition, tetranitromethane (NTP, 1990) and 3-nitro-3-hexene (Deichman *et al.*, 1963) did not cause hepatocellular neoplasms in mice. No reports of carcinogenicity studies in mice were found in the literature for nitroethane, 1-nitropropane, or 2-nitropropane.

A number of studies have been conducted to investigate the possible mechanism of 2-nitropropane-induced hepatic neoplasms and the lack of such effects by primary nitroalkanes in rats. The primary and secondary mononitroalkanes exist in a state of equilibrium between the protonated neutral nitroalkanes, the nonprotonated nitronic acid and its anion, or nitronate in aqueous solution. Löfroth *et al.* (1986) have demonstrated that nitrite is not the major cause of mutagenicity of nitroalkanes in various *Salmonella* strains. At cellular pH, the secondary nitroalkanes have a much higher relative concentration of the nitronate anion and nitronic acid forms than the primary nitroalkanes; therefore, Löfroth *et al.* (1986) hypothesized that the anion or the acid may be the ultimate mutagen. In general, the primary nitroalkanes nitromethane, nitroethane, 1-nitropropane, and 1-nitrobutane and their nitronates are not mutagenic (Conaway *et al.*, 1991a; Davis, 1993). 2-Nitropropane, its nitronate 2-propyl-2-nitronate, and the nitronates of 2-nitrobutane and 3-nitropentane are mutagenic (Conaway *et al.*,

1991a). The nitronates were more powerful mutagens than their respective parent compounds. Nitroethane, 1-nitropropane, and 2-nitropropane (Davis *et al.*, 1993) do not induce micronuclei in erythrocytes from mice, similar to nitromethane in the 13-week studies. In human lymphocytes exposed in culture, 2-nitropropane, but not 1-nitropropane, induces chromosomal aberrations and sister chromatid exchanges. Tetranitromethane does induce sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells (NTP, 1990); however, as presented in this report, nitromethane did not induce either. 2-Nitropropane, but not 1-nitropropane, has been shown to induce DNA repair synthesis in rat hepatocytes (Andrae *et al.*, 1988) *in vitro* and *in vivo* and cause *in vivo* oxidative damage to rat liver DNA and RNA (Conaway *et al.*, 1991b), as indicated by the increase in 8-hydroxydeoxyguanosine and 8-hydroxyguanosine. The primary nitroalkanes 1-nitropropane, 1-nitrobutane, and 1-nitropentane did not produce DNA or RNA damage; however, the secondary nitroalkanes 2-nitropropane, 2-nitrobutane, and 2-nitropentane did. The 2-nitroalkanes produce electrochemically active species, presumably modified nucleosides. Conaway *et al.* (1991b) concluded that the metabolites were responsible for the DNA and RNA damage. Tetranitromethane has been shown to nitrate hydroxyl groups of proteins, primarily of tyrosine residues (Riordan and Vallee, 1972).

Several nitroalkanes have been shown to be metabolized by cytochrome P_{450} in rat and mouse NADPH-dependent hepatic microsomes (Ullrich *et al.*, 1978, Sakurai *et al.*, 1980; Marker and Kulkarni, 1986; Dayal *et al.*, 1991). The specific activities of rat liver microsomes were greatest for 2-nitropropane nitronate and 2-nitropropane, followed by 1-nitropropane, nitromethane, and tetranitromethane. The substrate binding spectrum for nitromethane was different from the other nitro compounds in rat liver microsomes (Sakurai *et al.*, 1980) in that a possible cytochrome P_{450}-NO complex was formed. Formaldehyde and possibly nitric oxide were formed. Tetranitromethane had the same spectrum difference as nitromethane, but no formaldehyde was produced. 2-Nitropropane was metabolized to nitrite and acetone. In similar studies, Kuo and Fridovich (1986) have shown that the enzymatic denitrification of 2-nitropropane to acetone also results in the formation of free radicals, superoxide, and hydrogen peroxide. Cunningham and Matthews (1991) have demonstrated that 2-nitropropane given by gavage to F344/N rats causes hepatic cell proliferation, while 1-nitropropane does not. Chemicals with the aliphatic nitro group (-C-NO_2) have been added to a list of DNA-reactive subgroups recognized by the NTP for possible carcinogenic activity (Tennant and Ashby, 1991). It is not known whether the generation of reactive radicals directly or indirectly is involved in the mechanism of toxicity or carcinogenicity for some primary nitroalkanes and for nitromethane in particular.

Nitromethane exposure caused a significant increase in the incidence of alveolar/bronchiolar carcinomas in male mice exposed to 750 ppm, exceeding the historical control range. Although the incidence of alveolar/bronchiolar carcinomas was significant only in female mice exposed to 375 ppm, carcinomas were also found in the 188 and 750 ppm groups, while none were observed in the controls. In all exposed female groups the incidences of carcinoma were within the historical control range for female mice. The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was significantly increased in the 750 ppm group of females and was also elevated in the male 750 ppm group. The increased incidences of alveolar/bronchiolar neoplasms were considered to be treatment related in males and females. Exposure to tetranitromethane for 2 years caused increased incidences of alveolar/bronchiolar adenomas and carcinomas in male and female B6C3F_1 mice (2 ppm) and F344/N rats (5 ppm) and squamous cell carcinomas of the lung in male and female rats (NTP, 1990).

The only other lesions associated with nitromethane exposure were nonneoplastic nasal lesions that occurred in most exposed male and female mice. These lesions were similar to those observed in the 13-week study, except that they were much more severe following 2 years of exposure to nitromethane. Nasal lesions included degeneration and metaplasia of the olfactory epithelium and hyaline degeneration of the respiratory epithelium. No neoplasms of the nasal cavity were observed in exposed male or female mice. Exposure to tetranitromethane for 2 years caused hyperplasia and metaplasia of the nasal

respiratory epithelium in male and female mice (2 ppm) and rats (5 ppm) (NTP, 1990). There was no effect of tetranitromethane exposure on the olfactory epithelium in mice or rats, and no nasal cavity neoplasms were induced by exposure to tetranitromethane.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity** of nitromethane in male F344/N rats exposed to 94, 188, or 375 ppm. There was *clear evidence of carcinogenic activity* of nitromethane in female F344/N rats based on increased incidences of mammary gland fibroadenomas and carcinomas. There was *clear evidence of carcinogenic activity* of nitromethane in male B6C3F$_1$ mice based on increased incidences of harderian gland adenomas and carcinomas. There was *clear evidence of carcinogenic activity* in female B6C3F$_1$ mice, based on increased incidences of liver neoplasms (primarily adenomas) and harderian gland adenomas and carcinomas. Increased incidences of alveolar/bronchiolar adenomas and carcinomas in male and female mice exposed to nitromethane were also considered to be related to chemical administration.

Exposure to nitromethane by inhalation for 2 years resulted in increased incidences of nasal lesions including degeneration and metaplasia of the olfactory epithelium and degeneration of the respiratory epithelium in male and female mice.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH) (1995). *1995-1996 Threshold Limit Values (TLVs™) for Chemical Substances and Physical Agents and Biological Exposure Indices (BEIs™)*, p. 28. Cincinnati, OH.

Andrae, U., Homfeldt, H., Vogl, L., Lichtmannegger, J., and Summer, K.H. (1988). 2-Nitropropane induces DNA repair synthesis in rat hepatocytes *in vitro* and *in vivo*. *Carcinogenesis* 9, 811-815.

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

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity, and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* 257, 229-306.

Biocides, U.S.A. (1974). p. 156. Charles H. Kline and Company, Fairfield, NJ.

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.

Chiu, C.W., Lee, L.H., Wang, C.Y., and Bryan, G.T. (1978). Mutagenicity of some commercially available nitro compounds for *Salmonella typhimurium*. *Mutat. Res.* 58, 11-22.

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

Code of Federal Regulations (CFR) 29, § 1910.1000.

Conaway, C.C., Hussain, N.S., Way, B.M., and Fiala, E.S. (1991a). Evaluation of secondary nitroalkanes, their nitronates, primary nitroalkanes, nitrocarbinols, and other aliphatic nitro compounds in the Ames Salmonella assay. *Mutat. Res.* 261, 197-207.

Conaway, C.C., Nie, G., Hussain, N.S., and Fiala, E.S. (1991b). Comparison of oxidative damage to rat liver DNA and RNA by primary nitroalkanes, secondary nitroalkanes, cyclopentanone oxime, and related compounds. *Cancer Res.* 51, 3143-3147.

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

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology: Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Princeton, NJ.

Cunningham, M.L., and Matthews, H.B. (1991). Relationship of hepatocarcinogenicity and hepatocellular proliferation induced by mutagenic noncarcinogens vs carcinogens. *Toxicol. Appl. Pharmacol.* 110, 505-513.

Dahl, A.R., and Hadley, W.M. (1983). Formaldehyde production promoted by rat nasal cytochrome *P*-450-dependent monooxygenases with nasal decongestants, essences, solvents, air pollutants, nicotine, and cocaine as substrates. *Toxicol. Appl. Pharmacol.* 67, 200-205.

Davis, R.A. (1993). Aliphatic nitro, nitrate, and nitrite compounds. In *Patty's Industrial Hygiene and Toxicology* (G.D. Clayton and F.E. Clayton, Eds.), 4th ed., Vol. 2A, pp. 599-662. John Wiley and Sons, New York.

Dayal, R., Gescher, A., Harpur, E.S., Pratt, I., and Chipman, J.K. (1989). Comparison of the hepatotoxicity in mice and the mutagenicity of three nitroalkanes. *Fundam. Appl. Toxicol.* **13**, 341-348.

Dayal, R., Goodwin, B., Linhart, I., Mynett, K., and Gescher, A. (1991). Oxidative denitrification of 2-nitropropane and propane-2-nitronate by mouse liver microsomes: Lack of correlation with hepatocytotoxic potential. *Chem.-Biol. Interact.* **79**, 103-114.

Decker, J.R., Moss, O.R., and Kay, B.L. (1982). Controlled-delivery vapor generator for animal exposures. *Am. Ind. Hyg. Assoc. J.* **43**, 400-402.

Deichmann, W.B., MacDonald, W.E., Anderson, W.A.D., and Bernal, E. (1963). Adenocarcinoma in the lungs of mice exposed to vapors of 3-nitro-3-hexene. *Toxicol. Appl. Pharmacol.* **5**, 445-456.

Dellarco, V.L., and Prival, M.J. (1989). Mutagenicity of nitro compounds in *Salmonella typhimurium* in the presence of flavin mononucleotide in a preincubation assay. *Environ. Mol. Mutagen.* **13**, 116-127.

Dequidt, J., Vasseur, P., and Potencier, J. (1973). Experimental toxicological study of some nitroparaffins. 4. Nitromethane. *Bull. Soc. Pharm.* **1**, 29-35.

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.

Douay, O., and Kamoun, P.P. (1980). Serotonin in experimental histidinemia. *Neurochem. Res.* **5**, 897-903.

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.

Fiala, E.S., Czerniak, R., Castonguay, A., Conaway, C.C., and Rivenson, A. (1987). Assay of 1-nitropropane, 2-nitropropane, 1-azoxypropane and 2-azoxypropane for carcinogenicity by gavage in Sprague-Dawley rats. *Carcinogenesis* **8**, 1947-1949.

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.

Gibbs, W., and Reichert, E.T. (1891). A systematic study of the action of definitely related chemical compounds upon animals. *Amer. Chem. J.* **13**, 361-370.

Gocke, E., King, M.-T., Eckhardt, K., and Wild, D. (1981). Mutagenicity of cosmetics ingredients licensed by the European Communities. *Mutat. Res.* **90**, 91-109.

Gosselin, R.E., Smith, R.P., and Hodge, H.C. (1984). *Clinical Toxicology of Commercial Products*, 5th ed., p. II-213. Williams and Wilkins, Baltimore, MD.

Griffin, T.B., Stein, A.A., and Coulston, F. (1982). Inhalation exposure of rats to vapors of 1-nitropropane at 100 ppm. *Ecotoxicol. Environ. Safety* **6**, 268-282.

Griffin, T.B., Stein, A.A., and Coulston, F. (1988). Chronic inhalation exposure of rats to vapors of nitroethane. *Ecotoxicol. Environ. Safety* 16, 11-24.

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

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

The International Technical Information Institute (1979). *Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal with Toxicity and Hazard Data*, pp. 373-374. Tokyo.

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.

Kirk-Othmer Encyclopedia of Chemical Technology (1978). 3rd ed., Vol. 1, pp. 961-967. John Wiley and Sons, New York.

Kirk-Othmer Encyclopedia of Chemical Technology (1981). 3rd ed., Vol. 15, pp. 910-916 and 969-987. John Wiley and Sons, New York.

Kuo, C.F., and Fridovich, I. (1986). Free-radical chain oxidation of 2-nitropropane initiated and propagated by superoxide. *Biochem. J.* 237, 505-510.

Lewis, T.R., Ulrich, C.E., and Busey, W.M. (1979). Subchronic inhalation toxicity of nitromethane and 2-nitropropane. *J. Environ. Pathol. Toxicol.* 2, 233-249.

Löfroth, G., Nilsson, L., and Andersen, J.R. (1986). Structure-activity relationship of nitroalkane-induced mutagenicity in the Ames Salmonella assay. *Prog. Clin. Biol. Res.* 209B, 149-155.

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., and Langlois, R.G. (1983). A simple fluorescent staining procedure for micronuclei and RNA in erythrocytes using Hoescht 33258 and pyronin Y. *Mutat. Res.* 120, 269-275.

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

Machle, W., Scott, E.W., and Treon, J. (1940). The physiological response of animals to some simple mononitroparaffins and to certain derivatives of these compounds. *J. Ind. Hyg. Toxicol.* 22, 315-332.

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

Margolin, B.H., Risko, K.J., Frome, E.L., and Tice, R.R. (1990). A general purpose statistical analysis program for micronucleus assay data. Appendix 2: Micronucleus data management and analysis version 1.4a. Integrated Laboratory Systems, Research Triangle Park, NC.

Marker, E.K., and Kulkarni, A.P. (1986). Cytochrome P-450−mediated denitrification of 2-nitropropane in mouse liver microsomes. *J. Biochem. Toxicol.* 1, 71-83.

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.

The Merck Index (1983). 10th ed. (M. Windholz, Ed.), p. 6464. Merck and Company, Rahway, NJ.

The Merck Index (1989). 11th ed. (S. Budavari, Ed.), p. 1046. Merck and Company, Rahway, NJ.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

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

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986). *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutagen.* **8** (Suppl. 7), 1-119.

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 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 (1995a). Hazardous Substances Data Bank. Database maintained by the National Library of Medicine, available through the MEDLARS system. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD.

National Library of Medicine (1995b). 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) (1987). Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice, 10/31/82 version (updated December 1987). Research Triangle Park, NC.

National Toxicology Program (NTP) (1990). Toxicology and Carcinogenesis Studies of Tetranitromethane (CAS No. 509-14-8) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). Technical Report Series No. 386. NIH Publication No. 90-2841. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1994). Seventh Annual Report on Carcinogens. Summary 1994, pp. 275, 376. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Porter, D.J.T., Voet, J.G., and Bright, H.J. (1972). Nitromethane. A novel substrate for D-amino acid oxidase. *J. Biol. Chem.* **247**, 1951-1953.

Remington's Pharmaceutical Sciences (1975). 15th ed. (J.E. Hoover, Ed.), p. 773. Mack Publishing Company, Easton, PA.

Riordan, J.F., and Vallee, B.L. (1972). Nitration with tetranitromethane. *Methods Enzymol.* **25**, 515-521.

Sadtler Standard Spectra. IR No. 61 (Midget Edition); UV No. 29; NMR No. 9146M. Sadtler Research Laboratories, Philadelphia, PA.

Sakurai, H., Hermann, G., Ruf, H.H., and Ullrich, V. (1980). The interaction of aliphatic nitro compounds with the liver microsomal monooxygenase system. *Biochem. Pharmacol.* **29**, 341-345.

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

Seilkop, S.K. (1995). The effect of body weight on tumor incidence and carcinogenicity testing in B6C3F$_1$ mice and F344 rats. *Fundam. Appl. Toxicol.* 24, 247-259.

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

SRI, International (1980). *Chemical Economics Handbook*, pp. 573.9001C,P. Menlo Park, CA.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* 67, 233.

Subbotin, V.G. (1967). Hygienic assessment of nitromethane and other nitroparafins in connection with sanitary protection of water bodies [in Russian, English summary]. *Gig. Sanit.* 32, 9-13.

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

Tennant, R.W., and Ashby, J. (1991). Classification according to chemical structure, mutagenicity to Salmonella and level of carcinogenicity of a further 39 chemicals tested for carcinogenicity by the U.S. National Toxicology Program. *Mutat. Res.* 257, 209-227.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. *Science* 236, 933-941.

Ullrich, V., Hermann, G., and Weber, P. (1978). Nitrite formation from 2-nitropropane by microsomal monooxygenases. *Biochem. Pharmacol.* 27, 2301-2304.

U.S. International Trade Commission (USITC) (1994). Synthetic Organic Chemicals. United States Production and Sales, 1992, p. 3—28. USITC Publication 2720. Washington, DC.

Wade, A.E., Evans, J.S., Sternson, L.A., and Gammans, R.E. (1977). Interaction of nitromethane with reduced hepatic microsomal cytochrome P-450. *Biochem. Pharmacol.* 26, 963-967.

Weatherby, J.H. (1955). Observations on the toxicity of nitromethane. *AMA Arch. Ind. Health* 11, 102-106.

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.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* 16 (Suppl. 18), 1-14.

Zitting, A., Nickels, J., and Savolainen, H. (1982). Comparison of acute toxic effects of intraperitoneally injected nitromethane and nitroethane in rats. *Toxicol. Lett.* 13, 189-194.