Final Report on the Safety Assessment of Ethoxyethanol and Ethoxyethanol Acetate[1]

Ethoxyethanol is an ether alcohol described as a solvent and viscosity-decreasing agent for use in cosmetics. Ethoxyethanol Acetate is the ester of Ethoxyethanol and acetic acid described as a solvent for use in cosmetics. Although these ingredients have been used in the past, neither ingredient is in current use. Ethoxyethanol is produced by reacting ethylene oxide with ethyl alcohol. Ethoxyethanol Acetate is produced via an esterification of Ethoxyethanol and acetic acid, acetic acid anhydride, or acetic chloride. Ethoxyethanol is metabolized to ethoxyacetaldehyde, which is further metabolized to ethoxyacetic acid, which is also a metabolite of Ethoxyethanol Acetate. Low to moderate acute inhalation toxicity is seen in animals studies. Acute oral toxicity studies in several species reported kidney damage, including extreme tubular degeneration. Kidney damage was also seen in acute dermal toxicity studies in rats and rabbits. Minor liver and kidney damage was also seen in short-term studies of rats injected subcutaneously with Ethoxyethanol, but was absent in dogs dosed intravenously. Mixed toxicity results were also seen in subchronic tests in mice and rats. Ethoxyethanol and Ethoxyethanol Acetate were mild to moderate eye irritants in rabbits; mild skin irritants in rabbits, and nonsensitizing in guinea pigs. Most genotoxicity tests were negative, but chromosome aberrations and sister-chromatid exchanges were among the positive results seen. Numerous reproductive and developmental toxicity studies, across several species, involving various routes of administration, indicate that Ethoxyethanol and Ethoxyethanol Acetate are reproductive toxicants and teratogens. Mild anemia was reported in individuals exposed occupationally to Ethoxyethanol, which resolved when the chemical was not used. Reproductive effects have been noted in males exposed occupationally to Ethoxyethanol. Although there are insufficient data to determine the potential carcinogenic effects of Ethoxyethanol or Ethoxyethanol Acetate, there is evidence that these chemicals are absorbed across human skin and that they are reproductive and developmental toxicants via dermal exposure. Therefore, these ingredients are unsafe for use in cosmetic formulations.

INTRODUCTION

The toxicity of Ethoxyethanol and Ethoxyethanol Acetate is reviewed in this report. Ethoxyethanol is an ether alcohol and Ethoxyethanol Acetate is the ester of Ethoxyethanol and acetic acid. Both ingredients are listed in the *International Cosmetic Ingredient Dictionary*; however, currently, there are no reported cosmetic uses of these ingredients.

CHEMISTRY

Chemical and Physical Properties

Ethoxyethanol is the ether alcohol that conforms to the following formula (Wenninger and McEwen 1997):

$$CH_3CH_2OCH_2CH_2OH$$

Ethoxyethanol is a clear, colorless liquid (with essentially no odor) that is soluble in water, alcohol, ether and acetone (Wenninger and McEwen 1997). Other names for this chemical include Ethanol, 2-Ethoxy and Ethylene Glycol Monoethyl Ether (Wenninger and McEwen 1997). The CTFA (Cosmetic, Toiletry, and Fragrance Association) chemical description of Ethoxyethanol is included in Table 1 (Nikitakis and McEwen 1990). Additional properties of Ethoxyethanol are included in Table 2.

Ethoxyethanol Acetate is the ester of Ethoxyethanol (*q.v.*) and acetic acid that conforms to the formula (Wenninger and McEwen 1997):

$$CH_3C(=O)OCH_2CH_2OCH_2CH_3$$

Ethoxyethanol Acetate is described as a water-white liquid (with mild, characteristic odor) that is soluble in water (Nikitakis and McEwen 1990). Other names for this chemical include Ethanol, 2-Ethoxy-, Acetate; 2-Ethoxyethanol Acetate; and Ethylene Glycol Monoethyl Ether Acetylated (Wenninger and McEwen 1997) and Ethoxyethyl Acetate (Budavari 1989). The chemical description of Ethoxyethanol Acetate is included in Table 1. Additional properties of Ethoxyethanol Acetate are included in Table 2.

Methods of Production

Usually, Ethoxyethanol is produced by reacting ethylene oxide with ethyl alcohol. However, it may be produced by

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Wilbur Johnson, Jr., Senior Scientific Analyst. Address correspondence to him at Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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TABLE 1
CTFA chemical descriptions of Ethoxyethanol and Ethoxyethanol Acetate (Nikitakis and McEwen 1990)

Property	Ethoxyethanol	Ethoxyethanol Acetate
Color	As specified by the buyer	As specified by the buyer
Odor	As specified by the buyer	As specified by the buyer
Identification	Positive: Close match to a standard IR spectrum with no indication of foreign materials	Positive: Close match to a standard IR spectrum with no indication of foreign materials
Specific gravity at 20°/20°C	0.928 to 0.933	0.9748
Refractive index at 25°C	1.402 to 1.410	—
Boiling range	132.0°C to 137.0°C	—
Boiling point at 760 mm Hg	—	156.3°C
Freezing point	—	−61.7°C
Vapor pressure at 20°C	—	2 mm Hg

direct alkylation of ethylene glycol with an alkylating agent such as diethyl sulfate. Ethylene glycol monoalkyl ethers (e.g., Ethoxyethanol) are not produced as pure compounds, but must be separated from the diethers of diethylene glycol, triethylene glycol, and the higher glycols (National Institute for Occupational Safety and Health [NIOSH] 1991).

Ethylene glycol monoalkyl ether acetates (e.g., Ethoxyethanol Acetate) are produced via esterification of the particular glycol ether with acetic acid, acetic acid anhydride, or acetic chloride (NIOSH 1991).

Reactivity

Ethoxyethanol is highly reactive in the presence of strong oxidizers (Clapp et al. 1987). Reportedly, it does not react with ozone (Joshi, Dodge, and Bufalini 1982). Ethoxyethanol is combustible when exposed to heat or flame. Furthermore, a mixture of this chemical with hydrogen peroxide + polyacrylamide gel + toluene is explosive when dry (Lewis 1993).

Ethoxyethanol Acetate is flammable when exposed to heat or flame, and can react with oxidizing materials. A moderate explosion hazard exists when Ethoxyethanol Acetate is heated. Mild explosions have occurred at the end of distillations (Lewis 1993).

Analytical Methods

Ethoxyethanol has been analyzed by gas chromatography in conjunction with flame ionization and mass spectrometry detection systems (Giachetti et al. 1996). It has also been analyzed by oxidation/reduction titration and thin-layer chromatography, and identified by infrared (IR), ultraviolet/visible, and nuclear magnetic resonance spectroscopy (NMR) (National Toxicology Program [NTP] 1993).

Ethoxyethanol Acetate has been analyzed by gas chromatography in conjunction with flame ionization and mass spectrometry detection systems (Giachetti et al. 1996). It has also been identified by IR and NMR spectroscopy (NTP 1985).

TABLE 2
Properties of Ethoxyethanol and Ethoxyethanol Acetate (National Institute for Occupational Safety and Health 1991)

Property	Ethoxyethanol	Ethoxyethanol Acetate
Molecular weight	90.1	132.2
Specific gravity (25°/4°C)	0.926	0.975
Evaporation rate (butyl acetate = 1)	0.41	0.2
Boiling point (°C)	135.0	156.3
Freezing point (°C)	−100	−61.7
Vapor pressure (mm Hg, 25°C)	5.75	2.8
Refractive index	1.406	1.406
Flash point (°C), closed cup	43	52
Autoignition temperature (°C)	235	379
Flammability limits (vol. % in air)	1.70–15.6	1.7
Water solubility (% by weight)	Miscible	23
Vapor density (air = 1)	3.1	4.6
ppm in saturated air (25°C)	7600	3700
mg/m^3 at 25°C (760 mm Hg = 1ppm)	3.69	5.41
ppm at 25°C (760 mm Hg = 1 mg/m^3	0.27	0.19

USE

Purpose in Cosmetics

Ethoxyethanol is listed as a solvent and viscosity-decreasing agent and Ethoxyethanol Acetate is listed as a solvent in the *International Cosmetic Ingredient Dictionary and Handbook* (Wenninger and McEwen 1997).

Scope and Extent of Use in Cosmetics

Product formulation data submitted to the Food and Drug Administration (FDA) in 1998 indicated no reported uses of Ethoxyethanol or Ethoxyethanol Acetate in cosmetic products (FDA 1998). However, data submitted to FDA in 1984 included use concentration ranges for Ethoxyethanol in the following product types: Hair conditioners (1 product, 10% to 25%), Nail polish and enamel (1 product, 0.1% to 1%), and Nail polish and enamel removers (1 product, 10% to 25%). Ethoxyethanol Acetate was used in two nail polish and enamel removers (1% to 5%) and in two other nail polish and enamel removers (25% to 50%) (FDA 1984).

Noncosmetic Use

Glycol Ethers, such as Ethoxyethanol, are useful as solvents for lacquers, paints, varnishes, dyes, inks, resins, cleaning formulations, and liquid soaps. Glycol ethers also have utility as coupling agents for a variety of chemical specialties, and are used as intermediates in the production of plasticizers and other solvents. The higher molecular weight glycol ethers are the primary components of most brake fluids (Miller 1987).

The following uses have been reported for the acetates of glycol ethers: in jet fuel deicers, inks and coatings, photography, dyeing, and the manufacture of printed circuit boards and plasticizers (Wess 1992). Specifically, Ethoxyethanol Acetate has been used as a blush retardant in lacquers, as a solvent for nitrocellulose, oils, and resins, in wood stains and varnish removers, and in products for the treatment of textiles and leathers (American Conference of Governmental Industrial Hygienists [ACGIH] 1991). It is also used as a solvent in the processes of welding nosepads to eyeglass frames and laminating plastic sheets (Fisher 1973; Rietschel and Fowler 1995).

Ethoxyethanol can be safely used as a diluent in color additive mixtures for food use that are exempt from certification (21CFR73.1). Additionally, Ethoxyethanol is listed among the components of rubber articles intended for repeated use that can be safely used in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food. The total amount of Ethoxyethanol is not to exceed 5% by weight of the rubber product (21CFR177.2600).

Ethoxyethanol Acetate and Ethoxyethanol are listed among the ingredients used in adhesives that can be safely used as components of articles intended for use in packaging, transporting, or holding food (21CFR175.105).

BIOLOGICAL PROPERTIES

Absorption, Distribution, Metabolism, and Excretion

Ethylene glycol ethers (e.g., Ethoxyethanol) are rapidly oxidized into acids, through transient aldehydic metabolites. Ethoxyethanol is metabolized by an alcohol dehydrogenase to ethoxyacetaldehyde, which is then metabolized by an aldehyde dehydrogenase to ethoxyacetic acid (Hoflack et al. 1995).

Ethoxyethanol and Ethoxyethanol Acetate have 2-ethoxyacetic acid as a common metabolite. The ester bonding of the acetate is hydrolyzed in blood, with a half-time of a few minutes. Ethoxyacetic acid is created by oxidation of the free primary hydroxyl group of Ethoxyethanol by alcohol dehydrogenase, and is excreted in the urine (Sohnlein et al. 1993).

There is experimental evidence that the in vivo biotransformation of Ethoxyethanol by alcohol dehydrogenase can occur in the liver and testes of Sprague-Dawley rats and Syrian Golden hamsters (Moslen et al. 1995).

The results of additional metabolism studies are summarized below.

The metabolism of Ethoxyethanol was evaluated using three rats. One of the rats (sex and strain not stated) was exposed (inhalation) to an atmosphere containing 37 g/m^3 Ethoxyethanol for 1 hour. The remaining two rats received 9.3 or 93 mg Ethoxyethanol, respectively, by gavage. Urine samples were collected for 24 to 48 hours post dosing and urine extracts analyzed by gas chromatography–mass spectrometry. Ethoxyacetic Acid and *N*-ethoxyacetylglycine were the two urinary metabolites identified. The combined excretion of these two metabolites was estimated at approximately 30% (Ethoxyethanol milligram equivalents) of the administered oral dose (Jonsson, Pedersen, and Steen 1982).

In another study, a single oral dose (230 mg/kg) of either [ethanol-1,2-^{14}C] Ethoxyethanol or [ethoxy-1-^{14}C] Ethoxyethanol was administered to male Sprague-Dawley rats (weights = 190 to 210 g). Excreta were collected for up to 96 hours post dosing. The results for rats dosed with ethanol-labeled Ethoxyethanol indicated that 80.6% of the administered dose was excreted in the urine. Rats dosed with ethoxy-labeled Ethoxyethanol excreted 75.5% of the administered dose in the urine. In both cases, most of the radioactivity in the urine was recovered during the first 24 hours after dosing. Approximately 11.7% of the ethoxy-labeled Ethoxyethanol was recovered as $^{14}CO_2$, whereas only 4.6% of the ethanol-labeled dose was recovered as $^{14}CO_2$. Less than 5% of the dose of each radiolabeled material was recovered in the feces, and less than 5% remained in the carcass at 96 hours post dosing. Total recoveries of radioactivity were 99.3% and 94.4% for the [ethanol-1,2-^{14}C]- and [ethoxy-1-^{14}C]-Ethoxyethanol studies, respectively. As a result of using a variety of analytical techniques, gas chromatography–mass spectrometry included, ethoxyacetic acid and *N*-ethoxy acetyl glycine were considered the predominant urinary metabolites. These two metabolites combined accounted for approximately 75% of the administered radioactivity. Ethoxyacetic

Acid accounted for 43.4% of the ethanol-labeled Ethoxyethanol dose and 44.9% of the ethoxy-labeled Ethoxyethanol dose. The glycine conjugate amounted to 32.1% and 28% of the ethanol-labeled and ethoxy-labeled doses, respectively. Analyses of extracts from the testes indicated a single radioactive moiety that was subsequently identified as ethoxyacetic acid (Cheever et al. 1984).

The urinary excretion of ethoxyacetic acid after dosing with Ethoxyethanol (in distilled water) was evaluated using an inbred strain of white male Wistar rats (weights = 240 to 320 g). Ethoxyethanol was administered as single oral doses ranging from plausible occupational human exposures (0.5 to 1 mg/kg) to doses reported in the literature (100 mg/kg). Five rats were used per dose administered. Urinary excretion of Ethoxyacetic Acid and its glycine conjugate was monitored up to 60 hours post dosing. (These data were compared with findings in humans. See section on Clinical Assessment of Safety.) For all doses administered, the mean elimination half-life of free and conjugated Ethoxyacetic Acid was 7.2 hours (Groeseneken et al. 1988).

In another study, Ethoxyethanol was administered to male Fe44/N rats (11 to 12 weeks old) at concentrations of 220, 650, and 1940 ppm in drinking water over a period of 24 hours. Ethoxyethanol was administered as 2-Ethoxy[U-^{14}C]ethanol, and each rat received ~15 μCi of radioactivity. Exhaled air was removed from each cage via a vacuum pump and collected in a series of gas traps. Both urine and feces were collected over a period of 72 hours. Most of the ^{14}C was either excreted in the urine or exhaled as CO_2. Of the dose administered, 25% to 40% was eliminated as ethoxyacetic acid, and 20% was eliminated as CO_2. Ethylene glycol was also excreted in the urine, representing 18% of the dose (Medinsky et al. 1990).

The absorption of Ethoxyethanol was evaluated using 16 male Dutch rabbits (5 to 6 months old). Following a 12-hour fast, the animals were dosed orally with 1000 mg/kg Ethoxyethanol. Blood samples were collected from the marginal ear vein and semen samples were also obtained. Four blood and semen samples per time point (total of 4 time points) were collected. Samples were analyzed using gas chromatography and a flame ionization detector. Blood concentrations of Ethoxyethanol peaked between 1 and 1.5 hours, and then rapidly declined. The time course for concentrations in the semen closely paralleled with that noted for Ethoxyethanol in the blood. The concentrations of Ethoxyethanol in the semen were slightly greater than those in the blood at all time points measured; blood/semen ratios appeared relatively constant. The actual concentrations of Ethoxyethanol in the blood and semen were not stated (Cincinnati University 1984).

The effect of dose on the distribution of Ethoxyethanol was evaluated using male F344/N rats (11 to 12 weeks old; mean body weight = 238 ± 16 g). The number of rats used was not stated. Rats were anesthetized, and 2-ethoxy[U-^{14}C]ethanol (30-μl aliquot in acetone) was applied to clipped skin of the back (area = 2 cm in diameter). The test substance was applied to three sites on the back at doses of 510, 1600, and 3700 μmol/kg, respectively. To prevent oral uptake from application sites, each site was covered with a perforated tissue capsule that was glued in place. After dosing, the rats were placed in glass metabolism cages. Regardless of the dose, approximately 20% to 25% of dermally applied Ethoxyethanol was absorbed and metabolized. The absorption and metabolism of Ethoxyethanol was defined as the amount of dermally applied 2-ethoxy[U-^{14}C]ethanol equivalents present in the carcass (excluding skin at application site) or excreta by 72 hours post exposure. A small amount of the applied dose remained at the application site at 72 hours post dosing. The majority of the metabolized 2-ethoxy[U-^{14}C]ethanol equivalents was excreted in the urine. There was no significant effect of dose on the excretion of 2-ethoxy[U-^{14}C]ethanol equivalents. Ethoxyacetic Acid was the major urinary metabolite. The metabolite profile of Ethoxyethanol was not affected by dose. Thus, the results of this study indicated that the percentage of administered Ethoxyethanol absorbed and metabolized and the profile of major metabolites were independent of the dermal dose (Sabourin et al. 1992).

An erratum to the preceding study was published in 1993 because of a problem with the high-performance liquid chromatograph (HPLC) analysis that was used. Specifically, the pH of the mobile phase (50 mM sodium formate, pH 4.0) used in the HPLC analysis of urinary metabolites was unstable due to the evaporation of formic acid during sparging of the solvent. As a result, the retention times of the Ethoxyethanol metabolites were very sensitive to changes in the pH of the mobile phase, which increased with time as formic acid evaporated. This caused shorter retention times for the Ethoxyethanol metabolites. Thus, in the preceding study, *N*-Ethoxyacetyl glycinate (EAG) was not detected in the urine by HPLC. EAG is the glycine conjugate of ethoxyacetic acid. When urine samples were reanalyzed using an improved analytical procedure, more accurate data resulted and EAG was detected. However, the results of this analysis did not alter the main conclusions in the original study (Sabourin et al. 1993).

The effect of dose on the absorption, metabolism, and excretion of Ethoxyethanol (as 2-ethoxy[U-^{14}C]ethanol) was evaluated using male F344/N rats (11 to 13 weeks old; 2 groups of 8). The rats were exposed to 2-ethoxy[U-^{14}C]ethanol at a concentration of 5 ppm for 40 minutes and at a concentration of 46 ppm for 6 hours using nose-only tubes. Significant percentages of the retained doses were exhaled as $^{14}CO_2$ during exposure (22%) and after exposure (16%). At both exposure concentrations, 46% of the retained dose was excreted in the urine, and approximately 10% of the retained dose was detected in the carcass at 66 hours post exposure. Ethoxyacetic acid was the major urinary metabolite, and the amount excreted was linearly related to the exposure concentration. The minor metabolites excreted in the urine were ethylene glycol and *N*-ethoxyacetyl glycinate. The researchers stated that the results of this study suggest that the toxicity of inhaled Ethoxyethanol should be directly proportional to exposure concentrations up to 46 ppm if the toxicity of Ethoxyethanol is due to ethoxyacetic acid (Kennedy et al. 1993).

In another inhalation experiment, four beagle dogs (weights = 10 to 13 kg) were exposed to Ethoxyethanol Acetate in an exposure facility that was supplied with filtered air at a rate of 10,000 l/min. The time-weighted average exposure concentration, calculated from the output of the hydrocarbon analyzer, was 50.8 ± 1.1 ppm. A breath sample was collected from each dog prior to placement in the inhalation chamber and after the following durations of exposure: 10, 20, 40, 60, 90, 120, 180, 240, and 300 minutes of exposure. After the dogs were removed from the chamber, breath samples were collected at 10, 20, 40, 60, 90, 120, and 180 minutes post exposure. Breath and room atmosphere samples were analyzed using gas chromatography. Study results indicated that Ethoxyethanol Acetate was rapidly absorbed through the lungs. Breath concentrations indicated a rapid increase with the duration of exposure, and a plateau was reached after approximately 3 hours. Following 10 minutes of exposure, 9 ppm Ethoxyethanol Acetate was detected in the breath (~80% absorption). The Ethoxyethanol Acetate concentration reached a plateau at approximately 16 ppm, indicating that 68% of the inhaled compound was absorbed. Postexposure breath samples indicated a rapid, exponential decrease in the concentration of Ethoxyethanol Acetate. The investigators stated that the data generated could be used to estimate Ethoxyethanol Acetate uptake after human exposure, assuming that the rate of absorption in humans was similar to that found in dogs. Thus, after inhalation exposure for 1 hour at a concentration of 50 ppm, approximately 220 mg of Ethoxyethanol Acetate would be absorbed through the lungs (equivalent to approximately 2 g of material in an 8-hour day) (Guest et al. 1984).

In addition to the preceding experiment, the absorption and elimination of Ethoxyethanol Acetate following dermal application and intravenous (IV) dosing were also evaluated using groups of male beagle dogs (weights = 10 to 13 kg). Study results are summarized below (Guest et al. 1984).

In IV administration experiments, Ethoxyethanol Acetate ([ethyl-1,2-^{14}C]2-ethoxyethylacetate) was added to a solution of the nonradioactive compound in saline, yielding a final concentration of 5 to 10 mg/ml. Approximately 3 ml of this solution (containing ~10 μl) was administered at a dose of 1 mg/kg using an in-dwelling forelimb intravenous catheter. Expired air was collected continuously to allow for the absorption of volatile organic materials and CO_2 in silica gel and sodium hydroxide traps. Blood samples were collected in heparinized tubes at intervals up to 8 hours, and urine samples were collected at 4- and 8-hour intervals using a urethral catheter. Overnight urine samples (8 to 24 hours) were collected from conscious dogs housed in stainless steel metabolism cages after recovery. Study results indicated that after IV administration of [ethyl-1,2-^{14}C]2-ethoxyethylacetate, 61% of the radioactivity was excreted in the urine within 24 hours. Approximately 20% of the administered dose was eliminated during the first 4 hours. After an initial, rapid distribution phase (~30 minutes in duration) the concentration of radioactivity in the blood decreased exponentially throughout the collection period (elimination half-life = 7.9 hours). Small amounts of radioactivity (<1% of the dose) were detected as $^{14}CO_2$ during the 4- and 8-hour collection periods. Trace amounts of radioactivity (<0.1% of the dose) were expired as volatile organic materials (Guest et al. 1984).

For the dermal absorption experiment, an appropriate quantity of Ethoxyethanol Acetate ([ethyl-1,2-^{14}C]2-ethoxyethylacetate) was added to 25 ml of undiluted, nonlabeled compound. The solution (40 μCi, 5 ml) was placed in a sealed glass absorption cell that was secured to the dog's thorax (skin contact surface area = 55.6 cm^2) with surgical tape and adhesive. The duration of exposure was 30 minutes (two beagle dogs) or 60 minutes (three dogs). Radioactivity was detected in the urine at 4, 8, and 24 hours after 30- and 60-minute exposures. Urinary excretion of radioactivity was similar during the first and second collection periods, and continued to be substantial overnight. Urinary excretion patterns were similar to the respective excretion patterns noted after IV dosing. Radioactivity was not measurable in the blood of the three dogs exposed for 60 minutes. Small amounts of $^{14}CO_2$ were detected in expired air. The percutaneous absorption rates at 30 and 60 minutes were 219.3 and 109.6 nmole-cm^2/min, respectively. Rates of percutaneous absorption were calculated after correcting for appropriate % excretion after IV administration. The investigators stated that the data generated could be used to estimate Ethoxyethanol Acetate uptake after human exposure, assuming that the rate of absorption in humans was similar to that found in dogs. Thus, after immersion of both hands for 30 to 60 minutes, approximately 640 mg of Ethoxyethanol Acetate would be absorbed through the skin (Guest et al. 1984).

The in vitro percutaneous absorption of Ethoxyethanol Acetate ([ethyl-1,2-^{14}C]2-ethoxyethylacetate) was also evaluated in this study using the Franz diffusion cell. At necropsy, whole skin was excised from the thorax of each of two beagle dogs. Subcutaneous fat was then removed from the skin. The rate of absorption across the skin was determined by placing an excess amount of the test compound (0.3 ml, 1.5 μCi) in the upper chamber of the cell and monitoring its presence in the lower chamber hourly from 2 to 7 hours. A lag time (time taken to reach steady state absorption) of 1.6 hours was reported. The test compound was absorbed across the skin at a rate of 2.3 mg/cm^2/h (Guest et al. 1984).

The in vitro percutaneous absorption of [^{14}C]Ethoxyethanol Acetate (undiluted) through full thickness rat skin and human stratum corneum was also evaluated using Franz-type diffusion cells. The absorption rate for the test compound through full thickness rat skin was 2.41 ± 0.81 mg/cm^2/h (permeability constant = 2.47 ± 0.83 × 10^{-3} cm/h; n = 12), and 1.41 ± 0.29 mg/cm^2/h (permeability constant = 1.45 ± 0.29 × 10^{-3} cm/h; n = 5) for the test compound through human stratum corneum. The ratio of permeability constants (rat/human) was 1.7, indicating that the test compound penetrated through rat skin more rapidly. The authors concluded that rat skin was more

permeable to Ethoxyethanol Acetate than human skin (Barber et al. 1992).

In another study, the percutaneous absorption of Ethoxyethanol in vitro was evaluated according to the method of Franz (1975) using full thickness abdominal skin obtained at autopsy. The donors were men under 60 years of age. The rate of absorption of Ethoxyethanol was described as great. Ethoxyethanol flux was 0.8202 mg/cm^2/h, with a lag time of 59.3 ± 5.6 minutes. The percutaneous absorption of Ethoxyethanol was enhanced in the presence of acetone (30% Ethoxyethanol mixed with 70% acetone). This mixture had a flux of 0.8328 mg/cm^2/h, with a lag time of 43.1 ± 7.7 minutes. After 90 minutes, the concentration of Ethoxyethanol (from mixture) that penetrated the skin was twice the concentration of pure Ethoxyethanol (132 ± 82.29 μg/cm^2 for mixture vs. 76.85 ± 38.85 μg/cm^2 for pure Ethoxyethanol) (Larese et al. 1994). An absorption rate of 0.8 mg/cm^2/h through isolated human epidermis was reported for Ethoxyethanol in an earlier study (Dugard et al. 1984).

TOXICOLOGY

Cytotoxicity

The cytotoxicity of Ethoxyethanol and Ethoxyacetic Acid (Ethoxyethanol metabolite) was evaluated using Chinese hamster ovary (CHO-K1) cell cultures without metabolic activation. The chemical concentrations (EC_{50} values) that allowed approximately 50% of the seeded cells to form colonies after 16 hours of incubation were determined. EC_{50} values for Ethoxyethanol and Ethoxyethanol Acetate were approximately 21.5 mg/ml (0.22 mmol/ml) and 4.64 mg/ml (0.04 mmol/ml), respectively (Jackh, Gelbke, and Helmstadter 1985).

Biocidal activity of Ethoxyethanol against the following strains/types of fungi and bacteria has been noted within the following concentration ranges: 2% to 5% (*Candida* species, fungus), 5% to 10% (*Cladosporium resinae*, fungus), 10% to 17% (*Gliomastix* species, fungus), and 5% to 10% (*Pseudomonas aeruginosa* and sulfate-reducing bacteria). For each concentration range, viability was detectable at the lower, but not the higher, concentration (Neihof and Bailey 1978).

The blockage of junction-mediated intercellular communication by Ethoxyethanol was evaluated using an in vitro assay that depends on the transfer of metabolites via gap junctions (i.e., metabolic cooperation). Wild-type chinese hamster V79 cells (6-TG^s cells) and a mutant line of V79 cells (6-TG^r cells) that lack hypoxanthineguanine phosphoribosyltransferase (HGPRT) were plated together and incubated with Ethoxyethanol and 6-thioguanine (6-TG). The enzyme-deficient cells (6-TG^r cells) cannot phosphoribosylate 6-TG. Ethoxyethanol was tested at a total of six doses (up to 8 mg/ml, 10 plates per dose). At the end of the incubation period, the number of colonies per plate was determined. Each colony represented the survival and proliferation of a 6-TG^r cell, and the increased number of recovered colonies over background was indicative of the blockage of junction-mediated intercellular communication. Ethoxyethanol was described as an effective blocker of intercellular communication. The researchers noted that the blockage of a specific type of intercellular communication, mediated by gap junctions, has been proposed as a mechanism of action of some teratogens (Loch-Caruso, Trosko, Corcos 1984). Ethoxyethanol-induced inhibition of metabolic cooperation in Chinese hamster V79 cells also has been demonstrated in other studies (Chen et al. 1984; Welsch and Stedman 1984).

Hematotoxicity

The hematotoxicity of Ethoxyethanol and Ethoxyethanol Acetate was evaluated using the following five cell lines: (1) the mouse myelomonocytic cell line WEH1-3b D$^+$; (2) the rat promyelocytic cell line IPC-81, derived from the BN rat leukemia (BNML); (3) human promyelocytic leukemia NB4; (4) murine factor–dependent cell line NFS-60; and (5) murine factor–dependent cell line DA1. Cell viability was judged by morphological intactness of cells under phase contrast microscopy. Morphological intactness was determined by the ability of cells to exclude trypan blue under bright-field microscopy, and by the dimethylthiazoldiphenyl tetrazolium (MTT) bromide colorimetric assay for mitochondrial dehydrogenase enzymic activity (Mossman 1983). Hematotoxicity after 48 hours of incubation was expressed as an IC_{50} value for each cell line. The IC_{50} (median inhibitory concentration, mM) for 48 hours of incubation corresponded to the concentration for which the cell viability in treated cultures was 50% of the viability in untreated control cultures. The IC_{50} values (in mM) for Ethoxyethanol were as follows: 1.6 (NB4 cell line), 1.5 (IPC-81), 9.0 (WEHI-3b), 11.3 (DA1), and 2.6 (NFS-60). IC_{50} values (in mM) for Ethoxyethanol Acetate were 4.3 (NB4 cell line), 17.5 (IPC-81), 6.5 (WEHI-3b), 8.0 (DA1), and 7.9 (NFS-60) (Ruchaud et al. 1992).

Acute Inhalation Toxicity

Following 4 hours of exposure to Ethoxyethanol (rats), an LC_{50} of >4000 ppm was calculated. Ethoxyethanol was classified as having low toxicity. The number and strain of animals tested and animal weights were not stated (Kennedy and Graepel 1991). In the same report, the acute LC_{50} for Ethoxyethanol Acetate in rats (8-hour exposure) was >1500 ppm, classifying it as slightly toxic.

In another study, the acute inhalation toxicity of Ethoxyethanol Acetate was evaluated using two groups of 10 rats (male and female; weights = 220 to 240 g) and four rabbits (two males, two females; weights = 2.2 to 2.5 kg). The animals were exposed to a saturated air-vapor mixture of the test substance (2000 ppm) for 4 hours. Exposure was followed by a 14-day observation period. No deaths occurred during the 4-hour exposure period. Slight and transient hemoglobinuria and/or hematuria was observed only in rabbits and were not present beyond 24 to 48 hours post exposure. Gross lesions were not observed in rabbits necropsied at the end of the observation period (Truhaut et al. 1979).

Six rats survived exposure to 1500 ppm Ethoxyethanol Acetate for 4 hours. Two of the six animals died after 8 hours of exposure (ACGIH 1991).

The acute inhalation toxicity of Ethoxyethanol Acetate has been described as moderate. This classification was based on the observation that guinea pigs survived exposure to essentially saturated vapor for 1 hour (ACGIH 1991).

Acute Oral Toxicity

The acute oral toxicity of Ethoxyethanol was evaluated using two groups of 25 Charles River COBS, CD, BR male rats (weights = 150 to 200 g) and two groups of 25 Charles River COBS, CD-1 male mice (weights = 15 to 17 g). The acute oral LD_{50} for Ethoxyethanol was 3527 mg/kg (95% confidence interval = 2511–4950 mg/kg) in fasted rats and 8103 mg/kg (95% confidence interval = 5769–11,376 mg/kg) in fed rats. In mice, the LD_{50} was 2451 mg/kg (95% confidence interval = 1431–4185 mg/kg) for fasted animals and 5346 mg/kg (95% confidence interval = 3609–7911 mg/kg) for fed animals. Clinical signs noted in fasted and fed animals of both species included inactivity, labored breathing, rapid respiration, anorexia, weakness, tremors, prostration, and death. Hematuria was observed only in fasted rats (Eastman Kodak Company 1981a).

In another study, the acute oral toxicity of Ethoxyethanol was evaluated using mostly groups of 10 mice (13 groups), rats (13 groups), and guinea pigs (5 groups). The ages, weights, and strain were not included. For each group of animals, the maximum dose tested did not exceed 6.0 cc/kg, and the minimum dose was not lower than 2.5 cc/kg. Ethoxyethanol did not produce immediate signs of toxicity. Even with moderate doses, death was sometimes delayed for 4 to 6 days. With high doses, death usually occurred at 24 to 36 hours post dosing. Hematuria was reported for practically all animals, and, after death, the bladders remained distended with blood-colored urine. Extreme tubular degeneration, with almost complete necrosis of nearly all of the cortical tubules, was observed in kidneys from some of the animals. In the other tubules, the changes were chiefly of the hyaline droplet or colloid droplet type, rather than hydropic. Approximately one third of the Bowman's spaces was distended and contained proteinaceous material. Extensive renal changes of this type were not frequent; however, mild changes were always observed. Additionally, hemorrhagic areas in the stomach and intestines and very mild hepatic damage were observed (Laug et al. 1939).

The acute oral toxicity of Ethoxyethanol Acetate was evaluated using male and female Wistar rats (weights = 220 to 240 g). The test substance, diluted in pure neutralized olive oil, was administered by intragastric administration at a dose volume of 10 ml/kg. Dosing was followed by a 2-week observation period. The LD_{50} was 3900 ± 100 mg/kg for male rats and 2900 ± 100 mg/kg for female rats. Ethoxyethanol was classified as moderately toxic. For each animal, ingestion was followed by hemoglobinuria and/or hematuria, which generally decreased progressively. However, occasionally, these signs persisted for more than a week. At microscopic examination, lesions of necrotizing acute glomerulotubular necrosis were noted in kidneys from rats that died early in the study (24 to 72 hours post dosing). Microscopic examination of kidneys from animals killed at the end of the observation period indicated tubular nephrosis of toxic origin, with different degrees of severity (Truhaut et al. 1979).

An acute oral LD_{50} of 3000 mg/kg has been reported for Ethoxyethanol in rats, classifying this chemical as slightly toxic. The number and strain of animals involved in this study and animal weights were not stated (Kennedy and Graepel 1991). In the same report, an acute oral LD_{50} of 5100 mg/kg was reported for Ethoxyethanol Acetate in rats, classifying this chemical as having low toxicity.

In another study, the acute oral toxicity of Ethoxyethanol and Ethoxyethanol Acetate was evaluated using groups of 10 male albino Wistar rats (weights = 90 to 120 g) and groups of 10 guinea pigs (strain not stated; weights = 250 to 300 g). Single doses of each test substance (concentrations up to 50% in distilled water) were administered by stomach tube. Generally, 10 animals per dose were tested. Acute oral LD_{50} values for Ethoxyethanol and Ethoxyethanol Acetate in rats were 2.46 g/kg and 5.10 g/kg, respectively. In guinea pigs, the LD_{50} values for Ethoxyethanol and Ethoxyethanol Acetate were 0.95 g/kg and 1.91 g/kg, respectively. Both test substances caused narcosis, but only at the LD_{50} or above (Smyth, Seaton, and Fischer 1941).

Acute Dermal Toxicity

The acute dermal toxicity of Ethoxyethanol was evaluated using five male, New Zealand white rabbits (weights not stated). A pad containing the test substance was applied to closely clipped abraded skin of the back, held in close contact with the skin by an occlusive wrap of dental dam (secured with adhesive tape), and removed after 24 hours. The following clinical signs were noted at the smaller administered doses: anorexia, slight depression, cyanosis, ataxia, and soft feces. Clinical signs at larger doses administered included salivation, nasal discharge, iritis, significant depression, labored breathing, and prostration. Skin irritation effects are included in the section on Skin Irritation later in this report. An LD_{50} of 3527 mg/kg (95% confidence interval = 2511–4950 mg/kg) was reported (Eastman Kodak Company 1981b).

The acute dermal toxicity of Ethoxyethanol Acetate ($\geq$99%) was evaluated using groups of six New Zealand rabbits (weights = 2.2 to 2.5 kg). Four groups of animals were tested with four different doses, respectively. Doses ranged from 19,500 to 48,750 mg. In each group, the test substance was applied (under successive layers of gauze, cotton-wool, a sheet of rubber [maintained with a bandage] and a second sheet of rubber fastened around the trunk) to clipped skin for 24 hours. (The weight difference between these elements plus solvent before and after skin contact corresponds to the absorbed quantities of the test substance.) The animals were then observed for 2 weeks. Study results are summarized below (Truhaut et al. 1979).

At the lowest dose applied (19,500 mg), the mean value for absorbed quantities of the test substance was 5,600 ± 700 mg/kg (six rabbits). At the highest dose applied (48, 750 mg), the mean value for absorbed quantities of the test substance was 14,350 ± 1000 mg/kg (six rabbits). An acute dermal LD_{50} of 10,500 mg/kg was reported. Generally, animals died between 24 and 48 hours post application. Hemoglobinuria and/or hematuria were observed. The decrease in red blood cells did not exceed 15% to 20%, and little variations in blood hemoglobin were noted. However, a considerable decrease (50% to 70%) in the white blood cell count was noted; values gradually returned to normal in surviving animals. Ketone bodies were present in the urine. Necropsy findings included bloody kidneys and large quantities of blood in the urinary bladder. No gross lesions were found in surviving animals that were killed at the end of the 2-week observation period. At microscopic examination, renal lesions were observed in animals that died early (up to 48 hours). Kidneys from animals killed after the 2-week observation period had fibroinflammatory alterations and a few dilated and atrophic tubules (Truhaut et al. 1979).

Acute Subcutaneous Toxicity

Ethoxyethanol (50% aqueous) was injected subcutaneously into rats (number, strain, and weights not stated) at doses ranging from 2.5 to 5.0 cc/kg and at a dose of 10 cc/kg. At microscopic examination of kidneys, acute nephrosis was observed. The subcutaneous injection of aqueous Ethoxyethanol at a larger dose of 10 cc/kg resulted in degenerative changes as well as marked filling of the intracapsular spaces and the tubules with blood. Similar results were reported following dosing with Ethoxyethanol Acetate according to the same procedure (von Oettingen and Jirouch 1931).

Acute Intraperitoneal Toxicity

Ten female albino Carworth mice (weights = between 18 and 27 g) were each injected intraperitoneally with undiluted Ethoxyethanol. The animals were observed for up to 7 days post injection. An acute LD_{50} of 1.71 g/kg was reported. At microscopic examination (7 days post injection) of tissues from animals that died or were killed, no significant lesions were found in the lungs, spleen, and lymphoid tissue. Moderate lesions of the kidneys were observed (Karel, Landing, and Harvey 1947).

Short-Term Oral Toxicity

The short-term oral toxicity of Ethoxyethanol was evaluated using groups of five male and female F344/N rats and groups of five male and female $B6C3F_1$ mice. Untreated groups of rats and mice served as controls. Five groups of five male rats and the same number of female rats were dosed by gavage for two weeks (5 times/week) with doses of Ethoxyethanol up to 5 g/kg/day. Deaths were noted at doses of 2.5 g/kg (3 of 5 males; 4 of 5 females) and 5.0 g/kg (5 of 5 males; 5 of 5 females). No deaths occurred at doses of 1.25 g/kg or less. When groups of five mice (same number of groups; same doses) were tested according to the same procedure, deaths were noted in male mice only at the highest dose tested (5 of 5 males died). For female mice, deaths were noted only at doses of 2.5 g/kg (2 of 5 mice) and 5.0 g/kg (5 of 5 mice) (Melnick 1984).

In another study, the short-term oral toxicity of Ethoxyethanol was evaluated using six groups of F344 rats (5 males, 5 females/group; 5 to 7 weeks old). Mean body weights for the six groups of male rats ranged from 107 to 108 g, and 107 to 110 g for the six groups of female rats. Five groups received the following doses of Ethoxyethanol in deionized water, respectively, daily for 14 days: 300, 600, 900, 1500, and 2500 mg/kg. The control group received deionized water during the dosing period. At the end of the study, the animals were killed and any organs with gross lesions submitted for microscopic examination. None of the rats died prior to the end of the study. The final mean body weights and mean body weight changes for male rats of all dose groups were variably lower when compared to the control group. For female rats, final body weights and mean body weight changes in the 1500- and 2500-mg/kg dose groups were notably lower when compared to control values (NTP 1993). Additional study results are summarized below.

With the exception of changes in weight of the thymus (and testis), most of the changes in absolute and relative organ weights were related to low final body weights. Dose-related decreases in absolute and relative thymus weights were reported for male and female rats. No test substance–related lesions were observed in male or female rats. Male reproductive effects reported in this study are included in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report (NTP 1993).

Five groups of $BC3F_1$ mice (5 to 7 weeks old; 5 males, 5 females/group) were dosed with Ethoxyethanol according to the procedure in the preceding experiment (same doses). A sixth group served as the control. Mean body weights for the six groups of male mice ranged from 21.7 to 22.6 g, and from 18.3 to 19.1 g for the six groups of female mice. One male mouse in the 900-mg/kg dose group died on day 10 of the study. The animal was hypoactive and dehydrated prior to death. No additional clinical signs were observed in mice of any of the treatment groups. The final mean body weights and mean body weight changes for males and females from all dose groups did not differ from control values. Changes in organ weight were described as minimal. In male and female mice, both absolute and relative thymus weights were similar to those noted for controls. No test substance–related gross lesions were found in male and female mice. A microscopic evaluation of the tissues was not performed (NTP 1993). Male reproductive effects reported in this study are included in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report.

The short-term oral toxicity of Ethoxyethanol was evaluated using three groups of 30 albino rats of the CR, COBS, CD, BR strain (average body weight = 235.7 ± 15.1 g). The three groups received oral doses (by gavage) of 450, 900, and 1800 mg/kg undiluted Ethoxyethanol, respectively, 5 days per

week for 6 weeks. The 10 control rats were dosed with distilled water. Necropsy was performed on 10 animals per dose group at the end of the study. Prior to necropsy, blood was drawn from the inferior vena cava for hematologic and clinical chemistry determinations. Compared to controls, body weight gain and feed consumption were significantly reduced ($p \leq .05$) in all treatment groups. On day 2 of the study, blood in the urine was reported for all animals in the 1800-mg/kg dose group. Blood in the urine was also reported for one rat in the 450-mg/kg dose group (day 7). Other signs of toxicity reported for the 1800-mg/kg dose group included tremors, piloerection, and labored breathing after 4 weeks of dosing. No significant test substance–related clinical signs were noted in the 900-mg/kg dose group. Other toxic effects are summarized below (Eastman Chemical Company 1982).

Animals of the 900- and 1800-mg/kg dose groups had statistically significant reductions ($p \leq .05$) in mean hemoglobin concentration, packed cell volume, and total erythrocyte count. Significant effects on these red blood cell parameters were not noted in rats at a dose of 450 mg/kg. Ethoxyethanol also induced dose-related leukopenia, and the decrease in the number of leukocytes was statistically significant only for the 1800-mg/kg dose group. Calculated red cell indices (mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) reflected the abnormal red cell morphology observed in groups with statistically significant changes in red blood cell parameters. No significant trends were found regarding serum clinical chemistry values. Reduced weight of the testes and associated microscopic changes induced by Ethoxyethanol are mentioned in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report. Weight of the thymus was also reduced; thymic atrophy was noted at doses of 900 mg/kg (2 of 10 rats) and 1800 mg/kg (4 of 9 rats). Other organ weight changes (e.g., spleen and liver) were a reflection of the decreased body weight gain noted (Eastman Chemical Company 1982).

In another study, the short-term oral toxicity of Ethoxyethanol was evaluated using four groups of 30 male F344 rats. The rats were 5 to 7 months old, and mean body weights for the four groups ranged from 161 to 165 g. Three groups received 5000, 10,000, and 20,000 ppm Ethoxyethanol in drinking water, respectively, daily for 60 days. The fourth group received drinking water only. At the end of dosing, 10 rats in each dose group were killed (except in the case of early deaths). According to the protocol, if lesions were observed at necropsy, half of the remaining rats were killed after a 30-day recovery period, and the remaining half after a 56-day recovery period. In the 20,000-ppm dose group, 20 of 30 rats died or were killed before the end of the 60-day dosing period. Thus, the 10 surviving rats in this group were combined with the 5 rats (20,000-ppm dose group) that survived the 13-week dosing period in the subchronic oral toxicity study (NTP 1993) summarized later in this report. Compared to controls, mean body weights (at day 60) of rats of the 5000- and 10,000-ppm dose groups were at least 6% lower. The mean body weight (at day 60) was 48% lower for the 20,000-ppm dose group. The following clinical observations were reported for treatment groups: abnormal posture, diarrhea, emaciation, and polyuria (NTP 1993). Male reproductive effects reported in this experiment are included in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report.

Short-Term Parenteral Toxicity

Ethoxyethanol was administered subcutaneously to four groups of rats (approximately 9 animals/group; strain and weights not stated) over a period of 4-weeks. The four groups received doses of 100, 200, 400, and 800 μl/kg/day, respectively, during the treatment period. A fifth group (negative control) was dosed with NaCl solution. Dyspnea, somnolence, and slight ataxia were noted in rats of the two higher dose groups. Compared to controls (hemoglobin = 14.6 g %; hematocrit = 45%), hemoglobin was decreased at doses of 400 μl/kg/day (value = 13.25 g %) and 800 μl/kg/day (value = 12.4 g %) and, decreased hematocrit, at a dose of 800 μl/kg/day (value = 43%). These were the only data from clinical-chemical diagnostics that were presented because other clinical-chemical data were within the framework of biological and methodological variance and indicated no pathological changes. Additional study results are summarized below (Stenger et al. 1971).

Occasionally, minor chronic inflammation of the injection site was noted in rats of 100 and 200 μl/kg/day dose groups. Similar observations were reported for control rats. Minor chronic inflammation of the injection site was also observed in five male rats dosed with 400 μl/kg/day. Minor changes in the liver and kidneys were observed in all animals of this dose group. Dissociation of the lobular structure of the liver and occasionally swollen hepatocytes were noted. Epithelia of the renal tubules, particularly the convoluted tubules, appeared swollen and the lumina of the tubules were constricted. Edema and hemorrhages at the injection site were noted in animals dosed with 800 μl/kg/day. Changes in the liver and kidneys (all nine animals) in rats of this dose group were similar to those observed in rats of the 400-μl/kg/day dose group, but were more severe. Testicular damage observed in male rats at doses of 400 and 800 μl/kg/day is reported in the section on Reproductive and Developmental Toxicity (Subcutaneous Studies) later in this report (Stenger et al. 1971).

Two groups of four dogs (breed and weights not stated) were injected intravenously with Ethoxyethanol at doses of 100 and 500 μl/kg/day over a period of 22 days. Untreated dogs served as controls. Irritation at the injection site was observed. In the 100-μl/kg/day dose group, inflammatory changes in the area of the injection site were noted in three of four dogs. Thrombosis of the veins was observed in two of the three. In the 500-μl/kg/day dose group, pronounced thrombophlebitis with a strong inflammatory reaction in the vicinity of the veins was observed. At microscopic examination, no changes in the internal organs were noted in dogs of either dose group (Stenger et al. 1971).

Subchronic Inhalation Toxicity

The subchronic inhalation toxicity of Ethoxyethanol was evaluated using groups of 30 Sprague-Dawley CD rats (15 males, 15 females/group; weights = 149 to 275 g) and groups of 20 New Zealand white rabbits (10 males, 10 females/group; weights = 2.1 to 3.3 kg). For rats and rabbits, three groups were exposed to Ethoxyethanol concentrations of 25 (92.5 mg/m^3), 100 (390 mg/m^3), and 400 ppm (1480 mg/m^3), respectively, 5 days per week (6 h/day) for 13 weeks. Nonexposed rats and rabbits served as controls. The only exposure-related changes noted in rats were a decreased pituitary to body weight ratio in high-dose males (400 ppm) and decreased absolute spleen weight in females from all test groups. Additionally, the spleen to body weight ratio was less than the control value in females from low- (25 ppm) and high dose (400 ppm) groups. The decrease in relative spleen weight was significant only at exposure concentrations of 25 and 400 ppm. Histopathological examination did not provide a reason for these changes in organ weight. A decrease in the leukocyte count was noted in high-dose females; however, this finding was said to have been of unknown biological significance. The concentration at which no biologically significant effects were observed in rats was 400 ppm (highest test concentration). The results for rabbits are summarized below (Barbee et al. 1984).

For male and female rabbits, growth was depressed slightly (compared to controls). The absolute weight of the testes and the organ (testes)-to-body weight ratio were decreased only at 400 ppm. At microscopic examination, slight focal degeneration of seminiferous tubules was noted in 3 of 10 rabbits. Hematocrit, hemoglobin concentration, and erythrocyte count were also decreased in rabbits exposed to 400 ppm. Hematological changes were not noted at lower concentrations. The concentration of Ethoxyethanol at which no biologically significant effects were observed was 100 ppm (Barbee et al. 1984).

In another study, two dogs (breed and weights not stated) were exposed to 840 ppm Ethoxyethanol 5 days per week for 12 weeks. Two other dogs, exposed to room air, served as controls. Animals were killed 5 weeks after the end of the exposure period, and tissues examined microscopically. A maximal decrease in erythrocyte counts and hemoglobin concentrations was observed after 4 to 6 weeks of exposure; values remained approximately the same until the end of the exposure period. Recovery was gradual. The anemia was classified as microcytic, based on a comparison of erythrocyte counts and packed cell volume. Microcytosis as well as hypochromia and polychromatophilia were noted in blood smears from both dogs. Generally, the leucocyte counts and proportions of granulocytes, lymphocytes, and monocytes were not greatly altered by experimental conditions. Regarding potential effects on renal function, apparently, experimental conditions did not influence urine output. Additionally, urinalyses did not establish any treatment-related effects. No damage was found at microscopic examination (Werner et al. 1943).

The following changes were noted in the liver and blood of rats, mice, or rabbits exposed to Ethoxyethanol concentrations in excess of 300 ppm (1104 mg/m^3) for up to 13 weeks: reduced cytoplasmic density, disruption of lobular structure, increased plasma fibrinogen, reduced serum proteins, and increased liver weights. Many of the observed changes were reversible (Miller et al. 1981, 1983; Stenger et al. 1971).

Subchronic Oral Toxicity

The subchronic oral toxicity of Ethoxyethanol was evaluated using five groups of male and female Sprague-Dawley rats. The test substance was administered (in drinking water) to four groups of animals at doses of 0.052 (6 rats), 0.213 (8 rats), 0.735 (11 rats), and 1.888 g/kg/day (16 rats), respectively, over a period of 90 days. The fifth group (13 rats) served as the control group. The mean number of days to death for the highest dose group (1.888 g/kg/day) was 18 days. Water and feed consumption in this group was statistically below normal. Rats of the 1.888 g/kg/day dose group had marked or general cloudy swelling of the renal tubules and cells of the liver and excessive pigment deposition in the spleen. The same degree of renal and hepatic damage observed in rats of the highest dose group was observed in rats from the control group that died from pneumonia. At necropsy, no lesions of pneumonia were found in any animals of the highest dose group. (Microscopic changes in the testes are described in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report) (Union Carbide Corporation 1947). Additional study results are summarized below.

The following findings were noted in animals of the 0.735-g/kg/day dose group. Statistically significant decreases in water and feed consumption and mean weight gain, body length, and body fat were found. Additionally, statistically significant increases in liver and kidney weights and blood urea nitrogen were observed. Regarding rats of lower dose groups (0.052 and 0.213 g/kg/day), no test substance-related changes were found in appetite, growth, or organs at microscopic examination. The blood urea nitrogen was slightly elevated in the 0.213-g/kg/day dose group. However, this was not considered a significant finding because there was no microscopic evidence of renal injury. The investigators concluded that 0.213 g/kg/day was the largest dose of Ethoxyethanol tested that was not toxic (Union Carbide Corporation 1947).

Ethoxyethanol was administered orally to five groups of rats (number of animals, strain, and weights not stated) over a period of 13 weeks. Three groups received doses of 50, 100, and 200 μl/kg/day, respectively, during the treatment period. The remaining two groups received doses of 100 and 200 μl/kg/day, respectively, up to day 59, after which doses were increased to 400 and 800 μl/kg/day, respectively, for the remainder of the treatment period. A sixth group served as the negative control group. Decreased hemoglobin and hematocrit values were reported in rats dosed with 400 and 800 μl/kg/day. At microscopic examination, no treatment-related changes were found in

any of the organs obtained from animals that received doses of 50, 100, and 400 μl/kg/day, respectively. However, deposits of hemosiderin, and, occasionally, individual hemopoietic foci in the spleen, were observed in rats dosed with 800 μl/kg/day. Also, doses of 200 and 800 μl/kg/day resulted in testicular changes. Details concerning these results are included in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report (Stenger et al. 1971).

When dogs (groups of six; breed and weights not stated) were dosed with Ethoxyethanol (doses of 50, 100, and 200 μl/kg/day, respectively) over a period of 13 weeks in the same study, decreased hemoglobin and hematocrit values were reported at each dose. Untreated dogs served as controls. No treatment-related gross or microscopic lesions were observed in any of the organs from dogs of the 50- and 100-μl/kg/day dose groups that were evaluated. However, in three (two males, one female) of six dogs of the 200-μl/kg/day dose group, minor changes in the kidneys were observed. The lumens of the convoluted tubules were widened and the epithelium was flattened. Additionally, changes in the testis were observed in the three male dogs from the 200-μl/kg/day dose group that were examined. Details relating to testicular changes are included in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report (Stenger et al. 1971).

The subchronic oral toxicity of Ethoxyethanol was evaluated using six groups of F344 rats (10 males, 10 females/group; 5 to 7 weeks old). Mean body weights for the six groups of male rats ranged from 142 to 146 g, and from 123 to 127 g for the six groups of female rats. Five groups received the following concentrations of Ethoxyethanol in deionized water, respectively, daily for 13 weeks: 1250, 2500, 5000, 10,000, and 20,000 ppm. The control group received deionized water during the dosing period. (For clinical pathological evaluations only, five additional groups [20 males, 20 females/group] exposed to the same doses, respectively, and a control group were used.) Five males and seven females in the 20,000-ppm treatment group either died or were moribund and killed early in the study. Because of the high mortality rate at this concentration, treatment of the remaining male and female rats in this group was discontinued during week 9. Compared to controls, notably decreased final mean body weights were observed in males exposed to a concentration of 10,000 or 20,000 ppm and in females exposed to concentrations of 5000 to 20,000 ppm Ethoxyethanol. Mean body weight gains in males and females receiving Ethoxyethanol concentrations of 5000 to 20,000 ppm were also notably lower when compared to the control group (NTP 1993). Additional study results are summarized below.

Clinical signs noted in treatment groups included the following: tremors, diarrhea, emaciation, abnormal posture, pallor, tachypnea, hypoactivity, and comatose state. In male rats, mild anemia (decreased red blood cell [RBC] count and hemoglobin concentrations) was observed at week 1 and mild to marked anemia (decreased hematocrit, hemoglobin concentration, and RBC count) was observed at weeks 3 and 13. In female rats, mild anemia was also noted at week 1, and mild to moderate anemia at weeks 3 and 13. Organ weight analyses were not performed for male and female rats of the 20,000-ppm exposure group because of the high mortality rate that was noted. However, for the remaining groups, changes in absolute and relative organ weights were observed. Excluding decreases in absolute and relative thymus (and testis) weights, it was stated that the absolute and relative organ weight changes could probably be attributed to the low final mean body weights. A concentration-related decrease in absolute and relative thymus weights was noted for males and females (NTP 1993). Male and female reproductive effects observed in this experiment are summarized in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report. Additionally, a statement to the effect that all microscopic lesions noted in this study were classified as nonneoplastic is included in the section on Carcinogenicity.

Five groups of $BC3F_1$ mice (5 to 7 weeks old) were dosed with Ethoxyethanol according to the procedure in the preceding experiment. Mean body weights for the six groups of male mice ranged from 22.7 to 23.9 g, and from 18.9 to 19.3 g for the six groups of female mice. The five groups (10 males, 10 females per group) received oral concentrations of 2500, 5000, 10,000, 20,000, and 40,000 ppm, respectively. A sixth group served as the control. (For clinical pathological evaluations only, five additional groups [20 males, 20 females/group] exposed to the same concentrations, respectively, and a control group were used.) None of the mice in any of the test groups or in the control group died or was killed before the end of the study. Compared to controls, body weight gains for groups of male and female mice that received 20,000 and 40,000 ppm Ethoxyethanol were lower. Emaciation was the only treatment-related clinical sign of toxicity noted in mice dosed with Ethoxyethanol (NTP 1993). Additional study results are summarized below.

Lesions were observed in the spleen (and testis) of male mice and the spleen and adrenal glands of female mice. A minimal to mild increase in hematopoiesis was observed in the spleen of female mice of the 20,000-ppm test group and in male and female mice of the 40,000-ppm test group. A minimal increase in splenic hematopoiesis was also noted in one female mouse from the 10,000-ppm test group. No apparent effect on the bone marrow (histologic sections) was found. Hypertrophy of the adrenal gland was noted in all test groups (NTP 1993). Male and female reproductive effects reported in this experiment are included in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report. Additionally, a statement to the effect that all microscopic lesions noted in this study were classified as nonneoplastic is included in the section on Carcinogenicity.

Chronic Inhalation Toxicity

The chronic inhalation toxicity of Ethoxyethanol Acetate was evaluated using four groups of either 10 male or 10 female Wistar rats (weights = 170 to 190 g) and 4 rabbits (2 males, 2 females; weights not stated). The animals were exposed to 200 ppm Ethoxyethanol Acetate 5 days per week (4 h/day) for

a period of 10 months. Twenty rats (10 males, 10 females) and 4 rabbits (2 males, 2 females) served as controls. No significant differences were found in body weight gain between test and control animals (rats and rabbits), and no evidence of hematuria, ketonuria, or hematological modifications was observed. All hemoglobin concentrations were within the normal range. Gross lesions were not noted at necropsy. At microscopic examination, focal lesions of tubular nephrosis (clear degeneration of the epithelium, with hyaline and granular tubular casts) were observed in male rats and rabbits. No microscopic alterations were found in female rats and rabbits (Truhaut et al. 1979).

Chronic Oral Toxicity

The chronic oral toxicity of Ethoxyethanol was evaluated using groups of F344/N rats and $B6C3F_1$ mice (50 animals per sex per species). Rats and mice were 7 and 8 weeks old at the beginning of the study, respectively. Three groups per species per sex were to have received doses of 0.5, 1.0, and 2.0 g/kg/day, respectively, by gavage over a period of 2 years (103 consecutive weeks). However, because of the large number of deaths in groups of mice and rats receiving doses of 2.0 g/kg/day, these groups were terminated at 17 to 18 weeks of the study. The test substance was administered in dose volumes of 5 ml/kg (rats) and 10 ml/kg (mice). Deionized water was administered to control groups according to the same procedure. The animals were observed for 1 week after the last week of dosing. Moribund animals and those that survived to the end of the study were killed by exsanguination (while under phenobarbital anesthesia) and immediately subjected to necropsy. Study results are summarized below (Melnick 1984).

Compared to the control group, the survival of male rats of the 1.0-g/kg/day dose group was significantly reduced ($p < .05$). However, no statistically significant difference in survival between the lower dose group (0.5 g/kg/day) and the control group was observed. No statistically significant difference in survival was noted when females dosed with 1.0 g/kg/day were compared to controls. Conversely, a significant increase in survival ($p < .01$) was reported for the 0.5-g/kg/day dose group. Contrary to the results noted in rats, no significant differences in the survival of male or female mice were found at either dose. Dose-related reduction in mean body weight was noted in groups of male and female rats. However, mean body weights of mice did not differ markedly from those of the respective control groups (Melnick 1984). Additional results are summarized below.

Compared to control male rats, the incidence of enlarged adrenal glands was increased in male rats dosed with Ethoxyethanol. This finding was not noted in female rats or male and female mice. Also, compared to controls, pituitary alterations (enlargement, presence of a mass, and/or discoloration) that are relatively common in the aging Fischer 344/N rat were decreased in incidence in groups of male and female rats dosed with Ethoxyethanol. The incidence of subcutaneous tissue masses in the region of the mammary gland was decreased (compared to control female rats) in female rats dosed with Ethoxyethanol. In male rats dosed with Ethoxyethanol, a decreased incidence (compared to control males) of the common testicular changes associated with the male Fischer 344/N rat (enlarged testis with or without evidence of a mass) was noted. Testis size was generally reduced in male mice dosed with Ethoxyethanol (Melnick 1984).

The conclusions of the preceding study were stated as follows: "Repeated administration of Ethoxyethanol at a dose of 2.0 g/kg was lethal to rats and mice. Early deaths in the high-dose groups of rats and mice appeared to be due to gastric ulcers. Ethoxyethanol caused testicular atrophy in male rats and mice. This lesion was apparent in high-dose male rats that died early in the two-year study and in the medium- and high-dose male mice. Gross observations indicate that chronic treatment with Ethoxyethanol at doses of 0.5 or 1.0 g/kg body weight caused an apparent enlargement of the adrenal glands in male rats and interfered with the development of spontaneous gross lesions of the spleen, pituitary, and testis that commonly occur in the aging male Fischer 344 rat. Chronic treatment with Ethoxyethanol also caused a decrease in the incidences of enlarged spleens and pituitaries and of subcutaneous masses in the mammary gland region in the aging female Fischer 344 rat." (Melnick 1984).

The chronic oral toxicity of Ethoxyethanol was evaluated using 20 albino rats from an inbred strain (3 weeks old; weights not stated). The animals were fed Ethoxyethanol in the diet at a concentration of 1.45% over a 2-year period, after which the animals were killed. The animals received approximately 725 mg/kg/day for 2 years. The negative control group was fed basal diet only. No statistically significant differences were found in weekly growth rates and feed consumption between test and control animals. At microscopic examination, testes were enlarged and edematous in two thirds of the animals, and slight, chronic renal damage was beyond that noted in control animals. No evidence of chronic liver damage was found. Additional information on testicular findings are included in the section on Reproductive and Developmental Toxicity—Oral Studies later in this report (Morris, Nelson, and Calvery 1942).

Ocular Irritation

The ocular irritation potential of undiluted Ethoxyethanol was evaluated using rabbits (number and strain not stated). The test substance (one drop) was instilled into the conjunctival sac of one eye of each animal; untreated eyes served as controls. Following instillation, hyperemia of the conjunctiva (positive reaction) and slight edema (positive reaction) were noted (von Oettingen and Jirouch 1931). When Ethoxyethanol Acetate was tested according to the same procedure, hyperemia of the conjunctiva (positive reaction), with pus formation and edema of the conjunctiva (positive reaction) and nictitating membrane resulted (von Oettingen and Jirouch 1931).

The ocular irritation potential of Ethoxyethanol in vitro was evaluated using the corneal opacity assay. Ethoxyethanol (40%, 60%, 80%, and 100% *v/v*; volume = 0.5 ml) was applied to the epithelial surface of the corneas for 30 minutes. Each cornea was

secured in a corneal holder, in which the cornea was inserted between two chambers (anterior and posterior compartments, respectively). Fresh minimal essential medium (MEM) +1% fetal bovine serum was placed in the posterior compartment, and the test substance in the anterior compartment (epithelial side). The corneas were incubated in a horizontal position in order to allow maximum contact of the chemical with epithelial cells, after which an initial determination of corneal opacity was made. A second assessment of corneal opacity was made following another 2-hour incubation period. The corneal holder apparatus could determine the severity of corneal opacity by measuring differences in light transmission between treated and control corneas. Opacity values were classified into four groups corresponding approximately to the following grades: mild (0 to 20 opacity units), mild/moderate (21 to 40), moderate (41 to 70), and severe (71 and over). The largest mean opacity value (31.2; 6 corneas) was noted at the highest test concentration of Ethoxyethanol (100%). Thus, the opacity reading for 100% Ethoxyethanol was in the mild/moderate range (Gautheron et al. 1992).

The ocular irritation potential of undiluted Ethoxyethanol Acetate and 30% *w/w* Ethoxyethanol Acetate in polyethylene glycol was evaluated using two groups of four to six New Zealand rabbits, respectively. In both groups, the test substance (0.1 ml) was instilled into the conjunctival sac of one eye of each animal. Untreated eyes served as controls. Reactions were scored according to the Draize scale (0 to 110) at 24, 48, and 72 hours and 7, 10, 14, and 21 days post instillation. Only results at 24 hours were reported. Corneal thickness was measured several hours prior to instillation of the test material and, thereafter, at intervals coincident with the Draize scoring. The average Draize score and value for corneal swelling in each group were reported along with the coefficient of variation (CV = [standard deviation/mean] $\times$ 100). For 100% Ethoxyethanol Acetate, the average Draize score was 15 (CV = 100%) and corneal swelling was 139% (CV = 30%). For 30% *w/w* Ethoxyethanol Acetate in polyethylene glycol, the average Draize score was 3 (CV = 66%) and corneal swelling was 101% (CV = 4.8%). At concentrations of 30% and 100%, Ethoxyethanol Acetate was classified as a mild and mild/moderate ocular irritant, respectively. The researchers stated that the large CV for the Ethoxyethanol Acetate Draize score makes these data suspect (Kennah et al. 1989).

The ocular irritation potential of Ethoxyethanol Acetate (99%) was evaluated using four New Zealand white rabbits. The test substance was instilled (0.1 ml or the equivalent weight) into the conjunctival sac, and reactions were scored at 4, 48, and 72 hours post instillation according to the Draize scale (0 to 110). The maximum average score (MAS) was obtained by averaging the individual animal weighted scores during observation periods of 48 and 72 hours post instillation, and then selecting the highest (maximum) of these averages. An MAS of 15 was reported for Ethoxyethanol Acetate (Bagley et al. 1992).

In another study, the ocular irritation potential of Ethoxyethanol Acetate ($\geq$99%) was evaluated according to the procedure of Draize (1959) using six New Zealand rabbits. The test substance was totally nonirritating (Truhaut et al. 1979).

Skin Irritation

Skin irritation was noted in an acute dermal toxicity study of Ethoxyethanol (five male New Zealand white rabbits tested) that is summarized earlier in this report (see section on Acute Dermal Toxicity). In this study, the test substance was injected into a pad that was held in close contact with the skin by an occlusive wrap of dental dam secured with adhesive tape. The pad was applied to closely clipped, abraded skin of the back and removed after 24 hours. Dermal responses were scored on days 1, 3, 7, 10, and 14 according to the Draize scale. At most, only slight skin irritation was observed (Eastman Kodak Company 1981b).

The primary skin irritation potential of Ethoxyethanol was evaluated using guinea pigs (number of animals and strain not stated). The test substance was applied (under an occlusive wrap) to depilated abdominal skin at doses up to 18.6 g/kg. The occlusive wrap was removed at the end of the 24-hour contact period. Only slight skin irritation was observed at the largest dose tested (Eastman Kodak Company 1981b).

The skin irritation potential of Ethoxyethanol Acetate ($\geq$99%) was evaluated according to the method of Draize (1959) using six New Zealand rabbits. At 24 hours, very slight erythema was observed in two of six rabbits; skin irritation was not observed at 72 hours. A primary irritation index of 0.08 was reported (Truhaut et al. 1979).

The skin irritation potential of Ethoxyethanol and Ethoxyethanol Acetate in adult New Zealand albino rabbits was evaluated using the Draize protocol and the EEC (European Economic Community) test method. In the Draize protocol (six rabbits), Ethoxyethanol (0.5 ml) was applied to shaved skin of one flank and to scarified skin of the other flank. The test sites were covered with an occlusive patch during the 24-hour application period. Reactions were scored at 24 and 72 hours according to the following scales: 0 (no erythema) to 4 (severe erythema: crimson red, with slight eschar formation [injuries in depth]) and 0 (no edema) to 4 (severe edema: area raised more than 1 mm and extending beyond area of application). The primary irritation index (PII) was then determined. Ethoxyethanol Acetate was also applied to each animal according to the same procedure. The PII for Ethoxyethanol Acetate was 1.9 (slightly irritating). The PII for Ethoxyethanol was not calculated (Zissu 1995). The EEC test results are summarized below.

The EEC test method was similar to the Draize protocol, with the exception that three adult rabbits were used, the occlusive patch remained in place for 4 hours, and the test substances were not applied to a second (scarified) site on the opposite flank. Additionally, reactions were scored at 24, 48, and 72 hours. Either test material was to have been classified as an irritant based on the following criteria: either erythema or eschar formation, or edema equivalent to a mean value of 2 or more observed in two or more animals. Ethoxyethanol and Ethoxyethanol Acetate were classified as nonirritants (Zissu 1995).

Skin Sensitization

The skin sensitization potential of Ethoxyethanol and Ethoxyethanol Acetate was evaluated in the Magnusson-Kligman maximization test using 30 Dunkin-Hartley albino guinea pigs (weights < 500 g). Ten of the 30 animals served as controls. On day 1 of the first week, each test substance (with Freund's adjuvant) was injected intradermally immediately behind the shoulders (first induction). During the second induction (day 8), each test substance was applied topically for 48 hours. The second induction was followed by a 1-week nontreatment period. On day 24, each test substance (0.5 ml) was applied to the left sheared flank at the maximal nonirritating concentration (10%, determined in preliminary study). Occlusive challenge patches remained in place for 48 hours. Challenge reactions were scored 24 and 48 hours after patch removal according to the following scale: 0 (no reaction) to 3 (marked erythema and edema). At 24 hours post application, macroscopic results (challenge site) were confirmed by microscopic examination. Only eczematous reactions were considered positive. Evaluation of the sensitization potential of Ethoxyethanol and Ethoxyethanol Acetate was based on the number of guinea pigs with positive reactions, rather than reaction intensity. No evidence of delayed cutaneous hypersensitivity (i.e., cell-mediated sensitization) was observed in any of the animals tested (Zissu 1995).

Immune System Effects

The effect of Ethoxyethanol on cell-mediated immunity was evaluated in an allograft rejection assay using allogeneic mice, $B6C3F_1$ (C57BL/6 female × C3H male), and syngeneic mice, $CD2F_1$ (BALB/c female × DBA/2 male). The mice were 5 to 6 weeks old and weighed 17 to 21 g. Test and control groups consisted of six to eight mice. Mouse lymphoid leukemia L1210 cells (tumor cells) were implanted intraperitoneally (IP) into allogeneic and syngeneic mice on day 0. Ethoxyethanol was administered orally to three groups of allogeneic mice at doses of 600, 1200, and 2400 mg/kg, respectively. Doses (dose volume = 0.01 ml/g body weight) were administered daily from day −12 to day −8 and from day −5 to day −1. Three groups of syngeneic mice received the same doses of Ethoxyethanol daily on days 1 to 5 and days 8 to 12. Control allogeneic and syngeneic mice were dosed orally with distilled water on the days indicated for Ethoxyethanol dosing, respectively. Allogeneic positive control mice were injected IP with 180 mg/kg cyclophosphamide on day −1. No positive control group for the syngeneic mice was included. An allogeneic tumor challenge model was used in this study. In this model, mice that are allogeneic in relation to the leukemic cell tumor used will not die when challenged unless they have been immunosuppressed, whereas in syngeneic mice, the tumor will grow and kill the animals unless there is a direct cytotoxic effect as a result of chemical treatment of the tumor cells. On the last day of dosing and on the day of death (or at 43 days after tumor implantation for allogeneic mice), smears were made from retroorbital sinus blood from each animal. Differential cell counts were made. Necropsies were performed to determine the presence of leukemic ascites, splenomegaly, and hepatomegaly. The study results are summarized below (Houchens, Ovejera, and Niemeier 1984).

The challenge of negative control (distilled water) syngeneic mice with 1×10^5 L1210 cells resulted in death (median survival time [MST] = 8.0 days). It was stated that the 8-day survival time falls within the acceptable range (8 to 11 days) for the L1210 tumor at this challenge level. However, no effect on the MST of syngeneic mice dosed with Ethoxyethanol was found, indicating no direct antitumor effect of this chemical (Houchens, Ovejera, and Niemeier 1984).

All allogeneic mice dosed with either distilled water or cyclophosphamide and also challenged with 3×10^6 tumor cells died with ascites. However, when mice dosed with Ethoxyethanol were challenged with 3×10^6 tumor cells, no more than one mouse per group died. The researchers stated that these results indicated that either Ethoxyethanol had prophylactic activity or that the immune system was stimulated (Houchens, Ovejera, and Niemeier 1984).

Differential counts of blood smears from allogeneic mice indicated monocytosis, which was considered indicative of monocytic leukemia, in mice that did not survive to the end of the study. Of the allogeneic mice dosed with Ethoxyethanol and necropsied on day 43, 7% had cholecystitis (Houchens, Ovejera, and Niemeier 1984).

The researchers stated that the results of this study suggest that Ethoxyethanol could have an antitumor effect through increased immunological competence or immunomodulation (Houchens, Ovejera, and Niemeier 1984).

The plaque-forming cell (PFC) response to trinitrophenyl-lipopolysaccharide (TNP-LPS) in Fischer 344 rats (CDF[F344/CrlBr] strain; 8 to 10 weeks old) was used to evaluate the immunotoxic potential of Ethoxyethanol. The rats were immunized with a single intravenous injection of 0.5 ml of 40 μg/ml TNP-LPS in sterile saline. Following immunization, four groups of six adult male, Fischer 344 rats were dosed orally with Ethoxyethanol in distilled water at doses of 50, 100, 200, and 400 mg/kg/day, respectively, on 2 consecutive days. The volume administered per dose was 2.5 ml/kg. Control rats were dosed with distilled water. Three days later, the primary immune response to TNP-LPS was determined using the direct PFC assay by Smialowicz et al. (1987). The immunotoxic potential of Ethoxyacetic Acid (Ethoxyethanol metabolite) was also evaluated using four groups of rats according to the same test procedure. For all doses administered, the exposure of rats to Ethoxyethanol or its principal metabolite, ethoxyacetic acid, failed to alter the antibody response to TNP-LPS (Smialowicz et al. 1992).

GENOTOXICITY

Genotoxicity data from over 20 studies on Ethoxyethanol and Ethoxyethanol Acetate are summarized in Table 3. All test results in nonmammalian systems were negative. However,

TABLE 3
Genotoxicity of Ethoxyethanol and Ethoxyethanol Acetate

Bacterial strains/cells tested	Test procedure	Test results	Reference
Ethoxyethanol			
Salmonella typhimurium strains (specific strains not listed)	*Salmonella*/microsome (Ames) test with and without metabolic activation	Not genotoxic at concentrations up to 93.3 mg/plate	Guzzie et al. 1986
Salmonella typhimurium strains TA1537, TA1535, TA100, and TA98	Plate incorporation assay with and without metabolic activation	No induction of reversions at test concentrations up to 23 mg/plate	EPA 1985
Salmonella typhimurium strains TA1535, TA1537, TA98, and TA100	Preincubation modification (Haworth et al. 1983) of the plate incorporation assay (Ames, McCann, and Yamasaki 1975) with and without metabolic activation	Not mutagenic at doses up to 10 mg/plate	Zeiger et al. 1985
Salmonella typhimurium strain TA1538	—	No significant mutagenic response	McGregor 1984
E. Coli strain scl-4-73	—	No significant mutagenic response	McGregor 1984
Drosophila melanogaster (male flies)	Sex-linked recessive lethals assay	Ethoxyethanol (in aqueous 5% sucrose solution) not mutagenic. Questionable or equivocal results with Ethoxyethanol in 0.7% saline solution.	Valencia et al. 1985
Drosophila melanogaster (male flies)	Sex-linked recessive lethals assay	Equivocal results in preceding assay challenged. Ethoxyethanol in 0.7% saline judged to be nonmutagenic	Mason, Valencia, and Zimmering 1992
Cloned Chinese hamster ovary cells (CHO-W-B1)	Chromosome aberrations assay with and without metabolic activation	Chromosome aberrations (without metabolic activation only) at concentration of 3170 μg/ml	Galloway et al. 1987
Chinese hamster ovary cells	Chromosome aberrations assay with and without metabolic activation	Statistically significant and concentration-related increases in chromosome aberrations (maximum increase of 5.6-fold) without metabolic activation). No significant effects observed with metabolic activation	Guzzie et al. 1986
Human lymphocytes	Chromosome aberrations assay	No clastogenic activity. No significant differences in incidence of chromosomal breaks at concentrations of 500, 1000, and 3000 ppm	Villalobos-Pietrini et al. 1989

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TABLE 3
Genotoxicity of Ethoxyethanol and Ethoxyethanol Acetate *(Continued)*

Bacterial strains/cells tested	Test procedure	Test results	Reference
Cloned Chinese hamster ovary cells (CHO-W-B1)	Sister-chromatid exchange assay with and without metabolic activation	Positive results with and without metabolic activation at a concentration of 3170 g/ml	Galloway et al. 1987
Chinese hamster ovary cells	Sister-chromatid exchange assay with and without metabolic activation	Statistically significant and dose-related increases in sister-chromatid exchanges with and without metabolic activation	Guzzie et al. 1986
Human lymphocytes	Sister-chromatid exchange assay	Frequency of sister-chromatid exchanges increased with increasing test concentrations (250 to 1500 ppm). Frequency was significantly different ($p < .001$) at each test concentration.	Villalobos-Pietrini et al. 1989
Chinese hamster ovary cells	CHO (CHO/HGPRT) mutation assay [CHO = Chinese hamster ovary cells; HGPRT = hypoxantine guanine phosphoribosyl transferase]	Not genotoxic at concentrations up to 42 mg/ml	Guzzie et al. 1986
Swiss-Webster mice	Peripheral blood micronucleus test	No statistically significant increases in number of micronuclei in peripheral blood PCEs following single IP. injections of 25%, 50%, or 80% of the LD_{50} in mice (2589 mg/kg)	Guzzie et al. 1986
L5178Y $TK^{+/-}$ mouse lymphoma cells	L5178Y $TK^{+/-}$ mouse lymphoma mutagenesis assay	Not mutagenic	Myhr, Bowers, and Caspary 1986
L5178Y mouse lymphoma cells	L5178Y cell mutation assay with and without metabolic activation	Negative results without metabolic activation. Weakly positive in two of three trials with metabolic activation: Positive results at concentration of 4 μl/ml (trial 1) and at 3 and 5 μl/ml (trial 2)	NTP 1993
	Ames test (Maron and Ames 1983)	Ethoxyethanol: Not mutagenic at concentrations up to 9 mg per plate. Ethoxyacetaldehyde: Not mutagenic at concentrations up to 9 mg/plate Ethoxyacetic acid: Not mutagenic at concentrations up to 2 mg/plate	Hoflack et al. 1995

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TABLE 3
Genotoxicity of Ethoxyethanol and Ethoxyethanol Acetate *(Continued)*

Bacterial strains/cells tested	Test procedure	Test results	Reference
Ethoxyethanol Acetate			
Salmonella typhimurium strains (specific strains not listed)	Ames test	No significant increase in gene mutations	Slesinski, Guzzie, and Tyler 1988
Chinese hamster ovary cells	CHO/HGPRT test system with and without metabolic activation	Negative results with and without metabolic activation	Slesinski, Guzzie, and Tyler 1988
Swiss-Webster mice	Peripheral blood micronucleus test	No remarkable clastogenicity	Slesinski, Guzzie, and Tyler 1988

Ethoxyethanol induced chromosome aberrations in two assays and sister-chromatid exchanges in three mammalian cell assays. Ethoxyethanol was weakly positive in the L5178Y cell mutation assay.

CARCINOGENICITY

All microscopic lesions (internal organs) observed in subchronic oral toxicity studies summarized earlier in the report text (NTP 1993) were classified as nonneoplastic. The two studies involved rats and mice. In one of the studies, the oral toxicity of Ethoxyethanol was evaluated using six groups of F344 rats (10 males, 10 females/group; 5 to 7 weeks old). Mean body weights for the six groups of male rats ranged from 142 to 146 g, and from 123 to 127 g for the six groups of female rats. Five groups received the following concentrations of Ethoxyethanol in deionized water, respectively, daily for 13 weeks: 1250, 2500, 5000, 10,000, and 20,000 ppm. (For clinical pathological evaluations only, five additional groups [20 males, 20 females/group] exposed to the same concentrations, respectively, and a control group were used.) In the other study, five groups of $BC3F_1$ mice (5 to 7 weeks old) were dosed with Ethoxyethanol according to the same procedure. Mean body weights for dosed with Ethoxyethanol according to the same procedure. Mean body weights for the six groups of male mice ranged from 22.7 to 23.9 g, and from 18.9 to 19.3 g for the six groups of female mice. The five groups (10 males, 10 females per group) received oral concentrations of 2500, 5000, 10,000, 20,000, and 40,000 ppm, respectively (NTP 1993). Additionally, the following statement, which relates to Ethoxyethanol, is also included in this report: "There have been no adequate carcinogenicity studies conducted with any of the glycol alkyl ethers." (NTP 1993). The Environmental Protection Agency (EPA) has classified Ethoxyethanol as a group D chemical, meaning that the data are insufficient for determining the carcinogenicity of 2-ethoxyethanol in humans (EPA 1985). This classification is based on 2-year chronic oral toxicity studies (Morris, Nelson, and Calvery 1942; Melnick 1984) that are summarized earlier in this report. According to the EPA document (1985), no tumors were observed in the study by Morris, Nelson, and Calvery 1942, and the tumor incidence was not increased in the study by Melnick (1984). These observations are based on groups of rats that were tested.

Anti-Carcinogenic Effect

Two groups of syngeneic, male Fischer F344/N rats (6 weeks old) with leukemia transplants received oral doses of 2.5 and 5.0 mg/ml Ethoxyethanol in water daily up to 60 days post transplant. Untreated animals (without transplants or Ethoxyethanol treatment) and animals receiving transplants but no Ethoxyethanol served as controls. Test and control groups consisted of 8 to 10 rats. The effect of Ethoxyethanol on the progression of leukemia transplants was determined by comparing the results for test animals with those for untreated rats with transplants. The expression of leukemia was quantified by measurement of relative spleen weight (i.e., the extent of splenomegaly). Ethoxyethanol induced small and variable decreases in RBC indices at both doses, and minor reductions in white blood cell counts at the high dose (5.0 mg/ml). As determined by the extent of splenomegaly, Ethoxyethanol delayed the progression of leukemia in transplant recipients in a dose-related manner. At both doses, the results for test animals were significantly different ($p < .01$) from those of control transplant recipients that were not dosed with Ethoxyethanol (Dieter et al. 1990).

In an earlier study, leukemic spleen cells from Fischer 344/N rats were injected subcutaneously into syngeneic recipients (8-week-old F344 rats; four groups of four), and the effects of Ethoxyethanol on tumor progression evaluated 70 days after transplantation. Two groups received doses of 2.5 and 5.0 mg/ml Ethoxyethanol, respectively; doses were administered ad libitum in drinking water. Rats (with leukemia transplants) in the third group served as untreated controls, and a fourth untreated control group received neither transplants nor Ethoxyethanol. Group 3 was used for statistical comparisons. Fischer rats given syngeneic leukemia cell transplants usually develop

immune-mediated hemolytic anemia and thrombocytopenia, which cause a 50% reduction in RBCs, hemoglobin, hematocrit, and platelets (Dieter et al. 1991). Test results are summarized below.

Ethoxyethanol slowed tumor growth in rats injected with leukemic spleen cells. At both doses, the progression of splenomegaly and leukoblastosis was significantly ($p < .05$) delayed at day 65 post implantation, at which time spleen weights and white blood cell counts were indistinguishable from untreated control values. Decreases in plate counts in transplant recipients were also ameliorated by Ethoxyethanol treatment. The reductions in RBC count, hemoglobin concentration, and hematocrit were not entirely prevented by treatment with Ethoxyethanol. Histopathological evaluation confirmed that mononuclear cell leukemia was expressed in the spleen and liver of rats given leukemic transplants. Responses were proportionally reduced in test rats, compared to responses in transplanted rats that were not exposed to Ethoxyethanol. Leukemia did not occur in rats that did not receive leukemia transplants nor Ethoxyethanol (Dieter et al. 1989).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

According to the European Community Classification of chemicals for reproductive toxicity, Ethoxyethanol is in category 2 on the basis of animal studies supporting evidence that adverse effects would be likely with human exposure (Petrelli and Traina 1995). Animal studies (inhalation, oral, dermal, and subcutaneous studies) on the reproductive and developmental toxicity of Ethoxyethanol and Ethoxyethanol Acetate are summarized below.

Inhalation Studies

Studies on the reproductive and developmental toxicity of Ethoxyethanol and Ethoxyethanol Acetate are summarized in Table 4 and described in the text. In most of the studies, reproductive toxicity was observed following inhalation exposure.

The developmental toxicity of Ethoxyethanol was evaluated using three groups of adult female Wistar rats (weights ≈150 g) and three groups of female New Zealand white rabbits (weights ≈3.5 kg). Female rats were mated with sexually mature males, and the day on which sperm were detected in the vaginal lavage of female rats was referred to as day 1 of gestation. Female rabbits (29 per group) were artificially inseminated. Semen samples were collected, pooled, and diluted from at least three bucks daily. The day of insemination was considered day 0 of gestation.

Two groups of rabbits were inseminated and exposed to mean Ethoxyethanol concentrations of 160 and 617 ppm (7 h/day), respectively, on days 1 through 18 of gestation. A third group was exposed to air only. At the beginning of exposure, the number of rabbits in each group ranged from 29 to 38 rabbits. Animals were exposed in heated, stainless steel vaporization chambers. Chamber atmospheres were generated by metering liquid Ethoxyethanol at control rates. The rabbits were killed with carbon dioxide on day 30 of gestation for necropsy. Gross examination was performed on the does and their offspring. All rabbit fetuses were examined for internal anomalies using a dissecting microscope. The results of this experiment are summarized below (Andrew and Hardin 1984).

Four does in the 617-ppm group, one doe in the 160-ppm group, and two air-exposed rabbits died during exposure. Based on necropsy findings, it was concluded that deaths in the 617-ppm group were caused primarily by chemical effects on appetite and gastrointestinal function. No treatment-related changes were found in the liver and kidneys. Slight to moderate infiltration of lymphocytes and/or heterophils was noted in the endometrium of the uterus. These changes were more prominent in rabbits exposed to 617 ppm, and were not observed in controls or rabbits exposed to 160 ppm. Compared to the control group, the corpora lutea of rabbits exposed to 617 ppm were smaller. No evidence that exposure to Ethoxyethanol altered fertility in either treatment group was found (Andrew and Hardin 1984).

The incidence of embryomortality in the 617-ppm exposure group was 100%; only early resorptions were observed. This finding was considered the most striking evidence of embryotoxicity in rabbits. Statistically significant results ($p < .05$) were also noted in the 160-ppm exposure group. The mean number of resorptions per litter was approximately six times that of controls. Compared to controls, no statistically significant findings with respect to fetal size (weight or length), placental weight, or sex ratios in the 160-ppm exposure group were found. However, significant ($p < .05$) increases in the incidence of the following defects were noted in this group: major malformations (ventral defects and fusion of aorta with pulmonary artery), minor anomalies (renal changes), and common skeletal variants (supernumerary ribs with associated vertebral variations and external defects). The researchers concluded that Ethoxyethanol was teratogenic in rabbits (Andrew and Hardin 1984).

In an experiment using rats (Andrew and Hardin 1984), initially, two groups of unmated rats were exposed to mean Ethoxyethanol concentrations of 150 and 649 ppm, respectively, 7h/day for 3 weeks (pregestational exposure). The females were then mated and exposed (7h/day) to mean Ethoxyethanol concentrations of 202 and 767 ppm, respectively, on days 1 through 19 of gestation. Air-exposed rats served as controls. At the beginning of exposure, the number of rats in each group ranged from 29 to 38. The method of inhalation exposure for rats was the same as that stated for rabbits earlier in this section. The rats were killed using carbon dioxide on day 21 of gestation. Gross examination was performed on the dams and their offspring. Half of all rat fetuses was examined for internal anomalies using a dissecting microscope. The results of this experiment are summarized below (Andrew and Hardin 1984).

Microscopic examination of the lungs, liver, and kidneys from rats did not detect any exposure-related lesions. Uterine involution was observed in 15 of 16 rats exposed to 767 ppm Ethoxyethanol during gestation, but not in other test groups.

TABLE 4
Reproductive and developmental toxicity studies—inhalation exposure

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (up to 767 ppm)	Two groups of female Wistar rats (29–38/group; weights = 150 g)	Three-week pregestational exposures (7 h/day) at 150 and 649 ppm, respectively. After mating, exposures at 202 and 767 ppm on gestation days 1–19, respectively.	Significant embryolethal effect at 767 ppm (25 × control value). Resorptions at 202 ppm = 2 × control value. Significantly increased ($p < .05$) incidence of cardiovascular defects in group subjected to pregestational air exposure followed by exposure to 202 ppm (gestation), but not in group exposed to 150 ppm (pregestation) and then 202 ppm (gestation). Minor skeletal defects predominated in 202-ppm exposure group	Andrew and Hardin 1984
Ethoxyethanol (up to 765 ppm)	Two groups of Wistar or Sprague-Dawley rats (weights = 250–300 g)	Exposure to 200 and 765 ppm, respectively, on gestation days 1–19 (6–7 h/day)	At 765 ppm, total resorption of all litters (fetotoxicity). Incidence of resorptions not significantly increased at 200 ppm. Also, significant increase ($p < .05$) in incidence of cardiovascular and skeletal defects (200 ppm), indicating teratogenicity	Hardin et al. 1981
Ethoxyethanol (up to 617 ppm)	Two groups of female New Zealand white rabbits (29–38/group; weights = 3.5 kg)	Exposure to 160 and 617 ppm (7 h/day), respectively, on gestation days 1–18	In 617-ppm exposure group, 100% incidence of embryomortality. In 160-ppm exposure group, mean number of resorptions per litter 6× that of controls. Significant increases in following defects: major malformations (ventral defects and fusion of aorta with pulmonary vessels), minor anomalies (renal changes), and common skeletal variants (supernumerary ribs with associated vertebral variations and external defects)	Andrew and Hardin 1984

(Continued on next page)

TABLE 4
Reproductive and developmental toxicity studies—inhalation exposure *(Continued)*

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (up to 615 ppm)	Two groups of New Zealand white rabbits	Exposure to 160 and 615 ppm, respectively, on gestation days 1–18 (6–7 h/day)	At 615 ppm, total resorption of all litters (fetotoxicity). Significant increase in number of resorptions ($p < .001$) and significantly increased ($p < .05$) incidence of renal, cardiovascular, and ventral body wall defects at 160 ppm (all indicative of teratogenicity)	Hardin et al. 1981
Ethoxyethanol (up to 400 ppm)	Three groups of 24 Dutch rabbits (5–7 months old; weights = 1.6–2.7 kg)	Exposure to 50, 150, and 400 ppm, respectively, on gestation days 6–18 (6 h/day)	Embryo/fetotoxicity (increased incidence of intrauterine deaths and reduced fetal weights) only at 400 ppm. No exposure-related macroscopic pathological abnormalities or external fetal abnormalities	Imperial Chemical Industries PLC 1983a
Ethoxyethanol (up to 250 ppm)	Female Alderly Park (Wistar derived; nulliparous specific pathogen-free) rats (11–13 weeks old; 206–208 g). Number per group not stated	Exposure to 10, 50, and 250 ppm, respectively, on gestation days 6–15	Fetotoxicity, but not teratogenicity, at 250 ppm. Fetotoxic effects included increased incidence of intrauterine deaths and reduced fetal ossification. Slight evidence of fetotoxicity at 50 ppm. No toxicologically significant effects at 10 ppm	Imperial Chemical Industries PLC 1983b
Ethoxyethanol (up to 250 ppm)	Nulliparous, specific pathogen–free Alpk/AP (Wistar–derived) rats (11–13 weeks old; weight range = 200–280 g). Three groups of 24	Exposure to 10, 50, and 250 ppm, respectively, on gestation days 6–18.	Not teratogenic at concentrations up to and including 250 ppm	Doe 1984a
Ethoxyethanol (200 ppm)	16 female Sprague-Dawley rats	Behavioral teratology test: Exposure (7 h/day) on gestation days 7–13	Compared to controls, rotorod performance (sophisticated test of neuromuscular ability) was decreased and open field activity (commonly used to measure exploratory activity) was depressed	Nelson, Brightwell, and Setzer 1982a
Ethoxyethanol (200 ppm)	16 female Sprague-Dawley rats	Behavioral teratology test: Exposure (7 h/day) on gestation days 7–13	Neurochemical changes in pup brains (all regions, except for brainstem)	Nelson et al. 1982b

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TABLE 4
Reproductive and developmental toxicity studies—inhalation exposure *(Continued)*

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (up to 175 ppm)	Female Dutch rabbits (5–7 months old; 3 groups of 24). Weight range = 1.7–2.8 g	Exposure to 10, 50, and 175 ppm, respectively, on gestation days 6–18.	Not teratogenic at concentrations up to and including 50 ppm in rabbits. 175 ppm considered marginal effect level for teratogenicity	Doe 1984a
Ethoxyethanol (up to 175 ppm)	Female Dutch rabbits (5–7 months old; 3 groups of 24)	Exposure on gestation days 6–18 (6 h/day)	At 175 ppm, incidences of skeletal variants and minor skeletal defects significantly increased. 50 ppm is clear no-effect level for embryo/fetotoxicity	Imperial Chemical Industries PLC 1983c
Ethoxyethanol (100 ppm)	Female Sprague-Dawley rats (Two groups of 15–20; weights = 200–300 g)	Behavioral teratology test: inhalation exposure on gestation days 7–13 (group 1) and 14–20 (group 2)	Significant decreases in rotorod performance and increase in latency of leaving central area of open field (compared to controls). Both indicative of some degree of embryotoxicity. Neurochemical changes in pup brains	Nelson, Brightwell, and Setzer 1984a
Ethoxyethanol (100 ppm) + 10% ethanol in drinking water		Behavioral teratology test: inhalation and oral exposure on gestation days 7–13 (group 3) and 14–20 (group 4)	Ethanol reduced the behavioral (neuromuscular effects) of Ethoxyethanol in group 3 and potentiated behavioral effects in group 4	Nelson, Brightwell, and Setzer 1984a
Ethoxyethanol (100 ppm)	Female Sprague-Dawley rats (2 groups of 14 and 15, respectively)	Behavioral teratology test: inhalation exposure (7 h/day) during gestation days 7–13 (15 rats) and 14–20 (14 rats)	Exposure on days 7–13 caused impaired performance on rotorod, and pups remained in central area of open field for significantly longer periods (compared to controls). Exposure on days 14–20 resulted in offspring with fewer differences in behavioral effects (compared to controls). Neurochemical changes in pup brains	Nelson et al. 1981

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TABLE 4
Reproductive and developmental toxicity studies—inhalation exposure *(Continued)*

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (100 ppm)	Groups of 15–20 female Sprague-Dawley rats	Behavioral teratology test: exposure on gestation days 7–13 or 14–20	Exposure on gestation days 7–13 caused impaired performance on rotorod and in open field test, and altered performance in avoidance conditioning. Exposure on days 14–20 caused impaired activity on the activity wheel (used to measure circadian activity) and in avoidance conditioning. Neurochemical changes in the brain (fewer effects after exposure on days 14–20)	Nelson and Brightwell 1984
Ethoxyethanol 17 mg/l (saturated vapor)	Male Alpk/Ap, Wistar-derived rats (number not stated)	3- or 4-h exposure followed by 14-day observation period	20% reduction in testicular weight. Hematuria in all animals	Doe 1984b
Ethoxyethanol Acetate (up to 600 ppm)	Female Sprague-Dawley rats (3 groups; weights = 200–300 g)	Exposure to 130 ppm (15 rats), 390 ppm (15 rats), and 600 ppm (9 rats) on gestation days 7–15 (7 h/day)	600 ppm caused resorption of all offspring. Heart defect in 130 ppm group. Because heart defects rarely occur spontaneously, these data indicate that either 130 or 390 ppm is teratogenic. Other data indicated that exposure to 130 and 390 ppm during organogenesis can cause growth retardation	Nelson et al. 1984b
Ethoxyethanol Acetate (up to 600 ppm)	Female rabbits (2 groups of 15)	Exposure to 200 and 600 ppm, respectively, on gestation days 7–15.	600 ppm caused skeletal and cardiovascular defects. Single defect (unnamed) noted at 200 ppm	Nelson et al. 1982c
Ethoxyethanol Acetate (up to 450 ppm)	Female Dutch rabbits (3 groups of 8; 5–7 months old). Weights = 1.4–3.1 kg.	Exposure to 100, 250, and 450 ppm, respectively, on gestation days 6–18 (6 h/day)	Fetotoxicity (reduced fetal weights) at all concentrations. Increased incidence of intrauterine deaths observed only at 450 ppm	Imperial Chemical Industries PLC 1983d

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TABLE 4
Reproductive and developmental toxicity studies—inhalation exposure *(Continued)*

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol Acetate (up to 400 ppm)	Female Dutch rabbits (3 groups of 23–24; weights = 1.7−2.8 kg). Ages were 5–7 months	Exposure to 25, 100, and 400 ppm, respectively, on gestation days 6–18 (6 h/day)	400 ppm caused fetotoxicity, and possible teratogenicity. 100 ppm caused slight fetotoxicity; 25 ppm did not. No evidence of teratogenicity at 25 or 100 ppm	Imperial Chemical Industries PLC 1983e
Ethoxyethanol Acetate (up to 400 ppm)	Female Dutch rabbits (5–7 months old; weight range = 1.7–2.9 g). Three groups of 24	Exposure to 25, 100, and 400 ppm, respectively, on gestation days 6–18.	No evidence of teratogenicity at 400 ppm. Slight fetotoxicity at 100 ppm. No effect at 25 ppm	Doe 1984a
Ethoxyethanol Acetate (up to 300 ppm)	Female Fischer 344 rats [COBS CDF (F-344)/CrlBR]. 4 groups of 30. Age range: 8–10 weeks old.	Exposure to 50, 100, 200, and 300 ppm, respectively, on gestation days 6–15 (6 h/day)	Developmental toxicity at 100 to 300 ppm. Developmental effects included teratogenicity at 200 to 300 ppm. No evidence of developmental toxicity, including teratogenicity, at 50 ppm	Tyl et al. 1988
Ethoxyethanol Acetate (up to 300 ppm)	Female New Zealand white rabbits (4 groups of 24; 5–5.5 months old)	Exposure to 50, 100, 200, and 300 ppm, respectively, on gestation days 6–18 (6 h/day)	Developmental toxicity at 100 to 300 ppm. Developmental effects included teratogenicity at 200 to 300 ppm. No evidence of developmental toxicity, including teratogenicity, at 50 ppm	Tyl et al. 1988

Significant regression of corpora lutea ($p < .05$) also was observed only in rats of the 767-ppm exposure group (9 of 16 rats). Changes in the corpora lutea were not as pronounced as those observed in rabbits. Ethoxyethanol did not appear to alter mating behavior, breeding performance, or fertility at either of the test concentrations (Andrew and Hardin 1984).

A significant embryolethal effect was noted in rats of the 767-ppm exposure group. The mean number of resorptions per litter in this group was 25 times that of air controls. Resorptions per litter in rats of the 202-ppm exposure group were approximately twice that of controls. Fetal body size was also significantly reduced ($p < .05$) in rats exposed to 202 ppm Ethoxyethanol during gestation. A significantly increased ($p < .05$) incidence of cardiovascular defects (transposed and retrotracheal pulmonary artery) was noted in rats subjected to 3 weeks of pregestational exposure to filtered air, followed by exposure to 202 ppm Ethoxyethanol during gestation, but not in rats subjected to 3 weeks of pregestational exposure to 150 ppm Ethoxyethanol, followed by exposure to 202 ppm Ethoxyethanol during gestation. The predominant effects noted in rats exposed to 202 ppm were minor skeletal defects. A significant increase ($p < .05$) in both minor skeletal anomalies and common skeletal variants was noted. The researchers concluded that Ethoxyethanol was teratogenic in rats (Andrew and Hardin 1984).

The teratogenicity of Ethoxyethanol was evaluated using two groups of pregnant Wistar or Sprague-Dawley rats and two groups of pregnant New Zealand white rabbits (number of animals not stated). Prior to mating, the weights of young adult, female rats ranged from 250 to 300 g. The weight range of rabbits in the study was not given. After mating, the two groups of rats were exposed to Ethoxyethanol (inhalation exposure) at concentrations of 200 and 765 ppm, respectively, 6 to 7 hours per day on days 1 to 19 of gestation. The two groups of rabbits were exposed to Ethoxyethanol at concentrations of 160 and 615 ppm, respectively, 6 to 7 hours per day on days 1 to 18 of gestation. Control rats and rabbits were exposed to air only according to the same procedures for test groups, respectively. Test results for rats and rabbits are summarized below (Hardin et al. 1981).

Initially, rabbits were to have been exposed on gestation days 1 to 24. However, gestation day 18 was the last day of exposure because severe anorexia and weight loss were reported for rabbits exposed to 615 ppm Ethoxyethanol. Five rabbits died, and the survivors recovered quickly after exposures were terminated. Maternal toxicity (increased liver weight and reduced feed consumption and body weight) was also noted in rats exposed to 160 ppm. Total resorption of all litters was reported for the 615-ppm test group. Compared to controls, the

number of live fetuses was significantly reduced, and the number of resorptions was significantly increased ($p < .001$) in the 160-ppm exposure group. The results of fetal morphological examinations indicated a significantly increased ($p < .05$) incidence of renal, cardiovascular, and ventral body wall defects in fetuses from the 160-ppm exposure group. Increases in certain minor skeletal variations were also noted (Hardin et al. 1981).

Signs of maternal toxicity (reduced liver weight and increased weights of the lungs and kidneys) were also observed in rats exposed to 765 ppm Ethoxyethanol during gestation. Maternal toxicity was not noted at the lower concentration of Ethoxyethanol (200 ppm). As was noted for rabbits, resorption was total for all litters of rats of the higher exposure group (765 ppm). However, the incidence of resorptions was not significantly increased at 200 ppm. Fetal toxicity (significant reduction in fetal body weight and crown-rump length) was noted at 200 ppm. Additionally, the results of fetal morphological examinations indicated a significant increase ($p < .05$) in the incidence of cardiovascular and skeletal defects in fetuses from females dosed with 200 ppm Ethoxyethanol. It was concluded that Ethoxyethanol was strongly fetotoxic at concentrations of 765 ppm (rats) and 615 ppm (rabbits), and teratogenic at concentrations of 200 ppm (rats) and 160 ppm (rabbits) (Hardin et al. 1981).

The teratogenicity of Ethoxyethanol was evaluated using four groups of 24 female, Dutch rabbits (5 to 7 months old; weights = 1.6 to 2.7 kg). After mating, three groups of pregnant females were exposed to concentrations of 50, 150, and 400 ppm, respectively, on days 6 through 18 of gestation (6 h/day). The fourth group (air-exposed) served as the control. With the exception of one rabbit (killed on gestation day 18), the animals were killed on day 21. Compared to controls, the mean corpuscular hemoglobin concentration in females exposed to 50 ppm Ethoxyethanol was statistically significantly smaller (at 5.0% level). This is the only hematological parameter that was significantly affected. At necropsy, no statistically significant differences in maternal splenic and thymic weights were found between test and control groups. Evidence of maternal toxicity (decreased body weight gain and feed consumption) was observed only in the 400-ppm exposure group. Reproductive effects are summarized below (Imperial Chemical Industries PLC 1983e).

Embryo/fetotoxicity (increased incidence of intrauterine deaths and reduced fetal weights) was also noted only in the 400-ppm exposure group. The increased incidence of early and late intrauterine deaths was significantly different at 1.0% and 5.0% levels, respectively, in this group. The reduction in fetal weight noted at 400 ppm was not statistically significant. Increased preimplantation losses were observed in all test groups; however, these observations were not related to exposure concentration. No gross lesions or external fetal abnormalities were attributed to Ethoxyethanol exposure (Imperial Chemical Industries PLC 1983a).

The teratogenicity of Ethoxyethanol was evaluated using four groups of Alderley Park (Wistar derived; nulliparous specific pathogen free) rats. Prior to mating, the rats were 11 to 13 weeks old, and weighed 206 to 280 g. Three groups were exposed to 10, 50, and 250 ppm Ethoxyethanol, respectively, on days 6 through 15 of gestation. The control group was exposed to air only. The animals were killed on day 21 of gestation and postmortem examination performed. No effects on maternal body weight, feed consumption, or feed utilization were observed at any dose. Compared to controls, statistically significant (at 5% level) reductions in hemoglobin, hematocrit, and mean cell volume were noted only in the 250-ppm exposure group. Hematological effects were not observed at lower concentrations. No gross lesions were found in dams at necropsy. Reproductive effects are summarized below (Imperial Chemical Industries PLC 1983b).

Fetotoxicity, but not teratogenicity, was observed in the 250-ppm exposure group. Fetotoxic effects included an increased incidence of intrauterine deaths, reduced fetal weights, and reduced fetal ossification. Compared to controls, greater preimplantation loss was observed in all exposure groups; however, the observation was statistically significant (at 1% level) only at concentrations of the 10 and 50 ppm. Differences in postimplantation losses were not statistically significant at any of the three test concentrations. Observations for fetal visceral, external, and skeletal defects were made. For minor external and visceral defects, the incidence was slightly increased in fetuses of the 10- and 250-ppm exposure groups. The proportion of fetuses affected was significantly increased (at 5% level) only in the 250 ppm exposure group. This increase was not significant when the 250-ppm exposure group was evaluated on a litter-by-litter basis. A statistically significant increase (at 1.0% level) in the proportion of fetuses with minor skeletal defects was observed only in the 250-ppm exposure group. This finding was due to increased partial/nonossification of parts of the skull, sternebrae, and vertebrae. Statistically significant skeletal alterations were also noted in the 10- and 50-ppm exposure groups. One or more skeletal variants was/were observed in the majority of fetuses examined. A statistically significant increase in the proportion of fetuses with each specific skeletal variant (except partial ossification of the 5th sternebra) was observed only at 250 ppm (Imperial Chemical Industries PLC 1983b).

The conclusion for the preceding study was stated as follows: Ethoxyethanol (250 ppm) was fetotoxic, but not teratogenic. At 50 ppm, there was evidence for slight fetotoxicity, but there were no toxicologically significant effects at 10 ppm (Imperial Chemical Industries PLC 1983b).

The teratogenicity of inhaled Ethoxyethanol was evaluated using nulliparous, specific pathogen–free female rats of the Alpk/AP (Wistar-derived) strain (11 to 13 weeks old; weight range = 200 to 280 g) and unmated female Dutch rabbits (5 to 7 months old; weight range = 1.7 to 2.8 g). The teratogenicity of inhaled Ethoxyethanol Acetate was also evaluated using rabbits of the same strain. In all experiments, animals (groups of 24) were exposed to Ethoxyethanol on days 6 to 18 of gestation. Untreated animals served as controls. The experimental procedures and results are summarized below (Doe 1984a).

Three groups of rabbits were exposed to Ethoxyethanol concentrations of 10, 50, and 175-ppm, respectively. Maternal toxicity or embryotoxicity was not observed. Compared to controls, the incidence of skeletal defects was statistically significantly greater ($p < .05$) in the 175-ppm exposure group, and this finding was due largely to retarded ossification of the skeleton and an increased incidence of 27 presacral vertebrae. The incidence of skeletal variants in the 175-ppm exposure group was also statistically significantly greater, and was due entirely to an increased number of fetuses with extra ribs, both short and of normal length. Skeletal defects constituted the only significant findings in rabbit fetuses. Ethoxyethanol was not teratogenic at concentrations up to and including 50 ppm (Doe 1984a).

Three groups of rats were exposed to Ethoxyethanol at concentrations of 10, 50, and 250 ppm, respectively. The only evidence of treatment-related, maternal toxicity was statistically significant reductions in hemoglobin, hematocrit, and RBC volume in the 250-ppm exposure group. Marked increases in the incidence of uterine deaths and in the proportion of dams affected, indicative of increased postimplantation loss, were also observed in this exposure group. Increased postimplantation loss was not observed at lower concentrations of Ethoxyethanol. Compared to controls, the proportion of fetuses with minor external and visceral defects was statistically significantly greater ($p < .05$) in the 250-ppm exposure group. However, when these findings were considered on a litter basis, the increase was not statistically significant. Cardiovascular abnormalities were not noted at either of the test concentrations. With respect to skeletal findings, the only statistically significant increase ($p < .05$) reported related to the proportion of fetuses with partially ossified lumbar vertebral process in the 10-ppm exposure group (Doe 1984a).

Three groups of rabbits were exposed to Ethoxyethanol Acetate at concentrations of 25, 100, and 400 ppm, respectively. Maternal toxicity was noted only in the 400-ppm exposure group. A marginal reduction in blood hemoglobin concentration was one of the findings. Compared to the control group, a statistically significant increase ($p < .05$) in the proportion of fetuses with minor external/visceral defects was observed in the 400-ppm exposure group. Significant increases in the proportion of fetuses with pelvic dilation, opaque/empty gall bladders, and pale and reduced spleens were noted. The proportion of fetuses with pale spleen was also statistically significantly increased ($p < .05$). Statistically significant changes in the proportion of fetuses with external or visceral defects were not noted in the 25-ppm exposure group. Relative to skeletal defects, there was a statistically significant increase ($p < .05$) in the proportion of fetuses with minor skeletal defects in the 400-ppm exposure group. This finding was indicative of retarded ossification. Significant increases in partial ossification of the first cervical centrum and the second sternebrae were noted in the 100-ppm exposure group. Additionally, the incidence of an extra center of ossification above the first sternebrae was significantly greater ($p < .05$) in the 25-ppm exposure group (Doe 1984a).

The conclusions for the preceding study are stated as follows: Ethoxyethanol was not teratogenic at concentrations up to and including 250 ppm in rats and 50 ppm in rabbits, whereas 175 ppm Ethoxyethanol was considered a marginal effect level for teratogenicity in rabbits. Fetotoxicity was noted at 250 ppm Ethoxyethanol, and possibly at 50 ppm Ethoxyethanol in rats. No evidence of teratogenicity (vertebral malformations) was found at a concentration of 400 ppm Ethoxyethanol Acetate, and 100 ppm Ethoxyethanol Acetate induced slight fetotoxicity. No effect was noted at 25 ppm Ethoxyethanol Acetate. In considering the study results for rats and rabbits, the researchers stated that concentrations of 175 to 250 ppm Ethoxyethanol were near the threshold level for teratogenicity (Doe 1984a).

The behavioral teratology of Ethoxyethanol was evaluated using 16 pregnant female Sprague-Dawley rats. The animals were exposed to 200 ppm Ethoxyethanol (7 h/day) on days 7 to 13 of gestation. Twenty-one control rats were sham-exposed. A significant reduction ($p < .01$) in weight gain was noted in dams exposed to Ethoxyethanol, and gestation was prolonged. No significant differences in birth weights were observed between test and control groups. With respect to pup behavioral activities, Ethoxyethanol caused a consistent pattern of deviations. Rotorod performance (sophisticated test of neuromuscular ability) was decreased and open field activity (commonly used measure of exploratory activity) was depressed. Avoidance conditioning, particularly the number of shuttles across the apparatus, was also decreased. Avoidance conditioning is a simple, aversively motivated test of learning ability. The researchers stated that these effects are consistent with a general pattern of decreased neuromotor function (Nelson et al. 1982a).

In another experiment, 15 pregnant Sprague-Dawley rats were exposed to 200 ppm Ethoxyethanol on gestation days 7 to 13. Exposure durations were 7 hours per day. Two groups of control rats were sham exposed during gestation. Ten pups (21 days old, no more than 2 per litter) from each test group were selected for neurochemical analyses of the brain. Exposure to Ethoxyethanol (200 ppm) on days 7 to 13 of gestation induced changes in neurotransmitter concentrations in all regions of the brain except for the brainstem. In particular, the cerebrum was affected, where acetylcholine, dopamine, and norepinephrine were increased (Nelson et al. 1982b).

The teratogenicity of Ethoxyethanol was evaluated using four groups of 24 Dutch rabbits. Prior to mating, the animals were 5 to 7 months old and weighed 1.7 to 2.8 kg. The three test groups were exposed to 10, 50, and 175 ppm Ethoxyethanol, respectively, on days 6 through 18 of gestation (6 hours per day). The control group was exposed to air only. The animals were killed on day 29 of gestation for necropsy. No effects on maternal body weight gain, feed consumption, or clinical condition were observed at either of the test concentrations. At necropsy, no treatment-related maternal macroscopic changes, alterations in spleen weight, or effects on bone marrow were observed. In the fetuses, the incidences of skeletal variants and minor skeletal defects were significantly increased only in the 175-ppm group,

compared to controls. The increase in skeletal variants in this group was statistically significant at the 1.0% level. Increases in minor skeletal defects were statistically significant at the 1.0% level and at the 5.0% level. Increases in skeletal variants and minor skeletal defects were noted at exposures of 10 and 50 ppm; however, these results were not considered treatment related (Imperial Chemical Industries PLC 1983c).

The conclusions for the preceding study were stated as follows: Ethoxyethanol (175 ppm) should be considered as a marginal effect level for teratogenicity. Ethoxyethanol (50 ppm) is a clear no-effect level for embryo/fetotoxicity and teratogenicity in Dutch rabbits (Imperial Chemical Industries PLC 1983c).

The reproductive toxicity of Ethoxyethanol (with and without ethanol) was evaluated using groups of female Sprague-Dawley rats (15 to 20 per group; weights = 200 to 300 g prior to mating). Ethoxyethanol was inhaled, whereas ethanol was administered in drinking water. Four of the treatment groups were described as follows: (1) One group of rats was exposed to 100 ppm Ethoxyethanol on days 7 through 13 of gestation. (2) The second group was exposed to 100 ppm Ethoxyethanol on days 14 through 20 of gestation. (3) The third group was exposed to 100 ppm Ethoxyethanol (7 h/day) + 10% ethanol (in drinking water) on days 7 through 13 of gestation. (4) The fourth group was exposed to 100 ppm Ethoxyethanol (7 h/day) + 10% ethanol (in drinking water) on days 14 through 20 of gestation. Two groups of controls were sham-exposed on gestation days 7 to 13 and 14 to 20, respectively. Ethanol was administered in drinking water during periods in which the animals were not in inhalation chambers. One female and one male pup per litter were used for each behavioral test. Ten pups per treatment group were killed on day 21 and brains collected for microscopic evaluation. Study results are summarized below (Nelson et al. 1984a).

The results of behavioral tests (following exposure on days 7 through 13 of gestation) indicated that ethanol appeared to reduce the behavioral (neuromuscular) effects of Ethoxyethanol. Exposure to Ethoxyethanol alone during this gestational period caused significant decreases in rotorod performance and an increase in the latency of leaving the central area of an open field. When behavioral effects resulting from exposure later in the gestation period (days 14 to 20) were evaluated, ethanol appeared to potentiate the behavioral effects of Ethoxyethanol, depressing both activity and learning (Nelson et al. 1984a).

The results of neurochemical assays indicated that Ethoxyethanol (exposure on gestation days 7 to 13) increased cerebral acetylcholine (Ach); dopamine (DA); midbrain Ach, NE, and protein; cerebellar Ach; and brainstem NE in 21-day-old pups, and decreased NE in newborn pups. Ethanol early in gestation (days 7 to 13) appeared to moderate these neurochemical effects of Ethoxyethanol. Ethoxyethanol (exposure on gestation days 14 to 20) increased levels of cerebral Ach, DA, and 5-hydroxytryptamine (5-HT) in 21-day-old pups, and decreased levels of NE in newborn pups. Ethanol late in gestation (days 14 to 20) altered the pattern of and appeared to enhance the neurochemical effects of Ethoxyethanol (Nelson et al. 1984a).

The investigators of the preceding study concluded that Ethoxyethanol was embryotoxic, and that some type of interaction could have occurred between ethanol and Ethoxyethanol (Nelson et al. 1984a).

The behavioral teratology of Ethoxyethanol was evaluated using four groups of pregnant Sprague-Dawley rats. Two groups were exposed to 100 ppm Ethoxyethanol on gestation days 7 to 13 (15 rats) and 14 to 20 (14 rats), respectively. Exposure durations were 7 hours per day. The two groups of control rats were sham exposed during gestation. Ten pups (21 days old, no more than 2 per litter) from each test group were selected for neurochemical analyses of the brain. Ethoxyethanol was evaluated at a concentration of 100 ppm based on the results of a dose range-finding study. The results of the range-finding study are summarized below (Nelson et al. 1981).

In the range-finding study, pregnant females were exposed to the following concentrations of Ethoxyethanol: 300 ppm (two rats), 600 ppm (six rats), 900 ppm (three rats), and 1200 ppm (four rats). Complete resorptions of litters were noted at concentrations of 900 and 1200 ppm, and an approximately 34% incidence of pup mortality was noted at 300 ppm (Nelson et al. 1981).

In the actual study, birth weights of pups in test groups (100 ppm Ethoxyethanol) were not significantly different from controls. Numerous deviations in behavior (compared to controls) were noted in pups from dams dosed on gestation days 7 to 13. Performance on the rotorod was impaired, and pups remained in the central "start" area of the open field for significantly longer periods. More of the 34-day-old offspring learned the avoidance conditioning task. Neurochemical analyses indicated that Ach was significantly increased in the cerebrum, cerebellum, and midbrain, but not in the brainstem, of 21-day-old pups. NE was decreased in the whole brains of neonates, but increased in the cerebrum, brainstem, and midbrain. DA and protein were increased in the cerebrum and midbrain, respectively (Nelson et al. 1981).

Compared to the preceding results, the offspring of dams exposed during gestation days 14 to 20 had fewer differences in behavioral effects when compared to controls. The offspring were less active on the activity wheel, and received more shocks and an increased duration of shock. Reduced concentrations of NE were noted in whole brains from neonates. In 21-day-old pups, only the cerebrum had neurochemical differences when compared to the control. Significant elevations in concentrations of Ach, DA, and 5-HT were noted in the cerebrum. Study results indicate that Ethoxyethanol (100 ppm) induced numerous behavioral and neurochemical deviations in offspring from rats exposed either on gestation days 7 to 13 or 14 to 20. These deviations were greater in rats exposed to Ethoxyethanol earlier in gestation (Nelson et al. 1981).

The behavioral teratology of Ethoxyethanol was evaluated using groups of 15 to 20 pregnant Sprague-Dawley rats (ages and weights not stated). The animals were exposed (inhalation)

to 100 ppm on gestation days 7 to 13 or 14 to 20. Two sets of controls were sham-exposed during the same days of gestation. Ethoxyethanol was evaluated at a concentration of 100 ppm based on the results of a dose range–finding study (numbers of animals not stated). The results of the range-finding study are summarized below (Nelson and Brightwell 1984).

In the range-finding study, none of the fetuses survived after dams were exposed to 1200 ppm Ethoxyethanol on gestation days 7 to 13; all fetuses were resorbed. Three fourths of the fetuses of dams exposed to 600 ppm during the same period died; no pups were born alive. After dams were exposed to 600 ppm on days 14 to 20 of gestation, one of six dams had pups alive. The pups survived for only 10 days and appeared runtlike. The exposure of dams to 200 ppm Ethoxyethanol on gestation days 14 to 20 resulted in the death of approximately one third of the offspring within the first week of life. At a concentration of 100 ppm, Ethoxyethanol induced neither death nor weight reductions in offspring. Maternal toxicity also was not observed at this concentration. The results of the behavioral teratology study on 100 ppm Ethoxyethanol are summarized below (Nelson and Brightwell 1984).

Following the exposure of dams to 100 ppm Ethoxyethanol on gestation days 7 to 13, offspring had impaired performance on the rotorod and in the open field, and had altered performance in avoidance conditioning. Following exposure on gestation days 14 to 20, offspring had impaired activity on the activity wheel and in avoidance conditioning. Activity wheels are used to measure circadian activity. Based on assays for determining neurotransmitter concentrations in brains from 21-day-old offspring, numerous alterations were noted following exposure to 100 ppm Ethoxyethanol on gestation days 7 to 13. Fewer effects were noted in rats exposed on gestation days 14 to 20. The investigators concluded that maternal exposure of rats to 100 ppm Ethoxyethanol during gestation produce some alterations in the central nervous system (CNS) of offspring (Nelson and Brightwell 1984).

Male Alpk/Ap, Wistar-derived rats (weights = 250 g) were exposed to saturated Ethoxyethanol vapor (17 mg/l) for 3 or 4 hours and then observed for 14 days. The animals (number not stated) were killed on day 15 and testes examined macroscopically. Compared to controls, Ethoxyethanol exposure resulted in a 20% reduction in testicular weight. Hematuria was also observed in treated animals. The investigators concluded that Ethoxyethanol induced testicular atrophy (Doe 1984b).

The teratogenicity of Ethoxyethanol Acetate was evaluated using female Sprague-Dawley rats (weights = 200 to 300 g). After mating, the rats were exposed to Ethoxyethanol Acetate concentrations of 130 ppm (15 rats), 390 ppm (15 rats), and 600 ppm (9 rats) on gestation days 7 through 15 (7 h/day). The animals were killed on day 20 of gestation and the fetuses examined for skeletal and visceral abnormalities. The untreated control group consisted of 34 female rats. No signs of maternal toxicity were observed. All fetuses from rats of the 600-ppm exposure group were resorbed. Compared to controls, a significant increase ($p < .05$) in resorptions and a 21% reduction in fetal weights ($p < .05$) was noted in the 390-ppm group. Slight, but significant ($p < .05$), decreases in fetal weight were noted at the 130-ppm dose. Skeletal and visceral defects are summarized below (Nelson et al. 1984b).

Compared to controls, visceral malformations of the heart and umbilicus were observed in fetuses from the 390-ppm exposure group ($p < .01$). A heart defect was observed only in one fetus from the 130-ppm exposure group. The researchers reported that because heart defects rarely occur spontaneously and there were no heart defects in control fetuses, these data indicate that the inhalation of either 130 or 390 ppm Ethoxyethanol Acetate was teratogenic to the rat. Compared to controls, three fetuses of the 390-ppm group had malformations of the ribs ($p < .01$). However, skeletal malformations were not noted in fetuses from dams exposed to 130 ppm. Although the number of skeletal malformations was significantly different at 390 ppm, the researchers agreed that it cannot be positively concluded that these malformations are treatment related, because of the low incidence (Nelson et al. 1984b).

Compared to controls, a statistically significant increase in the frequency of visceral variants was noted in fetuses of the 390-ppm exposure group ($p < .01$). A statistically significant increase in the number of skeletal variants was observed in fetuses at exposures of 130 ppm ($p < .05$) and 390 ppm ($p < .01$). The investigators reported that these data indicate that the inhalation of Ethoxyethanol Acetate at either concentration during the period of organogenesis can cause growth retardation in the rat (Nelson et al. 1984b). The teratogenicity of Ethoxyethanol Acetate was evaluated using two groups of 15 pregnant rats (strain and weights not stated). Ethoxyethanol Acetate was vaporized and the two groups were exposed to concentrations of 200 ppm and 600 ppm, respectively, on gestation days 7 to 15. A group of 33 rats served as sham-exposed controls. At a concentration of 600 ppm, Ethoxyethanol Acetate induced skeletal and cardiovascular defects; reduced fetal weights were also noted. Only a single defect was observed following exposure to 200 ppm Ethoxyethanol Acetate (Nelson et al. 1982c).

The teratogenicity of Ethoxyethanol Acetate was evaluated using four groups of eight female Dutch rabbits. Prior to mating, ages ranged from 5 to 7 months old and weights ranged from 1.4 to 3.1 kg. Three groups were exposed to 100, 250, and 450 ppm, respectively, on days 6 through 18 of gestation (6 hours per day). The control group was exposed to air only. Necropsy was performed on day 21. Maternal toxicity (reduced body weight gain and food consumption) was found in the three test groups during the first few days of exposure. Changes in clinical condition, hematological parameters, or spleen weights were not observed in any of the test groups. No treatment-related maternal lesions were found. Reproductive effects are summarized below (Imperial Chemical Industries PLC 1983d).

Both the group mean percentage of preimplantation losses and the percentage of litters with any preimplantation loss were greater than was noted for controls. Statistically significant

differences were observed only at 100 (significant at 1.0% level) and 450 ppm (significant at 5.0% level). A concentration-related increase (not statistically significant) in the percentage of litters with any postimplantation loss was noted over the entire range of exposure concentrations. However, the group mean percentage of litters with post-implantation loss was increased (not significant) only in the 450-ppm exposure group. The mean number of live fetuses per litter was reduced (not statistically significant) only in the 450-ppm group. A reduction (concentration-related and statistically significant) in mean fetal weights was noted in all exposure groups. This observation was accompanied by a reduction in mean gravid uterine weights. No statistically significant treatment-related external abnormalities were found in any treatment group. It was concluded that Ethoxyethanol was fetotoxic (reduced fetal weights) at all test concentrations, and that an increase in the incidence of intrauterine deaths was observed only at 450 ppm Ethoxyethanol Acetate (Imperial Chemical Industries PLC 1983d).

The teratogenicity of Ethoxyethanol Acetate was evaluated using four groups of mated female Dutch rabbits (23 to 24 per group). Prior to mating, the rabbits were 5 to 7 months old and weighed 1.7 to 2.8 kg. Three groups were exposed (6 h/day) to Ethoxyethanol Acetate at concentrations of 25, 100, and 400 ppm, respectively, on days 6 to 18 (inclusive) of gestation. The control group was exposed to air only according to the same procedure. The animals were killed on day 29 of gestation; ovaries and uterine contents were examined. A concentration-related increase in maternal body weight was reported over the range of concentrations tested. However, this finding was statistically significant ($p < .05$) only in females of the 400-ppm exposure group. A statistically significant reduction ($p < .05$) in hemoglobin concentration was also noted in rabbits of the 400-ppm exposure group. Slight reductions (not statistically significant) in the following RBC parameters were noted at 100 ppm and 400 ppm: hematocrit, RBC count, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration. No effects on red blood cells were observed in rabbits of the 25-ppm exposure group. A concentration-related (not statistically significant) increase in the white blood cell count was reported over the range of concentrations tested. No changes in clinical condition or behavior due to Ethoxyethanol Acetate exposure were observed. Reproductive effects are summarized below (Imperial Chemical Industries PLC 1983c).

A statistically significant increase in the incidence of fetal deaths in utero was observed only in females of the 400-ppm exposure group. This finding was associated with a statistically significantly greater percentage of postimplantation loss and lower mean number of live fetuses per litter when compared to controls. The increased incidence of fetal deaths was likely due to the three animals with total resorptions. These were omitted and the data were reanalyzed. At reanalysis, no statistically significant differences in the percentage of postimplantation loss and the mean number of live fetuses per litter were present. No statistically significant differences in the incidence of major fetal external/visceral and skeletal defects between control and test groups were found. For the minor external/visceral defects (400-ppm group), statistically significant increases in the proportion of fetuses with pelvic dilatation ($p < .01$), opaque/empty gall bladders ($p < .01$), and small pale spleens ($p < .01$) were found. A statistically significant increase in the proportion of fetuses with pale spleens also was observed in the 100-ppm exposure group ($p < .01$). A statistically significant increase in the proportion of fetuses with minor skeletal defects ($p < .01$ mostly) was noted only in fetuses of the 400-ppm exposure group. Significant skeletal defects (due to retarded ossification) were observed. Retarded ossification, considered indicative of fetotoxicity, was not statistically significant after exposure to 100 ppm. However, there was no evidence of fetotoxicity in the 25-ppm exposure group (Imperial Chemical Industries PLC 1983e).

The researchers reported that the incidence of six major skeletal malformations (compared to one in controls) provided evidence of possible teratogenicity at 400 ppm Ethoxyethanol Acetate. It was also reported that the incidences of major external and visceral abnormalities were not considered evidence of teratogenicity at 400 ppm. Furthermore, no evidence of teratogenicity was found at either 100 ppm or 25 ppm Ethoxyethanol Acetate. (Imperial Chemical Industries PLC 1983e).

The conclusion for the preceding study was stated as follows. Exposure of pregnant rabbits to 400 ppm Ethoxyethanol Acetate caused maternal toxicity, fetotoxicity, and possible teratogenicity. Exposure to 100 ppm Ethoxyethanol Acetate caused slight fetotoxicity, but exposure to 25 ppm Ethoxyethanol Acetate did not. No evidence of teratogenicity was found at either 100 ppm or 25 ppm Ethoxyethanol Acetate (Imperial Chemical Industries PLC 1983e).

The developmental toxicity of Ethoxyethanol Acetate was evaluated using mated Fischer 344 rats of the [COBS CDF (F-344)/CrlBR] strain (5 groups of 30; 8 to 10 weeks old) and mated female New Zealand white rabbits (5 groups of 24; 5 to 5.5 months old). Four groups of rats were exposed (inhalation) to 50, 100, 200 and 300 ppm Ethoxyethanol Acetate, respectively, on days 6 through 15 of gestation (6 h/day). On gestation day 21, blood samples were obtained from anesthetized rats (CO_2 inhalation). The rats were then killed by CO_2 asphyxiation. Four groups of rabbits were exposed (inhalation) to the same concentrations of Ethoxyethanol Acetate, respectively, on gestation days 6 through 18 (6 h/day). On gestation day 29, blood samples were collected after rabbits had been restrained; the rabbits were then killed by cervical dislocation. For rats and rabbits, one of the five groups served as the negative control group. Study results are summarized below (Tyl et al. 1988).

Ethoxyethanol Acetate exposure resulted in changes in maternal organ weights in rabbits. Compared to controls, a significant reduction in gravid uterine weight was noted at concentrations of 200 ppm ($p < .05$) and 300 ppm ($p < .001$). These findings were supported by 5 of 19 resorbed litters in the 200 ppm exposure group and 16 of 19 resorbed litters in the 300-ppm exposure group. Increased absolute (but not relative) liver weight

($p < .05$) was observed in females of the 300-ppm exposure group. In rats, a significant increase in maternal absolute liver weight (p values not stated) was noted in all exposure groups (50, 100, 200, and 300 ppm). Relative liver weight (expressed as % of corrected body weight) was increased in rats of the 100- to 300-ppm exposure groups (Tyl et al. 1988).

The results of evaluations of rabbit blood samples indicated a significant increase ($p < .01$) in mean corpuscular volume at 300 ppm. A significant (p value not stated), concentration-related decrease in the number of platelets was reported in rabbits of the 100- and 300-ppm exposure groups. Rats of the 200-ppm ($p < .001$) and 300-ppm ($p < .001$) exposure groups had a significant increase in the white blood cell count. Significant reductions in RBC count, hemoglobin, packed RBC volume, and RBC size (mean corpuscular volume) also were noted. With the exception of mean corpuscular volume ($p < .01$), the p values for RBC parameters were all $<.001$. A significant reduction in platelet count was observed in rats of the 200- and 300-ppm exposure groups (Tyl et al. 1988).

Regarding gestational parameters in rabbits, a significant decrease in the number of viable and nonviable implants per litter ($p < .05$) was noted in females of the 200-ppm exposure group. Also, compared to controls, significant increases or decreases ($p < .05$) in the following parameters were noted in females of the 300-ppm exposure group: corpora lutea/doe (decreased), viable implants/litter (decreased), nonviable implants/litter (increased), and early resorptions/litter (increased). In rats, a significant increase in the number of nonviable implants per litter was observed at a concentration of 300 ppm. A significant decrease ($p < .001$) in fetal body weight per litter was noted for all rat fetuses (males and females). Teratogenic findings for rabbits and rats are summarized below (Tyl et al. 1988).

For rabbits, a significant increase ($p < .05$) in the incidence of a number of tail defects, low-set ears, and total external malformations was observed in fetuses from the 200- and 300-ppm exposure groups. A significant increase ($p < .05$) in the incidence of total visceral malformations was observed in fetuses of the 200-ppm exposure group, but not in fetuses of the 300-ppm exposure group. Visceral malformations included cardiovascular, pulmonary, and renal defects. Regarding skeletal variations in rabbits, total malformations increased significantly ($p < .05$) in fetuses of the 200- and 300-ppm exposure groups. Also, an increased incidence of skeletal variations (indicative of toxicity, but not involving reduced ossification) was observed in fetuses of the 100- and 200-ppm exposure groups (Tyl et al. 1988).

With the exception of cranial ecchymosis ($p < .05$), no external malformations were observed in rat fetuses. A significant increase ($p < .05$) in the incidence of visceral malformations was noted in fetuses of the 300-ppm exposure group, whereas a significant increase ($p < .05$) in the incidence of skeletal malformations was noted in fetuses of the 200- and 300-ppm exposure groups. Visceral malformations included cardiovascular and renal defects. Numerous skeletal malformations (increased incidences at 100, 200, and 300 ppm) were observed, presenting a pattern indicative of fetotoxicity (Tyl et al. 1988).

The investigators in the preceding study concluded that in rats and rabbits, inhalation exposure to Ethoxyethanol Acetate during organogenesis produced maternal toxicity and developmental toxicity at concentrations of 100 to 300 ppm. Developmental effects included teratogenicity at 200 to 300 ppm. No evidence of maternal or developmental toxicity, including teratogenicity, was found at a test concentration of 50 ppm (Tyl et al. 1988).

Oral Studies

Studies on the reproductive and developmental toxicity of Ethoxyethanol and Ethoxyethanol Acetate (oral studies) are summarized in Table 5. Overall, the data indicate that both chemicals induce reproductive toxicity following oral exposure.

Ethoxyethanol was tested in a continuous breeding reproductive toxicity study involving male and female CD-1 mice (11 weeks old). The study was divided into four phases: (1) dose range finding, (2) continuous breeding phase, (3) identification of the affected sex, and (4) offspring assessment. In phase 1, Ethoxyethanol was administered in drinking water to five groups of mice (8 males, 8 females/group) at concentrations of 0.2%, 0.4%, 0.75%, 1.5%, and 3.0%, respectively, for 2 weeks. The only death reported in phase 1 was one male mouse in the 3.0% dose group. No significant signs of toxicity were observed in animals that survived. Based on results from the dose range finding phase, 2.0% was chosen as the maximum test concentration for the remaining phases. It was considered likely that 3.0% Ethoxyethanol in the diet could have resulted in significant dehydration due to reduced water consumption (NTP 1984). The test procedures and results from the remaining study phases are summarized below.

In the continuous breeding phase, three groups of 11-week-old CD-1 mice (20 males, 20 females/group) received concentrations of 0.5%, 1.0%, and 2.0% Ethoxyethanol in drinking water, respectively, during a 7-day premating period. The vehicle control group consisted of 40 male and 40 female mice. After the premating period, the animals were cohabited for approximately 14 weeks. Newborn litters were evaluated and then killed. The findings from the continuous breeding phase (phase 2) indicated that Ethoxyethanol caused a profound effect on reproductive function in CD-1 mice at test concentrations of 1.0% and 2.0% Ethoxyethanol. In the 2.0% test group, no litters were delivered. Similar results were reported for 2 of the 20 mating pairs dosed with 1.0% Ethoxyethanol. Also, in the 1.0% test group, a significant reduction was found in the number of live pups per litter, the proportion of pups born alive, and mean values for live pup weight. All mating pairs in the control group and the 0.5% test group delivered at least one litter. The researchers stated that the effects of Ethoxyethanol on female mice could be related to embryotoxicity, fetotoxicity, or to a direct antifertility effect. It was also stated that the teratogenicity of 1.0% Ethoxyethanol could have been responsible for the decreased

TABLE 5
Reproductive and developmental toxicity studies—oral exposure

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (up to 2.0% in drinking water)	CD-1 mice (3 groups, 20 males, 20 females/ group; 11 weeks old)	Doses of 0.5%, 1.0%, and 2.0%, respectively, in drinking water during 7-day premating period (males and females). Dosing followed by 14-week mating period.	No litters delivered after dosing with 2.0%. Embryotoxicity/ fetotoxicity at 1.0% dose; significant reduction in number of live pups/litter. No effects at 0.5% dose. In adult males, increased incidence of abnormal sperm and treatment-related testicular lesions	National Toxicology Program 1984
Ethoxyethanol (up to 2.0% in drinking water)	COBS Crl:CD-1 (ICR) BR outbred Swiss albino mice (3 groups, 20 males and 20 females/group; 6 weeks old)	Doses of 0.5%, 1.0%, and 2.0%, respectively, in drinking water during 7-day mating period (males and females). Dosing followed by 98-day mating period	No litters delivered after dosing with 2.0%. Significant decrease ($p < .05$) in proportion of pups born alive only at 1.0% dose. (Same results for 1.0% ethoxyacetic acid, Ethoxyethanol metabolite)	Morrissey et al. 1989
Ethoxyethanol Acetate (up to 2.0% in feed or drinking water)	COBS Crl:CD-1 (ICR) BR outbred Swiss albino mice (3 groups, 20 males and 20 females/group; 6 weeks old)	Doses of 0.5%, 1.0%, and 2.0%, respectively, in drinking water during 7-day mating period (males and females). Dosing followed by 98-day mating period	Significant decrease ($p < .05$) in proportion of pups born alive at doses of 1.0 and 2.0%	Morrissey et al. 1989
Ethoxyethanol (up to 2.0% in drinking water)	COBS Crl:CD-1 (ICR) BR outbred Swiss albino mice (3 groups, 20 males and 20 females/group; 6 weeks old)	Doses of 0.5%, 1.0%, and 2.0%, respectively, in drinking water continuously during 14-week mating period (males and females)	Significant adverse effects on fertility at 1.0 and 2.0% doses, but not at 0.5% dose. The mating of test animals with control animals resulted in significant effects on fertility and reproduction in males and females from 1.0 and 2.0% dose groups. Testicular atrophy noted in treated males	Lamb et al. 1984

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TABLE 5
Reproductive and developmental toxicity studies—oral exposure *(Continued)*

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol Acetate (up to 2.0% in drinking water)	CD-1 mice (3 groups, 40 males and 40 females/group; 11 weeks old)	Doses of 0.5%, 1.0%, and 2.0%, respectively, in drinking water for 7 days prior to 14-week mating period (males and females)	Reproductive toxicant in CD-1 mice. Significant decrease ($p < .01$) in number of litters per fertile pair and the proportion of pups born alive at doses of 1.0% and 2.0%. Degeneration of seminiferous tubules in second generation male mice. No significant reproductive effects at 0.5%	National Toxicology Program 1985
Ethoxyethanol (1.45% in diet)	20 male albino rats (inbred strain, 3 weeks old)	Ethoxyethanol (1.45% in diet) fed over 2-year period	Enlarged edematous testes in two thirds of animals. Testicular lesions more frequently bilateral and consisted of marked interstitial edema and marked tubular atrophy	Morris, Nelson, and Calvery 1942
Ethoxyethanol (doses up to 4200 mg/kg)	Virus antibody free female CD-1 mice (5 groups, 8–10 weeks old; weights = 17–33 g)	Doses of 1000, 1800, 2600, 3400, and 4200 mg/kg, respectively, in distilled water on gestation days 8–14	Significant reduction in mean fetal body weight with increasing doses. Increased incidence of embryo resorption. Significant increase in mean number of malformed fetuses in 1800 and 2600-mg/kg dose groups	Wier, Lewis, and Traul 1987
Ethoxyethanol (doses up to 4000 mg/kg)	Male JCL-ICR mice (4 groups, 6 weeks old)	Doses of 500, 1000, 2000, and 4000 mg/kg, respectively, 5 days/week for 5 weeks	Testicular atrophy in all groups. Dose-related atrophy of seminiferous epithelium at histopathological examination	Nagano et al. 1984
Ethoxyethanol Acetate (doses up to 4000 mg/kg)	Male rats	Doses of 500, 1000, 2000, and 4000 mg/kg, respectively, 5 days/week for 5 weeks	Testicular atrophy in all groups. Dose-related atrophy of seminiferous epithelium at histopathological examination	
Ethoxyethanol (3605 mg/kg)	Female CD-1 mice (50 animals; 6–8 weeks old)	Doses of 3605 mg/kg/day on gestation days 7–14	No viable litters	Schuler et al. 1984

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TABLE 5
Reproductive and developmental toxicity studies—oral exposure *(Continued)*

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (doses up to 2808 mg/kg)	Female Long-Evans hooded rats (3 groups of 4; 90–100 days old)	Doses of 936, 1872, and 2808 mg/kg, respectively, in distilled water. Animals killed at week 16	At week 16, no apparent lesions in testes (all dose groups). However, residual lesions in males from 1872-mg/kg dose group. Acute Ethoxyethanol exposure caused reversible impairment in testicular function	Oudiz et al. 1984
Ethoxyethanol (doses up to 2808 mg/kg)	Male Long-Evans hooded rats (3 groups of 4)	Doses of 936, 1872, and 2808 mg/kg, respectively, for 5 consecutive days. Semen evaluations up to 14 weeks post dosing. In second experiment, males dosed with 936 mg/kg 5 days/week for 6 weeks	Significantly decreased sperm counts in all dose groups, with varying degrees of recovery. In second experiment, sperm counts significantly depressed and sperm motility depressed	Zenick, Oudiz, and Niewenhuis 1984
Ethoxyethanol (doses up to 2500 mg/kg)	F344 male rats (5 groups of 5; weights = 107–108 g)	Doses of 300, 600, 900, 1500, and 2500 mg/kg, respectively, in deionized water daily for 14 days	Dose-related decreases in absolute and relative testis weights. Degeneration of seminiferous tubules in 2 highest dose groups	NTP 1993
Ethoxyethanol (doses up to 2500 mg/kg)	BC3F1 male mice (5 groups of 5; 5–7 weeks old)	Doses of 300, 600, 900, 1500, and 2500 mg/kg, respectively, in deionized water daily for 14 days	Relative testis weight significantly lower ($p \leq .05$) in 2500 mg/kg dose group	NTP 1993
Ethoxyethanol (doses up to 1.89 g/kg)	Male Sprague-Dawley rats (4 groups)	Doses of 0.052 (6 rats), 0.213 (8 rats), 0.735 (11 rats), and 1.89 g/kg/day (16 rats), respectively, for 90 days	Complete absence of sperm in testes of males from 1.89 g/kg dose group (for all 3 males examined)	Union Carbide Corporation 1947
Ethoxyethanol (doses up to 1800 mg/kg)	CR, COBS, CD, BR strain rats (3 groups of 30; average body weight = 235.7 ± 15.1 g)	Doses of 450, 900, and 1800 mg/kg/day, respectively, 5 days/week for 6 weeks	Significant decreases in absolute and relative testes weights in 900- and 1800-mg/kg dose groups. Microscopic evidence of testicular atrophy in all dose groups	Eastman Chemical Company 1982
Ethoxyethanol (doses up to 1000 mg/kg)	Male Sprague-Dawley rats (3 groups; 4 weeks old)	Doses of 250, 500, and 1,000 mg/kg body weight/day, respectively, up to 11 days. Six rats per dose group were killed after dosing. For each group, animals were killed according to the following schedule: 6 and 24 h after a single dose and after 2, 4, 7, and 11 days of repeated dosing	Doses of 500 and 1000 mg/kg caused degeneration of dividing and early-pachytene spermatocytes. No effect on middle and late stages of pachytene development at these doses. No spermatogenic abnormalities at 250 mg/kg	Creasy and Foster 1984

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TABLE 5
Reproductive and developmental toxicity studies—oral exposure *(Continued)*

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (doses up to 1000 mg/kg)	Male Sprague-Dawley rats (3 groups of 36; 4 weeks old)	Doses of 250, 500, and 1,000 mg/kg/day, respectively, for 11 days	Significant differences in testicular weight in 500 mg/kg ($p < .01$) and 1000-mg/kg ($p < .05$) dose groups. Spermatocyte degeneration (restricted to later stages of primary spermatocyte development and secondary spermatocytes) in both groups. (Similar pattern and severity of spermatocyte degeneration in rats dosed daily for 11 days, 500 mg/kg/day, with ethoxyacetic acid)	Foster et al. 1983
Ethoxyethanol (doses up to 1000 mg/kg)	Male rats (2 groups of 36)	Doses of 500 and 1,000 mg/kg/day, respectively, for 11 days	Severe spermatocyte degeneration. (Similar pattern of spermatocyte degeneration in rats dosed with ethoxyacetic acid, 500 mg/kg/day, for 11 days)	Foster et al. 1984
Ethoxyethanol (dose of 936 mg/kg)	Adult male Long-Evans hooded rats (10 animals; 70–80 days old)	Dose of 936 mg/kg 5 days per week for 6 weeks	At weeks 5 and 6, sperm count and % normal morphology significantly decreased ($p < .001$). Sperm motility significantly decreased ($p < .01$) at week 6. Significant decreases ($p < .01$) in weight of testes, caudae epididymides, and epididymides	Oudiz and Zenick 1986
Ethoxyethanol (doses up to 550 mg/kg)	Groups of rats	Doses ranging rom 210 to 550 mg/kg/day between days 7 and 17 of gestation	Embryomortality: 31% of implants (15 litters from dams receiving doses between 210 and 270 mg/kg/day), 69% of implants (19 litters from dams receiving doses between 270 and 400 mg/kg/day), and 100% embryomortality (8 litters from dams receiving doses between 400 and 550 mg/kg/day)	Chester, Hull, and Andrew 1986

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TABLE 5
Reproductive and developmental toxicity studies—oral exposure *(Continued)*

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (doses up to 500 mg/kg)	Sprague-Dawley rats (males and females; groups of 9–10)	Doses of 250 and 500 mg/kg/day for 2, 5, or 7 weeks. After 5 weeks of dosing, males dosed for 7 weeks were mated with untreated females	No motile sperm in males dosed with 500 mg/kg/day for 7 weeks. Significant decreases in % progressively motile sperm in males dosed with 250 mg/kg/day for 5 and 7 weeks. Reductions in sperm count in males dosed with 250 and 500 mg/kg/day for 5 and 7 weeks. Testis weight significantly reduced in males dosed with 500 mg/kg/day for 2 weeks. Significant decreases in pregnancy index and numbers of implantations and fetuses in 500 mg/kg/day group. No effects in 250 mg/kg dose group	Horimoto et al. 1996
Ethoxyethanol (doses of 500 mg/kg/day)	Groups of male Sprague-Dawley rats	Dosed with 500 mg/kg/day for 7 days	At 4 weeks post dosing, % motile and progressively motile sperm and total sperm count significantly reduced. Decreased testicular weight at 1, 2, and 4 weeks post dosing. Marked reduction or disappearance of spermatids at 4 weeks post dosing	Ninomiya et al. 1995
Ethoxyethanol Acetate (doses of 400 mg/kg)	Male mice	Dosed with 400 mg/kg/day, 5 days/week for 5 weeks	Testicular atrophy	Nagano, Nakayama, and Koyano 1979
Ethoxyethanol (doses up to 300 mg/kg)	Long-Evans male rats (72 animals; 80–90 days old)	Doses of 150 or 300 mg/kg/day, 5 days/week for 6 weeks	Significant reductions ($p \leq .05$) in testis weight and cauda epididymal and accessory organ weight only in males mated bidaily (doses of 300 mg/kg/day only). Significant decrease ($p \leq .05$) in spermatid count in mated and nonmated rats dosed with 300 mg/kg/day. Significant decreases ($p \leq .05$) in epididymal sperm count and % normal morphology, only in nonmated animals, at doses of 300 mg/kg; same results in males mated bidaily and dosed with 150 mg/kg	Hurtt and Zenick 1986

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TABLE 5
Reproductive and developmental toxicity studies—oral exposure *(Continued)*

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (doses up to 800 μl/kg)	Five groups of rats	Three dosed with 50, 100, and 200 μl/kg/day, respectively, over 13-week period. Two remaining groups dosed with 100 and 200 μl/kg/day, respectively, up to day 59, after which doses increased to 400 and 800 μl/kg/day, respectively, for remainder of dosing period	Testicular changes in 200 μl/kg/day dose group included occasional softening of interstitium with edema. Pronounced testicular damage in five male rats from 800 μl/kg/day dose group	Stenger et al. 1971
Ethoxyethanol (doses up to 400 μl/kg)	Six groups of female rats	Doses of 12.5, 25, 50, 100, 200, and 400 μl/kg/day, respectively during gestation	Dose-dependent increase in skeletal abnormalities, starting at 100 μl/kg/day	Stenger et al. 1971
Ethoxyethanol (doses up to 200 μl/kg)	Six groups of male dogs	Doses of 50, 100, and 200 μl/kg/day, respectively, over 13-week period	Testicular changes in 3 dogs from 200 μl/kg/day dose group that were examined. In many tubules, last maturation stages of seminal epithelium were missing	Stenger et al. 1971
Ethoxyethanol (doses up to 40000 ppm)	Five groups of $BC3F_1$ mice (10 males, 10 females/group, 5–7 weeks old). Weight ranges: 22.7–23.9 g (males) and 18.9–19.3 g (females)	Doses of 2500, 5000, 10,000, 20,000, and 40,000 ppm, respectively, for 13 weeks	Significantly decreased absolute testis weights in mice from 20,000- and 40,000-ppm dose groups; marked testicular degeneration. No histopathologic changes in mice from lower dose groups. Significantly longer estrous cycles in females from all dose groups	NTP 1993
Ethoxyethanol (doses up to 20,000 ppm)	Male F344 rats (3 groups of 30, 5–7 months old; weights = 161–165 g)	Doses of 5000, 10,000, and 20,000 ppm, respectively, daily for 60 days	Significantly lower absolute and relative testis and epididymal weights in 10,000- and 20,000-ppm dose groups. Moderate to marked testicular degeneration in these two groups, but not in the 5000-ppm dose group, at end of dosing period	NTP 1993
Ethoxyethanol (doses up to 20,000 ppm)	Five groups of F344 rats (10 males, 10 females/group; 5–7 weeks old). Weight ranges: 142–146 g (males) and 123–127 g (females)	Doses of 1250, 2500, 5000, 10,000, and 20,000 ppm, respectively, daily for 13 weeks	Testicular degeneration in all male rats receiving doses of 5000 ppm or greater	NTP 1993

pup survival, and that the decreased fertility in the female mice could have been related to gonadal, endocrine, or reproductive tract toxicity (NTP 1984).

Compared to controls, cauda epididymal sperm counts in male mice were reduced in the 1.0% test group; however, the pairwise comparisons were not statistically significant. Sperm motility values in the 2.0% test group were significantly lower, when compared to controls, and the number of abnormal sperm was considerably increased. Treatment-related lesions were noted in the testis (NTP 1984).

Mice of the 1.0% and 2.0% test groups in the continuous breeding phase were tested in a crossover mating trial (phase 3) to determine whether the males or females (or both sexes) had compromised reproductive performance when matched with control mice. No fertile matings were reported for females of the 2.0% test group cohabited with control males, and a decrease (not significant) in the percentage of fertile matings was reported for females of the 1.0% test group mated with control males. Compared to control mating pairs, the cohabitation of males of the 2.0% test group with control females resulted in significantly fewer fertile matings. A statistically significant decrease in the percentage of fertile matings was noted for females of the 1.0% Ethoxyethanol test group mated with control females. The investigators reported that the results of the crossover mating trial indicated that Ethoxyethanol did not affect reproduction in one sex to the exclusion of affecting the other, and that the no-observed-effect level for both sexes seemed to have been approximately 0.5% Ethoxyethanol in drinking water (NTP 1984).

The reproductive toxicity of Ethoxyethanol and its metabolite, 2-ethoxyacetic acid, was evaluated in a continuous breeding reproduction study using COBS Crl:CD-1 (ICR) BR outbred Swiss albino mice (6 weeks old). The three treatment groups per test material consisted of 20 mice per sex, and the control group consisted of 40 mice per sex. Initially (continuous breeding phase), the treatment groups were exposed to test materials during a 7-day premating period. Ethoxyethanol was administered to three groups of mice at concentrations of 0.50%, 1.0%, and 2.0% in feed or water, respectively. 2-Ethoxyacetic acid was also administered to three groups of mice at concentrations of 0.20%, 0.60%, and 1.0%, respectively, in feed or water. The animals were then randomly grouped as mating pairs, and cohabited and treated continuously for 98 days. Data were collected on all newborns during the 98-day cohabitation period. At the end of the cohabitation period, the mating pairs were separated; however, treatment continued. During the next 21 days, any final litters were delivered and kept for at least 21 days (Morrissey et al. 1989).

The reproductive performance of mice during the continuous breeding phase is summarized as follows. For Ethoxyethanol-treated mice, a significant decrease ($p < .05$) in the fertility index was noted only in the highest exposure group (2.0% Ethoxyethanol); no fertile mating pairs were observed in this group. A significant decrease ($p < .05$) in the proportion of pups born alive was noted only in the mid-concentration test group (1.0% Ethoxyethanol). For 2-ethoxyacetic acid–treated mice, nonsignificant differences in the fertility index were noted between the control group and all three treatment groups (0.2%, 0.6%, and 1.0%). A significant decrease in the proportion of pups born alive was noted in the highest exposure group (Morrissey et al. 1989).

The occurrence of significant reproductive effects on fertility during the continuous breeding phase signaled the need for a crossover mating trial to determine the affected sex. High-exposure mice of each sex (Ethoxyethanol- and 2-ethoxyacetic acid–treated mice) were mated with control mice of the opposite sex. High-exposure mice were selected in order to increase the possibility of detecting effects in the crossover mating trial. The three combinations of control and treated mice used were: control males with control females, high-exposure males with control females, and control males with high-exposure females. The results were compared to matings within the control group. Significant decreases ($p < .05$) in the fertility index were noted for matings between high-exposure males (2.0% Ethoxyethanol) and control females and between control males and high-exposure females. No fertile mating pairs (control males mated with high-exposure females) were found. For matings between high-exposure males and control females, the proportion of pups born alive was nonsignificant when compared to matings between control males and control females. For matings of male and female 2-ethoxyacetic acid–treated mice (high test concentration = 1.0%) with control mice, no significant differences in the fertility index or the proportion of pups born alive were found. However, a significant decrease in the mean number of live pups per litter was noted for litters resulting from the mating of high-exposure females with control males (Morrissey et al. 1989).

The reproductive toxicity of Ethoxyethanol Acetate was also evaluated in the preceding study using COBS Crl:CD-1 (ICR) BR outbred Swiss albino mice (6 weeks old). The test substance was administered in feed or water at concentrations of 0.5%, 1.0%, and 2.0%. A significant decrease ($p < .05$) in the fertility index was observed only in the highest exposure group. A significant decrease ($p < .05$) in the proportion of pups born alive was noted in 1.0% and 2.0% test groups. In the crossover mating trial (test procedure in preceding section on Ethoxyethanol), a significant decrease ($p < .05$) in the fertility index was noted for matings between high-exposure females (2.0% Ethoxyethanol) and control males. A significant decrease ($p < .05$) in the proportion of pups born alive was also noted for these mating pairs (Morrissey et al. 1989).

An offspring assessment of reproductive function was performed after Ethoxyethanol Acetate exposure (0.50 and 1.0% test concentrations). The dams were dosed through weaning and F_1 mice were dosed until they were mated at 74 ± 10 days of age. Male offspring were mated with female offspring from the same treatment group ($n = 20$/group/sex). F_2 litters were examined for litter size, sex, and pup weight. The only significant finding was a significant decrease ($p < .05$) in the

mating index at a test concentration of 1.0% (Morrissey et al. 1989).

The reproductive toxicity of Ethoxyethanol was evaluated using male and female COBS Crl:CD-1 (ICR) BR outbred albino mice (6 weeks old) according to the NTP fertility assessment by continuous breeding protocol. Groups of male and female mice (20 males, 20 females per group) were housed as mating pairs, and received 0.5%, 1.0%, and 2.0% Ethoxyethanol in drinking water, respectively, continuously for 14 weeks. Significant adverse effects on fertility were noted at concentrations of 1.0% and 2.0%, but not at 0.5%. At the end of the continuous breeding phase, treated males were mated with control females and treated females mated with control males. Fertility and reproduction were compared to the corresponding pairs of control males and control females. Significant effects on fertility and reproduction were observed in males and females of the 1.0% and 2.0% exposure groups. Testicular atrophy, decreased sperm motility, and increased abnormal sperm were observed in treated males. However, no specific anomalies were observed in females. The no-effect level for Ethoxyethanol-induced reproductive effects in male and female mice was set at approximately 0.5% (Lamb et al. 1984).

The reproductive toxicity of Ethoxyethanol Acetate was evaluated in a continuous breeding study using four groups of CD-1 mice (40 males, 40 females; 11 weeks old). In a preliminary dose range–finding study (phase 1), the test substance did not induce death or any significant physical signs of toxicity over the range of concentrations tested, 0.25% to 5.0% *w/w* (8 males, 8 females/group tested in phase 1). During the continuous breeding phase of the study (phase 2), three groups of mice were tested with 0.5%, 1.0%, and 2.0% Ethoxyethanol Acetate (in drinking water), respectively, for 7 days prior to initiation of a 14-week mating period. An untreated group of mice served as the control. A maximum test concentration of 2.0% was chosen because it was considered likely that 5.0% Ethoxyethanol Acetate could cause significant dehydration due to reduced water consumption. The results for the continuous breeding phase of the study are summarized as follows: Values for the fertility index (% of the number of pairs that delivered at least one litter/total cohabited) were 95, 100, and 74 for 0.5%, 1.0%, and 2.0% test groups, respectively. A value of 95 was reported for the control group. Compared to the control group, a significant decrease ($p < .01$) in the number of litters delivered per fertile pair was noted in the 1.0% and 2.0% Ethoxyethanol Acetate test groups. The number of live pups per litter was also significantly reduced ($p < .01$) at these two concentrations (NTP 1985). Results for the remaining phases of the study are summarized below.

In the third phase of the study, mice of the 2.0% test group were evaluated in a crossover mating trial with control mice to determine whether reproductive performance had been compromised. Females dosed with 2.0% Ethoxyethanol Acetate and mated with control males had 27% fertile matings, whereas 59% fertile matings were reported for control males mated with control females. All male and female animals were killed for necropsy at the end of the third phase. Compared to controls, group mean whole body, liver, and kidney weights for female mice were essentially the same ($p > .05$). In male mice, significant differences ($p < .05$) in whole body and right testis weights were noted. However, no significant differences ($p > .05$) regarding liver, kidney, seminal vesicles, right cauda, right epididymis, and prostate weights were reported for male mice (NTP 1985).

Also, in males from the third phase of the study, cauda epididymal sperm counts were essentially identical for control and test (2.0% Ethoxyethanol Acetate) mice. Sperm motility values for both groups were greater than 90.0%. However, compared to controls, the incidence of abnormal sperm was significantly increased ($p < .05$). After reviewing test results for male and female mice, the researchers reported that the results of the crossover mating trial (phase 3) indicate that the reproductive capacity of female mice was relatively more susceptible to the effects of Ethoxyethanol Acetate when compared to males under the same exposure conditions. Furthermore, the investigators reported that this observation was related to embryotoxicity, fetotoxicity, or to a direct effect on fertility because of a definitive decrease in the average number of litters per pair, the proportion of pups born alive, and litter size (NTP 1985).

Phase 4 of the study was designed to evaluate the reproductive performance in offspring (second generation) from litters resulting from the continuous breeding phase (phase 2). Pups delivered by control, 0.5%, and 1.0% Ethoxyethanol Acetate test groups were used. Pups from the 0.5% and 1.0% test groups continued to receive the test substance through lactation and, later, in drinking water. Twenty second generation male mice were randomly mated with 20 second generation female mice in all three groups (control, 0.5%, and 1.0%). All second generation animals were necropsied, at which time sperm morphology and vaginal cytology studies were performed. Results indicated a concentration-related decrease in the number of live pups per litter; however, the difference was not statistically significant ($p > .05$). Compared to controls, group mean liver weights in animals exposed to 1.0% Ethoxyethanol Acetate were significantly decreased ($p < .05$). Additional results for phase 4 are summarized below (NTP 1985).

Except for a significant increase in liver weight ($p < .05$) in male mice (0.5% exposure group) and a significant decrease ($p < .01$) in right cauda weight in male mice (1.0% exposure group), whole body and other organ weights were similar for test and control mice. At a concentration of 0.5% Ethoxyethanol Acetate, no statistically significant effect ($p > .05$) on the incidence of abnormal sperm and average sperm motility was observed. However, at a concentration of 1.0% Ethoxyethanol Acetate, a significant ($p < .01$), concentration-related decrease in sperm density was noted. In female mice, no significant differences in the relative frequency of different estrous stages were observed between test and control groups (NTP 1985).

Given the effect of Ethoxyethanol Acetate on fertility and reproduction noted above for CD-1 mice, a detailed histopathologic

evaluation of the following mice was completed: (1) female mice of the control group and 2.0% exposure group killed at the end of phase 3; (2) second generation female mice of the control group and 1.0% exposure group; and (3) second generation male mice of the control group and 1.0% exposure group. The results of these evaluations are summarized as follows. No significant treatment-related gross and histopathologic changes were observed in the control group or in females of the 2.0% exposure group. Dosing with 1.0% Ethoxyethanol Acetate also did not induce any gross or histopathologic changes in second generation female mice. However, treatment-related histopathologic changes were noted in a number of male mice. Mainly, lesions were observed in the testes and epididymis and consisted of degeneration of the seminiferous tubules, interstitial cell hyperplasia, reduction of sperm content, and the accumulation of fluid and degenerated cells in the epididymis (NTP 1985).

The researchers of the above study concluded that Ethoxyethanol Acetate was a reproductive toxicant in CD-1 mice, as evidenced by significant decreases in the number of litters per fertile pair, decreased live pups per litter, and a decreased proportion of pups born alive, especially at the highest concentration of 2.0% Ethoxyethanol Acetate (NTP 1985).

Twenty male albino rats of an inbred strain (3 weeks old; weights not stated) were fed 1.45% Ethoxyethanol in the diet over a 2-year period, after which animals were killed. The negative control group was fed basal diet only. At microscopic examination, enlarged edematous testes were reported for two-thirds of the animals. The testicular lesions were more frequently bilateral, and consisted of marked interstitial edema and marked tubular atrophy. Additional microscopic finding(s) in test animals are included in the section on Chronic Oral Toxicity earlier in this report (Morris, Nelson, and Calvery 1942).

The developmental toxicity of Ethoxyethanol was evaluated using random-bred, virus antibody–free CD-1 mice. In the teratology probe, groups of mated female mice (8 to 10 weeks old; 17 to 33 g) received the following doses of Ethoxyethanol, respectively, on days 8 through 14 of gestation: 1000, 1800, 2600, 3400, and 4200 mg/kg. The test substance was administered orally in distilled water at a dose volume of 10 ml/day. Control mice were dosed with distilled water. Mated female mice were killed via carbon dioxide inhalation on day 18. Necropsy was performed and uterine contents were examined for the numbers of implantation sites, resorptions, and live and dead fetuses. Fetuses were examined for external abnormalities. In the postnatal study, only sublethal doses of Ethoxyethanol (800 and 1200 mg/kg dose groups) were administered, and the dams were allowed to give birth. The number of live offspring was determined and each pup was examined externally for anomalies. All surviving dams and offspring were killed via carbon dioxide inhalation on postnatal day 22. Study results are summarized below (Wier, Lewis, and Traul 1987).

In the teratology probe, dose-related increases in maternal mortality and morbidity were noted in mated females. The following were taken into consideration in the morbidity assessment: lethargy, failure to right, uneven gait, abnormal breathing, and/or cold to the touch. Additionally, mean changes in maternal body weight during treatment were significantly decreased. During days 14 to 18 gestation, significantly reduced maternal weight gains were associated with the increased incidence of embryo resorption and fewer live fetuses at term. A significant reduction in mean fetal body weights was observed with increasing doses of Ethoxyethanol. A significant increase in the mean number of malformed fetuses was noted in the 1800- and 2600-mg/kg dose groups. Malformations noted in test groups were described as follows. Fused (syndactyly), small, or missing (oligodactyly) digits of the forepaw were noted in 5/6 litters (19/41 fetuses) of the 1800-mg/kg group, 2/2 litters (10/12 fetuses) of the 2600-mg/kg dose group, and 1/1 litter (1/4 fetuses) of the 3400-mg/kg dose group. Exencephaly was observed in the control group (1/5 litters, 1/53 fetuses), the 1800-mg/kg dose group (5/6 litters, 8/41 fetuses), the 2600-mg/kg dose group (1/2 litters, 1/12 fetuses), and the 3400-mg/kg dose group (1/1 litter, 1/4 fetuses). Cleft palate was noted in 4/6 litters (9/41 fetuses) of the 1800-mg/kg dose group and 1/2 litters (1/12 fetuses) of the 2600-mg/kg dose group. Prematurely open eye(s) were observed in the 1800- and 2600-mg/kg dose groups along with the other malformations (Wier, Lewis, and Traul 1987).

In the postnatal study, the mean number of live-born pups was significantly reduced in the 1200-mg/kg dose group. Additionally, litter size continued to decrease during the postnatal period. Mean pup weights were significantly reduced on postnatal day 1, but not during the remainder of the postnatal period. Malformations of the forepaw (fused or missing digits) and kinked tail were noted at external examination of offspring from the 800- and 1200-mg/kg dose groups. Also, for both groups, the percentage of pups with kinked tail increased with postnatal age (Wier, Lewis, and Traul 1987).

The results of the preceding study indicated that Ethoxyethanol induced embryo lethality and malformations, and decreased fetal weight at a dose that was not maternally toxic (Wier, Lewis, and Traul 1987).

Ethoxyethanol and Ethoxyethanol Acetate were administered orally to groups of male JCL-ICR mice (6 weeks old; number per group not stated) 5 days per week for 5 weeks. Each test substance was administered at doses of 500, 1000, 2000, and 4000 mg/kg body weight. Testicular atrophy was noted in groups dosed with Ethoxyethanol and Ethoxyethanol Acetate. Histopathological evaluation indicated dose-related, but variable, atrophy of the seminiferous epithelium. A significant decrease in white blood cell count was also noted in Ethoxyethanol and Ethoxyethanol Acetate dose groups. Decreased red cell count, packed cell volume, and/or hemoglobin content were noted at greater doses of Ethoxyethanol Acetate (Nagano et al. 1984).

In another study, the reproductive toxicity of Ethoxyethanol was evaluated using an in vivo mouse screening bioassay. Fifty pregnant, CD-1 mice (6 to 8 weeks old) were dosed orally with Ethoxyethanol (3605 mg/kg/day; dose volume = 10 ml/kg body weight) on days 7 to 14 of gestation. Day 1 of gestation was

defined as the day on which a copulatory plug was observed. Fifty control pregnant mice were dosed with distilled water according to the same procedure. None of the pregnant mice dosed with Ethoxyethanol had viable litters. The percentage of viable litters in the control group was 97%. Maternal mortality was 10% (5 of 50 mice) for the test group. No deaths were observed in the control group (Schuler et al. 1984).

The reproductive toxicity of Ethoxyethanol was evaluated using 16 (4 groups of 4; 90 to 100 days old) male Long-Evans hooded rats. Prior to dosing, the males were allowed to mate with ovariectomized, hormonally primed females over a period of 2 weeks. Three groups of males were then intubated with 936, 1872, and 2808 mg/kg Ethoxyethanol, respectively. Ethoxyethanol was diluted with water such that the rats received equivalent volumes of 3 ml/kg. The control group was dosed with distilled water. At day 2 post dosing, the animals were allowed to mate again, and copulatory behavior and semen samples evaluated. At 15 minutes after copulation, females were killed and semen samples obtained from uteri. The male animals were killed at week 16 for microscopic examination of tissues. Ethoxyethanol caused a significant decrease ($p \leq .01$) in sperm count in all dose groups. The evaluation of sperm motility data at weeks 4, 7, and 10 was confounded by either severe oligozoospermia or azoospermia in rats of the 1872- and 2808-mg/kg dose groups. Thus, insufficient numbers of sperm were available for evaluation during this period. However, no differences in sperm motility were observed at week 1 or week 14 (all dose groups). Compared to controls, a significant decrease in the percentage of sperm with normal morphology was noted in the 936-mg/kg dose group. The analysis of sperm morphology in the higher dose groups was complicated by severely depressed sperm counts at weeks 7 and 10. Analyses of reproductive and other organ weights indicated a significant decrease ($p < .05$) in epididymal weight in males of the 1872-mg/kg dose group. This was the only significant difference in organ weight. Differences in weight of epididymides between controls and the 2808-mg/kg dose group approached significance. At week 16 (when males were killed), no lesions were apparent in the testes of treated males. However, residual lesions were observed in the testes of males of the 1872-mg/kg dose group. These lesions were characterized by depleted or disorganized seminiferous tubules interspersed with normal tubules. Thus, the study results indicated that acute Ethoxyethanol exposure induced a reversible impairment of testicular function (Oudiz et al. 1984).

In another study, three groups of adult male Long-Evans hooded rats received oral doses of 936, 1872, and 2808 mg/kg Ethoxyethanol, respectively, for 5 consecutive days. Semen evaluations were conducted up to 14 weeks post dosing. Male rats were also dosed orally with Ethoxyethanol (936 mg/kg) 5 days per week for 6 weeks in a second experiment. Untreated males served as controls. In the first experiment, sperm counts were decreased in the two highest dose groups (by week 4). By week 7, the males were essentially azoospermic. Males of the lowest dose group also had decreased sperm counts and significantly increased numbers of sperm with abnormal shapes. The decreased sperm counts in all three test groups were described as significant. All males had varying degrees of recovery during the remainder of the study, as indicated by increased sperm counts. In the second experiment, sperm counts were significantly depressed (by week 5; $p \leq .01$), and the number of abnormal sperm was increased. By week 6, sperm motility was reduced. Dosing over a period of 6 weeks did not appear to alter the spermatogonial population, suggesting the potential for recovery. Increased brain and spleen weights ($p \leq .05$) were also observed in rats dosed for 6 weeks; hemoglobin and hematocrit were significantly decreased ($p \leq .05$) (Zenick, Oudiz, and Niewenhuis 1984).

The reproductive toxicity of Ethoxyethanol was evaluated in a study involving six groups of F344 male rats (5 males/group; 5 to 7 weeks old). Mean body weights for the six groups ranged from 107 to 108 g. Five groups received the following doses of Ethoxyethanol in deionized water, respectively, daily for 14 days: 300, 600, 900, 1500, and 2500 mg/kg. The control group received deionized water during the dosing period. At the end of the study, the animals were killed and any organs with gross lesions submitted for complete histopathologic examination (NTP 1993). Study results are summarized below.

With the exception of changes in weight of the testis (and thymus), most of the changes in absolute and relative organ weights were related to low final body weights. Dose-related decreases in absolute and relative testis weights were reported. At microscopic examination, degeneration of the seminiferous tubules was observed in males of the two highest dose groups (1500 and 2500 mg/kg). At the largest dose (2500 mg/kg), the severity of testicular degeneration ranged from moderate to marked. The severity of testicular degeneration ranged from minimal to mild in the 1500-mg/kg dose group. Testicular effects were not observed in any of the three smaller dose groups (NTP 1993). Other test results (unrelated to reproductive toxicity) reported in this experiment are included in the section on Short-Term Oral Toxicity earlier in this report.

Five groups of $BC3F_1$ mice (5 to 7 weeks old; 5 males/group) were dosed with Ethoxyethanol according to the procedure in the preceding experiment (same doses). A sixth group served as the control. Mean body weights for the five groups ranged from 21.7 to 22.6 g. Compared to the control group, relative testis weight was significantly lower ($p \leq .05$) in males of the 2500-mg/kg dose group. A microscopic evaluation of the tissues was not performed (NTP 1993). Other test results (unrelated to reproductive toxicity) reported in this experiment are included in the section on Short-Term Oral Toxicity earlier in this report.

In another experiment from the preceding study, the reproductive toxicity of Ethoxyethanol was evaluated using six groups of F344 rats (10 males, 10 females/group; 5 to 7 weeks old). Mean body weights for the six groups of male rats ranged from 142 to 146 g, and from 123 to 127 g for the six groups of female rats. Five groups received the following concentrations of

Ethoxyethanol in deionized water, respectively, daily for 13 weeks: 1250, 2500, 5000, 10,000, and 20,000 ppm. The control group received deionized water during the dosing period. (For clinical pathological evaluations only, five additional groups [20 males, 20 females/group] exposed to the same concentrations, respectively, and a control group were used) (NTP 1993). Study results are summarized below.

Organ weight analyses were not performed for male and female rats of the 20,000-ppm exposure group because of the high mortality. However, for the remaining groups, changes in absolute and relative organ weights were observed. Excluding decreases in absolute and relative testis (and thymus) weights, it was stated that the absolute and relative organ weight changes could probably be attributed to the low final mean body weights. A significant reduction in absolute ($p \leq .01$) and relative ($p \leq .05$) testis weights was noted for males of the 10,000-ppm exposure group. It is important to note that the only test substance–related gross lesion noted in rats was a reduction in testis size observed in males of the 10,000- and 20,000-ppm exposure groups. At microscopic examination, testicular degeneration was observed in all male rats that received concentrations of 5000 ppm Ethoxyethanol, or greater. The severity of testicular degeneration was moderate to marked only at the two highest concentrations (10,000 and 20,000 ppm) (NTP 1993). Sperm morphology and vaginal cytology evaluations were performed on rats of the control group and the 2500-, 5000-, or 10,000-ppm exposure groups. The results of these evaluations are summarized below.

Compared to controls, all spermatozoal measurements were significantly less in the 10,000-ppm treatment group. Sperm concentration also was significantly less in the semen of males of the 2500- and 5000-ppm exposure groups. A significant decrease in length of the estrous cycle was reported for females dosed with 10,000 ppm Ethoxyethanol (NTP 1993). Other test results (unrelated to reproductive toxicity) reported in this experiment are included in the section on Subchronic Oral Toxicity earlier in this report.

Five groups of $BC3F_1$ mice (5 to 7 weeks old) were dosed with Ethoxyethanol according to the procedure in the preceding experiment. Mean body weights for the six groups of male mice ranged from 22.7 to 23.9 g, and from 18.9 to 19.3 g for the six groups of female mice. The five groups (10 males, 10 females per group) received Ethoxyethanol at concentrations of 2500, 5000, 10,000, 20,000, and 40,000 ppm in drinking water, respectively. A sixth group served as the control. (For clinical pathological evaluations only, five additional groups [20 males, 20 females/group] exposed to the same concentrations, respectively, and a control group were used) (NTP 1993). Study results are summarized below.

Significantly decreased absolute testis weights were reported for mice of the two highest exposure groups (20,000 and 40,000 ppm). Test substance–related gross lesions included small testes and epididymides in mice of the 40,000-ppm exposure group. Histopathologic changes were observed in the testis (and spleen) of male rats and the spleen and adrenal glands of female mice. Degeneration of the testis in male mice was described as a marked, diffuse loss of germinal epithelium in the seminiferous tubules. Histopathologic changes were not noted in the testis of mice of the lower exposure groups. Sperm morphology and vaginal cytology evaluations were performed on mice of the 5000-, 10,000-, and 20,000-ppm exposure groups and the control group. Compared to controls, male mice of the high-exposure group (20,000 ppm) had significantly lower epididymal and testicular weights. Also, in this group, values for sperm motility, spermatid heads per testis, and spermatid count were significantly smaller. Compared to controls, significantly longer estrous cycles were reported for females of all exposure groups (NTP 1993). Other test results (unrelated to reproductive toxicity) reported in this experiment are included in the section on Subchronic Oral Toxicity earlier in this report.

In another experiment from the preceding study, the subchronic oral, reproductive toxicity of Ethoxyethanol was evaluated using four groups of 30 male F344 rats. The rats were 5 to 7 months old, and mean body weights for the four groups ranged from 161 to 165 g. Three groups received 5000, 10,000, and 20,000 ppm Ethoxyethanol in drinking water, respectively, daily for 60 days. The fourth group received drinking water only. At the end of dosing, 10 rats in each exposure group were killed (except in the case of early deaths). According to the protocol, if lesions were observed at necropsy, half of the remaining rats would be killed after a 30-day recovery period, and, the remaining half, after a 56-day recovery period. In the 20,000-ppm exposure group, 20 of 30 rats died or were killed before the end of the 60-day dosing period. Thus, the 10 surviving rats of this group were combined with the 5 rats (20,000-ppm exposure group) that survived the 13-week dosing period in the subchronic oral, reproductive toxicity study (NTP 1993) summarized earlier in this section. Study results are summarized below.

In rats of the 10,000- and 20,000-ppm exposure groups, absolute and relative right testis and epididymal weights were significantly smaller (compared to controls). This was true after dosing for 60 days and after 30 and 56 days of recovery. In males of the 5000-ppm exposure group, the absolute testis weight was significantly smaller than the control value after 56 days of recovery. At the end of the dosing period (day 60), moderate to marked testicular degeneration was noted in rats of the 10,000- and 20,000-ppm treatment groups, but not in rats of the 5000-ppm treatment group. Recovery from these lesions did not occur during the 30- or 50-day recovery periods. Although, at the end of exposure, testicular degeneration was not evident in rats of the 5000-ppm exposure group, degeneration was minimal in most males during the 30- and 50-day recovery periods (NTP 1993). Other test results (unrelated to reproductive toxicity) reported in this experiment are included in the section on Subchronic Oral Toxicity earlier in this report.

Changes in the testis were also observed in another subchronic oral toxicity study that appears earlier in the text of this report (see section on Subchronic Oral Toxicity). Four groups of male Sprague-Dawley rats received oral doses of 0.052 (6 rats),

0.213 (8 rats), 0.735 (11 rats), and 1.89 g/kg/day (16 rats) for 90 days. Changes in the testis (complete absence of sperm in testes from all three male rats) were observed at the largest dose tested, 1.89 g/kg/day Ethoxyethanol (Union Carbide Corporation 1947).

Changes in the testes were observed in a short-term oral toxicity study that appears earlier in this report (see section on Short-Term Oral Toxicity). Three groups of 30 albino rats of the CR, COBS, CD, BR strain (average body weight = 235 ± 15.1 g) received oral doses (by gavage) of 450, 900, and 1800 mg/kg Ethoxyethanol 5 days per week for 6 weeks. The results are summarized here as follows. Significant decreases in absolute and relative testes weights were observed in males of 900 and 1800 mg/kg Ethoxyethanol dose groups. The incidence of small testes was as follows. Two of 10 (450 mg/kg dose), 5 of 10 (900 mg/kg dose) and 6 of 10 (1800 mg/kg dose). Testicular atrophy was reported for all dose groups and usually was accompanied by degenerated spermatozoa in the epididymides and hypospermia (Eastman Chemical Company 1982).

The reproductive toxicity of Ethoxyethanol was evaluated using young male Sprague-Dawley rats (4 weeks old). The animals were randomly assigned to three treatment groups that received oral doses of 250, 500, and 1000 mg/kg body weight/day, respectively. The control group received an equal volume of the water vehicle (5 ml/kg body weight/day). Six rats from each dose group were killed according to the following schedule: 6 and 24 hours after a single dose and after 2, 4, 7, and 11 days of repeated daily doses (24 hours after the last dose). At necropsy, the testes and epididymides were prepared for light microscopy. Ethoxyethanol doses of 500 and 1000 mg/kg induced degeneration of the dividing and early-pachytene spermatocytes, but had no effect on the middle and late stage of pachytene development. It was noted that these changes developed more rapidly in the 500 mg/kg dose group. This dose also induced a more extensive lesion after 2 days of dosing, when compared to the largest dose. However, prolonged dosing resulted in the reversal of this trend. The smallest dose (250 mg/kg) did not induce any spermatogenic abnormalities (Creasy and Foster 1984).

The testicular toxicity of Ethoxyethanol was evaluated using three groups of male Sprague-Dawley rats (4 weeks old; 36 rats per group). The three groups received oral doses of 250, 500, and 1000 mg/kg/day, respectively, for 11 days. Untreated rats served as controls. At day 11, significant differences in testicular weight were noted in males of the 500-mg/kg ($p < .01$) and the 1000-mg/kg ($p < .05$) dose groups. Prior to day 11, no significant decreases in testicular weight were observed. Isolated occurrences of significantly decreased liver weight were also recorded at day 4 (500-mg/kg dose group; $p < .01$) and at day 11 (500-mg/kg dose group; $p < .05$). However, no histological or ultrastructural alterations were noted in the liver. Generally, greater changes in relative organ weights were noted in the 500-mg/kg dose group. Spermatocyte degeneration was observed in the testes of male rats of the 500- and 1000-mg/kg dose groups. After day 11, the onset of spermatocyte degeneration was more rapid in testes of males of the 500-mg/kg dose group. Degeneration was restricted to the later stages of primary spermatocyte development and secondary spermatocytes. Additionally, the following cell types were unaffected, apart from partial maturation depletion of early stage spermatids: Sertoli and Leydig cells, spermatogonia, prepachytene spermatocytes, and spermatids. No testicular abnormalities were observed in rats of the 250-mg/kg dose group. A similar pattern and severity of spermatocyte degeneration and depletion was observed in rats dosed daily for 11 days with ethoxyacetic acid (500 mg/kg/day) (Foster et al. 1983). In a later study, the oral dosing of male rats (two groups of 36; strain not stated) with doses of 500 and 1000 mg/kg/day, respectively, for 11 days resulted in severe spermatocyte degeneration. A similar pattern of spermatocyte degeneration was also observed in rats dosed with ethoxyacetic acid (500 mg/kg/day) for 11 days (Foster et al. 1984).

The spermatotoxicity of Ethoxyethanol (in water) was also evaluated in another study using 10 adult male, Long-Evans hooded rats (70 to 80 days old). The rats were dosed orally with Ethoxyethanol (936 mg/kg) 5 days per week for 6 weeks. Ten control male rats were dosed with water only. The males were allowed to mate with ovariectomized, hormonally primed female rats. During the exposure period, semen samples were collected from female rats immediately after mating. The samples were analyzed for sperm count, morphology, and motility. None of the males died during the study; none had overt signs of toxicity. At weeks 5 and 6, sperm count and percentage of normal morphology were significantly decreased ($p < .001$). Sperm motility was significantly decreased ($p < .01$) at week 6. Significant decreases ($p < .01$) in testes, epididymides, and caudae epididymides weights in treated males were also noted (Oudiz and Zenick 1986).

Ethoxyethanol, in drinking water, was administered to groups of rats (number, ages, strain, and weights not stated) between days 7 and 17 of gestation. The doses ranged from approximately 210 to 550 mg/kg body weight per day. For the 15 litters from dams that received doses between 210 and 270 mg/kg/day, the incidence of embryomortality was 31% of implants; ethoxyethanol had no apparent effect on pup body weights. For the 19 litters from dams that received doses between 270 and 400 mg/kg/day, the incidence of embryomortality was 69% of implants. Pup weights were 50% to 89% of those reported for controls; signs of delayed development, but no malformations, were observed. In the eight litters from dams that received doses between 400 and 550 mg/kg/day, embryomortality was 100%; no signs of maternal toxicity were noted (Chester, Hull, and Andrew 1986).

The effect of Ethoxyethanol on male fertility was evaluated using three groups of 9 to 10 Sprague-Dawley rats. The three groups received oral doses of 0, 250, 500 mg/kg, respectively, daily for 2, 5, or 7 weeks. After 5 weeks of dosing, males dosed for 7 weeks were mated with untreated females. Following each treatment period, the males were killed and preparations of caudal epididymal sperm were used in the assessment of sperm motility and count. Females were killed on day

14 of gestation, and numbers of implantation, resorption, and live fetuses recorded. The treatment of males with 500 mg/kg Ethoxyethanol for 5 weeks affected all sperm parameters, and no motile sperm were present in males receiving this dose daily for 7 weeks. In males that received 250 mg/kg doses for 5 and 7 weeks, the percentage of progressively motile sperm was significantly decreased. Reductions in sperm count were observed in males that received 250 and 500 mg/kg doses for 5 and 7 weeks. Testis weight was slightly reduced in rats receiving 500 mg/kg doses for 2 weeks. Significant decreases in the pregnancy index and numbers of implantations and fetuses were noted in the 500-mg/kg dose group; but, no effects were found in the 250-mg/kg dose group (Horimoto et al. 1996). In another study, groups of male Sprague-Dawley rats were dosed orally with Ethoxyethanol (500 mg/kg/day) for 7 days. At 4 weeks post dosing, the percentage of motile and progressively motile sperm and the total sperm count were significantly reduced. Decreased testicular weight was noted at 1, 2, and 4 weeks post dosing. Histopathological evaluation of the testes indicated a marked reduction or disappearance of spermatids at 4 weeks post dosing (Ninomiya et al. 1995).

Testicular atrophy and leukopenia were observed in male mice dosed orally with 400 mg/kg Ethoxyethanol Acetate. Doses were administered daily, 5 days per week for 5 weeks (Nagano, Nakayama, and Koyano 1979).

The spermatotoxicity of Ethoxyethanol was evaluated using 72 Long-Evans male rats (80 to 90 days old) and female rats of the same strain. The male rats were randomly assigned to mated (mated every other day) or nonmated (sexually rested) groups. Initially, males of the mated group were mated several times with ovariectomized, hormonally primed females over a period of 2 weeks. During the third and fourth weeks and the remainder of the experiment, the males (mated group) were mated every other day (twice daily, 3-hour sessions). Males from mated and nonmated groups were randomly assigned for oral dosing with 150 or 300 mg/kg Ethoxyethanol in distilled water (dose volume = 2 ml/kg). Doses were administered daily 5 days per week for 6 weeks (weeks 5 through 10 of experiment). Control males were dosed with distilled water according to the same procedure. All males were killed by carbon dioxide asphyxiation at week 11. Sperm counts were determined and sperm morphology evaluated. Analysis of variance was the statistical method used. Study results are summarized below (Hurtt and Zenick 1986).

The rats dosed with Ethoxyethanol were clinically indistinguishable from control rats and no significant differences in body weight gain were noted. Three animals died during the study (two controls and one of the 150-mg/kg group) for reasons unrelated to test substance administration. Ethoxyethanol (300 mg/kg) caused a significant reduction ($p \leq .05$) in testis weight, irrespective of the mating program. Ethoxyethanol (300 mg/kg) also caused significant reductions ($p \leq .05$) in cauda epididymal and accessory organ weight only in males mated bidaily. These significant changes (testis, cauda epididymis, and accessory organs) were not noted at the smaller dose of 150 mg/kg. Dosing with Ethoxyethanol had no effect on vas deferens weight. Ethoxyethanol (300 mg/kg) also induced a significant decrease ($p \leq .05$) in spermatid count in mated and nonmated rats. Epididymal sperm count and percentage normal morphology were significantly decreased ($p \leq .05$) only in nonmated animals of the 300-mg/kg dose group. Significant reductions in both parameters also were induced in males of the 150-mg/kg dose group mated bidaily (Hurtt and Zenick 1986).

Ethoxyethanol was administered orally to five groups of male rats (total number of animals, strain and weights not stated) over a period of 13 weeks. Three groups received doses of 50, 100, and 200 μl/kg/day, respectively, during the treatment period. The remaining two groups received doses of 100 and 200 μl/kg/day, respectively, up to day 59, after which doses were increased to 400 and 800 μl/kg/day, respectively, for the remainder of the treatment period. A sixth group served as the negative-control group. Changes in the testis were observed in five male rats of the 200-μl/kg/day dose group that were evaluated. The interstitium was sometimes edematous. Also, for a typical arrangement of the nuclear epithelia in the tubules (as far as the spermatocytes), absence of the last maturation stages was observed. Pronounced testicular damage was observed in five male rats of the 800-μl/kg/day dose group that were evaluated. The interstitium was sometimes edematous. Also, in the seminiferous tubules, parent spermatogonia and Sertoli cells were visible (typical basal arrangement); however, they often formed individual cells relative to the lumen. In some of the testes, layers of spermatogonia were oriented toward the lumen; further maturation stages were lacking. Tubular lumens (reduction in diameter noted) often appeared empty. Additional results for this subchronic study are included in the section on Subchronic Oral Toxicity earlier in this report (Stenger et al. 1971).

In the same study, groups of male dogs (six per group; breed and weights not stated) were dosed with Ethoxyethanol (doses of 50, 100, and 200 μl/kg/day, respectively) over a period of 13 weeks. Untreated dogs served as controls. Changes in the testis were observed in three dogs of the 200-μl/kg/day dose group that were examined. In one dog, the lumens of the seminiferous tubules appeared dilated. In many tubules, the last maturation stages of the seminal epithelium were missing. In the second dog, constriction of the seminiferous tubules was apparent, with preservation of the lower layers of the seminal epithelium (parent and dust spermatogonia). In the third dog, a conspicuous flattening of the squamous cell epithelium, with complete absence of the upper layers was apparent. In some tubules, the parent epithelium was also absent. Additional results for this subchronic study are included in the section on Subchronic Oral Toxicity earlier in this report (Stenger et al. 1971).

In the last experiment of this study, the teratogenicity of Ethoxyethanol was evaluated using six groups of pregnant rats (number per group, strain, and weights not stated). During gestation, the test substance was administered orally to the six groups at doses of 12.5, 25, 50, 100, 200, and 400 μl/kg/day, respectively. Untreated rats served as controls. A dose-dependent

increase in skeletal abnormalities was noted, starting at 100 μl/kg/day (Stenger et al. 1971).

Dermal Studies

Reproductive and developmental toxicity studies on Ethoxyethanol (dermal/subcutaneous exposure) and Ethoxyethanol Acetate (dermal exposure) are summarized in Table 6 and in the following text. Ethoxyethanol caused reproductive toxicity following subcutaneous injection (mice and rats) or application to the skin (rats). Reproductive toxicity in rats was also observed following the dermal application of Ethoxyethanol Acetate.

The developmental toxicity of Ethoxyethanol was evaluated using 76 pregnant SPF Sprague-Dawley rats. The 76 sperm-positive females resulted from the mating of 150 virgin females (weights = 200 to 225 g) with breeder males. Pregnant females were randomly divided into two test groups and one control group. Ethoxyethanol was applied at doses of 0.25 ml (25 rats) and 0.5 ml (26 rats) four times per day on gestation days 7 to 16. The four daily applications (approximately 2.5 hours apart) were made to the shaved interscapular region of each pregnant female. Twenty-five pregnant rats dosed with water served as controls. Ataxia in the higher dose group (2.0 ml/day total, last days of dosing) was the only sign of maternal toxicity. Compared to controls, maternal liver weights were significantly reduced ($p < .001$) and kidney weights were significantly increased ($p < .01$) in this group. Embryonic death (100%) also was noted in the higher dose group. In the other test group (1.0 ml/day total), the incidence of resorptions was significantly increased ($p = .0005$) over that noted in the negative control group; only 11 females had live fetuses. Visible malformations were observed only in two fetuses of the lower dose group (1.0 ml/day total). One fetus had umbilical hernia and the other did not have a tail. Significantly increased incidences of cardiovascular malformations ($p < .05$) and various types of deviations from normal skeletal development ($p < .05$) also were observed (Hardin et al. 1982).

Prior to initiation of the preceding study, a dose range–finding study was conducted using nonpregnant rats. The following total daily doses of undiluted Ethoxyethanol were applied (shaved interscapular region) to six groups of five rats, respectively, for 10 consecutive days: 0.35, 0.50, 0.70, 1.0, 1.4, and 2.0 ml/day. Five control females received water, total daily dose of 1.4 ml/day (0.35 ml four times per day), for 10 consecutive days. The animals were killed 5 days after the last dose. Liver and kidney weights were not altered in either of the test groups. Ataxia was noted on days 9 and 10 in the highest dose group (0.5 ml four times daily) and on day 10 in the 1.4-ml/day (0.35 ml four times daily) dose group. These were the only toxic signs that were noted (Hardin et al. 1982).

The developmental toxicity of Ethoxyethanol and Ethoxyethanol Acetate was evaluated using two groups of pregnant SPF Sprague-Dawley rats, respectively. Animal weights prior to study initiation were not stated. Ethoxyethanol (0.25 ml) was applied four times daily on days 7 to 16 of gestation. Ethoxyethanol Acetate (0.35 ml) was applied according to the same procedure. The volumes administered were equimolar (2.6 mmole per treatment). Water was applied to 17 control rats. Compared to controls, a reduction in the body weight of dams was noted in both treatment groups. This reduction in body weight was associated with completely resorbed litters and significantly fewer live fetuses per litter. Significant reductions in fetal body weights were also noted in these groups. At gross examination of the fetuses, the only malformations observed were three fetuses with acaudia and imperforate anus in the Ethoxyethanol treatment group. The researchers stated that the results of this study confirm that Ethoxyethanol can be absorbed in embryotoxic, fetotoxic, and teratogenic quantities through the intact skin of pregnant rats (Hardin, Goad, and Burg 1984).

Ethoxyethanol was used as a positive control in a dermal teratogenicity study involving groups of 10 to 13 pregnant Crl:CD BR rats. Animals in the positive-control group were dosed dermally with 0.1 ml Ethoxyethanol on days 6 through 15 of gestation. Reduced body weight, cardiovascular defects, delayed skeletal development, and skeletal malformations were observed in fetuses from the positive-control group. Ethoxyethanol was a dermal teratogen (Ryan et al. 1988).

Subcutaneous Studies

Ethoxyethanol was administered subcutaneously to four groups of male rats (approximately 9 animals/group; strain and weights not stated) over a period of 4 weeks. The four groups received doses of 100, 200, 400, and 800 μl/kg/day, respectively, during the treatment period. A fifth group (negative control) was dosed with NaCl solution. Testicular damage was observed in five males of the 400-μl/kg/day dose group and in four males of the 800-μl/kg/day dose group. In the five males from the smaller dose group, the interstitium was edematous and parent spermatogonia were present in the tubules between typical Sertoli cells. In some tubules, multinuclear cells were present, and dust spermatogonia and spermatocytes were found in several locations. Severe testicular damage was observed in the four males of the 800-μl/kg/day dose group. The interstitium was edematous. Also, in the seminiferous tubules, parent spermatogonia and Sertoli cells were visible (typical basal arrangement); however, they often formed individual cells relative to the lumen. In some of the testes, layers of dust spermatogonia were oriented toward the lumen; further maturation stages were lacking. Tubular lumens often appeared empty. Additional results for this short-term study are included in the section on Short-Term Parenteral Toxicity earlier in this report (Stenger et al. 1971).

The teratogenicity of Ethoxyethanol was evaluated using groups of pregnant mice, rats, and rabbits (number of animals, strain, and weights not stated). The test substance was administered subcutaneously, during gestation, to two groups of mice (50 and 100 μl/kg/day), three groups of rats (25, 50, and 100 μl/kg/day), and one group of rabbits (25 μl/kg/day). Untreated animals served as controls. Serious malformations were not observed in any species. However, in the rat, the increase in

TABLE 6
Reproductive and developmental toxicity studies—dermal/subcutaneous exposure

Test substance	Animals tested	Test procedure	Test results	Reference
Ethoxyethanol (total dose up to 2.0 ml/day)	Female SPF Sprague-Dawley rats (2 groups: weights = 200–225 g)	Applied to skin at doses of 0.25 ml (25 rats) and 0.5 ml (26 rats), 4 times/day on gestation days 7–16. Total daily doses of 1.0 ml/day (25 rats) and 2.0 ml/day (26 rats)	Embryonic death (100%) in 2.0 ml/day dose group. In 1.0-ml/day dose group, incidence of resorptions significantly increased ($p = .0005$) over that noted in negative control group; 11 females had live fetuses. Visible malformations only in 2 fetuses from 1.0-ml/day group. Significantly increased incidences of cardiovascular malformations ($p < .05$) and various types of deviations from normal skeletal development ($p < .05$)	Hardin et al. 1982
Ethoxyethanol (total dose of 1.0 ml/day)	Female SPF Sprague-Dawley rats (1 group)	Applied to the skin at doses of 0.25 ml, 4 times/day on gestation days 7–16. Total dose of 1.0 ml/day	Significant reductions in fetal body weights. Completely resorbed litters and significantly fewer live fetuses per litter. Acaudia and imperforate anus (3 fetuses); only gross malformations observed	Hardin, Goad, and Burg 1984
Ethoxyethanol Acetate (total dose of 1.40 ml/day)	Female SPF Sprague-Dawley rats (1 group)	Applied to the skin at doses of 0.35 ml, 4 times/day on gestation days 7–16. Total dose of 1.40 ml/day	Significant reductions in fetal body weights. Completely resorbed litters and significantly fewer live fetuses per litter. No gross malformations	Hardin, Goad, and Burg 1984
Ethoxyethanol (0.1 ml dose)	Female Crl:CD® BR rats (10–13 animals)	As positive control, applied to skin (0.1 ml) on gestation days 6–15	Reduced fetal body weight, cardiovascular defects, delayed skeletal development, and skeletal malformations. Dermal teratogen	Ryan et al. 1988
Ethoxyethanol (doses up to 800 μl/kg)	Male rats (4 groups of 9/group)	Doses of 100, 200, 400, and 800 μl/kg/day, respectively, injected subcutaneously over 4-week period	Testicular damage in 5 males from 400-μl/kg/day dose group. Pronounced testicular damage in 4 males from 800-μl/kg/day dose group	Stenger et al. 1971
Ethoxyethanol (doses up to 100 μl/kg/day)	Mice (2 groups), rats (3 groups), and rabbits (1 group)	Subcutaneously injected doses: Mice (50 and 100 μl/kg/day, respectively); rats (25, 50, and 100 μl/kg/day, respectively); and rabbits (25 μl/kg/day)	No serious malformations in either species. Compared to controls, a large increase in skeletal abnormalities in rats was observed at doses of 100 μl/kg/day	Stenger et al. 1971

skeletal abnormalities was great when the 100-μl/kg/day dose group was compared to controls (Stenger et al. 1971).

In Vitro Studies

Studies on the reproductive and developmental toxicity of Ethoxyethanol in vitro are summarized in Table 7 and in text. Ethoxyethanol induced embryotoxicity and testicular toxicity in these assays.

The embryotoxicity of Ethoxyethanol was evaluated in an in vitro assay. At a concentration of 100 mM, Ethoxyethanol completely inhibited the embryonic development of albino rat embryos. Ethoxyethanol (50 mM) induced a high frequency of unrotated embryos, and dysmorphic effects at the level of the telencephalon were noted at a concentration of 25 mM. Gross anomalies were not observed in embryos exposed to 12.5 mM Ethoxyethanol. The no-effect concentration for embryotoxicity was 6.25 mM Ethoxyethanol (Giavini et al. 1993).

At test concentrations ranging from 0.3 up to 1 mg/ml, Ethoxyethanol induced no apparent effects on growth or development parameters in Sprague-Dawley rat embryo cultures. An increased incidence of growth-retarded forelimb buds was noted at the greatest test concentration (1 mg/ml) (Bowden et al. 1995).

The embryotoxicity of Ethoxyethanol was evaluated in an in vitro study involving post-implantation rat embryos. The embryos were explanted on day 10.5 of gestation and cultured in heat-inactivated rat serum containing 1.0 to 15.0 μl/ml Ethoxyethanol. Ethoxyethanol induced embryotoxicity, and this effect was dose-dependent. Embryonic growth and development were retarded. The minimum embryotoxic concentration of Ethoxyethanol was 7.3 ± 0.2 μl/ml (75.5 ± 2.3 μmol/ml) (Brown-Woodman et al. 1994).

TABLE 7
Reproductive and developmental toxicity—in vitro studies

Test substance	Cell cultures tested	Test procedure	Test results	Reference
Ethoxyethanol (at concentrations up to 100 mM)	Albino rat embryos	In vitro embryotoxicity assay. Test concentrations of 6.25, 12.5, 25, 50, and 100 mM	No embryotoxicity (6.25 mM). No gross morphological anomalies (12.5 mM). Dysmorphic effects at the level of the telencephalon (25 mM). High frequency of unrotated embryos (50 mM). Complete inhibition of embryonic development	Giavini et al. 1993
Ethoxyethanol (at concentrations up to 1 mg/ml)	Sprague-Dawley rat embryos	In vitro embryotoxicity assay. Test concentrations of 0.3–1 mg/ml culture	Increased incidence of growth-retarded forelimb buds at 1 mg/ml	Bowden et al. 1995
Ethoxyethanol (at concentrations up to 15 l/ml)	Rat embryos	In vitro embryotoxicity assay. Ethoxyethanol tested at concentrations ranging from 1.0–15.0 l/ml culture	Dose dependent embryotoxicity. Minimum embryotoxic concentration = 7.3 ± 0.2 l/ml	Brown-Woodman et al. 1994
Ethoxyethanol	Mixed cultures of Sertoli and germ cells from male Sprague-Dawley rat testes	In vitro testicular toxicity assay.	No morphological evidence of toxicity at concentrations up to 50 mM . (After incubation of cultures with the metabolite, ethoxyacetic acid (2–10 mM), degeneration of pachytene and dividing spermatocytes noted. Spermatocytes, spermatogonia, and Sertoli cells unaffected)	Gray et al. 1985

The testicular toxicity of Ethoxyethanol was evaluated using mixed cultures of Sertoli and germ cells from the testes of immature, male Sprague-Dawley rats (28 days old). Ethoxyethanol did not induce any morphological evidence of toxicity at concentrations up to 50 mM during a 72-hour incubation period. However, after incubation (up to 72 hours) of cultures with the metabolite Ethoxyacetic Acid (2 to 10 mM), degeneration of the pachytene and dividing spermatocytes was noted. Spermatocytes, spermatogonia, and Sertoli cells were unaffected (Gray et al. 1985).

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation

Patch tests were performed on one to 20 patients with or suspected of having contact allergy to cosmetic products. Ethoxyethanol was tested at a concentration of 2% in petrolatum. Details concerning the experimental procedure were not stated. No irritant reactions were observed (de Groot 1994).

Case Report

The following effects were observed in a 44-year-old woman who ingested approximately 40 ml of Ethoxyethanol: vertigo, unconsciousness, effects on the central nervous system, metabolic acidosis, renal insufficiency, signs of hepatic damage, and neurasthenia. Recovery from most symptoms was noted within 44 days. Neurasthenia was the only symptom that was noted at the time of recovery (Fucik 1969).

OCCUPATIONAL EXPOSURE

NIOSH has recommended that Ethoxyethanol be regarded in the workplace as having the potential to cause adverse reproductive effects in male and female workers (NIOSH 1983). These recommendations were based on the results of animal studies available at that time that demonstrated dose-related embryotoxicity and other reproductive effects in several species of animals exposed by different routes of administration. Of particular concern were those studies in which exposure of pregnant animals to concentrations of Ethoxyethanol at or below Occupational Safety and Health Administration (OSHA) permissible exposure limits (PELs) (in 1981, OSHA PEL for occupational exposure to Ethoxyethanol was 200 ppm [740 mg/m^3], as a time-weighted average [TWA] for an 8-hour workshift) led to increased incidences of embryonic death, teratogenesis, or growth retardation, and exposure in male animals resulted in testicular atrophy and sterility. Therefore, NIOSH determined that appropriate controls should be instituted to minimize worker exposure to both compounds (NIOSH 1983), Ethoxyethanol and Ethoxyethanol Acetate.

More recently, after reviewing and evaluating the available data on the toxicity and health effects of Ethoxyethanol and Ethoxyethanol Acetate, NIOSH recommended that exposure to Ethoxyethanol and Ethoxyethanol Acetate be limited to 0.5 ppm (1.8 mg Ethoxyethanol/m^3 and 2.7 mg Ethoxyethanol Acetate/m^3) as a 10-hour TWA. NIOSH also stated that dermal contact is prohibited because Ethoxyethanol and Ethoxyethanol Acetate are readily absorbed through the skin (NIOSH 1991). Limitations established by other organizations are indicated below.

OSHA lists 200 ppm as the PEL, expressed as an 8-hour TWA, for Ethoxyethanol. In the United Kingdom, the allowable exposure limit for Ethoxyethanol is 25 ppm (Browning and Curry 1994).

American Conference of Governmental Industrial Hygienists (ACGIH) has recommended that the ambient air TWA for Ethoxyethanol be less than 5 ppm and that skin contact be avoided (Smallwood et al. 1988).

The Japan Society for Occupational Health has recommended occupational exposure limits for Ethoxyethanol and Ethoxyethanol Acetate as reference values for preventing adverse effects on workers. An occupational exposure limit-mean (OEL-M) value of 5 ppm has been determined for both chemicals. The OEL-M for the mean concentration of a chemical substance is defined as the reference value to the mean exposure concentration at or below which adverse health effects caused by the substance do not appear in most workers working for 8 hours per day (40 hours per week) under a moderate workload (Japan Society for Occupational Health 1996).

The potential human hazards of adverse developmental effects posed by exposure to Ethoxyethanol and Ethoxyethanol Acetate were evaluated in a risk assessment. The risk assessment relates to the current use of these ingredients by workers in the manufacture of semiconductors. Approximately 400 air samples from the workplace of seven different companies were used to determine the extent of inhalation exposure to Ethoxyethanol and Ethoxyethanol Acetate during the manufacture of silicon wafers. The geometric mean concentrations of Ethoxyethanol and Ethoxyethanol Acetate in workplace air samples were 0.36 and 0.02 ppm, respectively. The present risk assessment involved the calculation of an A/D ratio (A is defined as the lowest dose that produces signs of overt toxicity in adults; D is defined as the lowest dose that produces any one of the three signs of developmental toxicity in the offspring). The investigators stated that the results of developmental toxicity tests (animal studies) in the published literature coupled with workplace exposure data indicate that workers in the semiconductor industry are exposed to concentrations of Ethoxyethanol and Ethoxyethanol Acetate that should not place them or their offspring at risk for adverse effects. The human hazard posed by these chemicals was described as moderate (A/D $\approx$ 5) (Paustenbach 1988).

In an earlier risk assessment on Ethoxyethanol, an AIS (acceptable intake subchronic) of 4.8 mg/day and an AIC (acceptable intake chronic) of 3.5 mg/day were reported, based on teratology and subchronic inhalation data, respectively (Environmental Protection Agency 1984).

The doses of Ethoxyethanol that are projected to be associated with different reproductive risks in humans at a frequency of 10^{-6} or 10^{-4} are summarized in Table 8. This table is taken from

TABLE 8
Summary of doses of ethoxyethanol that are projected to be associated with different reproductive risks in humans at a frequency of 10^{-6} or 10^{-4} (Ballew and Hattis 1989)

Reproductive effect	Projected reproductive risk frequency for effect	Doses (ppm Ethoxyethanol) projected to be associated with the risk*	
		Lower limit	Best estimate
Miscarriages	10^{-6}	0.00056	0.53
	10^{-4}	0.0061	1.8
Minor skeletal defects	10^{-6}	4.4×10^{-6}	0.022
	10^{-4}	4.8×10^{-5}	0.073
External malformations	10^{-6}	0.0011	1.1
	10^{-4}	0.012	3.5
Digit or limb malformations	10^{-6}	—	—
	10^{-4}	—	—
Total malformations	10^{-6}	—	—
	10^{-4}	—	—
Infant mortality (projected from fetal weight changes)	10^{-6}	—	0.069
	10^{-4}	—	6.8

*Values in the lower limit column represent the doses associated with the indicated level of effect under a more pessimistic assumption about the degree of interindividual variability in susceptibility of the human population (log probit slope of 1). Values in the best estimate column represent calculations using the investigators' "best-estimate" assumption of the degree of interindividual variability in susceptibility for the quantal reproductive effects (log probit slope of 2).

a report that represents an experimental, pioneering analysis aimed at producing quantitative estimates of risk for female reproductive effects that are more traditionally treated using the NOEL (no-effect level)/uncertainty factor paradigm (Ballew and Hattis 1989).

Excretion

Ethoxyacetic Acid has been detected in the urine of varnish production plant workers exposed to a mixture of glycol ethers, which included Ethoxyethanol. The average urinary concentrations of ethoxyacetic acid (12 male workers) before and after a shift in the varnish production area were 128.5 mg/l and 167.8 mg/l, respectively (Angerer et al. 1990). Most of the glycol ethers were taken up through the skin. The high concentrations of ethoxyacetic acid in preshift urine samples (compared to postshift samples) was attributed to the high level of Ethoxyethanol exposure on the day before the examination, and the fact that alkoxyacetic acids are excreted very slowly (Angerer et al. 1990). Reportedly, oxalic acid is a secondary metabolite of alkoxyacetic acids, such as ethoxyacetic acid, in humans. Oxalic acid has been detected in the urine of silk printing workers exposed to glycol ethers (Ethoxyethanol and Ethoxyethanol Acetate included), and has also been referred to as the eventual metabolite of ethylene glycol ethers once the ether bond is broken by oxidative reactions in the body (Laitinen et al. 1994).

In an earlier study, 19 subjects were exposed to Ethoxyethanol, along with other glycol ethers, in a varnish production plant. The average urinary concentrations of ethoxyacetic acid before and after a shift in the varnish production area were 53.2 mg/l and 53.8 mg/l, respectively. To study the kinetics of this metabolite, the excretion of ethoxyacetic acid in the urine during an exposure-free weekend was evaluated using 17 subjects. The median values for the calculated half-times were 57.4 and 63.4 hours, respectively. On the basis of the study results, it was calculated that the limit value should not exceed 50 mg ethoxyacetic acid per liter of urine, which is the current German biological tolerance value for ethoxyacetic acid in the urine (Sohnlein et al. 1993).

The urinary excretion of ethoxyacetic acid during daily exposure to Ethoxyethanol and Ethoxyethanol Acetate was evaluated using a group of five female workers (29 to 54 years old). The women were employed as operators of silk-screen printing installations for the surface marking of a variable assortment of plastic containers. The women were exposed to both chemicals during 5 days of normal production and 7 days after a 12-day production stop. The mean combined exposure concentration of Ethoxyethanol and Ethoxyethanol Acetate (expressed in equivalent weight of Ethoxyethanol) was 14.0 mg/m^3. Clearly, the urinary excretion of ethoxyacetic acid increased during the work week. Furthermore, even after a period of nonexposure (12 days), traces of ethoxyacetic acid were detectable (Veulemans et al. 1987).

Ethoxyacetic Acid has also been detected in the urine of 30 male employees of a silk-screen shop. Eight-hour breathing zone air samples indicated concentrations of Ethoxyethanol

Acetate as great as 34 ppm in the silk-screen print room. Urinary ethoxyacetic acid concentrations (expressed as mg ethoxyacetic acid/g creatinine) in exposed workers were as follows: 14.0 ± 7.3 (silk-screen operations), 7.9 ± 5.6 (screen-cleaners and others), 8.7 ± 3.5 (ink-mixing room workers), and 1.5 (Indicia room operators). In the Indicia room, hand silk-screening of labels for small parts was done (Lowry et al. 1993).

Five subjects breathed air containing 10, 20, or 40 mg/m^3 Ethoxyethanol through a respiratory mask. Over a 200-minute period of exposure, these concentrations resulted in the administration of doses of 0.25, 0.5, and 1 mg/kg, respectively. The elimination of ethoxyacetic acid in the urine was described as far from complete after 48 hours of exposure, at which time 23% (average value) of inhaled Ethoxyethanol was excreted as ethoxyacetic acid in the urine. Ethoxyacetic acid conjugates were absent from the urine. The average elimination half-life of ethoxyacetic acid was 42 hours (Groeseneken et al. 1988).

In an earlier study, ethoxyacetic acid was detected in the urine within the first hour of respiratory exposure to Ethoxyethanol. The quantity of ethoxyacetic acid excreted in the urine increased continually up to 3 to 4 hours post-exposure, at which time there was an exponential decline. The elimination half-life for urinary ethoxyacetic acid was 21 to 24 hours. Traces of ethoxyacetic acid remained detectable in the urine 12 days after the cessation of exposure. A linear correlation was noted between the average Ethoxyethanol exposure over the 5-day work week and urinary ethoxyacetic acid excretion at the end of the week (Groeseneken, Veulemans, and Masschelein 1986a).

The results of an inhalation study involving ten subjects (ages = 19 to 28 years old) indicated that Ethoxyethanol was rapidly absorbed through the lungs. Approximately 64% of the inhaled vapor was retained at rest. The subjects were exposed to concentrations up to 40 mg/m^3 for 4 hours (Groeseneken, Veulemans, and Masschelein 1986b).

The pulmonary absorption and elimination of Ethoxyethanol Acetate was evaluated using 10 subjects (21 to 30 years old). Subjects were exposed to concentrations up to 50 mg/m^3 for 4 hours. Samples of mixed expired air during exposure contained Ethoxyethanol Acetate as well as small amounts of Ethoxyethanol. It was stated that the presence of Ethoxyethanol in expired air could have resulted from the metabolic conversion of Ethoxyethanol Acetate by plasma esterases. Furthermore, expired Ethoxyethanol increased proportionally with greater exposure concentrations of Ethoxyethanol Acetate. The respiratory elimination of Ethoxyethanol Acetate accounted for $\leq 0.5\%$ of the total body uptake (Groeseneken et al. 1987a).

In another study, 10 subjects (21 to 30 years old) were exposed to Ethoxyethanol Acetate concentrations up to 50 mg/m^3 for 4 hours, and the excretion of ethoxyacetic acid in the urine monitored. After 1 hour of exposure to Ethoxyethanol Acetate, ethoxyacetic acid was detected in the urine. The rate of excretion of ethoxyacetic acid increased with the duration of exposure; the half-life for appearance was 2.3 ± 0.1 hours. The maximum rate of excretion occurred 3 to 4 hours after the cessation of exposure. As the exposure concentration of Ethoxyethanol Acetate increased, its uptake as well as ethoxyacetic acid excretion increased (Groeseneken et al. 1987b).

Hematotoxicity

A cross-sectional, observational study of the effects of ethylene glycol ethers was conducted. The study participants included 94 shipyard painters and 55 controls. The painters were exposed to glycol ethers, as well as a myriad of other chemicals. Hemoglobin, hematocrit, red cell indices, total and differential white blood cell counts, and platelet counts were measured. The actual concentrations of exposure to glycol ethers for study participants was not stated in this study. However, data on past levels of exposure were provided. Painters were uniformly assumed to have had exposures within the observed range (0 to 80 mg/m^3 Ethoxyethanol, mean = 9.9 ± 15.7; plus additional exposure through the skin) on an average basis since 1978 (Sparer et al. 1988). Study results indicated that a significant proportion of painters was anemic (10%) and granulocytopenic (5%), although the means of all variables were comparable between the groups. It was suggested that these effects resulted from glycol ether exposure. Based on a review of company records, it was determined that most of these abnormalities were acquired during employment. These abnormalities could not be explained by either preexisting disease or other exposure. It is important to note that shipyard painters were actually exposed to a myriad of chemicals, and, of these, glycol ethers, benzene, and lead are the only chemicals that are known to be toxic to bone marrow or circulating blood cells. Effects of lead and benzene on the blood were ruled out either because of very low/negligible worker exposure (Welch and Cullen 1988).

Mild anemia (depressed hematocrit) was observed in 20 of 70 individuals (mainly young adult males, ages and weights not stated) exposed to Ethoxyethanol on the job daily. For most of the subjects, the decreased hematocrits were within the normal clinical range. No evidence of hepatic, renal, reproductive, or neurologic problems was found in any of the subjects. Approximately 6 months after Ethoxyethanol was replaced with another solvent, 12 of the 20 subjects returned for retesting. Test results indicated normal hematocrits in 11 subjects; however, decreased hematocrit was still observed in the 12th subject. Further study indicated that the 12th subject was receiving long-term antibiotic treatment (Lockheed Corporation 1992).

Cytogenetic Effects

Nineteen workers at a varnish production plant were involved in a study of the cytogenetic effects of glycol ethers, Ethoxyethanol included, in the work environment. Venous blood samples were collected on the Tuesday following a shift. Short-term lymphocyte cultures were prepared according to the method of Rudiger et al. (1988). In each case, sister chromatid exchange

was determined as the mean of 15 metaphases. Micronuclei were assessed by determining the number of double-nucleic cells after cytokinesis block, up to a total of 20 micronuclei, and relating it to 500 double-nucleic cells. Fifteen subjects who were not occupationally exposed to glycol ethers underwent cytogenetic examinations for reference purposes. For exposed subjects, the number of micronuclei averaged 7.1 per 500 double-nucleic cells. This value was similar to the value of 7.7 per 500 double-nucleic cells in the reference group. Additionally, the mean sister-chromatid exchange rates for exposed and nonexposed subjects were identical (value = 11.2). Therefore, no exposure-related cytogenetic effects were detected in this assay (Sohnlein et al. 1993).

Reproductive and Developmental Toxicity

The effect of long-term exposure to Ethoxyethanol on semen quality was evaluated using 37 male workers who had the potential for exposure to Ethoxyethanol on a daily basis at a metal castings company and 39 nonexposed workers. The workers were exposed to Ethoxyethanol during its use as a binder slurry in a metal castings process. Eight-hour TWA breathing zone exposures to Ethoxyethanol ranged from nondetectable to 24 ppm (geometric mean = 6.6 ppm). All but two urine samples had detectable amounts of ethoxyacetic acid. The average sperm count per ejaculate among workers exposed to Ethoxyethanol was significantly smaller than that of nonexposed workers ($p = .05$). Additionally, exposed men had a significantly greater proportion of abnormal sperm shapes (immature forms) and a significantly smaller proportion of double headed forms when compared to controls. No statistically significant differences in semen volume, sperm concentration, semen pH, viability, motility, velocity, and normal morphology or testicular volume were noted among exposed and non-exposed men (Ratcliffe et al. 1989).

Male reproductive effects were observed in a population of 73 painters exposed to glycol ethers (Ethoxyethanol included) and a myriad of other chemicals at a large shipbuilding facility. The workers were exposed to Ethoxyethanol at a TWA of 0 to 80.5 mg/m^3 (mean exposure = 9.9 mg/m^3). Forty nonexposed workers served as controls. For sperm density determinations, oligospermia was defined as a concentration of $\leq$20 million sperm per milliliter of semen. Three of the 73 men were azoospermic and one was essentially azoospermic (sperm concentration = 0.2 million/cc). The proportion of painters with a sperm count of $\leq$20 million/cc (oligospermia) was 13%. The investigators suggested that these effects resulted from exposure to glycol ethers. It was noted that, generally, lead and epichlorohydrin are the only other chemicals in the environment of shipyard workers that reportedly affect sperm counts in humans and animals. However, it was determined that these chemicals were not responsible for the male reproductive effects observed for the following reasons: Epichlorohydrin was not detected in air samples, and the highest mean blood lead concentration reported was 40 μg%. This concentration of lead has not been documented to cause depressed sperm counts (Welch et al. 1988).

A case-control study was conducted using 1019 first-time patients at a clinic for reproductive disorders. The 1019 cases (mean age = 29.1 years) were defined as patients diagnosed as infertile or subfertile, based on a spermiogram. The control group consisted of 475 patients (mean age = 29.5 years) who were diagnosed as fertile (normal) using the same procedure. The presence of ethoxyacetic acid in the urine served as an indicator of possible exposure to Ethoxyethanol. Ethoxyacetic acid was detected in 39 cases and six controls; this gave an odds ratio of 3.11 ($p = .004$). It was stated that the absence of a significant correlation between the concentration of urinary ethoxyacetic acid and the various assessments of sperm quality could be explained by the anticipated latent period between exposure and observed effects. It was noted that if, like in animals, the toxic effects of Ethoxyethanol manifest themselves at the early stages of spermatogenesis, a latent period of 10 weeks should be taken into account between the onset of exposure and clinical disturbances in the spermiogram. When the study group was divided according to sperm concentration corrected for motility and morphology, a highly significant clustering of ethoxyacetic acid–positive patients was noted among the subcategories representing complete azoospermia and severe oligozoospermia (chi square probability of .0087). Thus, the main finding in the study was the highly significant association between a diagnosis of impaired fertility and the detection of ethoxyacetic acid in the urine (Veulemans et al. 1993).

SUMMARY

Ethoxyethanol (Ethylene Glycol Monoethyl Ether) is an ether alcohol and Ethoxyethanol Acetate is the ester of Ethoxyethanol and acetic acid. Both ingredients are listed in the International Cosmetic Ingredient Dictionary; however, currently, there are no reported cosmetic uses of these ingredients in the FDA database.

Ethoxyethanol is usually produced by reacting ethylene oxide with ethyl alcohol. Ethylene glycol monoalkyl ether acetates, such as Ethoxyethanol Acetate, are produced via esterification of the particular glycol ether with acetic acid, acetic acid anhydride, or acetic chloride.

Ethylene glycol ethers (e.g., Ethoxyethanol) are rapidly oxidized into acids through transient aldehydic metabolites. Ethoxyethanol is metabolized by an alcohol dehydrogenase to ethoxyacetaldehyde, which is then metabolized by an aldehyde dehydrogenase to ethoxyacetic acid. Ethoxyethanol and Ethoxyethanol Acetate have 2-ethoxyacetic acid as a common metabolite.

The percutaneous absorption of [^{14}C]Ethoxyethanol Acetate across human stratum corneum and skin samples from rats and dogs has been demonstrated in vitro.

In animal studies (rats and guinea pigs), low to moderate toxicity has been noted following acute inhalation exposure to Ethoxyethanol/Ethoxyethanol Acetate. Similar results were reported in subchronic inhalation studies involving rats, rabbits, and dogs and in a chronic inhalation study involving rats.

The following acute oral LD_{50} values have been reported for Ethoxyethanol: 3527 mg/kg (fasted rats), 8103 mg/kg (fed rats), 2451 mg/kg (fasted mice), and 5346 mg/kg (fed mice). In this study as well as another acute oral toxicity study involving mice, rats, and guinea pigs, signs of kidney damage were noted. Mild liver damage was also reported in the latter study.

In acute oral toxicity tests on Ethoxyethanol Acetate, LD_{50} values of 5100 mg/kg and 1910 mg/kg were reported for rats and guinea pigs, respectively. Narcosis was noted at or above the LD_{50}.

Acute dermal LD_{50} values of 3527 mg/kg and 10,500 mg/kg were reported for rabbits dosed with Ethoxyethanol and Ethoxyethanol Acetate, respectively. Signs of renal damage were reported in the study on Ethoxyethanol Acetate. Renal damage was also observed in rats after acute subcutaneous exposure to Ethoxyethanol and Ethoxyethanol Acetate, and in mice after acute intraperitoneal exposure to Ethoxyethanol.

In a short-term (14 days) oral toxicity study involving rats and mice, no test substance–related gross lesions were observed at doses up to 2500 mg/kg. However, in a longer duration (6 weeks) short-term oral toxicity study, blood in the urine (sign of renal damage) was reported for rats receiving doses up to 1800 mg/kg. Significant reductions in hemoglobin concentration, packed cell volume, and total erythrocyte count were also reported in this study.

In other short-term studies, minor damage to the kidneys and liver was noted in groups of rats dosed subcutaneously with Ethoxyethanol (up to 800 μl/kg/day) for 4 weeks, and the absence of internal organ lesions was reported for groups of dogs dosed intravenously with Ethoxyethanol (up to 500 μl/kg/day) for 22 days.

As noted in short-term toxicity studies on Ethoxyethanol, mixed results regarding chemical toxicity were also reported in subchronic oral toxicity tests. Marked or general renal and hepatic damage was noted in rats receiving doses of Ethoxyethanol up to 1.888 g/kg/day for 90 days. The same degree of damage to these organs was noted in control animals that died from pneumonia. However, evidence of pneumonia was not noted at necropsy of rats from the highest dose group. Treatment-related, systemic changes in organs were not observed in rats receiving Ethoxyethanol doses up to 200 μl/kg/day for 13 weeks. However, decreased hemoglobin and minor changes in the kidneys of dogs were noted at the highest dose tested (200 μl/kg/day). Decreased hemoglobin concentration was also noted in groups of rats receiving Ethoxyethanol concentrations up to 20,000 ppm daily for 13 weeks.

An increased incidence of enlarged adrenal glands was noted in male rats receiving doses up to 2.0 g/kg/day over a period of 2 years. This finding was not reported for female rats or male and female mice. Repeated administration at the highest dose administered was lethal to rats as well as mice. In another chronic oral toxicity study, chronic renal damage was noted in rats fed Ethoxyethanol at a concentration of 1.45% in the diet for 2 years. Chronic hepatic damage was not observed.

Ethoxyethanol and Ethoxyethanol Acetate were mild to moderately irritating to the eyes of rabbits.

In skin irritation tests, Ethoxyethanol and Ethoxyethanol Acetate were, at most, mild skin irritants in rabbits. Furthermore, neither substance induced delayed cutaneous hypersensitivity in guinea pigs when tested at a concentration of 10%.

The results of an allograft rejection assay using allogeneic mice and syngeneic mice indicated that Ethoxyethanol could exert an antitumor effect through increased immunological competence or immunomodulation. The purpose of this assay was to evaluate the effect of Ethoxyethanol on cell-mediated immunity.

The results of all nine nonmammalian mutagenicity assays and seven of 12 mammalian cell mutagenicity assays were negative for Ethoxyethanol/Ethoxyethanol Acetate. However, Ethoxyethanol induced chromosome aberrations in two assays and sister-chromatid exchanges in three assays. Ethoxyethanol was weakly positive in the L5178Y cell mutation assay.

All microscopic lesions (internal organs) observed in subchronic oral toxicity studies (mice and rats) on Ethoxyethanol by the NTP were classified as nonneoplastic. The duration of each study was 13 weeks.

The results of numerous reproductive and developmental toxicity animal studies involving various routes of test substance administration (inhalation, oral, dermal, and subcutaneous exposures) indicate that Ethoxyethanol and Ethoxyethanol Acetate are reproductive toxicants as well as teratogens. The animal species tested included rats, rabbits, mice, and dogs.

Ethoxyethanol (2%) did not induce skin irritation in 20 patients with or suspected of having contact allergy to cosmetic products.

Mild anemia was observed in 20 of 70 individuals exposed to Ethoxyethanol on the job daily. Neither of the subjects had evidence of hepatic, renal, reproductive, or neurologic problems. Reevaluation of a subset of the subjects with anemia who subsequently worked in an environment in which Ethoxyethanol was replaced with another solvent indicated normal hematocrits in 11 of 12 subjects. Additionally, reproductive effects have been noted in males exposed to Ethoxyethanol in the work environment.

DISCUSSION

The Cosmetic Ingredient Review (CIR) Expert Panel agreed with the NTP conclusion that "There have been no adequate carcinogenicity studies conducted with any of the glycol alkyl ethers." The EPA has also determined that the data are insufficient for determining the carcinogenicity of glycol alkyl ethers.

The Expert Panel noted that the available data indicate that Ethoxyethanol and Ethoxyethanol Acetate are well absorbed across human skin and that there is no uncertainty that they are reproductive and developmental toxicants via this route of exposure. The Panel noted that it may be possible that there is a threshold level for reproductive and developmental toxicity

following dermal exposure to Ethoxyethanol and Ethoxyethanol Acetate. Such a threshold has not been determined, however. Therefore, based on the demonstrated reproductive and developmental toxicity associated with dermal and other exposures, these ingredients are considered unsafe for use in cosmetic formulations.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that Ethoxyethanol and Ethoxyethanol Acetate are unsafe for use in cosmetic products.

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