National Toxicology Program
Toxicity Report Series
Number 52

NTP Technical Report
on Toxicity Studies of

Urethane in Drinking Water and Urethane in 5% Ethanol

Administered to F344/N Rats
and B6C3F$_1$ Mice

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Post Office Box 12233
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NIH Publication 96-3937
March 1996

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

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This NTP report on the toxicity studies of urethane in drinking water and urethane in 5% ethanol is based primarily on 13-week studies that took place from December 1990 through April 1991.

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PEER REVIEW

The draft report on the toxicity studies of urethane in drinking water and urethane in 5% ethanol was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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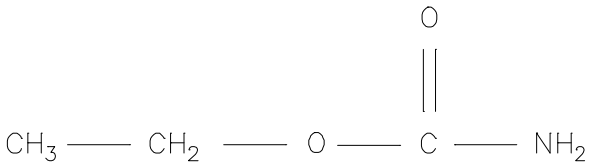
ABSTRACT

Urethane



CAS Number	51-79-6
Molecular Formula	$C_3H_7NO_2$
Molecular Weight	89.09
Synonyms	Urethan; ethyl carbamate; carbamic acid ethyl ester; ethyl urethan

Urethane, a byproduct of fermentation found in alcoholic beverages, is carcinogenic in rodents and is classified by the International Agency for Research on Cancer as a possible human carcinogen. The United States Food and Drug Administration nominated urethane for study because of the widespread exposure of humans through the consumption of fermented foods and beverages and because of a lack of adequate dose-response data about the carcinogenicity of urethane with and without the coadministration of ethanol. Comparative studies of urethane in drinking water and in 5% ethanol were conducted to investigate possible effects of ethanol on urethane toxicity. Toxicokinetic studies of urethane in drinking water and in 5% ethanol and genetic toxicity studies of urethane *in vivo* and *in vitro* were also conducted.

Groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice, 6 weeks of age, received 0, 110, 330, 1,100, 3,300, or 10,000 ppm urethane in drinking water or in 5% ethanol for 13 weeks. Toxicokinetic evaluations were performed for urethane in the plasma of male mice after 13 weeks of administration in drinking water or 5% ethanol. The mutagenicity of urethane in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 with and without S9 was tested at doses up to 16,666 µg/plate; urethane was also tested for induction of sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells and sex-linked recessive lethal mutations and chromosomal reciprocal translocations in *Drosophila melanogaster*. The frequency of

micronucleated erythrocytes induced in peripheral blood and bone marrow cells of mice by urethane in drinking water and in 5% ethanol was also evaluated.

In rats that received urethane in drinking water, seven males and four females administered 10,000 ppm and one female administered 3,300 ppm died before the end of the study; body weight gains were reduced at these concentrations. Two males and all females given 10,000 ppm urethane in 5% ethanol died during the study, and the body weight gains of males and females that received 3,300 ppm were lower than those of the controls. Relative right kidney, liver, and lung weights of males and females and relative right testis weights of males administered 1,100 ppm or greater were generally higher than those of the controls in each study. Leukopenia and lymphopenia were observed in rats receiving urethane in either drinking water or ethanol and occurred in males receiving 330 ppm or greater and females receiving 110 ppm or greater. Other differences in hematology and clinical chemistry variables were not considered to be biologically significant.

Lymphoid depletion of the spleen, lymph nodes, and thymus was observed in male and female rats receiving 1,100, 3,300, or 10,000 ppm urethane in drinking water. Cellular depletion of the bone marrow occurred in males and females in the 10,000 ppm groups. Hepatocellular fatty changes and clear cell foci of alteration were noted in the liver of males and females that received 3,300 or 10,000 ppm. The incidences of nephropathy were significantly increased in female rats that received 1,100 ppm or greater; the severity of this lesion in exposed males and females was greater than that in the controls. Females that received 330 ppm or greater had higher incidences of cardiomyopathy than the controls; the severity of this lesion was greater in males in the 10,000 ppm group and females in the 3,300 and 10,000 ppm groups than in the controls.

In rats that received urethane in 5% ethanol, lymphoid depletion occurred in males and females in the 3,300 and 10,000 ppm groups. Cellular depletion of the bone marrow was observed in males and females in the 10,000 ppm groups. Only males in the 10,000 ppm group had hepatocellular fatty change (8/10) and clear cell foci (1/10); the incidence and severity of nephropathy in males and females and cardiomyopathy in males were similar to those in rats administered urethane in drinking water; however, no cardiomyopathy was observed in females receiving urethane in ethanol. The estrous cycle length of females receiving urethane in ethanol appeared to be longer than that of females receiving urethane in drinking water. Because cycle length was longer in the 10,000 ppm groups than in the controls in both the drinking water and ethanol vehicle studies, this difference may represent an exacerbation of the toxicity of urethane. A longer estrous cycle may be a sign of reproductive impairment and correlates with a decrease in female fecundity.

All mice administered 10,000 ppm urethane in either vehicle died. All mice that received 3,300 ppm urethane in drinking water died, while only one male and four females receiving 3,300 ppm urethane in 5% ethanol died. Body weight gains of males and females in all 1,100 ppm groups were less than those of the respective controls, but the weight gains of mice receiving 1,100 ppm urethane in 5% ethanol were greater than those of mice receiving urethane in drinking water. The mean body weights of the lower exposure groups were similar to those of the respective controls, and there were no other differences between the body weights of mice receiving urethane in drinking water and those receiving urethane in 5% ethanol. Fluid consumption, and therefore total urethane intake, appeared lower in mice receiving the 5% ethanol vehicle than in those receiving the water vehicle. The relative right kidney, liver, and lung weights of males and females administered urethane in drinking water or ethanol were generally greater than those of the controls. Clearance of urethane from the plasma of male mice was complete within 2 hours after urethane was administered in water, but urethane was not cleared 12 hours after administration in 5% ethanol. At the end of 13 weeks of urethane administration, the plasma urethane elimination half-life was 0.8 hours; the kinetics were similar for concentrations of 110, 330, and 1,100 ppm urethane in water and in ethanol. However, at each exposure level, the plasma urethane concentration was four times greater for urethane administered in 5% ethanol than for urethane administered in drinking water, indicating a possible inhibition of urethane metabolism by ethanol. Kinetic measurements for elimination by female mice could not be obtained from the data collected.

In mice administered urethane in drinking water, lung inflammation occurred in males and females that received 1,100 ppm or greater. Alveolar epithelial hyperplasia occurred in the lungs of males in the 330 and 1,100 ppm groups and females in the 1,100 ppm group; one male mouse in the 330 ppm group had an alveolar/bronchiolar adenoma (see the following summary table). Mice receiving urethane in 5% ethanol had lower incidences and severity of lung inflammation but generally greater incidences and severity of alveolar epithelial hyperplasia than mice receiving the same concentrations of urethane in drinking water. Alveolar/bronchiolar adenomas occurred in four males and one female administered urethane in ethanol.

Incidence and Severity of Lung Lesions in B6C3F$_1$ Mice in the 13-Week Studies of Urethane in Drinking Water and Urethane in 5% Ethanol[1]

	Concentration (ppm)					
	0	110	330	1,100	3,300	10,000
DRINKING WATER VEHICLE						
Male						
Lung	10	10	10	10	10	10
Alveolar epithelial hyperplasia	0	0	3 (2.0)	1 (4.0)	0	0
Alveolar/bronchiolar adenoma	0	0	1	0	0	0
Female						
Lung	10	10	10	10	10	10
Alveolar epithelial hyperplasia	0	0	0	4 (1.0)	0	0
Alveolar/bronchiolar adenoma	0	0	0	0	0	0
5% ETHANOL VEHICLE						
Male						
Lung	10	10	10	10	10	10
Alveolar epithelial hyperplasia	0	1 (2.0)	2 (4.0)	3 (2.0)	5* (1.8)	0
Alveolar/bronchiolar adenoma	0	1	0	1	2	0
Female						
Lung	10	10	10	10	10	10
Alveolar epithelial hyperplasia	0	0	2 (2.0)	7** (1.1)	2 (2.5)	0
Alveolar/bronchiolar adenoma	0	0	1	0	0	0

[1] Ten mice per group were examined. In the urethane in drinking water study, all males administered 3,300 ppm died by Week 4, and all females administered 3,300 ppm died by Week 5. All mice administered 10,000 ppm urethane in either vehicle died by Week 3. Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test.

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

Nephropathy was observed in males and females that received urethane in either vehicle, and the lesions in female mice were more severe than those in male mice; ethanol did not appear to increase the incidence or severity of nephropathy. Cardiomyopathy occurred in males and females that received 1,100 or 3,300 ppm urethane in drinking water and in females that received 3,300 ppm urethane in ethanol. Lymphoid depletion occurred in mice that received 3,300 or 10,000 ppm urethane; 5% ethanol did not appear to enhance these effects. However, urethane in 5% ethanol induced ovarian atrophy; the incidence of this lesion was lower in females receiving urethane in drinking water. A concentration of 1,100 ppm urethane in either drinking water or ethanol effectively stopped estrous cycling.

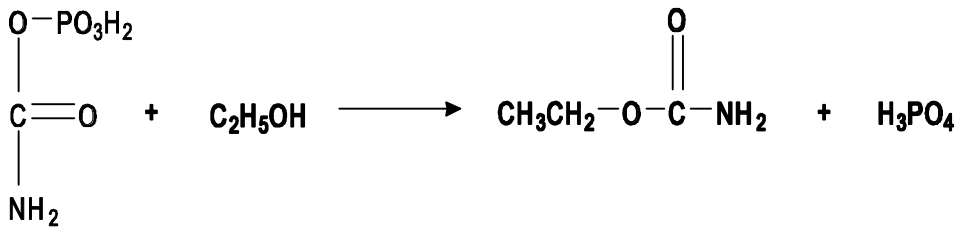
Urethane is clearly genotoxic *in vitro* and *in vivo*. *In vitro*, urethane induced mutations in *Salmonella typhimurium* strain TA1535 in the presence of liver S9 enzymes. Sister chromatid exchanges were induced in cultured Chinese hamster ovary (CHO) cells with and without S9. However, no induction of chromosomal aberrations was observed in CHO cells treated with urethane, with or without S9. *In vivo*, urethane induced sex-linked recessive lethal mutations and reciprocal translocations in germ cells of adult male *Drosophila melanogaster* fed urethane. Significantly increased frequencies of micronucleated erythrocytes were observed in peripheral blood obtained from male and female mice after 45 days of exposure and in bone marrow and peripheral blood obtained after 13 weeks of exposure to urethane in drinking water. There appeared to be no significant difference in the magnitude of the response in the peripheral blood micronucleus test between mice administered urethane in drinking water and mice administered urethane in 5% ethanol.

In summary, concentrations of 1,100 ppm urethane or greater in drinking water caused lymphoid and bone marrow cell depletion and hepatocellular lesions and increased the severity of nephropathy and cardiomyopathy in male and female rats. The lethal effects of 10,000 ppm urethane were slightly exacerbated by 5% ethanol in female rats. Urethane administered in drinking water induced lung inflammation, alveolar and bronchiolar hyperplasia, alveolar/bronchiolar adenomas, nephropathy, cardiomyopathy, lymphoid and bone marrow cell depletion, seminiferous tubule degeneration, and ovarian atrophy and follicular degeneration in mice. In female mice, 5% ethanol appeared to exacerbate ovarian atrophy. Mice administered urethane in 5% ethanol consumed less fluid, and therefore less urethane, than mice receiving urethane in drinking water. Coadministration of urethane and ethanol inhibited the clearance of urethane from plasma. Incidences and severity of alveolar epithelial hyperplasia and alveolar/bronchiolar adenoma appeared to be slightly enhanced in mice receiving urethane in 5% ethanol compared to mice receiving urethane in drinking water. However, the administration of urethane in 5% ethanol for 13 weeks did not enhance the frequency of micronucleated erythrocytes in the peripheral blood of male or female mice.

INTRODUCTION

Physical and Chemical Properties, Production, Use, and Exposure

Urethane, a white crystalline solid, is believed to be formed through the ethanolysis reaction of ethanol with carbamyl phosphate, produced by yeast during fermentation (Ough, 1976):



Yeast carbamyl phosphate synthetase catalyzes the reaction of ATP, ammonia, and carbon dioxide to the ethanolysis substrate.

The name "urethane" is sometimes applied to high-molecular-weight polyurethanes that are used as foams, elastomers, and coatings. These products are not made of urethane and should not be confused with urethane.

The primary exposure of humans to urethane is through consumption of alcoholic beverages and fermented foods. Ough (1976) measured the concentration of urethane in various foods and beverages; most wines and beers were found to contain less than 5 µg urethane per liter. An American beer contained the lowest concentration of urethane, 0.6 µg/L, and the highest concentration, 192 µg/L, was found in sake. White bread contained 0.9 µg/kg and sourdough bread 2.4 µg/kg; treating the bread with 1 N hydrochloric acid and heating increased the amount of extractable urethane 40-fold, presumably due to release from a bound form. A human weighing 100 kg who consumes 250 mL of a fermented beverage containing 100 µg urethane per liter is exposed to 0.25 µg urethane per kilogram body weight.

Disposition and Metabolism

Urethane is readily absorbed from the skin and gastrointestinal tract after administration. Urethane administered parenterally to rats and mice is rapidly distributed in body fluids throughout the body

(IARC, 1974; Nomura, 1976). It is not concentrated in any particular tissue in rats and mice (Nomeir *et al.*, 1989). Administered to pregnant mice, urethane is found in the body fluids of the fetuses.

In mice, up to 90% of the orally administered urethane is excreted within 24 hours as carbon dioxide in expired air, 4% to 10% is excreted in urine, less than 1% is excreted in the feces, and 6% remains in the body. In newborn mice, only 20% of the intraperitoneally administered urethane is eliminated in 24 hours. The rate of elimination increases slowly during the first 10 days after birth, then increases quickly 15 to 20 days after birth. The longer retention time of urethane in newborn mice is attributed to the lack of metabolizing enzymes (IARC, 1974).

Most of the absorbed urethane is hydrolyzed by microsomal esterase, producing ethanol, carbon dioxide, and ammonia. A small amount of urethane (about 6%) is metabolized by $P_{450}2E1$ to *N*-hydroxyethyl carbamate, *N*-hydroxyvinyl carbamate, and epoxyethyl carbamate. The vinyl carbamate and its epoxy derivative may be the proximate and ultimate electrophilic metabolites responsible for genotoxicity and carcinogenicity. Epoxyethyl carbamate interacts with DNA to form 7-(2-oxoethyl)guanine adducts (Miller and Miller, 1983; Gupta and Dani, 1989; Salmon and Zeise, 1991; Park *et al.*, 1993).

Coadministration of ethanol with urethane greatly affects the disposition of urethane. In experiments by Waddell *et al.* (1987), localization of radioactivity in organs of male A/JAX mice orally administered radiolabeled urethane in 12% ethanol was almost completely inhibited 1 hour after administration. In the presence of alcohol, the majority of the radiolabel remained confined to the lumen of the stomach and intestines. In mice administered urethane only, the radiolabel accumulated in the salivary, sebaceous, and harderian glands, the medullary bone, the pancreas, liver and bile, and the gastric and intestinal epithelium.

The kinetic parameters of urethane clearance are also altered by ethanol coadministration. In experiments reported by Yamamoto *et al.* (1988), male A/JAX mice orally coadministered 125 µmol [*ethyl*-1-^{14}C]-urethane with 5,000 mg ethanol per kilogram body weight had a peak blood concentration of urethane that was increased more than twofold over the concentrations occurring after administration of the same amount of urethane alone. The half-life ($t_{½}$) of urethane administered alone is about 2 hours. In contrast, the blood concentration of urethane coadministered with ethanol remained constant and high for 8 hours; after this time, the concentration of urethane declined according to a two-component curve. The first component had a $t_{½}$ of about 2 hours, a rate similar to that of urethane administered alone. When urethane concentrations declined to approximately one-third of peak levels, a second, slower

component with a $t_{½}$ of about 4 hours appeared. Based on the data presented by Yamamoto *et al.* (1988), urethane can be expected to be almost completely eliminated 24 hours after coadministration with ethanol.

The ethanol concentration in blood following bolus oral administration of 5,000 mg/kg remains high for about 2 hours and then declines linearly with time, with a $t_{½}$ of 4 hours. Yamamoto *et al.* (1988) found that in mice receiving 5,000 mg/kg ethanol, urethane excretion began only when blood ethanol levels were below 1.5 mg/mL. No data are available on the clearance of urethane from rats.

The delay in urethane excretion caused by ethanol was likely due to interference with urethane metabolism. According to this hypothesis, when ethanol concentrations exceed a critical point, urethane catabolism is inhibited. When the ethanol concentration declines below this point, urethane metabolism is thought to proceed as normal. In a series of *in vitro* experiments, the liver-mediated evolution of carbon dioxide from urethane tagged in the carbonyl moiety was examined; 40 mM ethanol (approximately 1.5 mg/mL) was found to reduce carbon dioxide production by 50% (Yamamoto *et al.*, 1988). Whether this observation is relevant to the metabolic pathway described above has not yet been determined. It is likely that ethanol administration has effects beyond simple alterations in metabolic pathways. For example, it is difficult to relate the difference in the physical distribution of urethane between mice treated with and without ethanol to this difference in metabolism.

Toxicity

TOXIC EFFECTS IN HUMANS

There are no reports in the literature of toxicity of urethane in humans.

TOXIC EFFECTS IN ANIMALS

The reported intraperitoneal LD_{50} of urethane for rats is 1,500 mg/kg (Chaube and Murphy, 1966); the oral LD_{50} of urethane for mice is 2,500 mg/kg (Osswald, 1959). No oral LD_{50} values for rats were available.

Urethane is known to induce several acute toxic reactions. In female C57BL/6J mice receiving subcutaneous injections of 4 mg urethane per kilogram body weight for 12 days, spleen and thymus weights and circulating leukocyte levels were depressed (Luebke *et al.*, 1987). The immunocompetence of dosed mice was also severely compromised, as measured by the delayed hypersensitivity reaction.

Female B6C3F$_1$ mice receiving a total dose of 4,000 µg/kg urethane by intraperitoneal injection in 14 days also had lower spleen and thymus weights than the controls, but peripheral blood cell counts were not affected (Luster *et al.*, 1982). The presence of nucleated erythrocytes in mice following urethane administration supports the possibility that blood-forming organs are targets of urethane toxicity (Heddle and Bruce, 1977). Urethane's hypnotic and anesthetic properties suggest neuropharmacological effects, which may become significant when the chemical is coadministered with ethanol (Salmon and Zeise, 1991).

REPRODUCTIVE TOXICITY AND TERATOGENICITY

The toxic and carcinogenic effects of urethane are transplacental and may be heritable as well. Subcutaneous administration of 1.0 mg/g urethane to pregnant ICR/Jcl mice on Day 17 of gestation caused embryonic deaths, malformation (skeletal defects and cleft palate), and neoplasms (mostly lung neoplasms) in the offspring (Nomura, 1975). Neural tube closure defects were observed in mice administered 15 mg urethane on the Day 7 of gestation (Shepherd, 1976), and a single injection of 1.5 mg/g urethane induced exencephaly in CBA and C3HeB mice at Day 8.5 of gestation (Tutikawa and Harada, 1972). Digital malformation developed in ICR/Jcl mice administered urethane by subcutaneous injection on Day 10 (1 mg/kg) or 11 (1.5 mg/kg) of gestation, but lung neoplasms were observed in the offspring of mice administered urethane on or after Day 13 of gestation (Nomura, 1974). Treatment of NMRI mice with a single intraperitoneal injection of 0.8 mg/g urethane on Day 14 of gestation caused increased incidences of polydactylism in fetuses examined on Day 18 of gestation (Burkard and Fritz-Niggli, 1987).

When the male and female offspring of ICR/Jcl mice that received 1.0 mg/g urethane subcutaneously were mated with untreated mice, the F_2 generation also developed a greater incidence of lung tumors (Nomura, 1975). The author concluded that urethane may damage the fetal germ cells, and the defect is transmitted to the next generation.

IMMUNOTOXICITY

Five- to seven-week-old female B6C3F$_1$ mice that received daily intraperitoneal injections of urethane (4 mg/g total dose) over a 14-day period had a depressed splenic lymphoproliferative response to Con A (a T lymphocyte mitogen) and a reduced number of primary antibody plaque-forming cells following sheep erythrocyte or *Escherichia coli* lipopolysaccharide (a T-independent antigen) challenge. Proliferation of pluripotent stem cells was markedly reduced, as was natural killer cell activity. The

number of metastatic lung foci and 125[I]UDR incorporation in lungs were significantly increased following injection of B16 melanoma cells (Luster *et al.*, 1982).

CARCINOGENICITY

Urethane is a multipotential carcinogen. Depending on species, strain, sex, and age, urethane can induce many types of neoplasms, notably lung adenomas, malignant lymphomas of the thymus, hepatomas, mammary gland carcinomas, hemangiomas, forestomach papillomas, and skin neoplasms. The latency period for most of these neoplasms is more than 1 year, although that for lung adenomas is 2 to 6 months and that for malignant lymphomas is 4 to 12 months (Mirvish, 1968; IARC, 1974; Nomura, 1976). Newborn or very young mice are more susceptible to urethane carcinogenesis than older mice, possibly due to the inability of the very young to metabolize (hydrolyze) the chemical.

EFFECTS OF ETHANOL ON LUNG ADENOMA INDUCTION IN MICE BY URETHANE

Kristiansen *et al.* (1990) administered urethane (0, 200, 500, or 1,000 ppm) in drinking water and in 5%, 10%, and 20% ethanol to female A/Ph mice for 12 weeks. All exposed mice developed lung adenomas at 12 weeks; analyses of the number of neoplasms per mouse indicated that 5% ethanol did not affect neoplasm development, and 10% or 20% ethanol inhibited lung adenoma formation. In male C3H/HeJ mice that received 0, 10, or 20 mg/kg urethane in drinking water or in 12% ethanol for 41 weeks, the number of lung adenomas per mouse was depressed in groups receiving ethanol compared to control mice receiving water only (Stoewsand *et al.*, 1991). However, in Han/NMRI mice that were administered 0, 18, 36, 90, or 180 mg/kg urethane in water or 20% ethanol by gavage daily for 8 weeks, then held for 8 weeks without dosing before being evaluated, ethanol had no effect on lung adenoma formation (Altmann *et al.*, 1991).

GENETIC TOXICITY

Urethane has been examined in a number of short-term genotoxicity tests; results from *in vivo* assays were generally positive, with the strongest responses seen in the mouse bone marrow micronucleus tests. The results of *in vitro* tests varied; the infrequent positive responses appeared to be achieved with very high doses of urethane in specific cell types under stringent conditions. No mammalian germ cell mutagenicity has been demonstrated for urethane. A review of the genotoxicity data for urethane was presented by de Serres and Ashby (1981). A brief summary of these results plus the results of more recent genotoxicity tests are presented below.

Urethane induced gene mutations in *Salmonella typhimurium*, generally at very high doses (10 mg/plate) and always with liver S9 activation enzymes (Richold and Jones, 1981; Skopek *et al.*, 1981; Venitt and Crofton-Sleigh, 1981; Zeiger *et al.*, 1992). Results of gene conversion experiments in *Saccharomyces cerevisiae* were mixed and possibly strain dependent, with negative results reported in strains D4 (Jagannath *et al.*, 1981) and D7 (Zimmermann and Scheel, 1981) and positive results noted in strain JD1 (Sharp and Parry, 1981). No induction of gene mutations was observed in L5178Y mouse lymphoma cells (Jotz and Mitchell, 1981; Amacher and Turner, 1982) or hamster V-79 cells (Allen *et al.*, 1982a) treated with urethane, with or without S9. Sister chromatid exchange (SCE) induction by urethane was reported in rat ascites hepatoma cells, but not in rat esophageal tumor cells (Abe and Sasaki, 1982) or cultured hamster cells (Evans and Mitchell, 1981; Perry and Thomson, 1981; Abe and Sasaki, 1982; Allen *et al.*, 1982b) treated with urethane with or without metabolic activation. Unscheduled DNA synthesis (Agrelo and Severn, 1981) and an increase in SCEs (Shiraishi, 1986) were observed in human cell cultures treated with urethane, with and without S9 activation.

In vivo, urethane was reported to induce a variety of genotoxic effects. Significant increases in the frequency of sex-linked recessive lethal mutations (Nomura, 1979; Foureman *et al.*, 1994) and reciprocal translocations (Foureman *et al.*, 1994) were reported in germ cells of male *Drosophila melanogaster* administered urethane by feeding. Results of mammalian bone marrow cell tests were uniformly positive. Micronuclei were induced in rats (Schlegel and MacGregor, 1984) and mice (Bruce and Heddle, 1979; Salamone *et al.*, 1981; Tsuchimoto and Matter, 1981). SCEs were induced in rats (Sharief *et al.*, 1984), mice (Cheng *et al.*, 1981; Allen *et al.*, 1982b; Conner and Cheng, 1983; Dragani *et al.*, 1983; Majone *et al.*, 1983; Goon and Conner, 1984; Sharief *et al.*, 1984; Sozzi *et al.*, 1985; Allen *et al.*, 1986), and hamsters (Sharief *et al.*, 1984). Chromosomal aberrations were induced in mice (Dean, 1969; Sugiyama *et al.*, 1981; Miyashita *et al.*, 1987). Effective doses for these mammalian *in vivo* investigations ranged from 300 to 1,000 mg urethane per kilogram body weight.

Despite the genetic effects seen in mammalian somatic cells *in vivo* following treatment with urethane, the results of an extensive test for induction of germ cell mutations (32,000 F_1 mice screened for specific locus mutations) were negative (Russell *et al.*, 1987). In this assay, a single intraperitoneal injection of urethane (1,750 mg/kg) was administered to male mice. In addition, exposure of differentiating mouse spermatogonia to urethane (five daily injections of 1,000 mg/kg) did not result in an increased frequency of sperm-head abnormalities (Wyrobek and Bruce, 1975; Bruce and Heddle, 1979; Topham, 1980). Finally, results of dominant lethal tests in mice were also negative (Bateman, 1967; Tutikawa, 1968; Epstein *et al.*, 1972; NTP, unpublished data). The possibility that urethane does not reach the testicular

tissue is not in accord with reports of dose-related increases in spermatogonial cell SCE frequencies (Roberts and Allen, 1980) and significant decreases in testicular DNA synthesis (Seiler, 1977) following exposure to urethane. These negative germ cell mutagenicity test results with urethane appear to contradict a report that, in mice, parental exposure to urethane prior to mating produced increased tumor incidences in the F_1 generation (Nomura, 1982).

Metabolites of urethane, particularly *N*-hydroxyurethane and vinyl carbamate, were also active in a variety of short-term genotoxicity assays (Dahl *et al.*, 1980; Allen *et al.*, 1982a; Sharief *et al.*, 1984), and the responses recorded for these compounds were often stronger than the responses seen with the parent compound, urethane.

Mutagenicity test data for ethanol show little if any activity in bacterial assays (Zeiger *et al.*, 1992). Chromosomal breakage and aneuploidy have occasionally been reported in eukaryotic cells exposed *in vitro* (Meisner *et al.*, 1970; Badr *et al.*, 1977; Alvarez *et al.*, 1980; de Raat *et al.*, 1983) or *in vivo* (Baraona *et al.*, 1981), particularly when experimental conditions permit generation of the genetically active metabolite acetaldehyde. Other laboratories, however, found no evidence of ethanol-induced clastogenicity in mammalian somatic cells (Chaubey *et al.*, 1977; Korte *et al.*, 1979; Jansson, 1982; Banduhn and Obe, 1985). There have been many studies of the reproductive effects of ethanol in mammals, including studies of the induction of germ cell mutagenic effects. As with other ethanol studies, results were mixed (Abel, 1982; Gavaler, 1983) and were possibly strain dependent. There are, however, several reports of induction of dominant lethal mutations in male rats (Klassen and Persaud, 1976; Mankes *et al.*, 1982) and mice (Badr and Badr, 1975; Anderson and Beyler, 1978; James and Smith, 1982; Berryman *et al.*, 1992) treated with ethanol. The mutagenicity data for ethanol and for acetaldehyde were thoroughly reviewed by Obe and Anderson (1987). There are no mutagenicity data from combined urethane/ethanol administration, other than the NTP data included in Appendix F of this report.

Study Rationale and Design

Urethane was nominated by the United States Food and Drug Administration for testing by the NTP because of the widespread exposure of humans through the consumption of fermented foods and beverages and because of a lack of adequate dose-response data for the carcinogenicity of urethane with and without the coadministration of ethanol. Because the oral route is the most common route of human exposure and because the effect of urethane toxicity in alcoholic beverages was to be investigated, urethane was administered in drinking water in studies of up to 13 weeks duration, with and without ethanol, which is known to delay urethane absorption and metabolism. Endpoints evaluated included histopathology in F344/N rats and B6C3F_1 mice and clinical pathology in F344/N rats. The effects of urethane and urethane in ethanol on the reproductive system were assessed by evaluation of testicular and spermatozoal parameters and by determination of the length of the estrous cycle. In addition, the genetic toxicity of urethane was assessed in studies in *S. typhimurium*, cultured Chinese hamster ovary cells, and *D. melanogaster*, and the genetic toxicity of urethane and urethane in ethanol was assessed in *in vivo* studies, by the determination of micronucleated erythrocytes in mouse bone marrow cells and peripheral blood.

MATERIALS AND METHODS

Procurement and Characterization of Urethane and Ethanol

Two lots of urethane (Lots JV 01929MT and TW 05211AW) were obtained from Aldrich Chemical Company (Milwaukee, WI). The 95% ethanol (Lot NBR.NONBO7) was obtained from Quantum Chemical (Cincinnati, OH). Initial identity, purity, and stability analyses of urethane were performed by Midwest Research Institute (MRI; Kansas City, MO). Initial identity and purity analyses of ethanol were performed by the study laboratory.

Urethane: The chemical, a white, crystalline solid, was identified as urethane by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy; spectra were consistent with the spectrum expected for the structure of urethane and with a literature reference (*Sadtler Standard Spectra*). The melting point of 50.4° to 50.9° C was also consistent with a literature reference (*Merck Index*, 1983). Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with theoretical values. Karl Fischer water analysis indicated 0.18% ± 0.05% water. Ester hydrolysis indicated a purity of approximately 100%. Thin-layer chromatography by two solvent systems indicated a major product spot only; gas chromatography with flame ionization detection indicated only a major peak by two systems, with no impurities with areas of 0.1% or greater relative to the major peak. The cumulative data indicated a purity greater than 99% for both lots.

Accelerated stability studies performed by MRI with gas chromatography indicated that urethane is stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. At the study laboratory, the bulk chemical was stored protected from light at 20° ± 5° C. The study laboratory monitored the stability of the bulk chemical throughout the studies with gas chromatography; no degradation of urethane was observed.

Ethanol: The chemical, a clear liquid, was identified as 95% ethanol by infrared spectroscopy; the spectrum was consistent with a literature reference (MRI, 1989). Gas chromatographic analysis indicated a major peak only, with no impurities with areas of 0.1% or greater relative to the peak area. Periodic analyses confirmed the integrity of the bulk ethanol, which was stored at 20° ± 5° C.

Dose Formulations

Drinking water solutions were prepared by adding urethane to deionized water or to a 5% (w/v) solution of ethanol in deionized water. The 5% ethanol solution was prepared in a precalibrated carboy, with the required volume of 95% (v/v) ethanol (calculated based on a density of 0.810) measured into the carboy and then diluted to volume with deionized water.

Stability studies of the dose formulations were performed by MRI with gas chromatography. The results indicated that 0.1 mg/mL solutions of urethane in water were stable for 3 weeks when stored in the dark at 5° C; solutions of 0.1 mg/mL urethane plus 5% ethanol in water were stable for 3 weeks in the dark at room temperature. Results of stability studies previously performed on ethanol (Lot A36968; not used in these studies) indicated that 5% (50 mg/mL) ethanol in drinking water is stable for 4 weeks when stored in the dark at room temperature. All solutions were stable for 96 hours under dosing conditions.

During the 13-week studies, the dose formulations were stored in sealed containers in the dark at 4° ± 3° C for no more than 3 weeks. The study laboratory periodically analyzed dose formulations taken from the dose preparation laboratory and solutions left after dosing (animal room samples) with gas chromatography. All dose formulations administered to rats and mice and all animal room samples were within 10% of the theoretical concentrations for urethane and ethanol.

Toxicity Study Designs

BASE STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY) and were 25 days old (rats) or 32 days old (mice) at receipt. Rats were quarantined 18 to 21 days and mice were quarantined 11 to 14 days; rats and mice were 43 to 46 days old when the studies began. Blood samples were collected from 10 rats and five mice of each sex at the beginning of the studies, from five untreated male mice 6 days after exposure began, and from five male and five female rats and mice from the untreated control groups at the end of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b); all results were negative. Additional details concerning the study design are provided in Table 1.

The doses selected for the 13-week studies were based on literature reports (Osswald, 1959). Groups of 10 rats and 10 mice per sex received 0, 110, 330, 1,100, 3,300, or 10,000 ppm urethane in drinking water *ad libitum*, with and without 5% ethanol added, 7 days a week for 13 weeks. Rats were housed

five per cage by sex and mice were housed individually. NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) was available *ad libitum*. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and at least 10 room air changes per hour.

Complete necropsies were performed on all base-study animals. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all rats and mice in the 0, 3,300, and 10,000 ppm groups in the urethane in drinking water and the urethane in 5% ethanol studies, all mice in the 1,100 ppm groups in the urethane in drinking water study, and all animals that died early. Gross lesions and selected target tissues of rats and mice in lower dose groups were examined microscopically. Organs weighed and tissues examined microscopically are listed in Table 1.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

SUPPLEMENTAL EVALUATIONS

Clinical Pathology

Clinical pathology studies were performed on rats designated for clinical pathology testing and on all base-study rats at the end of the 13-week study. Ten male and ten female rats per exposure level were evaluated. Blood for hematology and clinical chemistry evaluations was collected from clinical pathology study rats on Days 3 and 23; blood was collected from rats in the 10,000 ppm groups in the urethane in 5% ethanol study on Day 16 (males) or Day 15 (females) before these rats were killed. Blood was collected from base-study rats during Week 13. At all time points, animals were anesthetized with CO_2, and blood samples were drawn from the retroorbital sinus. Samples for hematology analysis were placed in collection tubes containing potassium EDTA anticoagulant; samples for clinical chemistry evaluations were placed in tubes without anticoagulant. The latter samples were allowed to clot; the samples were then centrifuged and serum was removed.

Hematologic determinations were made on a Sysmex TOA E-2500 hematology analyzer with reagents obtained from Baxter Scientific Products (McGaw Park, IL). The variables evaluated are listed in Table 1. Differential leukocyte counts and morphologic evaluation of blood cells were determined by light microscopy from blood smears stained with Wright-Giemsa. Smears made from blood samples stained with New Methylene Blue N were examined microscopically for quantitative determination of reticulocytes.

Clinical chemistry variables were measured with a Roche <Cobas> FARA automated centrifugal analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). The parameters that were evaluated are listed in Table 1. Reagents for analyses of sorbitol dehydrogenase and total bile acids were obtained from Sigma Chemical Company (St. Louis, MO); other reagents were obtained from the equipment manufacturer.

Sperm Motility and Vaginal Cytology in Rats and Mice

Vaginal cytology and sperm motility evaluations were performed on base-study rats and mice at the end of the studies. Ten male and 10 female rats and mice were evaluated per group. In the urethane in drinking water studies, male rats from the 0, 330, 1,100, and 3,300 ppm groups, female rats in the 0, 1,100, 3,300, and 10,000 ppm groups, and male and female mice in the 0, 110, 330, and 1,100 ppm groups were evaluated. In the urethane in 5% ethanol studies, rats and mice in the 0, 330, 1,100, or 3,300 ppm groups were evaluated. The parameters that were evaluated are listed in Table 1. Methods were those described in the NTP Statement of Work (April, 1987). Briefly, for the 12 days prior to sacrifice, the vaginal vaults of 10 females of each species per dose group were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to identify estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, and metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced and the tissue was incubated in the saline solution and

then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Urethane Elimination Kinetics

Plasma was analyzed for urethane and ethanol concentrations at multiple time points at the end of the 13-week mouse studies, after dosed water or ethanol was removed. Blood was collected from mice in the urethane in drinking water study 0, 1, 2, 3, and 5 hours after treatment ended and from mice in the urethane in 5% ethanol study 0, 2, 4, 6, and 8 hours after treatment ended. Three male and three female mice per group were evaluated at each time point except 0 hours, when no females were evaluated, and at 1 hour (urethane in drinking water study) or 2 hours (urethane in 5% ethanol study), when one female per group was evaluated.

Blood samples were collected in tubes containing sodium citrate. Ethanol concentrations in plasma were measured with a Roche <Cobas> FARA automated centrifugal analyzer. Reagent was obtained from Sigma Diagnostics. Urethane concentrations in plasma were measured by gas chromatography with flame ionization detection (concentrations above 5 µg/mL) or gas chromatography/mass spectrometry (concentrations below 5 µg/mL).

TABLE 1 Experimental Design and Materials and Methods in the 13-Week Studies of Urethane in Drinking Water and Urethane in 5% Ethanol

Urethane in Drinking Water Studies	Urethane in 5% Ethanol Studies
EXPERIMENTAL DESIGN	
Study Laboratory TSI Mason Laboratories (Worcester, MA)	Same as urethane in drinking water studies
Strain and Species F344/N rats $B6C3F_1$ mice	Same as urethane in drinking water studies
Animal Source Taconic Farms (Germantown, NY)	Same as urethane in drinking water studies
Size of Study Groups Base Studies: 10 males and 10 females Clinical Pathology Study: 10 male and 10 female rats	Same as urethane in drinking water studies
Doses 0, 110, 330, 1,100, 3,300, or 10,000 ppm urethane in drinking water, *ad libitum*, 7 days a week for 13 weeks	0, 110, 330, 1,100, 3,300, or 10,000 ppm urethane in 5% ethanol, *ad libitum*, 7 days a week for 13 weeks
Date of First Dose Rats: 21 January 1991 (males), 22 January 1991 (females) Mice: 10 December 1990 (males), 11 December 1990 (females)	Rats: 23 January 1991 (males), 24 January 1991 (females) Mice: 12 December 1990 (males), 13 December 1990 (females)
Date of Last Dose and Necropsy Rats: 23 April 1991 (males), 24 April 1991 (females) Mice: 12 March 1991 (males), 13 March 1991 (females)	Rats: 25 April 1991 (males), 26 April 1991 (females) Mice: 14 March 1991 (males), 15 March 1991 (females)
Type and Frequency of Observation Animals were observed twice daily and were weighed at the beginning of the study, weekly thereafter, and at necropsy. Clinical observations were recorded and water consumption by cage was measured weekly.	Same as urethane in drinking water studies
Necropsy Complete necropsies were performed on all animals in the base studies. The following organs were weighed: heart, right kidney, liver, lungs, right testis, and thymus.	Same as urethane in drinking water studies

TABLE 1 Experimental Design and Materials and Methods in the 13-Week Studies of Urethane in Drinking Water and Urethane in 5% Ethanol (continued)

Urethane in Drinking Water Studies	Urethane in 5% Ethanol Studies
EXPERIMENTAL DESIGN (continued)	
Histopathologic Examination	
Complete histopathologic evaluations were performed on all rats and mice in the 0, 3,300, and 10,000 ppm groups and on all mice in the 1,100 ppm groups, including rats and mice that died before the end of the studies. The following tissues were examined: adrenal glands, brain (three sections), clitoral glands, esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions and tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), liver (two sections), lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial glands, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spinal cord and sciatic nerve (if neurologic signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle (if neuromuscular signs were present), thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). Gross lesions of rats and mice in all lower dose groups were examined. The following target tissues were examined in the lower exposure groups: bone marrow, heart, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), spleen, and thymus in male and female rats and mice and ovaries, pancreatic islets, and testes in male and female mice.	Complete histopathologic evaluations were performed on all animals in the 0, 3,300, and 10,000 ppm groups and on all animals that died early. Tissues routinely examined were the same as those examined in the urethane in drinking water studies. The following target tissues were examined in lower exposure groups: bone marrow, kidneys, liver, lymph nodes, spleen, and thymus of male and female rats and mice, lungs and ovaries of mice, and heart of male rats and female mice.
Supplemental Evaluations	
Clinical Pathology Study:	
Blood for hematology and clinical chemistry evaluations was collected on Days 3 and 23 from rats in the clinical pathology study groups and at the end of the study from base-study rats. Ten male and ten female rats per exposure level were evaluated. Hematology variables included hematocrit (Hct), hemoglobin (Hgb) concentration, erythrocyte (RBC) count, reticulocyte count, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, and leukocyte (WBC) count and differential. Clinical chemistry variables included urea nitrogen (UN), creatinine, total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase, creatine kinase, sorbitol dehydrogenase (SDH), and total bile acids.	Same as urethane in drinking water studies. Supplemental rats in the 10,000 ppm groups were evaluated on Day 15 (females) or 16 (males).

TABLE 1 Experimental Design and Materials and Methods in the 13-Week Studies of Urethane in Drinking Water and Urethane in 5% Ethanol (continued)

Urethane in Drinking Water Studies	Urethane in 5% Ethanol Studies
EXPERIMENTAL DESIGN (continued)	
Supplemental Evaluations (continued)	
Sperm Motility and Vaginal Cytology Evaluations:	
Sperm motility and vaginal cytology evaluations were performed on base-study animals at the end of the studies. For male rats in the urethane study and all rats and mice in the urethane in 5% ethanol studies, the 0, 330, 1,100, and 3,300 ppm groups were evaluated. Female rats in the 0, 1,100, 3,300, and 10,000 ppm groups and male and female mice in the 0, 110, 330, and 1,100 ppm groups of the urethane studies were evaluated. Male rats and mice were evaluated for necropsy body and reproductive tissue weights, epididymal spermatozoal data, and spermatogenesis. Females were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in the various stages.	Same as urethane in drinking water studies
Urethane Elimination Kinetics:	
Plasma from mice was analyzed for urethane and ethanol concentrations at the end of the 13-week studies, 0, 1, 2, 3, and 5 hours after dosed water was removed. Three male and three female mice per exposure level were evaluated at each time point except 0 hours, when no females were evaluated, and 1 hour, when one female per group was evaluated.	Same as urethane in drinking water study, except sampling periods were 0, 2, 4, 6, and 8 hours posttreatment. No females were evaluated at 0 hours posttreatment; one female per group was analyzed at 2 hours.
ANIMAL MAINTENANCE	
Time Held Before Study	
Rats: 18 days (males), 19 days (females) Mice: 11 days (males), 12 days (females)	Rats: 20 days (males), 21 days (females) Mice: 13 days (males), 14 days (females)
Age When Study Began	
Approximately 6 weeks	Approximately 6½ weeks
Age at Necropsy	
18 weeks	18-19 weeks
Method of Animal Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weight.	Same as urethane in drinking water studies
Diet	
NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form and deionized water (City of Worcester) containing the appropriate doses were available *ad libitum*.	Same as urethane in drinking water studies
Animal Room Environment	
Rats were housed five animals per cage by sex and mice were housed individually. The temperature was maintained at 69° to 75° F and relative humidity at 35% to 65%, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.	Same as urethane in drinking water studies

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1992). Urethane was sent to the testing laboratory as a coded aliquot and was incubated with the *S. typhimurium* tester strains (TA97, TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37 ° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37 ° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of urethane. The high dose was limited by study design to 15,000 (Study 1) or 16,666 µg/plate (Study 2). All assays were repeated. Representative trials are listed in Table F1 (Appendix F); because the data have been published, not all trials are included.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). Urethane was supplied as a coded aliquot. The aliquot was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs) both in the presence and the absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of urethane; in the absence of toxicity, a high dose of 5,000 µg/mL was selected. A single flask per dose was used.

In the SCE test without S9, CHO cells were incubated for 26 to 26.5 hours with urethane in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 to 26.5 hours, the medium containing urethane was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with urethane, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no urethane, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind

and those from a single test were read by the same person. Up to 50 second-division metaphase cells were scored for frequency of SCEs per cell from each dose level.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with urethane for 11 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with urethane and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 11 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. Up to 200 first-division metaphase cells were scored at each dose level. Classes of aberrations recorded included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

DROSOPHILA MELANOGASTER TEST PROTOCOL

The assays for induction of sex-linked recessive lethal (SLRL) mutations and chromosomal reciprocal translocations (RTs) were performed with adult flies as described by Foureman *et al.* (1994). Urethane was supplied as a coded aliquot and was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because a positive response was obtained in the SLRL test, urethane was tested for induction of RTs by the same method of exposure.

For the SLRL test, Canton-S males were allowed to feed for 72 hours on a solution of urethane in 5% sucrose. Treated males were mated to three *Basc* females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings were treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution). If a cluster was identified, all data from the male in question were discarded. Presumptive lethal

mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

For the RT test, the exposure regimen was essentially the same as that for the SLRL test, except that Canton-S males were mass-mated to marker (X.Y,y; bw; st) females. The females were transferred to fresh medium every 3 to 4 days for a period of about 3 weeks to produce a total of six broods. The results of the SLRL test were used to determine the germ cell stages most likely to be affected by urethane. F_1 heterozygous males were backcrossed individually to bw;st females, and the F_2 progeny were screened for pseudolinkage, which results from the induction of a translocation in a germ cell of the parental male. Flies suspected of carrying RTs were retested to confirm the findings.

PERIPHERAL BLOOD AND BONE MARROW MICRONUCLEUS TEST PROTOCOLS

A detailed discussion of these assays is presented in MacGregor *et al.* (1990). Male and female B6C3F_1 mice received urethane in drinking water with or without 5% ethanol for up to 13 weeks. In the United States Department of Agriculture (USDA) study presented in Table F6, mice receiving urethane in drinking water were evaluated after 45 days of exposure (peripheral blood analyses) and at the end of 13 weeks of exposure (peripheral blood and bone marrow analyses). The data collected at Environmental Health Research and Testing (EHRT), Inc. (Tables F7 and F8) were taken from peripheral blood slides prepared at the end of the 13-week toxicity studies conducted by TSI Mason Laboratories. Bone marrow and peripheral blood smears were prepared immediately after the animals were killed; the smears were fixed in absolute methanol. The slides were later stained with a chromatin-specific fluorescent dye mixture (Hoechst 33258/pyronin Y or acridine orange) and coded. Slides were scanned at 630× or 1,000× magnification with a semi-automated image analysis system (USDA study) or manually (EHRT, Inc. study) to determine the frequency of micronuclei. At the USDA, 10,000 normochromatic erythrocytes (NCEs) and 2,000 polychromatic erythrocytes (PCEs) were scored per mouse; there were 12 mice per control group and eight mice per exposed group. At EHRT, Inc., 2,000 NCEs were scored in each of five mice per group. The criteria of Schmid (1976) were used to define micronuclei.

Statistical Methods

CALCULATION AND ANALYSIS OF LESION INCIDENCES

The incidences of lesions as presented in Appendixes A and B are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. The Fisher exact test, a procedure based on the overall proportion of affected animals, was used in the 13-week studies (Gart *et al.*, 1979).

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed with the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry, hematology, spermatid, and spermatozoal data, which typically have skewed distributions, were analyzed with the nonparametric multiple comparison methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose response (Dunnett's or Dunn's test). Trend-sensitive tests were used when Jonckheere's test was significant at a P-value less than 0.01.

Prior to analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel. Implausible values, extreme values from animals that were suspected of being sick due to causes other than treatment, and values that the study laboratory indicated as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across dose levels.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *Salmonella typhimurium* assay is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that was not dose related, not reproducible, or not of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF CHINESE HAMSTER OVARY CELL CYTOGENETICS DATA

For the SCE data, statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses indicated that the trial was positive. A statistically significant trend ($P<0.05$), in the absence of any responses reaching 20% above background, led to a call of equivocal (Galloway *et al.*, 1987).

Chromosomal aberration data are presented as the percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose-response curve and individual dose points (Galloway *et al.*, 1987). For a single trial, a statistically significant ($P<0.05$) difference for one dose point and a significant trend ($P<0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend, in the absence of a statistically significant increase at any one dose point, led to a conclusion of equivocal activity. Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

ANALYSIS OF *DROSOPHILA MELANOGASTER* DATA

Sex-linked recessive lethal data were analyzed by simultaneous comparison with the concurrent and historical controls with a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P-value was less than or equal to 0.01 and the mutation frequency in the tested group was greater than 0.10% or if the P-value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive

if the P-value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or if the P-value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P-value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

The translocation data were analyzed according to the conditional binomial response test of Kastenbaum and Bowman (1970).

ANALYSIS OF PERIPHERAL BLOOD AND BONE MARROW MICRONUCLEUS DATA

At the USDA, log transformation of the NCE data and testing for normality by the Shapiro-Wilk test and for heterogeneity of variance by Cochran's test were performed before statistical analyses. The frequency of micronucleated cells among NCEs was determined by analysis of variance with the SAS GLM procedure. The NCE data for each exposure group were compared with the concurrent solvent control by Student's *t*-test. The frequency of micronucleated cells among PCEs was analyzed by the Cochran-Armitage trend test (Armitage, 1971), and individual exposure groups were compared to the concurrent control group by Kastenbaum-Bowman's binomial test (Kastenbaum and Bowman, 1970).

At EHRT, Inc., the results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the control group (Margolin *et al.*, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, a trial was considered positive if the trend P-value was less than or equal to 0.025 or the *t*-test P-value for any exposed group compared to the control group was less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducible positive trials (as noted above). Results of the 13-week exposure studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call was determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Quality Assurance

The animal studies of urethane were performed in compliance with United States Food and Drug Administration Good Laboratory Practices regulations (21 CFR, Part 58). The Quality Assurance Unit of EG&G Mason Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

13-Week Studies in F344/N Rats

In rats that received urethane in drinking water, one female in the 3,300 ppm group and seven males and four females in the 10,000 ppm groups died before the end of the study (Table 2). In rats receiving urethane in 5% ethanol, two males and all females in the 10,000 ppm groups died before the end of the study (Table 3). The final mean body weights and mean body weight gains of male and female rats receiving 3,300 or 10,000 ppm and females receiving 1,100 ppm urethane in drinking water were less than those of the controls (Table 2 and Figure 1). The final mean body weights and mean body weight gains of males that received 3,300 or 10,000 ppm and females that received 3,300 ppm urethane in 5% ethanol were notably less than those of the controls (Table 3 and Figure 2).

Clinical signs including thinness, abnormal posture, and ruffled fur were observed in most males and all females administered 10,000 ppm urethane in drinking water and in all males and females administered 10,000 ppm urethane in ethanol. These signs were observed less frequently in rats in the lower exposure groups.

Water consumption by male and female rats that received 10,000 ppm urethane in drinking water was less than that by the controls (Table 2); water consumption by rats receiving urethane in ethanol decreased with increasing urethane concentration (Table 3). Compound consumption is shown in Tables 2 and 3.

TABLE 2 Survival, Weight Gain, Water Consumption, and Compound Consumption Data for F344/N Rats in the 13-Week Study of Urethane in Drinking Water

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average Water Consumption[3] (g/day)	Average Dose[3] (mg/kg/day)
		Initial	Final	Change			
MALE							
0	10/10	143	334	191		22.3	
110	10/10	142	319	177	96	20.8	8
330	10/10	141	324	183	97	21.3	23
1,100	10/10	142	321	178	96	20.9	78
3,300	10/10[4]	140	302	159	91	20.8	287
10,000	3/10[5]	143	254	109	76	13.2	622
FEMALE							
0	10/10	124	194	70		17.2	
110	10/10	126	194	68	100	18.7	11
330	10/10	125	201	76	104	18.6	33
1,100	10/10	126	184	58	95	18.4	114
3,300	9/10[6]	122	167	44	86	16.2	332
10,000	6/10[7]	123	128	4	66	8.0	525

[1] Number surviving at 13 weeks/number of animals per group. For groups with no survivors, no final mean body weights or body weight changes are given.
[2] (Dose group mean/control group mean) × 100.
[3] Average of individual consumption values for Weeks 1-13 for animals in the base study. Consumption data for Week 1 were not available for male rats in the 110, 330, and 1,100 ppm groups or female rats in the 10,000 ppm group; for these groups, average consumption values for Weeks 2-13 are given.
[4] Final body weights of three males were not available.
[5] Week of death: 11, 12, 13, 13, 13, 13, 13.
[6] Week of death: 13.
[7] Week of death: all deaths occurred during Week 13.

TABLE 3 Survival, Weight Gain, Water Consumption, and Compound Consumption Data for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average 5% Ethanol Consumption[3] (g/day)	Average Dose[3] (mg/kg/day)
		Initial	Final	Change			
MALE							
0	10/10	142	329	187		21.5	
110	10/10	146	319	172	97	20.5	9
330	10/10	143	320	177	97	20.3	27
1,100	10/10	143	314	172	95	19.3	87
3,300	10/10	141	307	166	93	16.1	221
10,000	8/10[4]	154	219	68	66	9.8	545
FEMALE							
0	10/10	120	192	71		16.8	
110	10/10[5]	128	198	68	103	17.0	10
330	10/10	122	194	71	101	15.9	28
1,100	10/10	125	191	66	100	13.5	79
3,300	10/10	123	174	51	91	10.1	201
10,000	0/10[6]	128	)	)	)	4.7	473

[1] Number surviving at 13 weeks/number of animals per group.
[2] (Dose group mean/control group mean) × 100.
[3] Average of individual consumption values for Weeks 1-13 for all animals in the base study. Consumption data for Week 1 were not available for male rats in the 10,000 ppm group or female rats in the 3,300 ppm group; for these groups, average consumption values for Weeks 2-13 are given. For females in the 10,000 ppm group, ethanol and urethane consumption values for Weeks 2-4 only are given.
[4] Week of death: 3, 6.
[5] The final body weight of one female was not available.
[6] Week of death: 3, 3, 3, 3, 3, 5, 5, 6, 6, 6.

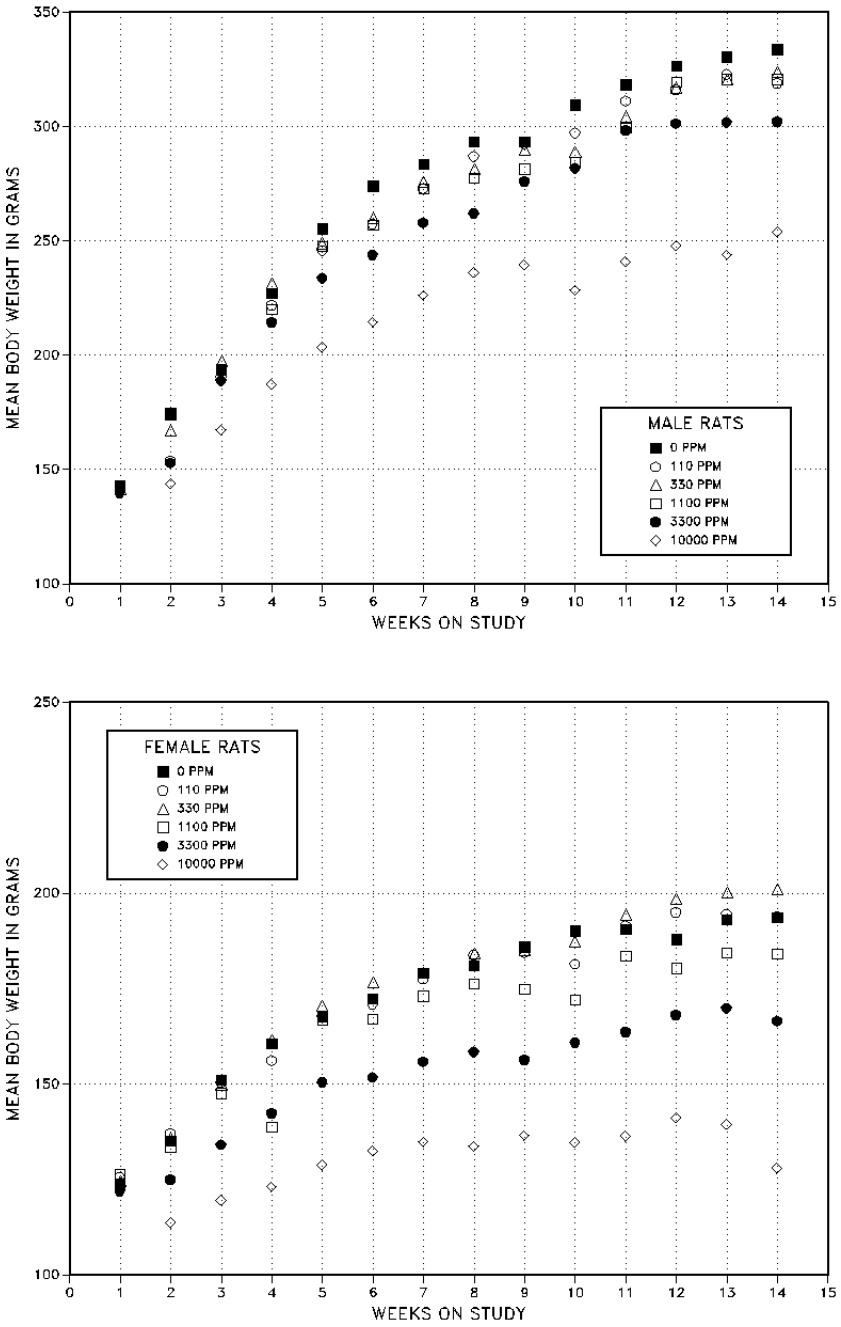


FIGURE 1 Body Weights of F344/N Rats Administered Urethane in Drinking Water for 13 Weeks

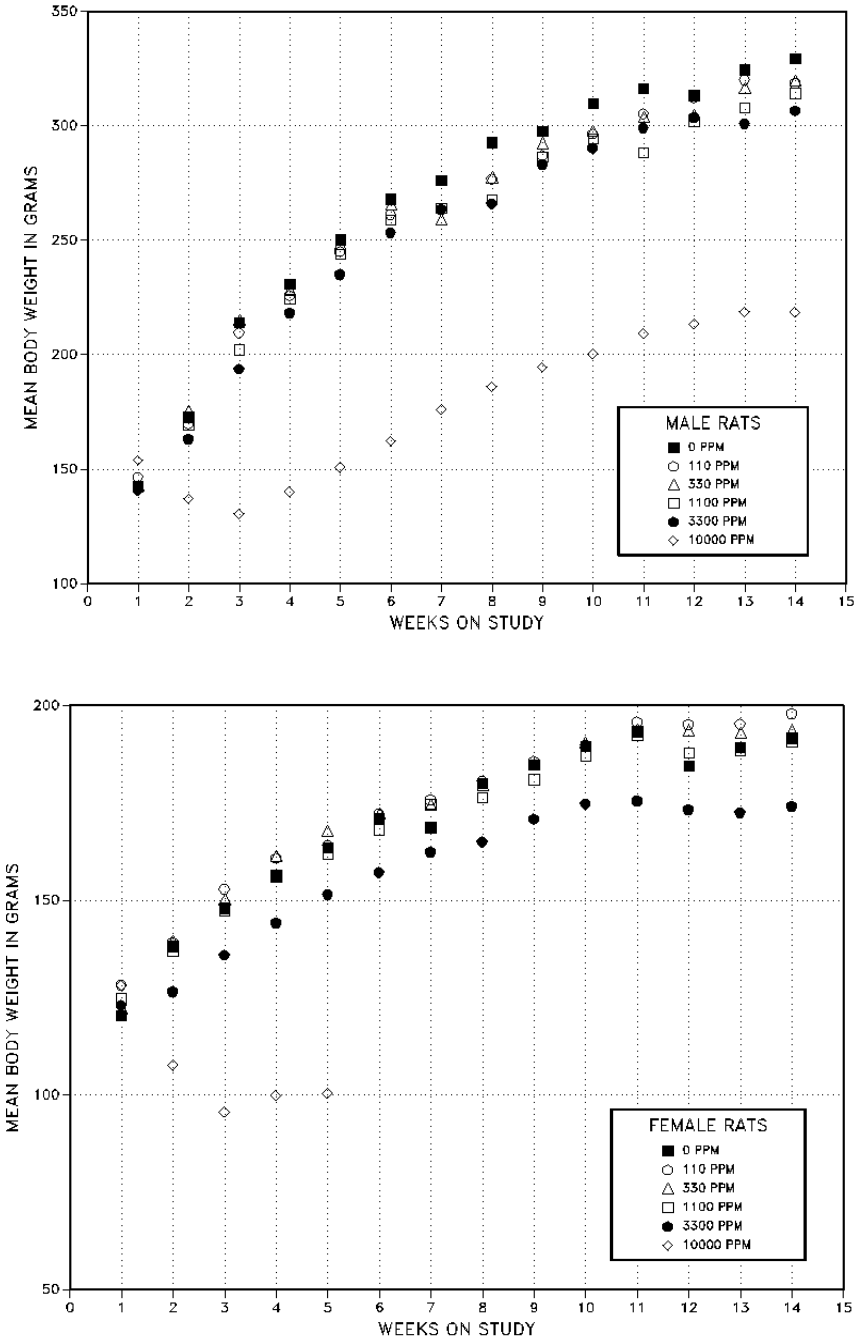


FIGURE 2 Body Weights of F344/N Rats Administered Urethane in 5% Ethanol for 13 Weeks

Urethane in Drinking Water: Statistically significant differences in absolute and relative organ weights occurred between control and exposed rats (Table C1). The relative right kidney and liver weights of males receiving 1,100 ppm or greater, the relative lung and right testis weights of males receiving 3,300 or 10,000 ppm, the right kidney, liver, and lung weights of females receiving 1,100 ppm or greater, and the relative heart weight of females receiving 10,000 ppm were significantly greater than those of the controls. These differences were considered secondary to the lower mean body weights of exposed male and female rats, although the greater relative weights of certain organs may have biologic significance. For example, relative kidney weights of rats are often increased in studies where water consumption is reduced.

A time- and concentration-dependent leukopenia, evidenced by decreased leukocyte (WBC) counts, occurred on Day 23 in males administered 1,100 ppm or greater and in females administered 330 ppm or greater (Tables 4 and D1). At the end of the study, leukopenia occurred in males receiving 330 ppm or greater and females receiving 110 ppm or greater. The leukopenia was characterized by lymphopenia, evidenced by decreased lymphocyte counts.

Hematocrit (Hct) values, hemoglobin (Hgb) concentrations, and erythrocyte (RBC) counts had minimal, inconsistent increases and decreases in various exposure groups at different time points (Tables 4 and D1). At the end of the study, morphologic changes were observed in the peripheral blood smears of males and females in the 10,000 ppm groups. These changes included lower WBC counts, with greater numbers of smudged or bare WBC nuclei, than in the controls. The incidences of polychromatophilic erythrocytes were increased in the 10,000 ppm groups, and the incidences of acanthocytes, schistocytes, microcytes, stomatocytes, target cells, and Howell-Jolly bodies were also slightly increased. A few giant platelets, up to 8 µm in diameter, were observed. In the 3,300 ppm groups, occasional acanthocytes and schistocytes were observed at the end of the study. No biologically significant changes occurred in the lower exposure groups at the end of the study or in any exposure group at earlier time points. Overall, the changes in the red cell variables and red cell morphology would indicate that a minimal responsive anemia was occurring that was masked in rats in the 10,000 ppm groups by dehydration attributed to decreased water consumption.

TABLE 4 Selected Hematology Data for F344/N Rats in the 13-Week Study of Urethane in Drinking Water[1]

	Concentration (ppm)					
	0	110	330	1,100	3,300	10,000
MALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 13	10	10	9	10	10	6
Hematocrit (%)						
Day 3	46.1 ± 0.4	44.6 ± 1.1	45.4 ± 0.6	44.0 ± 0.3*	44.8 ± 0.4	47.0 ± 0.4
Day 23	53.8 ± 0.4	49.4 ± 0.3**	50.5 ± 0.5*	53.2 ± 1.3	51.7 ± 0.4	54.0 ± 1.4
Week 13	49.8 ± 0.6	51.4 ± 0.7	50.5 ± 0.4	50.6 ± 0.3	51.9 ± 0.5*	45.9 ± 2.2
Hemoglobin (g/dL)						
Day 3	14.3 ± 0.1	13.9 ± 0.3	14.1 ± 0.2	13.8 ± 0.2	13.9 ± 0.1	14.6 ± 0.1
Day 23	17.2 ± 0.2	15.8 ± 0.1**	16.0 ± 0.2**	17.0 ± 0.4	16.4 ± 0.1	17.2 ± 0.4
Week 13	15.8 ± 0.2	16.6 ± 0.2*	16.2 ± 0.1	16.1 ± 0.1	16.6 ± 0.2*	14.2 ± 0.8
Erythrocytes (10^6/µL)						
Day 3	7.27 ± 0.08	7.12 ± 0.17	7.24 ± 0.10	6.97 ± 0.08	7.14 ± 0.09	7.45 ± 0.09
Day 23	8.91 ± 0.08	8.15 ± 0.06**	8.37 ± 0.10	8.97 ± 0.24	8.67 ± 0.07	9.14 ± 0.24
Week 13	9.47 ± 0.11	9.99 ± 0.15*	9.73 ± 0.10	9.70 ± 0.07	9.83 ± 0.10	8.25 ± 0.47
Leukocytes (10^3/µL)						
Day 3	8.25 ± 0.22	8.79 ± 0.36	8.86 ± 0.50	8.64 ± 0.56	9.32 ± 0.58	8.62 ± 0.50
Day 23	9.34 ± 0.39	9.43 ± 0.27	9.20 ± 0.33	7.53 ± 0.37**	6.60 ± 0.29**	4.46 ± 0.21**
Week 13	10.40 ± 0.33	10.51 ± 0.30	8.66 ± 0.61*	7.51 ± 0.50**	7.13 ± 0.36**	4.62 ± 0.32**
Lymphocytes (10^3/µL)						
Day 3	6.95 ± 0.21	7.18 ± 0.34	7.71 ± 0.56	7.20 ± 0.62	7.64 ± 0.43	7.19 ± 0.32
Day 23	8.05 ± 0.35	8.11 ± 0.25	7.72 ± 0.30	6.35 ± 0.30**	5.14 ± 0.25**	3.42 ± 0.24**
Week 13	8.50 ± 0.29	8.20 ± 0.35	6.84 ± 0.49*	5.89 ± 0.43**	5.51 ± 0.32**	3.27 ± 0.12**
FEMALE						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 3	44.7 ± 0.4	44.6 ± 0.3	44.8 ± 0.4	45.2 ± 0.2	44.6 ± 0.4	46.9 ± 0.5**
Day 23	53.5 ± 1.9	48.2 ± 0.4	48.0 ± 0.5	48.4 ± 0.3	52.5 ± 1.2	56.1 ± 0.7
Week 13	47.1 ± 0.5	47.0 ± 0.4	48.2 ± 0.4	48.8 ± 0.5	51.3 ± 1.3**	50.4 ± 1.2*
Hemoglobin (g/dL)						
Day 3	14.3 ± 0.2	14.2 ± 0.1	14.3 ± 0.1	14.4 ± 0.1	14.2 ± 0.1	15.1 ± 0.2**
Day 23	17.3 ± 0.6	15.6 ± 0.1	15.6 ± 0.2	15.5 ± 0.1	16.8 ± 0.4	17.9 ± 0.2
Week 13	15.5 ± 0.1	15.4 ± 0.1	15.8 ± 0.1	16.0 ± 0.2*	16.5 ± 0.4*	15.6 ± 0.4
Erythrocytes (10^6/µL)						
Day 3	7.31 ± 0.09	7.26 ± 0.05	7.33 ± 0.08	7.36 ± 0.06	7.29 ± 0.09	7.68 ± 0.10*
Day 23	8.77 ± 0.31	7.82 ± 0.06	7.93 ± 0.08	7.90 ± 0.07	8.60 ± 0.20	9.38 ± 0.12
Week 13	8.48 ± 0.13	8.59 ± 0.08	8.78 ± 0.07	8.83 ± 0.09	8.99 ± 0.25	8.40 ± 0.26
Leukocytes (10^3/µL)						
Day 3	9.97 ± 0.28	9.34 ± 0.17	9.09 ± 0.25	10.01 ± 0.35	8.84 ± 0.26	9.15 ± 0.40
Day 23	8.64 ± 0.45	8.41 ± 0.28	7.05 ± 0.24**	6.77 ± 0.19**	5.79 ± 0.19**	4.94 ± 0.27**
Week 13	9.43 ± 0.44	7.32 ± 0.30**	6.69 ± 0.24**	6.78 ± 0.28**	5.07 ± 0.19**	4.17 ± 0.19**
Lymphocytes (10^3/µL)						
Day 3	8.00 ± 0.25	7.57 ± 0.17	7.55 ± 0.23	8.09 ± 0.24	7.27 ± 0.17*	7.23 ± 0.36*
Day 23	7.36 ± 0.38	7.06 ± 0.29	5.97 ± 0.26*	5.60 ± 0.27**	4.55 ± 0.15**	3.50 ± 0.21**
Week 13	7.88 ± 0.41	6.01 ± 0.22**	5.34 ± 0.26**	5.17 ± 0.26**	4.10 ± 0.18**	3.23 ± 0.20**

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

Numerous differences in clinical chemistry variables occurred in various exposure groups at different time points (Table D3). These differences were generally minimal, with most values falling within physiologic ranges, and did not demonstrate strong exposure or time relationships or consistency between males and females. Increases in urea nitrogen (UN) concentration and decreases in alkaline phosphatase activity were probably related to decreased feed intake or dehydration. The differences in the biochemical markers of hepatic function/injury (alkaline phosphatase, sorbitol dehydrogenase, alanine aminotransferase, and bile acid) were within physiologic reference value ranges and are not considered biologically significant.

Gross necropsy observations of rats administered urethane in drinking water were limited to thin carcasses and/or fluid in the thoracic cavity in many of the male and female rats in the 10,000 ppm groups that died before the end of the study. Microscopically, treatment-related effects were observed in the lymphoid tissue, bone marrow, liver, kidneys, and heart (Table 5).

Lymphoid depletion was observed in the spleen, mandibular and mesenteric lymph nodes, and thymus (Table 5). Based on the frequency and severity of the lesion, the spleen was the most severely affected tissue in males and in females, followed by the lymph nodes and then the thymus. Lymphoid depletion was characterized in the spleen by loss of cells in the white pulp and was characterized in the lymph nodes and thymus by loss of cortical lymphocytes (Plates 1 and 2). Lymphoid depletion in the spleen occurred with increasing incidence and severity at the three highest exposure levels (1,100, 3,300, and 10,000 ppm), while in the lymph nodes and thymus, lymphoid depletion was observed primarily in the 10,000 ppm groups.

Cellular depletion of the bone marrow was present in several males and females in the 10,000 ppm groups (Table 5). This change consisted of a minimal to mild loss of both myeloid and erythroid hematopoietic cells (Plates 3 and 4).

TABLE 5 Incidence and Severity of Selected Lesions in F344/N Rats in the 13-Week Study of Urethane in Drinking Water[1]

	Concentration (ppm)					
	0	110	330	1,100	3,300	10,000
MALE						
Spleen						
Depletion lymphoid	0/10	0/10	0/10	7/10** (1.1)	10/10** (2.0)	9/10** (2.1)
Lymph node, mandibular						
Depletion lymphoid	0/10	0/10	0/10	0/10	1/10 (2.0)	5/10* (2.2)
Lymph node, mesenteric						
Depletion lymphoid	0/10	0/10	0/10	0/10	0/10	6/8** (2.3)
Thymus						
Depletion lymphoid	0/10	0/10	0/10	0/10	0/10	2/10 (2.0)
Bone marrow						
Depletion cellular	0/10	0/10	0/10	0/10	0/10	5/10* (2.0)
Liver						
Clear cell focus	0/10	0/10	0/9	0/10	2/10	1/10
Fatty change	0/10	0/10	1/9 (1.0)	0/10	0/10	5/10* (2.0)
Kidney						
Nephropathy	10/10 (1.2)	10/10 (1.5)	10/10 (1.7)	10/10 (1.7)	10/10 (2.0)	9/10 (1.8)
Heart						
Cardiomyopathy	10/10 (1.0)	10/10 (1.0)	10/10 (1.0)	10/10 (1.1)	10/10 (1.1)	10/10 (3.8)
FEMALE						
Spleen						
Depletion lymphoid	0/10	0/10	0/10	3/10 (1.0)	10/10** (1.8)	10/10** (2.6)
Lymph node, mandibular						
Depletion lymphoid	0/10	0/10	0/10	0/10	1/10 (2.0)	8/10** (2.3)
Lymph node, mesenteric						
Depletion lymphoid	0/10	0/10	0/10	0/10	1/10 (2.0)	5/10* (2.6)
Thymus						
Depletion lymphoid	0/10	0/10	0/10	0/10	0/10	1/10 (2.0)
Bone marrow						
Depletion cellular	0/10	0/10	0/10	0/10	1/10 (2.0)	9/10** (1.8)
Liver						
Clear cell focus	0/10	0/10	0/10	1/10	6/10**	3/10
Kidney						
Nephropathy	0/10	1/10 (1.0)	1/10 (1.0)	4/10* (1.3)	8/10** (1.3)	10/10** (1.8)
Heart						
Cardiomyopathy	4/10 (1.0)	6/10 (1.0)	10/10** (1.1)	10/10** (1.1)	10/10** (3.3)	10/10** (3.5)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test.

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

Liver lesions attributed to urethane administration included hepatocellular fatty change and clear cell foci of alteration (Table 5). Fatty change occurred in centrilobular cells of males in the 10,000 ppm group and consisted of hepatocytes with one or more discrete, clear intracytoplasmic vacuoles. Clear cell foci were observed in exposed males and females and consisted of randomly distributed, small clusters of hepatocytes with perinuclear clear zones (Plate 5). Clear cell foci were more common in exposed females than in males, and the incidence of this lesion was greatest in females in the 3,300 ppm group. Decreased survival in the 10,000 ppm groups may have accounted for the absence of a dose response in the incidence of clear cell foci.

Kidney effects attributed to urethane treatment occurred in males and females (Table 5). Qualitatively, the lesions were similar between males and females and consisted of scattered foci of regenerative tubules, intratubular hyaline casts, and inflammatory cell infiltration, collectively diagnosed as nephropathy. In males, the lesions were similar to the spontaneous nephropathy frequently seen in rats, but the severity was slightly greater in all groups of exposed males than in the controls. Spontaneous nephropathy changes are uncommon in young female rats; the clear increases in incidence and severity of nephropathy that occurred with increasing urethane concentration in this study were associated with chemical administration.

Treatment with urethane was associated with the exacerbation of spontaneous cardiomyopathy in males and females (Table 5). The spontaneous cardiomyopathy commonly seen in F344/N rats is a minimal change. In male rats receiving 10,000 ppm urethane, the severity of cardiomyopathy was markedly greater than that in the controls. In females, the incidence of cardiomyopathy was significantly increased at concentrations of 330 ppm and greater, and the severity of this lesion in the 3,300 and 10,000 ppm groups was greater than that in the controls (Plate 6).

Epididymal spermatozoal motility and concentration in male rats in the 1,100 and 3,300 ppm groups were significantly less than those in the controls (Table E1). The percentage of time spent in the various estrous stages of exposed females were similar to those of the controls (Table E2). The estrous cycle length of females in the 10,000 ppm group was longer than that of the controls; however, the difference was not significant.

Urethane in 5% Ethanol: Several statistically significant differences between exposed and control rats were observed in absolute and relative organ weights (Table C2). Relative right kidney, liver, lung, and right testis weights of exposed males and relative right kidney, liver, and lung weights of exposed females were generally greater than those of the controls. Most of these differences were considered secondary to the lower mean body weights of exposed animals. However, a number of the pathologic findings noted in these organs could have contributed to these differences.

Numerous alterations in hematology and clinical chemistry variables occurred in a pattern similar to that in the urethane in drinking water study, indicating that 5% ethanol had no effect on these changes. As in the urethane in drinking water study, a time- and concentration-dependent decrease in WBC and lymphocyte counts was observed (Tables 6 and D2). The decreased numbers of lymphocytes account for the decreased numbers of WBCs. Histologic evidence of lymphoid depletion in the lymph nodes, spleen, and thymus of exposed rats suggested lymphoid atrophy and decreased lymphopoiesis. The lymphopenia could be attributed to a decreased lymphopoiesis related to a stress-induced increase in endogenous corticosteroid production and/or a decreased nutritional status. Decreases in Hct values, Hgb concentrations, and RBC counts in exposed rats were minimal and transient, and increases in reticulocyte counts and mean cell volume suggested increased erythropoietic activity in the bone marrow or spleen. Most of the differences in clinical chemistry variables occurred in various exposure groups at different time points and were minimal, with most values falling within physiologic reference ranges, except for the values for moribund animals (Table D4). Increases in UN, total protein, and albumin concentrations were consistent with dehydration (due to low fluid intake) or decreased feed intake.

TABLE 6 Selected Hematology Data for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol[1]

	Concentration (ppm)					
	0	110	330	1,100	3,300	10,000
MALE						
n						
Day 3	10	10	10	10	10	10
Day 16	0	0	0	0	0	10
Day 23	10	10	10	10	10	0
Week 13	10	10	10	10	10	8
Hematocrit (%)						
Day 3	45.7 ± 0.4	45.0 ± 0.3	44.7 ± 0.5	45.6 ± 0.5	45.8 ± 0.2	49.6 ± 0.4**
Day 16	)	)	)	)	)	54.1 ± 0.5
Day 23	52.6 ± 0.8	50.9 ± 0.7	50.1 ± 0.3*	50.1 ± 0.4*	49.8 ± 0.3**	)
Week 13	49.5 ± 0.6	51.5 ± 0.5	51.0 ± 0.5	49.3 ± 0.6	50.4 ± 0.5	50.3 ± 0.5
Hemoglobin (g/dL)						
Day 3	14.4 ± 0.2	14.1 ± 0.1	14.0 ± 0.2	14.3 ± 0.2	14.3 ± 0.1	15.3 ± 0.1**
Day 16	)	)	)	)	)	17.7 ± 0.1
Day 23	16.5 ± 0.3	16.1 ± 0.3	15.9 ± 0.2	15.9 ± 0.1*	15.9 ± 0.1*	)
Week 13	15.8 ± 0.1	16.4 ± 0.2*	16.2 ± 0.1	15.9 ± 0.2	16.1 ± 0.2	15.9 ± 0.2
Erythrocytes (10^6/µL)						
Day 3	7.32 ± 0.07	7.14 ± 0.07	7.13 ± 0.09	7.30 ± 0.09	7.29 ± 0.04	7.87 ± 0.09**
Day 16	)	)	)	)	)	9.54 ± 0.10
Day 23	8.74 ± 0.12	8.38 ± 0.17	8.29 ± 0.10*	8.37 ± 0.08*	8.41 ± 0.08*	)
Week 13	9.43 ± 0.09	9.86 ± 0.09	9.77 ± 0.08	9.58 ± 0.11	9.61 ± 0.09	9.16 ± 0.10
Reticulocytes (10^6/µL)						
Day 3	0.49 ± 0.02	0.47 ± 0.01	0.48 ± 0.01	0.46 ± 0.03	0.47 ± 0.02	0.56 ± 0.01**
Day 16	)	)	)	)	)	0.09 ± 0.01
Day 23	0.25 ± 0.01	0.25 ± 0.02	0.21 ± 0.01*	0.21 ± 0.01	0.21 ± 0.01	)
Week 13	0.21 ± 0.01	0.27 ± 0.01**	0.23 ± 0.01*	0.27 ± 0.02**	0.25 ± 0.02*	0.46 ± 0.05**
Mean cell volume (fL)						
Day 3	62.5 ± 0.3	63.0 ± 0.4	62.8 ± 0.4	62.7 ± 0.3	62.9 ± 0.3	63.0 ± 0.4
Day 16	)	)	)	)	)	56.8 ± 0.3
Day 23	60.3 ± 0.2	61.0 ± 0.6	60.5 ± 0.5	59.9 ± 0.4	59.3 ± 0.4	)
Week 13	52.6 ± 0.3	52.2 ± 0.2	52.4 ± 0.3	51.7 ± 0.3	52.6 ± 0.4	55.0 ± 0.5**
Leukocytes (10^3/µL)						
Day 3	9.32 ± 0.39	8.16 ± 0.25	8.55 ± 0.50	8.93 ± 0.62	10.27 ± 0.44	9.15 ± 0.40
Day 16	)	)	)	)	)	6.81 ± 0.56
Day 23	10.19 ± 0.32	10.39 ± 0.39	8.79 ± 0.31**	8.09 ± 0.46**	5.88 ± 0.22**	)
Week 13	10.60 ± 0.43	10.19 ± 0.31	9.59 ± 0.46	8.71 ± 0.33**	6.94 ± 0.17**	4.95 ± 0.75**
Lymphocytes (10^3/µL)						
Day 3	7.50 ± 0.37	6.79 ± 0.30	6.85 ± 0.43	7.28 ± 0.41	7.94 ± 0.34	7.11 ± 0.34
Day 16	)	)	)	)	)	5.70 ± 0.51
Day 23	8.80 ± 0.31	8.64 ± 0.39	7.47 ± 0.34*	6.52 ± 0.33**	4.91 ± 0.20**	)
Week 13	8.86 ± 0.37	8.26 ± 0.24	7.59 ± 0.37*	6.87 ± 0.27**	5.33 ± 0.21**	3.60 ± 0.59**

TABLE 6 Selected Hematology Data for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol (continued)

	Concentration (ppm)					
	0	110	330	1,100	3,300	10,000
FEMALE						
n						
Day 3	10	10	10	10	10	10
Day 15	0	0	0	0	0	10
Day 23	10	10	10	10	10	0
Week 13	9	10	10	10	10	0
Hematocrit (automated) (%)						
Day 3	46.6 ± 0.4	46.5 ± 0.5	46.9 ± 0.3	46.4 ± 0.6	47.5 ± 0.5	50.8 ± 0.5**
Day 15	)	)	)	)	)	54.4 ± 1.0
Day 23	50.1 ± 0.4	53.9 ± 0.5	49.7 ± 0.6	49.4 ± 0.5	50.1 ± 0.7	)
Week 13	47.6 ± 0.6	46.3 ± 0.4	47.1 ± 0.4	48.4 ± 0.4	50.8 ± 0.5**	)
Hemoglobin (g/dL)						
Day 3	14.7 ± 0.1	14.7 ± 0.2	14.9 ± 0.1	14.6 ± 0.2	15.0 ± 0.1	16.0 ± 0.2**
Day 15	)	)	)	)	)	17.6 ± 0.3
Day 23	16.1 ± 0.2	17.3 ± 0.2	15.9 ± 0.2	15.7 ± 0.2	15.9 ± 0.3	)
Week 13	15.9 ± 0.2	15.4 ± 0.1	15.4 ± 0.1	15.6 ± 0.1	16.5 ± 0.2	)
Erythrocytes (10^6/µL)						
Day 3	7.61 ± 0.07	7.64 ± 0.09	7.69 ± 0.07	7.58 ± 0.12	7.74 ± 0.08	8.29 ± 0.09**
Day 15	)	)	)	)	)	9.24 ± 0.17
Day 23	8.16 ± 0.07	8.81 ± 0.09**	8.11 ± 0.09	8.02 ± 0.11	8.27 ± 0.14	)
Week 13	8.61 ± 0.10	8.26 ± 0.13	8.41 ± 0.06	8.61 ± 0.06	8.92 ± 0.09	)
Reticulocytes (10^6/µL)						
Day 3	0.39 ± 0.01	0.40 ± 0.02	0.39 ± 0.02	0.37 ± 0.02	0.38 ± 0.01	0.41 ± 0.01
Day 15	)	)	)	)	)	0.08 ± 0.01
Day 23	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.20 ± 0.02	)
Week 13	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.25 ± 0.01*	)
Mean cell volume (fL)						
Day 3	61.2 ± 0.3	61.0 ± 0.5	61.0 ± 0.4	61.6 ± 0.3	61.4 ± 0.4	61.4 ± 0.3
Day 15	)	)	)	)	)	59.1 ± 0.3
Day 23	61.4 ± 0.3	61.2 ± 0.3	61.5 ± 0.3	61.6 ± 0.4	60.6 ± 0.3	)
Week 13	55.2 ± 0.4	56.3 ± 0.6	56.0 ± 0.2	56.1 ± 0.1*	57.0 ± 0.2**	)
Leukocytes (10^3/µL)						
Day 3	9.51 ± 0.31	9.11 ± 0.31	9.49 ± 0.40	10.17 ± 0.49	10.09 ± 0.33	9.74 ± 0.18
Day 15	)	)	)	)	)	5.82 ± 0.31
Day 23	9.68 ± 0.33	8.92 ± 0.34	8.72 ± 0.54	6.84 ± 0.23**	6.16 ± 0.28**	)
Week 13	8.44 ± 0.37	6.21 ± 0.28**	6.88 ± 0.32**	6.85 ± 0.24**	6.48 ± 0.49**	)
Lymphocytes (10^3/µL)						
Day 3	8.04 ± 0.30	7.33 ± 0.27	7.70 ± 0.28	8.41 ± 0.45	7.91 ± 0.32	7.81 ± 0.11
Day 15	)	)	)	)	)	4.46 ± 0.24
Day 23	8.22 ± 0.38	7.64 ± 0.38	7.02 ± 0.45*	5.64 ± 0.18**	5.06 ± 0.19**	)
Week 13	7.04 ± 0.39	5.04 ± 0.24**	5.49 ± 0.31**	5.09 ± 0.26**	4.76 ± 0.29**	)

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

The only gross necropsy observation considered related to administration of urethane in ethanol was thinness of the carcasses of all females and several males in the 10,000 ppm groups. Microscopically, the tissues that were targets of toxicity in the urethane in 5% ethanol study were the same as those identified in the urethane in drinking water study (Table 5) and included the lymphoid tissue, bone marrow, liver, kidneys, and heart (Table 7). The effects in these tissues were morphologically similar to those in the urethane in drinking water study, but with some differences in the incidence and severity, as noted below.

Lymphoid depletion was present in the spleens of males and females in the 3,300 and 10,000 ppm groups (Table 7); this lesion occurred at higher exposure concentrations than in the urethane in drinking water study, in which rats in the 1,100, 3,300, and 10,000 ppm groups were affected. Lymphoid depletion of the mandibular and mesenteric lymph nodes occurred with greater frequency and severity in the urethane in 5% ethanol study than in the urethane in drinking water study, with generally moderate lesions affecting most rats in the 10,000 ppm groups and several rats in the 3,300 ppm group. As in the urethane in drinking water study, the thymus was mostly unaffected by lymphoid depletion, with only a single male and a single female in the 10,000 ppm groups having mild or moderate lesions.

Hematopoietic cell depletion in the bone marrow occurred in several males and females administered 10,000 ppm urethane in ethanol (Table 7); this lesion occurred in fewer males than in the urethane in drinking water study, but the severity of cellular depletion in males and females receiving urethane in ethanol was slightly greater.

In contrast to urethane administered in drinking water, urethane in 5% ethanol induced liver effects in male rats only. Fatty change of centrilobular hepatocytes similar to that seen in the urethane in drinking water study was present in most males in the 10,000 ppm group. Clear cell foci, observed primarily in the 3,300 and 10,000 ppm groups of females receiving urethane in water, occurred only in a single male receiving 10,000 ppm urethane in ethanol.

TABLE 7 Incidence and Severity of Selected Lesions in F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol[1]

	Concentration (ppm)					
	0	110	330	1,100	3,300	10,000
MALE						
Spleen						
Depletion lymphoid	0/10	0/10	0/10	0/10	10/10** (2.0)	10/10** (2.2)
Lymph node, mandibular						
Depletion lymphoid	0/10	0/10	0/10	0/10	3/10 (2.0)	10/10** (2.8)
Lymph node, mesenteric						
Depletion lymphoid	0/10	0/9	0/10	0/10	2/10 (2.0)	8/10** (2.1)
Thymus						
Depletion lymphoid	0/9	0/10	0/10	0/10	0/10	1/10 (2.0)
Bone marrow						
Depletion cellular	0/10	0/10	0/10	0/10	0/10	2/10 (2.5)
Liver						
Clear cell focus	0/10	0/10	0/10	0/10	0/10	1/10
Fatty change	0/10	0/10	0/10	0/10	0/10	8/10** (1.8)
Kidney						
Nephropathy	10/10 (1.4)	10/10 (1.5)	10/10 (1.2)	10/10 (1.4)	10/10 (1.9)	10/10 (1.7)
Heart						
Cardiomyopathy	9/10 (1.2)	10/10 (1.0)	10/10 (1.3)	10/10 (1.0)	10/10 (1.0)	9/10 (3.0)
FEMALE						
Spleen						
Depletion lymphoid	0/10	0/10	0/10	0/10	9/10** (1.6)	10/10** (2.1)
Lymph node, mandibular						
Depletion lymphoid	0/10	0/10	0/10	0/10	3/10 (2.3)	10/10** (2.8)
Lymph node, mesenteric						
Depletion lymphoid	0/10	0/9	0/10	0/10	2/10 (2.0)	10/10** (2.9)
Thymus						
Depletion lymphoid	0/10	0/10	0/10	0/10	0/10	1/10 (3.0)
Bone marrow						
Depletion cellular	0/10	0/10	0/10	0/10	0/10	10/10** (2.7)
Kidney						
Nephropathy	4/10 (1.0)	6/10 (1.0)	3/10 (1.0)	5/10 (1.0)	10/10** (1.8)	5/10 (1.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

The effects of urethane in 5% ethanol on the incidence and severity of nephropathy were similar to those of urethane in drinking water (Table 7). In males, this change included a slightly greater severity of nephropathy in the 3,300 and 10,000 ppm groups than in the controls; in females, the incidence and severity of nephropathy were greater in the 3,300 ppm group than in the controls. No effect was observed in females in the 10,000 ppm group, likely as a result of the early deaths of these rats.

As in the urethane in drinking water study, urethane in 5% ethanol caused an increased severity of cardiomyopathy in males the 10,000 ppm group (Table 7). However, in marked contrast to the

exposure-related increases in incidence and severity of cardiomyopathy in female rats in the urethane in drinking water study, no exacerbation of this lesion was observed in females receiving urethane in ethanol. This lesion was absent in females in the 10,000 ppm group (perhaps attributable to the early deaths of these rats), and the incidence and severity of cardiomyopathy in the 3,300 ppm group (6/10, minimal severity) were similar to those in the controls (5/10, minimal severity).

Epididymal spermatozoal motility in male rats in the 1,100 and 3,300 ppm groups and spermatozoal concentration in males in the 3,300 ppm group were significantly less than those in the controls (Table E3). The estrous cycle length of females that received 3,300 ppm was longer than that of the controls (Table E4).

13-Week Studies in B6C3F$_1$ Mice

All male and female mice that received 3,300 or 10,000 ppm urethane in drinking water died or were killed moribund; two control females in this study also died early (Table 8). Among mice administered urethane in 5% ethanol, two control females, one male receiving 110 ppm, one male and one female receiving 330 ppm, one male and four females receiving 3,300 ppm, and all mice receiving 10,000 ppm died or were killed moribund before the end of the study (Table 9). All deaths of mice in the 10,000 ppm groups in both studies occurred during or before Week 3. The final mean body weights and weight gains of males and females receiving 1,100 ppm urethane in drinking water (Table 8 and Figure 3) and of males and females receiving 1,100 or 3,300 ppm urethane in 5% ethanol (Table 9 and Figure 4) were notably less than those of the respective controls.

In both studies, clinical signs of toxicity were generally restricted to the 3,300 and 10,000 ppm groups and included thinness, lethargy, abnormal posture, and ruffled fur. No clinical signs of toxicity were observed in most mice surviving to the end of the study.

Water consumption data are shown in Tables 8 and 9. These values represent water removed from the bottles and are probably overestimates of water actually consumed; this is especially true for male mice that received 110 ppm urethane in drinking water. Water consumption by males and females administered 3,300 ppm urethane in ethanol was less than that by the controls (Table 9); water consumption by mice receiving 10,000 ppm urethane in drinking water or in 5% ethanol was also less than that by the controls (data not shown). For all other groups in both studies, water consumption was similar to or greater than that by the respective controls. The excessive water consumption values for female mice in the drinking water study was likely due to spillage rather than to actual water intake by the mice.

TABLE 8 Survival, Weight Gain, Water Consumption, and Compound Consumption Data for B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average Water Consumption[3] (g/day)
		Initial	Final	Change		
MALE						
0	10/10	23.4	40.8	17.4		5.1
110	10/10	23.7	41.6	17.8	102	15.5
330	10/10	23.8	42.3	18.6	104	5.0
1,100	10/10	24.0	31.0	7.0	76	5.4
3,300	0/10[4]	24.0	)	)	)	)
10,000	0/10[5]	23.5	)	)	)	)
FEMALE						
0	8/10[6]	20.0	35.3	15.3		7.5
110	10/10	19.5	34.6	15.1	98	9.5
330	10/10	19.7	34.3	14.6	97	10.1
1,100	10/10	19.2	23.2	4.0	66	10.8
3,300	0/10[7]	19.6	)	)	)	9.9
10,000	0/10[8]	19.4	)	)	)	)

[1] Number surviving at 13 weeks/number of animals per group.
[2] (Dose group mean/control group mean) × 100.
[3] Average of individual water consumption values for Weeks 2-13 for all animals in the base study; consumption values for male mice in the 3,300 and 10,000 ppm groups and males and females in the 10,000 ppm groups are not provided due to 100% mortality of mice in these groups early in the study. For females in the 3,300 ppm group, water consumption values for Weeks 2-4 only are given. Water consumption values represent water removed from bottles, not necessarily water consumed.
[4] Week of death: all deaths occurred during Week 4.
[5] Week of death: seven deaths occurred during Week 2; three deaths occurred during Week 3.
[6] Week of death: 12, 13.
[7] Week of death: one death occurred during Week 4; nine deaths occurred during Week 5.
[8] Week of death: five mice died during each of Weeks 2 and 3.

TABLE 9 Survival, Weight Gain, Water Consumption, and Compound Consumption Data for B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol

Dose (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls[2] (%)	Average 5% Ethanol Consumption[3] (g/day)
		Initial	Final	Change		
MALE						
0	10/10	26.0	43.7	17.7		8.5
110	9/10[4]	25.9	44.7	18.6	102	6.9
330	9/10[5]	25.7	42.3	16.8	97	9.9
1,100	10/10	25.6	36.0	10.4	82	5.5
3,300	9/10[6]	25.7	29.4	3.7	67	3.3
10,000	0/10[7]	25.0	)	)	)	)
FEMALE						
0	8/10[8]	20.3	33.9	13.3		6.4
110	10/10	21.3	36.7	15.4	108	6.0
330	9/10[9]	20.8	32.2	11.4	95	6.4
1,100	10/10	20.9	27.3	6.3	81	5.9
3,300	6/10[10]	20.6	21.5	0.8	64	3.7
10,000	0/10[7]	20.6	)	)	)	)

[1] Number surviving at 13 weeks/number of animals per group.
[2] (Dose group mean/control group mean) × 100.
[3] Average of individual ethanol consumption values for Weeks 2-13 for all animals in the base study; consumption data for males and females in the 10,000 ppm groups are not provided due to 100% mortality in these groups by Week 3. Ethanol consumption values represent ethanol removed from bottles, not necessarily ethanol consumed.
[4] Week of death: 3.
[5] Week of death: 12.
[6] Week of death: 1.
[7] Week of death: all deaths occurred during Week 3.
[8] Week of death: 12, 13.
[9] Week of death: 10.
[10] Week of death: 11, 12, 13, 13.

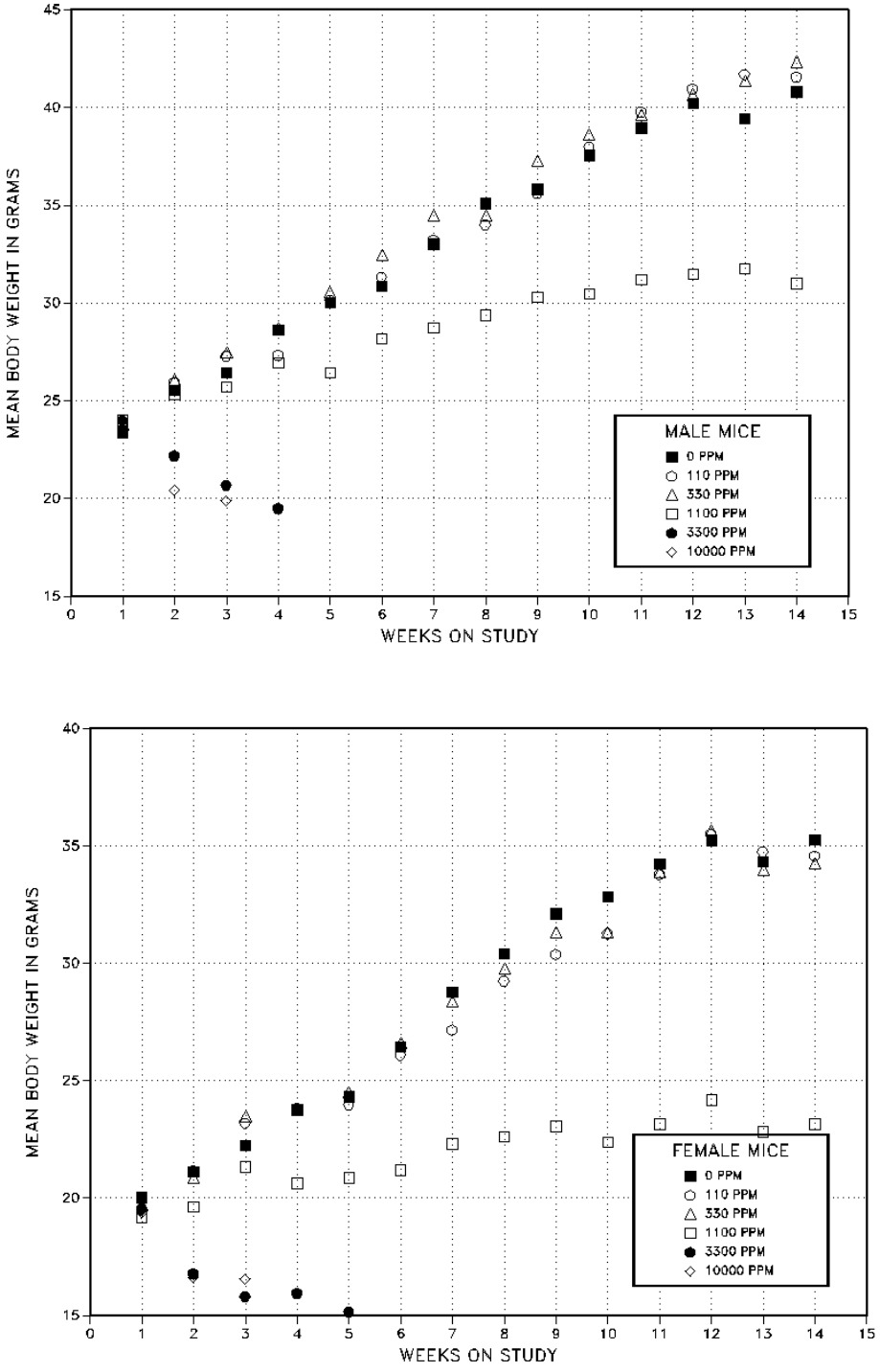


FIGURE 3 Body Weights of B6C3F$_1$ Mice Administered Urethane in Drinking Water for 13 Weeks

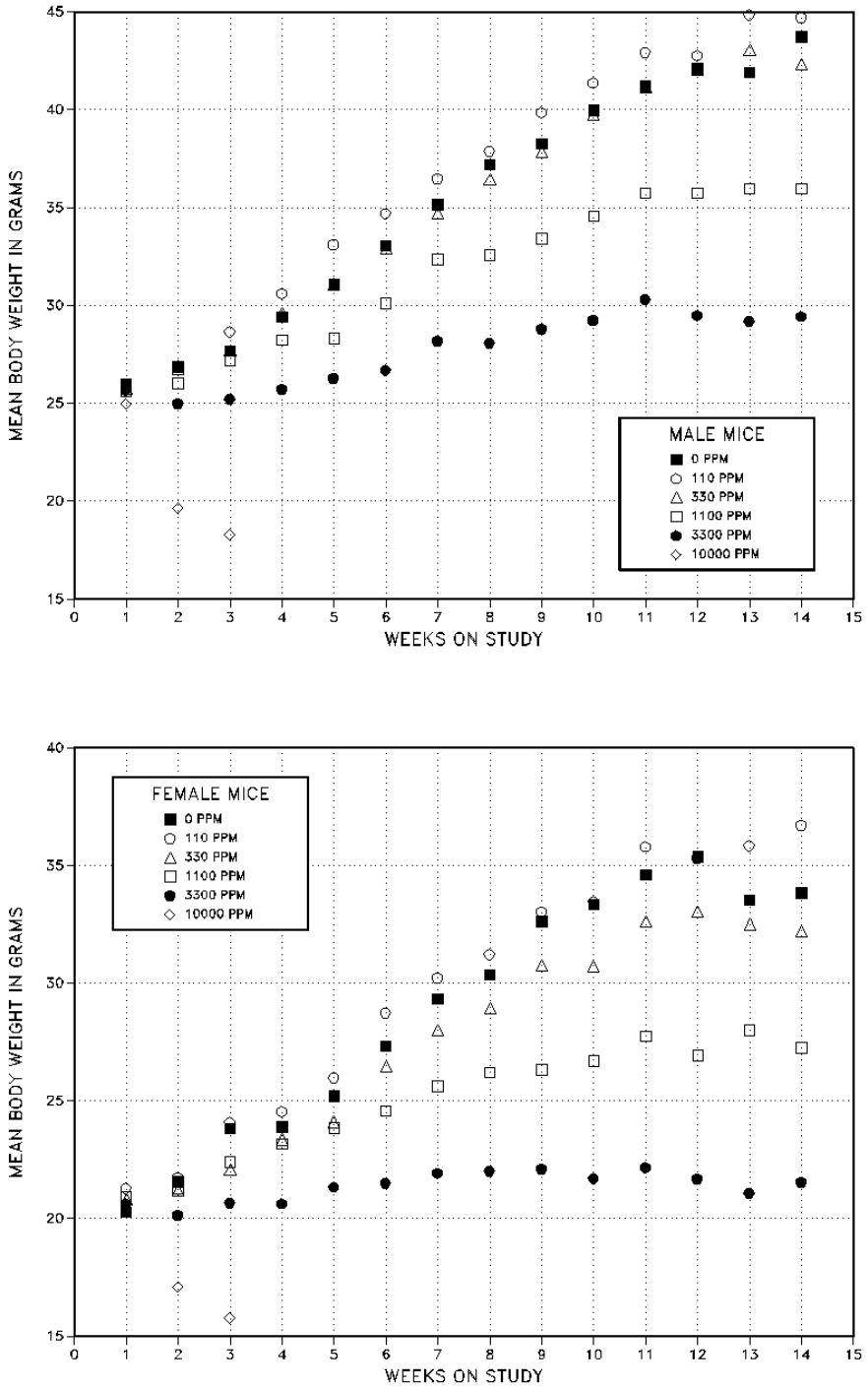


FIGURE 4 Body Weights of B6C3F$_1$ Mice Administered Urethane in 5% Ethanol for 13 Weeks

Urethane in Drinking Water: Male and female mice in the 1,100 ppm groups had slightly greater absolute lung weights and significantly greater relative lung weights than the controls (Table C3); relative liver and lung weights were also generally greater in exposed males and females than in the controls. Other statistically significant differences in organ weights between exposed and control groups were considered secondary to differences in mean body weights.

Gross necropsy observations in mice treated with urethane were limited to thin carcasses and/or fluid in the abdominal cavity in females receiving 1,100 ppm or greater. Microscopically, treatment-related effects were observed in the lungs, kidneys, heart, lymphoid tissue, bone marrow, ovaries, testes, liver, and pancreas (Table 10).

Inflammatory and proliferative changes were observed in the lungs of exposed mice (Table 10). Inflammation of the alveolar parenchyma occurred in mice receiving 1,100 ppm or greater. Minimal to mild inflammation, characterized by serofibrinous exudation and macrophage accumulation in alveolar lumens, was present in all mice that died before the end of the study. Another lesion observed in many of the mice that died early was bronchiolar hyperplasia. This lesion was a minimal to mild, diffuse proliferation of bronchiolar epithelial cells which caused increased cellularity, stratification, and overall thickness of the epithelial lining (Plates 7 and 8). Less severe inflammation, primarily accumulations of intra-alveolar macrophages, was present in several mice in the 1,100 ppm groups that survived to the end of the study. Proliferative changes of airway cells were also observed in surviving mice. Alveolar epithelial hyperplasia occurred in a three males in the 330 ppm group and in one male and four females in the 1,100 ppm groups. The severity of this change ranged from minimal, with one or more small foci of alveoli lined by slightly enlarged, monomorphic cuboidal cells, to marked, with multifocal lesions comprised of larger epithelial cells, occasionally with slight cellular atypia. A single alveolar/bronchiolar adenoma was diagnosed in a male in the 330 ppm group. The adenoma was a circumscribed lesion, cytologically similar to marked alveolar hyperplasia but more clearly expansile and compressive, and with alveolar lumens filled by proliferative epithelial cells.

TABLE 10 Incidence and Severity of Selected Lesions in B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water[1]

	Concentration (ppm)					
	0	110	330	1,100	3,300	10,000
MALE						
Lung						
Inflammation	0/10	0/10	0/10	4/10* (1.0)	10/10** (1.8)	10/10** (1.6)
Bronchiole, hyperplasia	0/10	0/10	0/10	0/10	10/10** (1.6)	1/10 (1.0)
Alveolar epithelium, hyperplasia	0/10	0/10	3/10 (2.0)	1/10 (4.0)	0/10	0/10
Alveolar/bronchiolar adenoma	0/10	0/10	1/10	0/10	0/10	0/10
Kidney						
Nephropathy	1/10 (1.0)	3/10 (1.0)	0/10	3/10 (1.3)	3/10 (1.3)	9/10** (2.1)
Heart						
Cardiomyopathy	0/10	0/10	0/10	1/10 (1.0)	3/10 (1.7)	0/10
Hemorrhage	0/10	0/10	0/10	0/10	1/10 (2.0)	9/10** (1.4)
Mineralization	0/10	0/10	0/10	0/10	3/10 (1.7)	3/10 (1.3)
Spleen						
Depletion lymphoid	0/10	0/10	0/10	0/10	9/10** (2.2)	10/10** (3.9)
Lymph node, mandibular						
Depletion lymphoid	0/10	0/10	0/10	0/10	8/10** (2.9)	8/8** (3.9)
Lymph node, mesenteric						
Depletion lymphoid	0/10	0/10	0/10	0/10	7/10** (2.6)	8/8** (3.9)
Thymus						
Depletion lymphoid	0/9	0/10	0/10	0/10	7/7** (3.3)	7/7** (4.0)
Bone marrow						
Depletion cellular	0/10	0/10	0/10	0/10	0/10	10/10** (2.7)
Testis						
Seminiferous tubule, degeneration	0/10	0/10	0/10	0/10	0/10	5/10* (1.6)

TABLE 10 Incidence and Severity of Selected Lesions in B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water (continued)

	Concentration (ppm)					
	0	110	330	1,100	3,300	10,000
FEMALE						
Lung						
Inflammation	0/10	0/10	0/10	7/10** (1.7)	10/10** (2.0)	10/10** (1.5)
Bronchiole, hyperplasia	0/10	0/10	0/10	0/10	5/10* (1.0)	3/10 (1.3)
Alveolar epithelium, hyperplasia	0/10	0/10	0/10	4/10* (1.0)	0/10	0/10
Kidney						
Nephropathy	0/10	0/10	1/10 (1.0)	8/10** (2.8)	2/10 (1.5)	4/10* (2.3)
Heart						
Cardiomyopathy	0/10	0/10	0/10	3/10 (2.3)	1/10 (1.0)	0/10
Hemorrhage	0/10	0/10	0/10	0/10	2/10 (2.5)	6/10** (2.3)
Mineralization	0/10	0/10	0/10	0/10	3/10 (2.0)	5/10* (1.6)
Spleen						
Depletion lymphoid	0/10	0/10	0/10	0/10	8/10** (2.6)	9/10** (3.8)
Lymph node, mandibular						
Depletion lymphoid	0/10	0/10	0/10	0/9	8/8** (3.6)	10/10** (3.7)
Lymph node, mesenteric						
Depletion lymphoid	0/10	0/10	0/10	2/10 (2.5)	8/9** (3.3)	7/7** (3.6)
Thymus						
Depletion lymphoid	0/10	0/10	0/10	1/9 (2.0)	8/8** (3.8)	7/7** (3.9)
Bone marrow						
Depletion cellular	0/10	0/10	0/10	1/10 (2.0)	2/10 (2.0)	8/10** (2.0)
Ovary						
Atrophy	0/10	0/10	0/10	7/10** (2.6)	0/10	0/10
Follicle, degeneration	0/10	0/10	0/10	0/10	9/10** (1.3)	8/10** (1.6)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test.

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

Exposed mice had kidney changes that were diagnosed as nephropathy (Table 10). Minimal lesions of nephropathy consisted of one to a few scattered foci of basophilic, regenerative tubule cells. In more severe lesions, the number of affected tubules was increased, and changes included tubule dilatation and the presence of intratubular hyaline casts. The severity of nephropathy was greater in exposed females than in males. Moderate nephropathy occurred in females in the 1,100 ppm group; females in the 3,300 and 10,000 ppm groups also had less severe lesions. In contrast, a significantly increased incidence of mild lesions was seen only in male mice in the 10,000 ppm group that died early.

Several treatment-related lesions were noted in the hearts of exposed mice. Hemorrhage and mineralization in the myocardium were observed in males and females in the 3,300 and 10,000 ppm groups (Table 10). These minimal to moderate lesions were focal to multifocal and were characterized by accumulations of free erythrocytes between myocardial fibers (hemorrhage) or the presence of granular basophilic material within degenerate myocytes (mineralization). Cardiomyopathy, a change of longer duration, was present in mice in the 1,100 and 3,300 ppm groups (Table 10). Cardiomyopathy was a multifocal change of minimal to moderate severity and was characterized by degeneration and necrosis of myofibers, variably associated with accumulations of mononuclear inflammatory cells and interstitial fibrosis.

Depletion of lymphocytes was a treatment-related effect in the lymphoid tissue (spleen, lymph nodes, and thymus) of mice receiving 3,300 or 10,000 ppm (Table 10); depletion in the thymus and mesenteric lymph nodes was also observed in females in the 1,100 ppm group. In males and females, the severity of the lesion increased with increasing urethane concentration. In the spleen, lymphoid depletion was characterized by mild to marked loss of lymphocytes in the white pulp; in the mandibular and mesenteric lymph nodes and the thymus, the lesion was characterized by a moderate to marked loss of cortical lymphocytes.

Cellular depletion of the bone marrow was also present in males in the 10,000 ppm group and females in the 3,300 and 10,000 ppm groups, but this lesion generally occurred in fewer mice and was less severe than the lymphoid depletion (Table 10). One female that received 1,100 ppm also had mild hematopoietic cell depletion of the bone marrow. Cellular depletion consisted of a minimal to mild loss of both myeloid and erythroid hematopoietic cells.

Cytoplasmic alteration was observed in the livers of all mice in the 3,300 and 10,000 ppm groups and in one male and seven females in the 1,100 ppm groups (Tables B1 and B2). This lesion, which

consisted of a loss of the normal cytoplasmic vacuolization present within hepatocytes, occurred primarily in periportal areas. Cytoplasmic alteration was considered to be the result of glycogen loss in mice that died or had markedly lower body weight gains than the controls and was not considered to be a direct effect of urethane administration.

In the pancreas of several males and females that received 10,000 ppm, the vascular spaces within and around the pancreatic islets were dilated. This lesion was diagnosed as angiectasis and was considered to be due to the moribund condition of the mice, with vascular stasis, and not a direct effect of urethane exposure.

Minimal to mild degeneration occurred in the testes of males administered 10,000 ppm and in the ovaries of females administered 3,300 or 10,000 ppm (Table 10). Degeneration of the seminiferous tubules, characterized by loss of germ cells and by the presence of a few to numerous spermatid giant cells within tubule lumens, was observed in five males receiving 10,000 ppm. Females administered 3,300 or 10,000 ppm had degenerative changes of the ovarian follicles consisting of greater amounts of cell debris within developing follicles than occurred in control females. The histopathologic changes in the testis and ovaries were considered secondary to the debilitated condition of the mice, as these changes occurred only in mice that died early. In seven females in the 1,100 ppm group, the ovaries were smaller than those of the controls as a result of decreased numbers of follicles and corpora lutea and the flattening of interstitial cells. Epididymal spermatozoal concentration was generally lower in exposed males than in the controls, and the difference was significant in the 110 and 1,100 ppm groups (Table E5). Spermatozoal motility was also lower in males in the 1,100 ppm group than in the controls. Females that received 1,100 ppm had effectively ceased to have an estrous cycle (Table E6). In nine females, no cyclicity was demonstrated, while in the remaining female, the percentage of diestrous smears was doubled.

Urethane in 5% Ethanol: Relative right kidney, liver, and lung weights of male and female mice generally increased with increasing exposure concentration, although the relative liver weights of exposed males were not significantly greater than those of the controls (Table C4). Other differences in organ weights between exposed and control mice were considered secondary to the lower mean body weights of exposed animals.

Gross necropsy observations were limited to thin carcasses and/or fluid in the abdominal cavity of several females in the 3,300 ppm group. Tissues identified microscopically as targets of toxicity of urethane in 5% ethanol were similar to those identified in the urethane in drinking water study and included the lungs, kidneys, heart, lymphoid tissue, bone marrow, ovaries, and liver (Table 11). Morphologically, the effects of urethane administered in ethanol were similar to those of urethane administered in drinking water, but some differences in the incidences and severity of lesions were observed, as noted below.

Administration of urethane in ethanol caused inflammatory and proliferative changes in the lung similar to those observed in mice in the urethane in drinking water study (Table 11). Minimal to mild inflammation characterized by serofibrinous exudation with associated macrophages was observed primarily in males and females in the 3,300 ppm groups and less frequently in males and females in the 10,000 ppm groups. Proliferative lesions of the airway epithelium occurred in surviving mice that received urethane in ethanol; these lesions were similar to those in the urethane in drinking water study but had a greater incidence and severity. Focal thickenings of alveolar walls due to epithelial hyperplasia (Plates 9 and 10) were present in one or more mice in each exposed group with survivors except females receiving 110 ppm. Incidences of alveolar/bronchiolar adenoma were greater in mice administered urethane in ethanol than in mice administered urethane in drinking water, and adenomas occurred in more males receiving urethane in ethanol than females (Table 11). Four male mice, two in the 3,300 ppm group and one each in the 110 and 1,100 ppm groups, had adenomas. One female in the 330 ppm group had an adenoma. Alveolar/bronchiolar adenomas in the 110, 330, and 1,100 ppm groups had typical morphology of a circumscribed, solid lesion comprised of monomorphic cuboidal epithelial cells filling alveolar spaces. Adenomas in males administered 3,300 ppm exhibited slight atypia; these adenomas were composed of irregular solid clusters of cells with round to ovoid nuclei and moderate amounts of eosinophilic cytoplasm (Plates 11 and 12).

TABLE 11 Incidence and Severity of Selected Lesions in B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol[1]

	Concentration (ppm)					
	0	110	330	1,100	3,300	10,000
MALE						
Lung						
Inflammation	0/10	0/10	0/10	1/10 (1.0)	7/10** (1.0)	5/10* (1.0)
Alveolar epithelium, hyperplasia	0/10	1/10 (2.0)	2/10 (4.0)	3/10 (2.0)	5/10* (1.8)	0/10
Alveolar/bronchiolar adenoma	0/10	1/10	0/10	1/10	2/10	0/10
Kidney						
Nephropathy	3/10 (1.0)	0/10	1/10 (1.0)	2/10 (1.0)	9/10** (2.0)	2/10 (1.0)
Spleen						
Depletion lymphoid	0/10	0/10	0/10	0/10	0/10	3/10 (2.0)
Lymph node, mandibular						
Depletion lymphoid	0/10	0/10	0/10	0/9	2/10 (2.0)	3/10 (2.0)
Lymph node, mesenteric						
Depletion lymphoid	0/10	0/10	0/10	0/10	3/10 (2.3)	7/10** (2.3)
Thymus						
Depletion lymphoid	0/10	0/10	0/10	0/10	1/9 (3.0)	8/9** (2.9)
Bone marrow						
Depletion cellular	0/10	0/10	0/10	0/10	1/10 (2.0)	5/10* (2.0)
FEMALE						
Lung						
Inflammation	0/10	0/10	0/10	1/10 (1.0)	8/10** (1.4)	2/10 (1.5)
Alveolar epithelium, hyperplasia	0/10	0/10	2/10 (2.0)	7/10** (1.1)	2/10 (2.5)	0/10
Alveolar/bronchiolar adenoma	0/10	0/10	1/10	0/10	0/10	0/10
Kidney						
Nephropathy	1/10 (1.0)	0/10	2/10 (1.0)	0/10	10/10** (3.1)	3/10 (1.0)
Heart						
Cardiomyopathy	0/10	1/10 (1.0)	0/10	0/10	6/10** (2.7)	0/10
Mineralization	0/10	0/10	0/10	0/10	2/10 (2.5)	0/10
Spleen						
Depletion lymphoid	0/10	0/10	0/10	0/10	6/9** (2.3)	6/10** (2.0)
Lymph node, mandibular						
Depletion lymphoid	0/10	0/10	0/10	0/10	4/9* (3.0)	2/10 (2.0)
Lymph node, mesenteric						
Depletion lymphoid	0/9	0/10	1/10 (2.0)	0/10	7/8** (2.6)	7/10** (2.0)
Thymus						
Depletion lymphoid	0/10	0/10	0/10	0/10	3/8 (3.3)	9/10** (2.2)
Bone marrow						
Depletion cellular	0/10	0/10	0/9	0/10	0/10	9/10** (2.0)
Ovary						
Atrophy	0/10	0/10	1/10 (1.0)	10/10** (1.8)	9/9** (3.4)	0/9
Follicle, degeneration	0/10	0/10	0/10	0/10	0/9	5/9* (1.2)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test.

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

The incidences of alveolar/bronchiolar adenoma were greater in males and females receiving urethane in ethanol than in mice receiving urethane in drinking water. Males receiving urethane in ethanol had a greater incidence of alveolar/bronchiolar adenoma than females (Table 11); four male mice, including two in the 3,300 ppm group and one each in the 110 and 1,100 ppm groups, had adenomas, while a single female in the 330 ppm group had a lung adenoma. Alveolar/bronchiolar adenomas in mice in the 110, 330, and 1,100 ppm groups had typical morphology and were composed of a circumscribed lesion consisting of monomorphic cells filling alveolar spaces. Adenomas in males in the 3,300 ppm group exhibited slight atypia, being composed of irregular solid clusters of cells with round to ovoid nuclei and moderate amounts of eosinophilic cytoplasm (Plates 11 and 12). No incidences of bronchiolar epithelial hyperplasia, as occurred in the urethane in drinking water study, were observed in the urethane in 5% ethanol study.

Treatment-related incidences of nephropathy occurred primarily in male and female mice in the 3,300 ppm groups (Table 11). This lesion consisted of multifocal degenerative and regenerative tubule changes. A few control mice had minimal nephropathy; however, the incidence ($P \leq 0.01$) and severity were significantly greater in mice receiving 3,300 ppm. The incidence of nephropathy was generally slightly greater in exposed females than in males, and the severity in females administered 3,300 ppm was greater than in males receiving this concentration of urethane.

The effects of urethane in ethanol on the heart were less prominent than those of urethane in drinking water and occurred only in exposed females. Mild to moderate cardiomyopathy occurred in females in the 3,300 ppm group (Table 11); two of the affected females also had multifocal mineralization of degenerate myofibers.

Mild to moderate lymphoid depletion of the splenic white pulp, mandibular and mesenteric lymph nodes, and thymus occurred in mice in the 3,300 and 10,000 ppm groups (Table 11). The incidence and severity of this lesion varied, and no consistent trends with exposure concentration, site, or sex were observed. Mild depletion of hematopoietic cells in the bone marrow was also present in several males and females in the 10,000 ppm groups (Table 11). The changes in lymphoid tissues and bone marrow cells were generally less severe in mice receiving urethane in ethanol than those in mice receiving urethane in drinking water.

Ovarian changes induced by urethane in ethanol were similar to those induced by urethane in drinking water. Females in the 10,000 ppm group had follicular degeneration with accumulation of cell debris within the follicle (Table 11). All females in the 1,100 and 3,300 ppm groups had minimal

to marked atrophy of the ovary (decreased size resulting from reduced numbers of follicles and corpora lutea). Atrophy occurred with greater incidence but was less severe in females receiving 1,100 ppm urethane in ethanol than in females in the 1,100 ppm group (the highest exposure group with survivors) in the urethane in drinking water study.

Cytoplasmic alteration of hepatocytes was observed in females administered 330 ppm or greater; the incidence of this lesion was not concentration related. Cytoplasmic alteration was characterized by loss of cytoplasmic vacuolization, presumably caused by glycogen loss, in periportal cells. The lesion was considered to be a secondary effect of morbidity.

Epididymal spermatozoal motility in exposed males was significantly less than that in the controls; left epididymal, cauda epididymal, and testis weights of males given 3,300 ppm were also lower than those of the controls (Table E7). Females receiving 3,300 ppm had ceased to cycle and produced constant diestrous smears (Table E8). Females in the 1,100 ppm group had a longer cycle length than the controls, although the difference was not significant; this was probably a less severe manifestation of the effects observed in the 3,300 ppm group.

Urethane Elimination Kinetics

The average half life for elimination of urethane, with and without 5% ethanol, by male mice was 0.77 hours and ranged from 0.6 to 0.9 hours. No kinetic calculations could be performed on data from female mice due to wide variation in readings. Generally, the concentrations of urethane in the plasma of males and females receiving urethane in ethanol were approximately the same as the concentrations in males receiving urethane in drinking water (Table 12). In mice receiving 1,100 ppm urethane in drinking water, females had higher urethane concentrations in plasma than males (Table 13). Elimination curves for urethane in plasma of male mice are shown in Figures 5 and 6. In nearly all plasma samples, the ethanol concentration was below the limits of detection.

TABLE 12 Urethane Concentrations in Plasma of Male B6C3F$_1$ Mice in the 13-Week Studies of Urethane in Drinking Water and Urethane in 5% Ethanol[1]

Time[2] (Hours)	Concentration (ppm)		
	110	330	1,100
URETHANE IN DRINKING WATER STUDY			
0	0.176 ± 0.094[3]	0.632 ± 0.437	0.877 ± 0.265
1	<LOD	0.259 ± 0.072[3]	0.356 ± 0.306
2	<LOD	<LOD	0.168 ± 0.045
3	<LOD	<LOD	<LOD
5	<LOD	<LOD	2.77[4]
URETHANE IN 5% ETHANOL STUDY			
0	0.626 ± 0.792	1.08 ± 0.32	4.09 ± 2.63
2	0.109[4]	0.160[4]	0.594 ± 0.453[3]
4	<LOD	0.171[4]	0.123[4]
6	<LOD	<LOD	<LOD
8	<LOD	<LOD	<LOD

[1] Urethane concentrations are given as µg urethane/mL plasma (mean ± standard deviation for groups of three mice). For data given as <LOD, at least one sample was available for analysis, and the urethane concentration in all samples was below the limit of detection. LOD is defined as three times the standard deviation of the blank response expressed as concentration (<0.90 µg/mL).

[2] Number of hours after bottle of dosed water was withdrawn.

[3] For one mouse, the urethane concentration was below the limit of detection.

[4] For two mice, urethane concentrations were below the limit of detection.

TABLE 13 Urethane Concentrations in Plasma of Female B6C3F$_1$ Mice in the 13-Week Studies of Urethane in Drinking Water and Urethane in 5% Ethanol[1]

Time[2] (Hours)	Concentration (ppm)		
	110	330	1,100
URETHANE IN DRINKING WATER STUDY			
1	<LOD	<LOD	4.16
2	<LOD	0.110[3]	7.05 ± 10.52
3	<LOD	0.100[3]	1.23 ± 0.48
5	<LOD	0.097[3]	16.7 ± 25.2
URETHANE IN 5% ETHANOL STUDY			
2	<LOD	0.100	4.64
4	<LOD	<LOD	0.118 ± 0.039
6	<LOD	0.204 ± 0.056	0.373 ± 0.291[4]
8	<LOD	<LOD[5]	0.399 ± 0.264

[1] Urethane concentrations are given as µg urethane/mL plasma (mean ± standard deviation for groups of three mice). For data given as <LOD, at least one sample was available for analysis, and the urethane concentration in all samples was below the limit of detection. LOD is defined as three times the standard deviation of the blank response expressed as concentration (<0.90 µg/mL). At the 1-hour (urethane in drinking water study) and 2-hour (urethane in 5% ethanol study) evaluations, only one plasma sample was collected per group.

[2] Number of hours after bottle of dosed water was withdrawn.

[3] For two mice, urethane concentrations were below the limit of detection.

[4] For one mouse, the urethane concentration was below the limit of detection.

[5] Two plasma samples were available; for both, the urethane concentration was below the limit of detection.

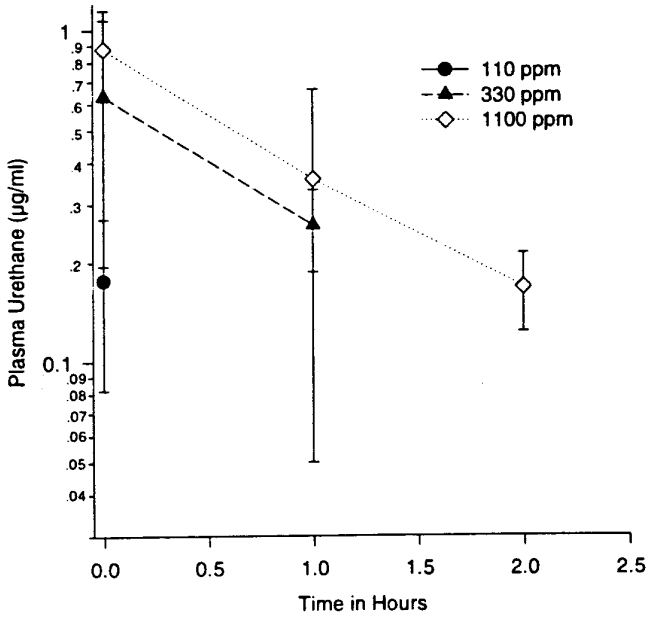


FIGURE 5 **Urethane Concentrations in Plasma of Male B6C3F$_1$ Mice Administered Urethane in Drinking Water for 13 Weeks (None that the ordinate is in log scale.)**

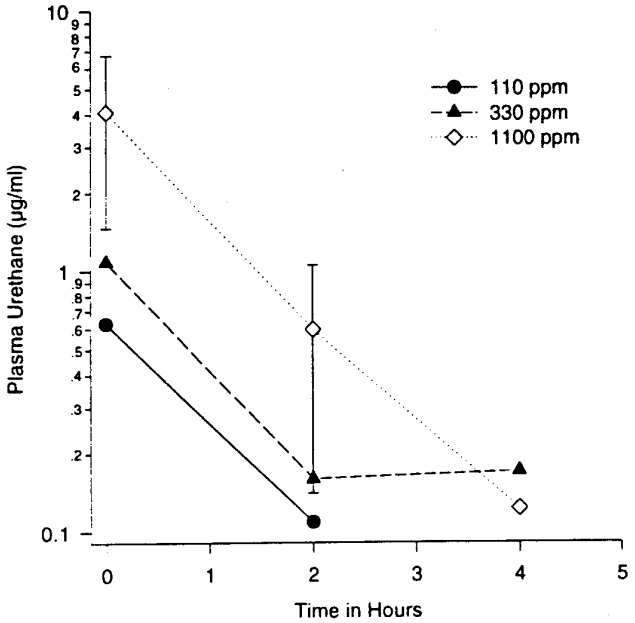


FIGURE 6 **Urethane Concentrations in Plasma of Male B6C3F$_1$ Mice Administered Urethane in 5% Ethanol for 13 Weeks (None that the ordinate is in log scale.)**

Genetic Toxicity

Urethane is clearly genotoxic *in vitro* and *in vivo*. Two *Salmonella typhimurium* studies were performed with urethane (Table F1; Zeiger *et al.*, 1992). In both studies, mutagenic activity was detected in strain TA1535 in the presence of hamster liver S9, but the magnitude of the responses differed between studies. In the first study, a positive response was obtained in each of two trials conducted with 30% hamster liver S9. In the second study, the mutagenic responses observed in TA1535 in trials conducted with various concentrations (5%, 10%, and 30%) of hamster liver S9 were not reproducible, and the results of this second study were considered equivocal overall. No mutagenic activity was detected in any other *S. typhimurium* tester strain in either of these two studies.

In cytogenetic tests with cultured Chinese hamster ovary (CHO) cells, urethane induced sister chromatid exchanges with and without S9 (Table F2). No induction of chromosomal aberrations was observed in CHO cells treated with up to 5,000 µg/mL urethane, with or without S9 (Table F3).

Urethane was shown to be a potent inducer of sex-linked recessive lethal mutations (Table F4) and reciprocal translocations (Table F5) in germ cells of male *Drosophila melanogaster* exposed as adults via feeding (Foureman *et al.*, 1994). In mice administered urethane in drinking water, the numbers of micronucleated erythrocytes were significantly greater in peripheral blood after 45 days or 13 weeks of exposure and in the bone marrow after 13 weeks of exposure than in the controls (Table F6). The micronucleus frequencies in both normochromatic and polychromatic erythrocytes were higher in exposed mice than in the controls. In 13-week studies designed to compare the effects of the addition of 5% ethanol to urethane in drinking water, no significant differences in the frequencies of induced micronuclei occurred between groups of mice administered urethane in water (Table F7) and mice administered urethane in ethanol (Table F8); analyses of peripheral blood smears showed strongly positive responses in male and female mice receiving urethane in drinking water or in 5% ethanol.

In conclusion, based on the results described above, urethane induces gene mutations in *S. typhimurium* and chromosomal damage in mammalian cells *in vitro* and *in vivo*. In addition, it is a potent inducer of germ cell genetic damage in male *D. melanogaster*. Ethanol did not appear to enhance or diminish the positive response that occurred in the *in vivo* mouse micronucleus test.

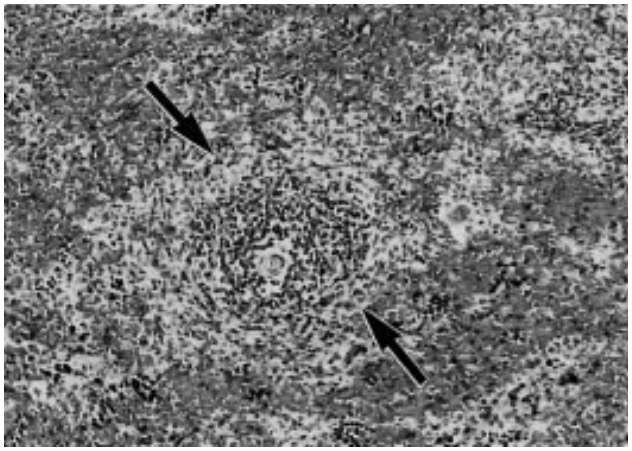


PLATE 1

Spleen of a female rat administered 10,000 ppm urethane in drinking water for 13 weeks. The white pulp (arrows) is diminished in size due to a loss of lymphocytes from the PALS and the marginal zone. Compare to the normal white pulp in Plate 2. H&E 130×.

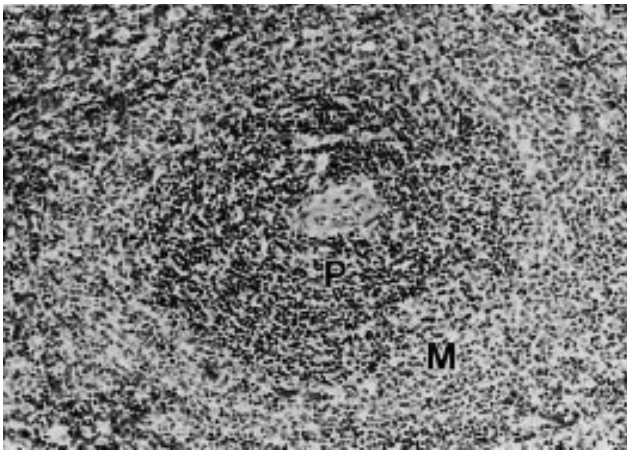


PLATE 2

Spleen of a control female rat. The white pulp is composed of an inner, darker staining PALS (P) and outer, paler staining marginal zone (M). H&E 130×.

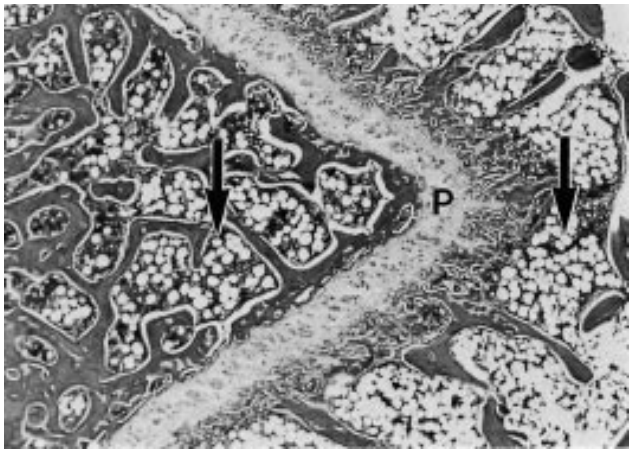


PLATE 3

Femoral bone marrow of a female rat administered 10,000 ppm urethane in drinking water for 13 weeks. There is a loss of cellularity in the marrow spaces (arrows) with resultant prominence of adipose cells. P = physis. Compare to normal bone marrow shown in Plate 4. H&E 32×.

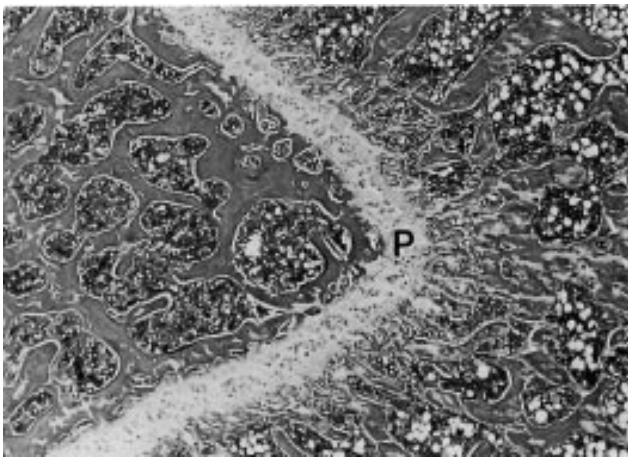


PLATE 4

Femoral bone marrow of a control female rat. Hematopoietic cells fill the marrow spaces between the bony trabeculae. P = physis. H&E 32×.

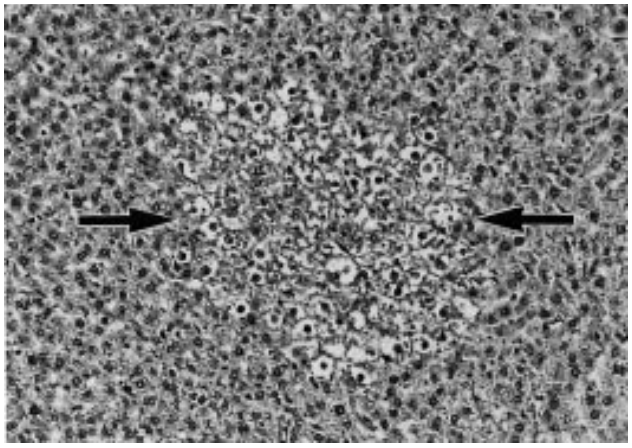


PLATE 5

Liver of a female rat administered 3,300 ppm urethane in drinking water for 13 weeks. A single clear cell focus (arrows) is composed of hepatocytes with central nuclei and perinuclear clear areas. H&E 130×.

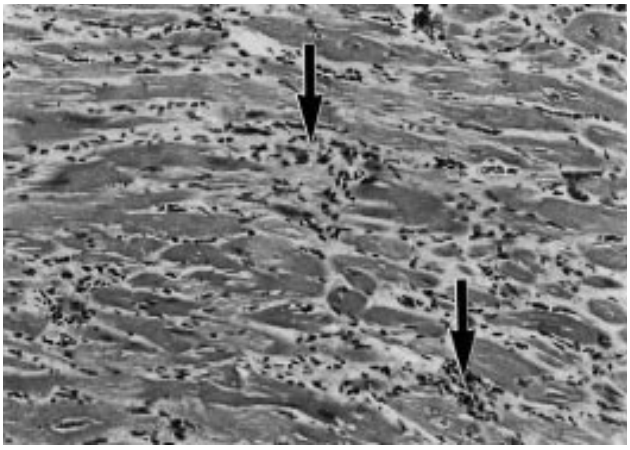


PLATE 6

Heart of a female rat administered 10,000 ppm urethane in drinking water for 13 weeks. Cardiac myofibers are characterized by focal loss of myofibers in the myocardium with replacement by chronic inflammatory cells and/or fibrosis (arrows). H&E 130×.

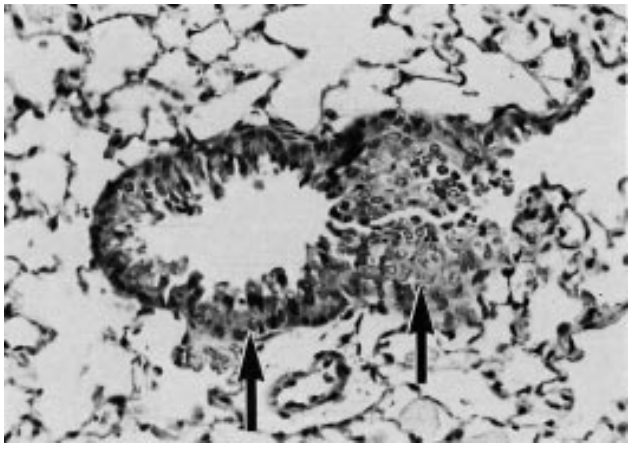


PLATE 7

Lung of a male mouse that died after 4 weeks of administration of 3,300 ppm urethane in drinking water. The epithelium of the terminal bronchiole is thickened due to an increased number and stratification of cells (arrows), with narrowing of the bronchiolar opening into the alveolar duct. Compare to the normal terminal bronchiole in Plate 8. H&E 210×.

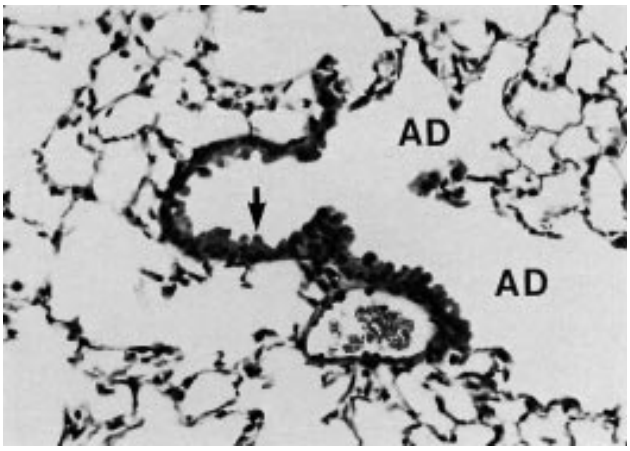


PLATE 8

Lung of a control male mouse. A terminal bronchiole is lined by low columnar epithelial cells with apical blebs (arrow) and bifurcates into alveolar ducts (AD). H&E 210×.

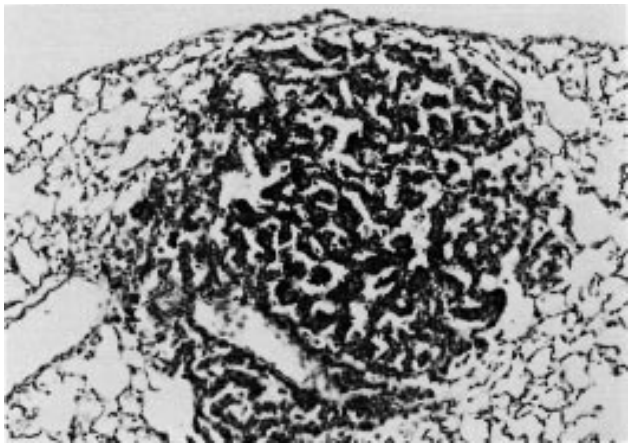


PLATE 9

Lung of a female mouse administered 3,300 ppm urethane in 5% ethanol for 13 weeks. Focal alveolar epithelial hyperplasia is characterized by a circumscribed area of parenchyma with thickened alveolar walls. H&E 100×.

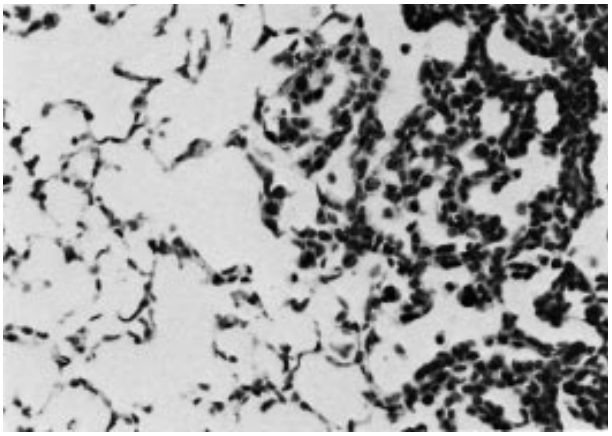


PLATE 10

Higher magnification of the lung in Plate 9, showing the border between focal alveolar epithelial hyperplasia on the right and normal alveolar parenchyma on the left. In contrast to the alveoli of normal parenchyma, alveoli in the hyperplastic lesion are lined by proliferative cuboidal epithelial cells. H&E 260×.

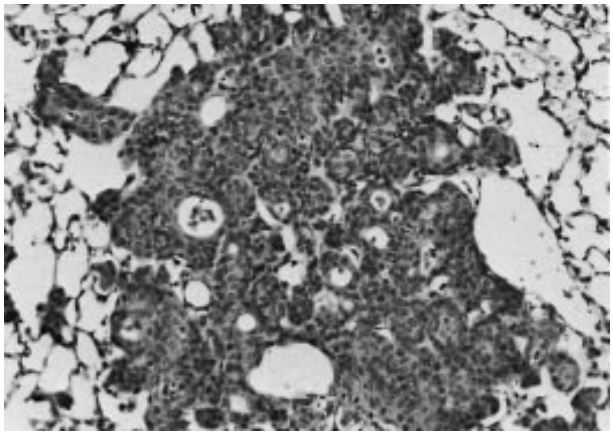


PLATE 11

Alveolar/bronchiolar adenoma in the lung of a male mouse administered 3,300 ppm urethane in 5% ethanol for 13 weeks. The mass has irregular margins and consists of solid clusters of epithelial cells obliterating alveolar spaces. H&E 130×.

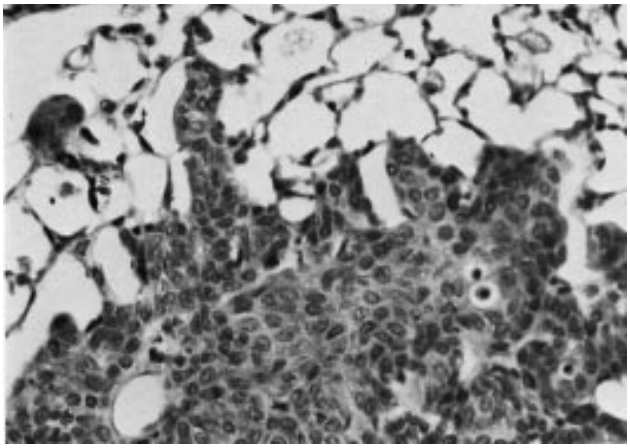


PLATE 12

Higher magnification of the border between normal parenchyma and the adenoma shown in Plate 17. Neoplasm cells are slightly pleomorphic, with round to elongated nuclei and moderate amounts of cytoplasm. H&E 260×.

DISCUSSION

Urethane was nominated for study because data on dose response were not available and information on the effect of ethanol on urethane toxicity was inadequate. The present report compares and contrasts the results of 13-week studies of urethane in drinking water and in 5% ethanol in rats and mice. These 13-week studies are also an attempt to determine dose levels for further evaluation of the effect of ethanol on urethane carcinogenesis in rats and mice.

Urethane in Drinking Water

RATS

Seven male and four female rats administered 10,000 ppm urethane in drinking water died before the end of the study, with most deaths of males and all deaths of females occurring during Week 13. Thus, the LD_{50} of urethane in drinking water for rats appears to be about 10,000 ppm. At this concentration, water consumption by male and female rats was less than that of the controls. The final mean body weight gains of male and female rats administered 1,100 ppm or greater were less than those of the controls.

Urethane induced an exposure concentration-related leukopenia and lymphopenia in male and female rats. The leukopenia was largely secondary to the lymphopenia. There was histologic evidence of lymphoid depletion in the lymph nodes and spleen. As the weight gains of exposed rats were less than those of the controls, the leukopenia would be consistent with a decreased lymphopoiesis related to a decreased nutritional status, possibly compounded by a stress-related increase in endogenous steroid production. However, the absence of thymic lymphoid depletion suggests that urethane may have a direct effect on the lymphoid tissues, as the incidence and severity of lymphoid depletion in the spleen and lymph nodes were concentration related. Further evidence of a direct urethane effect included the occurrence of lymphoid depletion in nondebilitated animals.

Liver clear cell foci were observed in rats administered 1,100 ppm or greater, especially in females in the 3,300 ppm group. The lower incidence of clear cell foci in females in the 10,000 ppm group compared to the 3,300 ppm group may be due to the lower water consumption, survival, and body weight gain of females administered 10,000 ppm (Table 2). The induction of clear cell foci suggests that urethane may have hepatocarcinogenic potential, as these foci rarely occur in 13-week studies.

Spontaneous nephropathy is a common lesion in male rats (Montgomery and Seely, 1990). Urethane administration appeared to exacerbate nephropathy in male rats, as the severity of nephropathy was closely related to the concentration of urethane administered. In female rats, the incidence and severity of nephropathy were exposure related. The significance of urethane enhancement of nephropathy is unknown.

Urethane also enhanced the severity of cardiomyopathy in exposed rats. In male rats administered 10,000 ppm, the enhanced severity may have been due to the debilitated condition of the rats, as it was seen only in rats that died early; however, the incidence and severity of cardiomyopathy in female rats were also exposure related. The significance of urethane enhancement of cardiomyopathy in female rats is unknown.

Epididymal spermatozoal motility and concentration were significantly less in males in the 1,100 and 3,300 ppm groups than in the controls.

MICE

Mice are more sensitive than rats to the toxic effects of urethane; all male and female mice administered 10,000 ppm died by Week 3, and all mice administered 3,300 ppm died during Weeks 4 and 5. Two control female mice also died early, although the cause of death was not clear. Body weight gains of males and females in the 1,100 ppm groups were lower than those of the controls. Water consumption by mice in the 10,000 ppm groups was less than that by the controls.

At concentrations of 3,300 and 10,000 ppm, urethane induced lymphoid depletion in the mandibular and mesenteric lymph nodes, spleen, and thymus of male and female mice; lymphoid depletion in the mesenteric lymph node and spleen was also observed in females in the 1,100 ppm group. Urethane induced bone marrow cell depletion in male and female mice in the 10,000 ppm groups and in females in the 1,100 and 3,000 ppm groups. The lymphoid tissues have been described as sites for urethane carcinogenesis in Swiss mice administered 0.4% urethane in drinking water (Toth *et al.*, 1961).

Females were also more susceptible to the induction by urethane of renal nephropathy, with greater incidences of this lesion observed in females than in males administered 1,100 ppm. The induction of nephropathy by urethane has not been reported in the literature. The present study also showed that urethane at concentrations of 1,100 and 3,300 ppm induced cardiomyopathy in male and female mice; induction of this lesion by urethane has not been reported in the literature.

Urethane induced concentration-related increases in the incidences of lung inflammation in mice administered 1,100 ppm or greater and alveolar epithelial hyperplasia in mice in the 330 and 1,100 ppm groups. The cause of lung inflammation is unknown, although it is very likely related to urethane toxicity. Bronchiole hyperplasia was observed in mice in the 3,300 and 10,000 ppm groups, all of which died early; this lesion may be the result of an early regenerative response to the site-specific bronchiolar toxicity of urethane. The morphology of the bronchiole hyperplasia was consistent with hyperplastic lesions described by Rehm and Kelloff (1991) following intratracheal instillation of 3-methylcholanthrene in $B6C3F_1$ mice; these mice were reported to undergo metaplastic change followed by development of squamous cell and adenosquamous carcinomas. In the current study, one alveolar/bronchiolar adenoma occurred in a male mouse in the 330 ppm group. Alveolar and bronchiole hyperplasia as well as alveolar/bronchiolar adenoma rarely occur in $B6C3F_1$ mice in the NTP 13-week studies. These findings show that the lung is a target of urethane toxicity in $B6C3F_1$ mice. Urethane has also been tested in other mouse strains that are more sensitive to pulmonary neoplasm induction than $B6C3F_1$ mice. Kristiansen *et al.* (1990) reported that 200 ppm urethane in drinking water induced a 100% incidence of pulmonary neoplasms in 6.5-week-old female A/Ph mice after 12 weeks; the control incidence in this study was 40%. In a study by Altmann *et al.* (1991), 65% of a group of 10-week-old female NMRI mice receiving daily gavage doses of 18 mg/kg urethane developed lung neoplasms after 8 weeks; 21% of the control females developed lung neoplasms.

Epididymal spermatozoal motility and concentration were lower in male mice in the 1,100 ppm group than in the controls; females administered 1,100 ppm urethane had ovarian atrophy and a longer diestrus than the controls. In females administered 3,300 or 10,000 ppm, urethane induced ovarian follicle degeneration.

Urethane in 5% Ethanol

RATS

Two males and all females in the 10,000 ppm groups died before the end of the study. The final mean body weight of the surviving male rats in the 10,000 ppm group was lower than that of the controls; males and females administered 3,300 ppm also had slightly lower final mean body weights than those of the controls. Water consumption decreased as the urethane concentration increased.

In general, the relative right kidney, liver, and lung weights of exposed males and females and the relative right testis weight of exposed males were greater than those of the controls. Urethane in 5%

ethanol enhanced the severity of nephropathy in males in the 3,300 and 10,000 ppm groups and enhanced the incidence and severity of nephropathy in females in the 3,300 ppm group. Clear cell foci were observed in the liver of one male and fatty change was observed in eight males in the 10,000 ppm group; these lesions did not occur in females. The severity of cardiomyopathy was enhanced in males administered 10,000 ppm but was not affected by urethane administration in females.

Leukopenia and lymphopenia were observed in all exposed groups of males and females except for males administered 110 ppm; the severity was not related to urethane concentration. As in the study of urethane in drinking water, leukopenia was mainly due to lymphopenia. The lymphopenia may have been the result of cellular depletion in the spleen, lymph nodes, and bone marrow.

Epididymal spermatozoal motility and concentration were significantly ($P \leq 0.05$) lower in exposed males than in the controls. The estrous cycle of females in the 3,300 ppm group was lengthened.

MICE

All male and female mice in the 10,000 ppm groups died during Week 3. Additionally, two control females, one male in the 110 ppm group, one male and one female in the 330 ppm groups, and one male and four females in the 3,300 ppm groups died early. The final mean body weights of mice receiving 1,100 or 3,300 ppm were less than those of the controls.

The relative right kidney, liver, and lung weights of male and female mice generally increased with increasing exposure concentration. Urethane induced nephropathy in males and females, particularly in the 3,300 ppm groups. Lymphoid depletion occurred in the 3,300 and 10,000 ppm groups, and the incidences were greater in females than in males.

Urethane in 5% ethanol also induced lung inflammation at concentrations of 3,300 and 10,000 ppm. The incidence of alveolar epithelial hyperplasia was also greater in exposed males and females than in the controls. Alveolar/bronchiolar adenomas occurred in one male each in the 110 and 1,100 ppm groups, two males in the 3,300 ppm group, and one female in the 330 ppm group; this neoplasm did not occur in the control mice.

Urethane in 5% ethanol also had effects on the reproductive system. In exposed male mice, epididymal spermatozoal motility was significantly lower than that in the controls, and left epididymal, cauda epididymal, and testis weights were lower than those of the controls. All females administered

1,100 or 3,300 ppm had ovarian atrophy; this lesion has been reported to predispose the ovary to neoplastic development (Maronpot, 1987). Females in the 3,300 ppm group also had a lengthened diestrus.

Comparison Between the Studies of Urethane in Drinking Water and Urethane in 5% Ethanol

RATS

The survival rates, body weight gains, hematology and clinical chemistry results, and nephropathy and cardiomyopathy incidences and severity were similar between control groups in the two rat studies.

At a concentration of 10,000 ppm, urethane appeared to be more toxic to females when administered in 5% ethanol than when administered in drinking water; all females administered 10,000 ppm urethane in 5% ethanol died by Week 6, compared to only four females administered 10,000 ppm in drinking water dying during Week 13. This effect of urethane in 5% ethanol on mortality was not apparent in the lower exposure groups of females or in any exposed groups of males.

At each urethane concentration, the final mean body weights of survivors in each study were similar. Relative right kidney, liver, lung, and right testis weights were greater in exposed male and female rats receiving urethane in drinking water or in 5% ethanol than in the controls; however, there were no differences in organ weights between rats receiving urethane in water and rats receiving urethane in 5% ethanol at any exposure concentration.

At each exposure concentration, rats administered urethane in 5% ethanol consumed less fluid, and therefore urethane, than rats receiving the same concentration of urethane in drinking water. This may explain the lower mortality of males administered 10,000 ppm urethane in 5% ethanol compared to males administered the same concentration in water. However, more female rats in the 10,000 ppm group in the 5% ethanol study died than females administered the same concentration in the drinking water study, in spite of the lower fluid consumption, and thereby urethane consumption, by females in the 5% ethanol study; the reason for this greater mortality is not clear.

Concentration-related reductions in leukocyte and lymphocyte counts were observed in males and females in each study; however, the differences from controls in the 5% ethanol study appeared to be less. This may also be related to the lower fluid consumption and ethanol intake by rats in the 5% ethanol study.

Urethane enhanced the severity of cardiomyopathy in male rats that received 10,000 ppm and female rats that received 3,300 or 10,000 ppm in the drinking water study. However, 5% ethanol appeared to reduce the severity of cardiomyopathy induced by urethane in female rats but not in male rats. Ethanol generally reduced the severity of urethane-induced nephropathy in female rats but not in male rats. Clear cell foci were induced in male and female rats administered 3,300 or 10,000 ppm urethane in drinking water, but these lesions were only observed in one male rat administered 10,000 ppm urethane in 5% ethanol. It is very likely that the reduced incidences and severity of cardiomyopathy, nephropathy, and liver clear cell foci in rats in the 5% ethanol study compared to the drinking water study were related to the lower urethane consumption by rats in the ethanol study.

Urethane in drinking water or 5% ethanol also induced lymphoid depletion in the mandibular and mesenteric lymph nodes and spleen and induced bone marrow cell depletion in male and female rats. Lymphoid depletion occurred at higher exposure concentrations (3,300 and 10,000 ppm) in rats in the 5% ethanol study than in the drinking water study (1,100 ppm or greater). Again, this effect may be partially explained by the result of the lower fluid, and therefore urethane, consumption by rats in the 5% ethanol groups.

Urethane in drinking water or 5% ethanol also reduced epididymal spermatozoal motility and concentration in male rats; ethanol did not appear to enhance the effect. Urethane in 5% ethanol lengthened the estrous cycle of females rats in the 3,300 ppm group; 10,000 ppm urethane in drinking water also slightly lengthened the estrous cycle.

MICE

The survival and body weight gains of control male and female mice in the drinking water study were similar to those of the controls in the 5% ethanol study. In each study, two female control mice died of unknown causes during Weeks 12 and 13.

According to calculations based on fluid consumption, exposed males and females in the 5% ethanol study drank less fluid, and thus received less urethane, than mice administered the same concentration of urethane in drinking water; however, the fluid consumption measurements recorded only fluid remaining in the bottles and not actual fluid consumption by the mice. The survival rates of males and females receiving 3,300 ppm urethane in 5% ethanol were greater than those of males and females receiving the same concentration of urethane in drinking water, presumably because urethane consumption in the 5% ethanol study was lower. The lower urethane consumption by mice in the ethanol study is also reflected by the generally greater weight gains of mice administered 110, 3,300, or 1,100 ppm in ethanol compared to mice receiving the same urethane concentrations in drinking water.

At concentrations of 3,300 and 10,000 ppm in drinking water or 5% ethanol, urethane induced lymphoid depletion in the mandibular and mesenteric lymph nodes, spleen, and thymus of male and female mice; however, among mice administered 1,100 ppm urethane, lymphoid depletion occurred only in female mice in the drinking water study. Nephropathy was observed in female mice in the 1,100 ppm group in the drinking water study but not in females administered the same concentration of urethane in 5% ethanol; this absence of nephropathy in the females receiving 5% ethanol may be related to the lower urethane intake as a result of lower fluid consumption by these females. Urethane in drinking water or 5% ethanol also decreased epididymal spermatozoal concentration and motility in male mice; the effect did not seem enhanced by the 5% ethanol vehicle. If 5% ethanol had any effect on urethane toxicity in the male reproductive system, the effect may have been masked due to the lower fluid, and therefore urethane, consumption by mice in the 5% ethanol study. Urethane in drinking water or in 5% ethanol also lengthened diestrus in females.

The 5% ethanol vehicle appeared to enhance urethane-induced ovarian atrophy. The pathway of ovarian atrophy induction by urethane is not clear. Ethanol may facilitate the effect of urethane on the ovary by disrupting the pituitary-gonadal axis (Taggart Davis, 1977). Ovarian atrophy has been shown to predispose the organ to neoplastic development (Maronpot, 1987). Long-term exposure to high doses of urethane in ethanol may enhance ovarian carcinogenesis.

After 13 weeks of continued urethane administration in drinking water or 5% ethanol, plasma levels of urethane were four times greater in mice in the 5% ethanol study than in the drinking water study. The time required to eliminate the plasma urethane was greater for mice receiving ethanol than for mice receiving drinking water. Yamamoto *et al.* (1988) demonstrated that the concentration of urethane in the blood did not begin to decrease in male mice after a single dose of urethane in ethanol until the blood ethanol concentration fell below 1.5 mg/mL. The presence of ethanol in the circulation may have inhibited urethane metabolism in male mice. Toxicokinetics data for female mice were not available.

Inhibition of urethane metabolism by 5% ethanol in mice appeared to promote lung carcinogenesis. Urethane administered in drinking water induced lung inflammation and alveolar epithelial hyperplasia; the incidences of these lesions increased with increasing exposure concentration. The 5% ethanol vehicle appeared to enhance the induction of alveolar epithelial hyperplasia and alveolar/bronchiolar adenoma in exposed male and female mice in spite of the lower consumption of urethane by these mice compared to mice in the drinking water study. In male and female mice receiving 1,100 ppm urethane in 5% ethanol, the incidences of alveolar epithelial hyperplasia and alveolar/bronchiolar adenoma were greater than those in mice receiving the same concentration of urethane in drinking water. The incidences of these lesions in female mice in the 330 ppm group and male mice in the 110 ppm group in the 5% ethanol study were also greater than the incidences in mice administered the same urethane concentrations in drinking water. The greater incidences of alveolar epithelial hyperplasia and alveolar/bronchiolar adenoma in males and females in the 3,300 ppm groups in the 5% ethanol study may have been due to the longer survival time of these mice compared with mice in the drinking water study; nevertheless, an increase in incidence with increasing urethane concentration is evident in the 5% ethanol study. However, the increases are not statistically significant due to the small number of mice in each exposure group.

The effects of ethanol on lung carcinogenesis in mice remain unclear; results reported in the literature are conflicting. Stoewsand *et al.* (1991) reported that 12% ethanol in drinking water inhibited urethane-induced lung Clara cell and alveolar adenoma development (as determined by incidences and number of lesions per mouse) in male C3H mice. Female A/Ph mice administered 200 to 1,000 ppm urethane in drinking water or in 5%, 10%, or 20% ethanol had a pulmonary neoplasm incidence of 100% (Kristiansen *et al.*, 1990); however, the number of neoplasms per mouse was lower in mice administered urethane in 10% or 20% ethanol than in mice administered urethane in drinking water. The 5% ethanol vehicle had no effect on the number of lung neoplasms per mouse. However, Altmann *et al.* (1991) reported that in female NMRI mice administered 18, 36, 90, or

180 mg/kg urethane by gavage daily for 8 weeks, lung adenoma formation (incidence and number per mouse) were similar between mice administered urethane in 20% ethanol and mice administered urethane in water.

In the present studies, the frequency of micronuclei induced in the peripheral blood of male and female mice after 13 weeks of exposure to urethane were similar between animals receiving the drinking water vehicle and those receiving the 5% ethanol vehicle. Choy *et al.* (1995) demonstrated that a high dose of ethanol (2,500 to 3,500 mg/kg) coadministered intraperitoneally with urethane suppressed micronuclei induction in male CD-1 mice. At a lower dose (1,250 to 2,250 mg/kg), ethanol appeared to stimulate micronuclei induction, but the results were not conclusive.

The data in the present studies showed that the daily fluid consumption at each concentration of urethane in water was different from the fluid consumption at the same concentration of urethane in 5% ethanol. This, in turn, affected the daily consumption of urethane by each exposed group. In the 3,300 ppm groups, male and female mice in the drinking water study had a higher fluid intake and, in turn, a higher urethane intake than mice in the 5% ethanol study. This led to a higher mortality rate in mice in the drinking water study. In the 1,100 ppm groups, the fluid, and therefore urethane, intake by male mice in the drinking water study was similar to that by males receiving urethane in ethanol. The fluid intake by females in the 1,100 ppm group in the drinking water study appeared higher than that by females in the ethanol study; however, the incidences of alveolar epithelial hyperplasia and alveolar/bronchiolar adenoma in males and females were greater in the 5% ethanol study than in the drinking water study. In the 330 ppm groups, urethane intake appeared similar between the mice in the two studies, and yet the incidences of alveolar epithelial hyperplasia and alveolar/bronchiolar adenoma were greater in the 5% ethanol study than in the drinking water study.

Urethane has been shown to be hydrolyzed by microsomal esterase to ethanol, carbon dioxide, and ammonia in mice, but a small amount of urethane (approximately 6%) is metabolized by P_{450}2E1 to *N*-hydroxyethyl carbamate, *N*-hydroxyvinyl carbamate, and epoxyethyl carbamate. The vinyl carbamate and its epoxy derivative may be the proximate and ultimate electrophilic metabolites responsible for genotoxicity and carcinogenicity (Gupta and Dani, 1989). P_{450}2E1 has been shown to be inducible by ethanol (Lieber, 1988). It is possible that ethanol delays urethane metabolism by inhibiting esterase activity. The resulting effective internal dose of urethane available for activation by P_{450}2E1 may become greater, producing more of the proximate and ultimate electrophilic metabolites and eventually enhancing carcinogenesis. This may be especially true during chronic

ethanol exposure, as P_{450}2E1 activity may be elevated. Further studies may clarify the effects of ethanol on urethane metabolism and carcinogenesis.

Immature mice are more sensitive to the induction of lung neoplasms by urethane than mature mice. Kaye (1960) reported that the concentration of urethane in the blood of 13-day-old Swiss mice was higher than that of 6-month-old Swiss mice administered an identical (0.75 mg/kg) intraperitoneal injection of urethane. The 13-day-old mice required 20 hours, compared to 12.5 hours for the older mice, to completely eliminate urethane from the blood. The sensitivity of the younger mice to lung carcinogenesis by urethane was attributed to this low catabolic rate. The present studies also show that urethane coadministered with ethanol is retained longer in the blood of mice than urethane administered in drinking water. It remains to be determined whether ethanol enhances the conversion of urethane to its ultimate electrophilic metabolites and the formation of DNA adducts; the induction of P_{450}2E1 by ethanol has been reported (Lieber, 1988).

In summary, concentrations of 1,100 ppm urethane or greater induced lymphoid and bone marrow cell depletion and hepatocellular lesions and increased the severity of nephropathy and cardiomyopathy in male and female rats. The lethal effects of 10,000 ppm urethane were slightly exacerbated by 5% ethanol in female rats. Concentrations of 1,100 ppm urethane or greater in drinking water induced lung inflammation, alveolar and bronchiolar epithelial hyperplasia, alveolar/bronchiolar adenoma, nephropathy, cardiomyopathy, lymphoid and bone marrow cell depletion, seminiferous tubule degeneration, and ovarian atrophy and follicular degeneration in mice. In female mice, 5% ethanol exacerbated ovarian atrophy. Mice administered urethane in 5% ethanol consumed less fluid, and therefore less urethane, than mice receiving urethane in drinking water. Coadministration of urethane and 5% ethanol inhibited the clearance of urethane from plasma. The incidences and severity of alveolar epithelial hyperplasia and alveolar/bronchiolar adenoma appeared to be slightly enhanced in mice receiving urethane in 5% ethanol compared to mice receiving urethane in drinking water. However, administration of urethane in 5% ethanol for 13 weeks did not enhance the frequency of micronucleated erythrocytes induced in the peripheral blood of male or female mice.

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APPENDIX A

Summary of Nonneoplastic Lesions in Rats

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Study of Urethane in Drinking Water[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental death						1
Natural deaths						6
Survivors						
Terminal sacrifice	10	10	10	10	10	3
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(9)	(10)	(10)	(10)
Clear cell focus					2 (20%)	1 (10%)
Congestion	1 (10%)					1 (10%)
Fatty change			1 (11%)			5 (50%)
Hepatodiaphragmatic nodule	1 (10%)	2 (20%)			1 (10%)	2 (20%)
Infiltration cellular, mononuclear cell			1 (11%)			
Thrombosis						1 (10%)
Mesentery			(1)			
Fat, necrosis			1 (100%)			
Pancreas	(10)				(10)	(9)
Acinus, atrophy	2 (20%)					
Salivary glands	(10)				(10)	(10)
Cytoplasmic alteration						1 (10%)
Stomach, forestomach	(10)				(10)	(10)
Hyperplasia, squamous	1 (10%)				2 (20%)	
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Cardiomyopathy	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Atrium, thrombosis						2 (20%)
Endocrine System						
Pituitary gland	(10)				(10)	(10)
Pars distalis, cyst	1 (10%)				1 (10%)	
General Body System						
None						
Genital System						
Preputial gland	(10)				(10)	(9)
Inflammation, chronic	1 (10%)					
Prostate	(10)			(1)	(10)	(10)
Concretion	3 (30%)				3 (30%)	
Inflammation, chronic	1 (10%)					1 (10%)
Testes	(10)				(10)	(10)
Seminiferous tubule, degeneration					2 (20%)	

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Study of Urethane in Drinking Water (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular						5 (50%)
Myelofibrosis				1 (10%)		
Lymph node				(1)		
Pancreatic, infiltration cellular, histiocyte				1 (100%)		
Lymph node, mandibular	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					1 (10%)	5 (50%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(8)
Depletion lymphoid						6 (75%)
Hyperplasia, lymphoid	1 (10%)			2 (20%)		
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Congestion						1 (10%)
Depletion lymphoid				7 (70%)	10 (100%)	9 (90%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid						2 (20%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Foreign body	1 (10%)	1 (10%)				
Hemorrhage		1 (10%)				
Inflammation, chronic	5 (50%)	2 (20%)		1 (10%)		
Nose	(10)				(10)	(10)
Inflammation, chronic	3 (30%)				3 (30%)	
Glands, inflammation, acute	2 (20%)				1 (10%)	
Special Senses System						
Eye		(1)				
Conjunctiva, inflammation, chronic active		1 (100%)				
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Nephropathy	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (90%)
Urinary bladder	(10)			(1)	(10)	(10)
Concretion				1 (100%)	1 (10%)	

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Study of Urethane in Drinking Water[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental deaths					1	4
Survivors						
Terminal sacrifice	10	10	10	10	9	6
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine large, colon	(10)				(10)	(10)
Inflammation, chronic	2 (20%)					
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Basophilic focus			1 (10%)	1 (10%)		
Clear cell focus				1 (10%)	6 (60%)	3 (30%)
Hepatodiaphragmatic nodule		1 (10%)	3 (30%)		1 (10%)	
Infarct					1 (10%)	
Pancreas	(10)				(10)	(10)
Acinus, atrophy	1 (10%)					1 (10%)
Stomach, forestomach	(10)				(10)	(10)
Hyperplasia, squamous	1 (10%)					2 (20%)
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Cardiomyopathy	4 (40%)	6 (60%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Endocrine System						
Pituitary gland	(10)				(10)	(10)
Pars distalis, cyst	1 (10%)					
Thyroid gland	(10)				(10)	(10)
Ultimobranchial cyst					1 (10%)	
General Body System						
None						
Genital System						
Ovary	(10)			(2)	(10)	(10)
Cyst				2 (100%)	1 (10%)	1 (10%)

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Study of Urethane in Drinking Water (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular					1 (10%)	9 (90%)
Myelofibrosis	1 (10%)					
Lymph node, mandibular	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					1 (10%)	8 (80%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					1 (10%)	5 (50%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid				3 (30%)	10 (100%)	10 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid						1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic	1 (10%)					
Nose	(10)				(10)	(10)
Inflammation, chronic	2 (20%)					
Trachea	(10)				(10)	(10)
Glands, ectasia	1 (10%)					1 (10%)
Special Senses System						
Eye		(2)		(1)		
Inflammation, chronic active		1 (50%)		1 (100%)		
Posterior chamber, hemorrhage		1 (50%)				
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic			1 (10%)			
Nephropathy		1 (10%)	1 (10%)	4 (40%)	8 (80%)	10 (100%)
Cortex, mineralization	7 (70%)	7 (70%)	7 (70%)	7 (70%)	5 (50%)	8 (80%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund sacrifice						2
Survivors						
Terminal sacrifice	10	10	10	10	10	8
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Clear cell focus						1 (10%)
Fatty change						8 (80%)
Hepatodiaphragmatic nodule	1 (10%)	1 (10%)		1 (10%)		
Bile duct, hyperplasia		1 (10%)				
Pancreas	(10)				(10)	(10)
Acinus, atrophy	1 (10%)				1 (10%)	1 (10%)
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Cardiomyopathy	9 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (90%)
Endocrine System						
Thyroid gland	(10)				(10)	(10)
Ultimobranchial cyst					1 (10%)	
General Body System						
None						
Genital System						
Epididymis	(10)				(10)	(10)
Aspermia	1 (10%)					
Dilatation	1 (10%)					
Inflammation, chronic	1 (10%)					
Preputial gland	(10)				(10)	(10)
Inflammation, chronic	1 (10%)					1 (10%)
Prostate	(10)				(10)	(10)
Inflammation, acute	1 (10%)					1 (10%)
Inflammation, chronic	1 (10%)					
Testes	(10)				(10)	(10)
Seminiferous tubule, degeneration	2 (20%)				1 (10%)	

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular						2 (20%)
Lymph node, mandibular	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					3 (30%)	10 (100%)
Lymph node, mesenteric	(10)	(9)	(10)	(10)	(10)	(10)
Depletion lymphoid					2 (20%)	8 (80%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					10 (100%)	10 (100%)
Thymus	(9)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid						1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)				(10)	(10)
Hemorrhage					1 (10%)	
Nose	(10)				(10)	(10)
Foreign body	2 (20%)					
Inflammation, acute	1 (10%)					
Inflammation, chronic					1 (10%)	
Glands, hyperplasia	1 (10%)					
Glands, inflammation, acute	1 (10%)					
Special Senses System						
Eye		(1)				
Inflammation, chronic active		1 (100%)				
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Nephropathy	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Urinary bladder	(10)	(1)	(1)		(10)	(10)
Concretion		1 (100%)	1 (100%)			1 (10%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund sacrifice						10
Survivors						
Terminal sacrifice	10	10	10	10	10	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(9)	(8)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	4 (40%)	2 (22%)	1 (13%)			1 (10%)
Mesentery					(1)	
Foreign body					1 (100%)	
Granuloma					1 (100%)	
Pancreas	(10)				(10)	(10)
Inflammation, chronic	1 (10%)					
Stomach, forestomach	(10)				(10)	(10)
Hyperplasia, squamous						1 (10%)
Stomach, glandular	(10)				(10)	(10)
Granuloma	1 (10%)					
Cardiovascular System						
Heart	(10)				(10)	(10)
Cardiomyopathy	5 (50%)				6 (60%)	
Endocrine System						
Pituitary gland	(10)		(1)		(10)	(10)
Pars distalis, cyst					1 (10%)	
Thyroid gland	(10)				(10)	(10)
Ultimobranchial cyst						1 (10%)
General Body System						
None						
Genital System						
Clitoral gland	(10)			(1)	(10)	(10)
Cyst				1 (100%)		
Ovary	(10)	(1)	(2)	(2)	(10)	(10)
Cyst		1 (100%)	1 (50%)	2 (100%)	1 (10%)	
Ovotestis			1 (50%)			

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular						10 (100%)
Lymph node					(1)	
Pancreatic, hyperplasia, lymphoid					1 (100%)	
Lymph node, mandibular	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					3 (30%)	10 (100%)
Lymph node, mesenteric	(10)	(9)	(10)	(10)	(10)	(10)
Depletion lymphoid					2 (20%)	10 (100%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					9 (90%)	10 (100%)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid						1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)			(2)	(10)	(10)
Inflammation, chronic				2 (100%)		
Nose	(10)				(10)	(10)
Glands, inflammation, acute	1 (10%)					
Special Senses System						
Eye		(1)				
Inflammation, chronic active		1 (100%)				
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Nephropathy	4 (40%)	6 (60%)	3 (30%)	5 (50%)	10 (100%)	5 (50%)
Cortex, mineralization	9 (90%)	9 (90%)	7 (70%)	10 (100%)	10 (100%)	8 (80%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

APPENDIX B

Summary of Nonneoplastic Lesions in Mice

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Moribund sacrifice					10	3
Natural deaths						7
Survivors						
Terminal sacrifice	10	10	10	10		
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Congestion					1 (10%)	
Cytoplasmic alteration			2 (20%)	1 (10%)	10 (100%)	10 (100%)
Eosinophilic focus					1 (10%)	
Fatty change		1 (10%)				
Pancreas	(10)			(10)	(10)	(10)
Inflammation, chronic				1 (10%)		
Stomach, glandular	(10)			(10)	(10)	(10)
Mineralization				1 (10%)		
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Cardiomyopathy				1 (10%)	3 (30%)	
Hemorrhage					1 (10%)	9 (90%)
Mineralization					3 (30%)	3 (30%)
Endocrine System						
Adrenal cortex	(10)			(10)	(10)	(10)
Capsule, hyperplasia	6 (60%)			1 (10%)	1 (10%)	
Islets, pancreatic	(4)	(10)	(10)	(4)	(9)	(10)
Angiectasis						5 (50%)
General Body System						
None						
Genital System						
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Seminiferous tubule, degeneration						5 (50%)

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular						10 (100%)
Lymph node, mandibular	(10)	(10)	(10)	(10)	(10)	(8)
Depletion lymphoid					8 (80%)	8 (100%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(8)
Depletion lymphoid					7 (70%)	8 (100%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					9 (90%)	10 (100%)
Thymus	(9)	(10)	(10)	(10)	(7)	(7)
Cyst	1 (11%)					
Depletion lymphoid					7 (100%)	7 (100%)
Integumentary System						
Skin	(10)			(10)	(10)	(10)
Subcutaneous tissue, congestion						1 (10%)
Musculoskeletal System						
None						
Nervous System						
Brain	(10)			(10)	(10)	(10)
Hemorrhage					1 (10%)	2 (20%)
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Hemorrhage	1 (10%)			1 (10%)		1 (10%)
Inflammation				4 (40%)	10 (100%)	10 (100%)
Inflammation, chronic			1 (10%)			
Alveolar epithelium, hyperplasia			3 (30%)	1 (10%)		
Bronchiole, hyperplasia					10 (100%)	1 (10%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Cyst	1 (10%)					
Nephropathy	1 (10%)	3 (30%)		3 (30%)	3 (30%)	9 (90%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental death	1					
Moribund sacrifice					8	7
Natural deaths	1				2	3
Survivors						
Terminal sacrifice	8	10	10	10		
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(9)
Cytoplasmic alteration				7 (70%)	10 (100%)	9 (100%)
Stomach, forestomach	(10)			(10)	(10)	(10)
Epithelium, hyperplasia						1 (10%)
Stomach, glandular	(10)			(10)	(10)	(10)
Hemorrhage						1 (10%)
Mineralization				1 (10%)	2 (20%)	1 (10%)
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Cardiomyopathy				3 (30%)	1 (10%)	
Hemorrhage					2 (20%)	6 (60%)
Mineralization					3 (30%)	5 (50%)
Endocrine System						
Adrenal cortex	(10)			(10)	(10)	(10)
Capsule, hyperplasia	10 (100%)			6 (60%)	5 (50%)	
Islets, pancreatic	(5)	(10)	(10)	(7)	(7)	(9)
Angiectasis						4 (44%)
Parathyroid gland	(7)			(6)	(9)	(7)
Ectopic thymus	1 (14%)					
Thyroid gland	(9)			(10)	(10)	(10)
Follicular cell, hyperplasia				1 (10%)		
General Body System						
None						
Genital System						
Ovary	(10)	(10)	(10)	(10)	(10)	(10)
Atrophy				7 (70%)		
Follicle, degeneration					9 (90%)	8 (80%)

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular				1 (10%)	2 (20%)	8 (80%)
Myelofibrosis	1 (10%)					
Lymph node, mandibular	(10)	(10)	(10)	(9)	(8)	(10)
Depletion lymphoid					8 (100%)	10 (100%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(9)	(7)
Depletion lymphoid				2 (20%)	8 (89%)	7 (100%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					8 (80%)	9 (90%)
Thymus	(10)	(10)	(10)	(9)	(8)	(7)
Cyst				1 (11%)		
Depletion lymphoid				1 (11%)	8 (100%)	7 (100%)
Integumentary System						
Mammary gland	(10)			(10)	(10)	(9)
Galactocele						1 (11%)
Skin	(10)			(10)	(10)	(10)
Subcutaneous tissue, hemorrhage						1 (10%)
Musculoskeletal System						
None						
Nervous System						
Brain	(10)			(10)	(10)	(10)
Hemorrhage						2 (20%)
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Congestion	1 (10%)					
Hemorrhage				1 (10%)	1 (10%)	3 (30%)
Inflammation				7 (70%)	10 (100%)	10 (100%)
Alveolar epithelium, hyperplasia				4 (40%)		
Bronchiole, hyperplasia					5 (50%)	3 (30%)
Nose	(10)			(10)	(10)	(10)
Glands, inflammation, acute	2 (20%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic	1 (10%)					
Nephropathy			1 (10%)	8 (80%)	2 (20%)	4 (40%)
Papilla, mineralization	1 (10%)					

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental death			1			
Moribund sacrifice						10
Natural deaths		1			1	
Survivors						
Terminal sacrifice	10	9	9	10	9	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Fatty change	2 (20%)					
Pancreas	(10)	(1)	(1)		(10)	(10)
Inflammation, chronic						1 (10%)
Stomach, forestomach	(10)	(1)	(1)		(10)	(10)
Inflammation, chronic	1 (10%)					
Stomach, glandular	(10)	(1)	(1)		(10)	(10)
Mineralization						4 (40%)
Cardiovascular System						
Heart	(10)	(1)	(1)	(10)	(10)	(10)
Hemorrhage				1 (10%)		
Endocrine System						
Adrenal cortex	(10)	(1)	(1)		(10)	(10)
Capsule, hyperplasia	4 (40%)		1 (100%)		1 (10%)	1 (10%)
Adrenal medulla	(10)	(1)	(1)		(9)	(10)
Hyperplasia	1 (10%)					
Parathyroid gland	(10)	(1)			(8)	(7)
Cyst	1 (10%)				1 (13%)	
Thyroid gland	(10)	(1)	(1)		(10)	(10)
Inflammation, chronic	1 (10%)					
General Body System						
None						
Genital System						
Prostate	(10)	(1)	(1)		(10)	(9)
Concretion					3 (30%)	
Testes	(10)	(1)	(1)		(10)	(10)
Cyst						1 (10%)

TABLE B3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Depletion cellular					1 (10%)	5 (50%)
Lymph node	(1)					
Inguinal, hyperplasia, lymphoid	1 (100%)					
Lymph node, mandibular	(10)	(10)	(10)	(9)	(10)	(10)
Depletion lymphoid					2 (20%)	3 (30%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid					3 (30%)	7 (70%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Depletion lymphoid						3 (30%)
Thymus	(10)	(10)	(10)	(10)	(9)	(9)
Depletion lymphoid					1 (11%)	8 (89%)
Ectopic parathyroid gland	1 (10%)					
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Foreign body					1 (10%)	
Hemorrhage	1 (10%)	2 (20%)	1 (10%)	1 (10%)	2 (20%)	1 (10%)
Inflammation				1 (10%)	7 (70%)	5 (50%)
Inflammation, chronic		1 (10%)	1 (10%)			
Alveolar epithelium, hyperplasia		1 (10%)	2 (20%)	3 (30%)	5 (50%)	
Trachea	(10)	(1)	(1)		(10)	(10)
Foreign body					1 (10%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Nephropathy	3 (30%)		1 (10%)	2 (20%)	9 (90%)	2 (20%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE B4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental deaths	2					
Moribund sacrifice					2	10
Natural deaths			1		2	
Survivors						
Terminal sacrifice	8	10	9	10	6	
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(9)	(10)	(10)	(10)
Cytoplasmic alteration			1 (11%)	6 (60%)	3 (30%)	9 (90%)
Fatty change	1 (10%)					
Inflammation, acute	1 (10%)					
Pancreas	(10)		(1)		(10)	(10)
Inflammation, acute	1 (10%)					
Inflammation, chronic	1 (10%)					1 (10%)
Acinus, atrophy	1 (10%)					
Stomach, glandular	(10)		(1)		(10)	(10)
Mineralization						2 (20%)
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Cardiomyopathy		1 (10%)			6 (60%)	
Mineralization					2 (20%)	
Endocrine System						
Adrenal cortex	(10)		(1)		(10)	(10)
Capsule, hyperplasia	10 (100%)		1 (100%)		8 (80%)	4 (40%)
Parathyroid gland	(9)				(9)	(7)
Cyst					1 (11%)	
General Body System						
None						
Genital System						
Ovary	(10)	(10)	(10)	(10)	(9)	(9)
Atrophy			1 (10%)	10 (100%)	9 (100%)	
Follicle, degeneration						5 (56%)
Uterus	(10)		(1)		(10)	(10)
Thrombosis					1 (10%)	

TABLE B4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
Hematopoietic System						
Bone marrow	(10)	(10)	(9)	(10)	(10)	(10)
Depletion cellular						9 (90%)
Lymph node, mandibular	(10)	(10)	(10)	(10)	(9)	(10)
Depletion lymphoid					4 (44%)	2 (20%)
Lymph node, mesenteric	(9)	(10)	(10)	(10)	(8)	(10)
Depletion lymphoid			1 (10%)		7 (88%)	7 (70%)
Spleen	(10)	(10)	(10)	(10)	(9)	(10)
Depletion lymphoid					6 (67%)	6 (60%)
Thymus	(10)	(10)	(10)	(10)	(8)	(10)
Depletion lymphoid					3 (38%)	9 (90%)
Ectopic parathyroid gland						1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Congestion	1 (10%)		1 (10%)			
Foreign body	1 (10%)		1 (10%)			
Hemorrhage	1 (10%)		1 (10%)	1 (10%)		1 (10%)
Inflammation				1 (10%)	8 (80%)	2 (20%)
Alveolar epithelium, hyperplasia			2 (20%)	7 (70%)	2 (20%)	
Nose	(10)		(1)		(10)	(10)
Congestion	1 (10%)		1 (100%)			
Foreign body	1 (10%)		1 (100%)			
Glands, inflammation, acute					1 (10%)	
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Casts	1 (10%)					
Nephropathy	1 (10%)		2 (20%)		10 (100%)	3 (30%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

APPENDIX C

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Study of Urethane in Drinking Water[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE						
n	10	10	10	10	10	3
Necropsy body wt	326 ± 5	315 ± 9	310 ± 4	313 ± 5	296 ± 6**	253 ± 3**
Heart						
Absolute	1.153 ± 0.028	1.211 ± 0.067	1.165 ± 0.030	1.218 ± 0.051	1.069 ± 0.031	0.888 ± 0.031**
Relative	3.54 ± 0.09	3.84 ± 0.18	3.76 ± 0.08	3.90 ± 0.16	3.63 ± 0.12	3.52 ± 0.16
Right kidney						
Absolute	1.238 ± 0.039	1.171 ± 0.041	1.223 ± 0.031	1.270 ± 0.043	1.297 ± 0.036	1.126 ± 0.028
Relative	3.79 ± 0.08	3.72 ± 0.06	3.95 ± 0.07	4.05 ± 0.08*	4.39 ± 0.09**	4.46 ± 0.15**
Liver						
Absolute	14.045 ± 0.483	12.977 ± 0.436	13.186 ± 0.279	14.560 ± 0.486	14.689 ± 0.351	13.308 ± 0.313
Relative	43.03 ± 0.94	41.22 ± 0.63	42.55 ± 0.57	46.44 ± 0.97**	49.76 ± 1.06**	52.72 ± 1.16**
Lungs						
Absolute	1.917 ± 0.054	1.843 ± 0.097	1.813 ± 0.092	1.759 ± 0.059	2.030 ± 0.080	1.653 ± 0.048
Relative	5.89 ± 0.15	5.88 ± 0.33	5.85 ± 0.29	5.62 ± 0.15	6.85 ± 0.17**	6.55 ± 0.27
Right testis						
Absolute	1.428 ± 0.021	1.427 ± 0.031	1.409 ± 0.016	1.429 ± 0.022	1.431 ± 0.029	1.422 ± 0.040
Relative	4.39 ± 0.07	4.55 ± 0.10	4.55 ± 0.07	4.57 ± 0.08	4.85 ± 0.06**	5.63 ± 0.14**
Thymus						
Absolute	0.337 ± 0.013	0.313 ± 0.009	0.286 ± 0.013*	0.312 ± 0.008*	0.246 ± 0.006**	0.253 ± 0.020**
Relative	1.04 ± 0.05	1.00 ± 0.03	0.93 ± 0.04	1.00 ± 0.02	0.84 ± 0.03**	1.00 ± 0.07*
FEMALE						
n	10	10	10	10	9	6
Necropsy body wt	190 ± 3	180 ± 3	189 ± 3	178 ± 3*	158 ± 3**	124 ± 5**
Heart						
Absolute	0.756 ± 0.024	0.714 ± 0.035	0.751 ± 0.018	0.724 ± 0.020	0.676 ± 0.023	0.629 ± 0.064**
Relative	3.98 ± 0.10	3.96 ± 0.18	3.96 ± 0.07	4.08 ± 0.12	4.31 ± 0.22	5.05 ± 0.45**
Right kidney						
Absolute	0.703 ± 0.014	0.691 ± 0.017	0.713 ± 0.020	0.713 ± 0.015	0.679 ± 0.019	0.625 ± 0.031*
Relative	3.71 ± 0.08	3.84 ± 0.08	3.77 ± 0.07	4.00 ± 0.07*	4.32 ± 0.16**	5.02 ± 0.18**
Liver						
Absolute	6.379 ± 0.324	6.317 ± 0.177	6.859 ± 0.148	7.109 ± 0.199	6.747 ± 0.178	5.035 ± 0.075**
Relative	33.59 ± 1.56	35.05 ± 0.75	36.28 ± 0.71	39.94 ± 0.97**	42.89 ± 1.42**	40.77 ± 1.78**
Lungs						
Absolute	1.192 ± 0.032	1.290 ± 0.033	1.264 ± 0.052	1.313 ± 0.050	1.433 ± 0.065**	1.162 ± 0.050
Relative	6.29 ± 0.18	7.18 ± 0.22	6.68 ± 0.26	7.39 ± 0.28**	9.09 ± 0.42**	9.36 ± 0.35**
Thymus						
Absolute	0.215 ± 0.009	0.229 ± 0.013	0.229 ± 0.011	0.211 ± 0.006	0.228 ± 0.023	0.116 ± 0.015**
Relative	1.13 ± 0.04	1.27 ± 0.07	1.21 ± 0.05	1.19 ± 0.03	1.45 ± 0.15*	0.93 ± 0.11

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE						
n	10	10	10	10	10	8
Necropsy body wt	312 ± 5	302 ± 5	307 ± 6	302 ± 5	296 ± 4*	214 ± 7**
Heart						
Absolute	1.204 ± 0.040	1.197 ± 0.038	1.140 ± 0.043	1.093 ± 0.042*	1.017 ± 0.022**	0.766 ± 0.032**
Relative	3.86 ± 0.11	3.97 ± 0.11	3.71 ± 0.14	3.62 ± 0.12	3.43 ± 0.04*	3.58 ± 0.12*
Right kidney						
Absolute	1.226 ± 0.025	1.159 ± 0.024	1.217 ± 0.035	1.206 ± 0.030	1.234 ± 0.025	0.999 ± 0.039**
Relative	3.93 ± 0.06	3.84 ± 0.07	3.96 ± 0.08	4.00 ± 0.08	4.16 ± 0.06*	4.67 ± 0.11**
Liver						
Absolute	13.325 ± 0.417	11.627 ± 0.321**	12.741 ± 0.339	13.637 ± 0.343	13.420 ± 0.249	10.996 ± 0.413**
Relative	42.63 ± 0.89	38.48 ± 0.59	41.46 ± 0.76	45.20 ± 0.71*	45.27 ± 0.52*	51.35 ± 0.88**
Lungs						
Absolute	1.940 ± 0.103	1.845 ± 0.074	1.814 ± 0.079	1.821 ± 0.071	2.211 ± 0.076	1.513 ± 0.077**
Relative	6.20 ± 0.28	6.10 ± 0.18	5.91 ± 0.23	6.04 ± 0.22	7.46 ± 0.22**	7.10 ± 0.38**
Right testis						
Absolute	1.375 ± 0.077	1.397 ± 0.029	1.416 ± 0.038	1.418 ± 0.029	1.405 ± 0.040	1.258 ± 0.041
Relative	4.40 ± 0.24	4.64 ± 0.12	4.60 ± 0.08	4.71 ± 0.10	4.75 ± 0.15	5.88 ± 0.12**
Thymus						
Absolute	0.279 ± 0.011	0.281 ± 0.016	0.266 ± 0.010	0.267 ± 0.010	0.273 ± 0.010	0.164 ± 0.018**
Relative	0.89 ± 0.03	0.93 ± 0.05	0.87 ± 0.03	0.88 ± 0.03	0.92 ± 0.04	0.76 ± 0.06
FEMALE						
n	10	10	10	10	10	0
Necropsy body wt	186 ± 2	190 ± 5	189 ± 2	186 ± 3	168 ± 2**	)
Heart						
Absolute	0.740 ± 0.019	0.787 ± 0.021	0.798 ± 0.022	0.742 ± 0.026	0.649 ± 0.018**	)
Relative	3.97 ± 0.09	4.16 ± 0.09	4.21 ± 0.10	4.00 ± 0.10	3.88 ± 0.12	)
Right kidney						
Absolute	0.703 ± 0.017	0.721 ± 0.020	0.698 ± 0.019	0.705 ± 0.020	0.699 ± 0.011	)
Relative	3.77 ± 0.07	3.80 ± 0.06	3.68 ± 0.08	3.80 ± 0.10	4.17 ± 0.04**	)
Liver						
Absolute	6.704 ± 0.150	7.045 ± 0.151	6.977 ± 0.177	7.561 ± 0.362*	6.207 ± 0.114	)
Relative	36.04 ± 0.73	37.17 ± 0.48	36.81 ± 0.70	40.91 ± 2.22*	37.02 ± 0.38	)
Lungs						
Absolute	1.213 ± 0.043	1.290 ± 0.078	1.272 ± 0.038	1.320 ± 0.073	1.330 ± 0.087	)
Relative	6.52 ± 0.23	6.78 ± 0.35	6.71 ± 0.18	7.13 ± 0.41	7.94 ± 0.52**	)
Thymus						
Absolute	0.210 ± 0.008	0.223 ± 0.008	0.222 ± 0.011	0.220 ± 0.010	0.193 ± 0.012	)
Relative	1.12 ± 0.03	1.18 ± 0.05	1.17 ± 0.05	1.18 ± 0.04	1.15 ± 0.07	)

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE						
n	10	10	10	10	0	0
Necropsy body wt	39.1 ± 0.7	39.3 ± 0.9	40.0 ± 0.8	29.4 ± 0.4**	)	)
Heart						
Absolute	0.188 ± 0.006	0.214 ± 0.016	0.208 ± 0.009	0.157 ± 0.007	)	)
Relative	4.80 ± 0.17	5.45 ± 0.38	5.20 ± 0.20	5.33 ± 0.19	)	)
Right kidney						
Absolute	0.288 ± 0.008	0.319 ± 0.006*	0.310 ± 0.009	0.265 ± 0.007	)	)
Relative	7.38 ± 0.16	8.14 ± 0.14*	7.73 ± 0.15*	9.00 ± 0.17**	)	)
Liver						
Absolute	1.758 ± 0.069	1.957 ± 0.062	1.902 ± 0.041	1.432 ± 0.048**	)	)
Relative	44.91 ± 1.50	49.74 ± 0.90*	47.59 ± 0.92	48.64 ± 1.24	)	)
Lungs						
Absolute	0.236 ± 0.013	0.293 ± 0.033	0.252 ± 0.010	0.261 ± 0.011	)	)
Relative	6.02 ± 0.27	7.41 ± 0.76	6.29 ± 0.22	8.86 ± 0.35**	)	)
Right testis						
Absolute	0.123 ± 0.002	0.129 ± 0.002	0.125 ± 0.003	0.121 ± 0.002	)	)
Relative	3.16 ± 0.05	3.29 ± 0.09	3.13 ± 0.08	4.12 ± 0.08**	)	)
Thymus						
Absolute	0.062 ± 0.004	0.057 ± 0.003	0.064 ± 0.006	0.044 ± 0.004*	)	)
Relative	1.58 ± 0.11	1.45 ± 0.08	1.57 ± 0.14	1.49 ± 0.12	)	)
FEMALE						
n	8	10	10	10	0	0
Necropsy body wt	33.1 ± 0.9	32.5 ± 0.7	32.0 ± 0.5	21.2 ± 0.7**	)	)
Heart						
Absolute	0.154 ± 0.007	0.156 ± 0.008	0.152 ± 0.004	0.137 ± 0.004	)	)
Relative	4.67 ± 0.25	4.80 ± 0.24	4.76 ± 0.13	6.53 ± 0.28**	)	)
Right kidney						
Absolute	0.191 ± 0.004	0.184 ± 0.007	0.189 ± 0.004	0.169 ± 0.004**	)	)
Relative	5.79 ± 0.13	5.66 ± 0.22	5.92 ± 0.12	8.04 ± 0.33**	)	)
Liver						
Absolute	1.414 ± 0.044	1.366 ± 0.039	1.387 ± 0.049	1.173 ± 0.034**	)	)
Relative	42.70 ± 0.47	42.13 ± 1.34	43.33 ± 1.37	55.82 ± 2.34**	)	)
Lungs						
Absolute	0.239 ± 0.016	0.228 ± 0.005	0.264 ± 0.023	0.249 ± 0.013	)	)
Relative	7.19 ± 0.38	7.04 ± 0.25	8.27 ± 0.74	11.75 ± 0.42**	)	)
Thymus						
Absolute	0.061 ± 0.003	0.058 ± 0.004	0.060 ± 0.003	0.031 ± 0.003**	)	)
Relative	1.86 ± 0.14	1.77 ± 0.11	1.89 ± 0.12	1.41 ± 0.13*	)	)

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE						
n	10	9	9	10	9	0
Necropsy body wt	41.6 ± 0.8	42.9 ± 0.9	41.1 ± 0.5	34.3 ± 0.4**	28.2 ± 0.4**	)
Heart						
Absolute	0.210 ± 0.007	0.215 ± 0.009	0.213 ± 0.007	0.192 ± 0.006	0.179 ± 0.009**	)
Relative	5.05 ± 0.15	5.01 ± 0.19	5.19 ± 0.17	5.62 ± 0.16*	6.34 ± 0.29**	)
Right kidney						
Absolute	0.323 ± 0.010	0.331 ± 0.010	0.336 ± 0.006	0.319 ± 0.012	0.306 ± 0.010	)
Relative	7.74 ± 0.13	7.73 ± 0.17	8.20 ± 0.21	9.30 ± 0.33**	10.84 ± 0.27**	)
Liver						
Absolute	2.081 ± 0.101	2.159 ± 0.107	1.917 ± 0.038	1.589 ± 0.033**	1.526 ± 0.059**	)
Relative	49.77 ± 1.59	50.12 ± 1.56	46.62 ± 0.74	46.43 ± 1.05	54.03 ± 1.38	)
Lungs						
Absolute	0.274 ± 0.009	0.274 ± 0.013	0.301 ± 0.017	0.282 ± 0.008	0.290 ± 0.010	)
Relative	6.57 ± 0.15	6.41 ± 0.35	7.32 ± 0.44	8.22 ± 0.21**	10.29 ± 0.34**	)
Right testis						
Absolute	0.130 ± 0.004	0.129 ± 0.005	0.131 ± 0.004	0.116 ± 0.006	0.119 ± 0.006	)
Relative	3.13 ± 0.11	3.00 ± 0.12	3.19 ± 0.11	3.38 ± 0.17	4.25 ± 0.22**	)
Thymus						
Absolute	0.058 ± 0.003	0.060 ± 0.006	0.054 ± 0.005	0.046 ± 0.004	0.042 ± 0.003*	)
Relative	1.39 ± 0.07	1.39 ± 0.14	1.31 ± 0.12	1.36 ± 0.12	1.48 ± 0.12	)
FEMALE						
n	8	10	9	10	6	0
Necropsy body wt	32.0 ± 0.6	34.9 ± 1.1	30.6 ± 0.9	26.2 ± 0.3**	20.8 ± 1.2**	)
Heart						
Absolute	0.157 ± 0.008	0.157 ± 0.006	0.153 ± 0.005	0.149 ± 0.005	0.143 ± 0.006	)
Relative	4.90 ± 0.24	4.53 ± 0.20	4.99 ± 0.15	5.72 ± 0.25*	6.99 ± 0.56**	)
Right kidney						
Absolute	0.194 ± 0.005	0.201 ± 0.009	0.193 ± 0.009	0.198 ± 0.005	0.197 ± 0.007	)
Relative	6.09 ± 0.19	5.77 ± 0.22	6.33 ± 0.28	7.54 ± 0.17**	9.56 ± 0.49**	)
Liver						
Absolute	1.441 ± 0.035	1.596 ± 0.039	1.420 ± 0.038	1.289 ± 0.032*	1.300 ± 0.066*	)
Relative	45.13 ± 1.25	45.86 ± 0.90	46.56 ± 1.30	49.19 ± 1.15	62.87 ± 3.04**	)
Lungs						
Absolute	0.255 ± 0.013	0.267 ± 0.013	0.251 ± 0.014	0.266 ± 0.011	0.250 ± 0.017	)
Relative	7.98 ± 0.41	7.62 ± 0.22	8.31 ± 0.60	10.14 ± 0.41**	12.02 ± 0.50**	)
Thymus						
Absolute	0.061 ± 0.007	0.066 ± 0.006	0.051 ± 0.003	0.050 ± 0.002	0.028 ± 0.005**	)
Relative	1.89 ± 0.21	1.87 ± 0.15	1.68 ± 0.11	1.90 ± 0.09	1.32 ± 0.18	)

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

APPENDIX D

Hematology and Clinical Chemistry Results

TABLE D1 Hematology Data for F344/N Rats in the 13-Week Study of Urethane in Drinking Water[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 13	10	10	9	10	10	6
Hematocrit (automated) (%)						
Day 3	46.1 ± 0.4	44.6 ± 1.1	45.4 ± 0.6	44.0 ± 0.3*	44.8 ± 0.4	47.0 ± 0.4
Day 23	53.8 ± 0.4	49.4 ± 0.3**	50.5 ± 0.5*	53.2 ± 1.3	51.7 ± 0.4	54.0 ± 1.4
Week 13	49.8 ± 0.6	51.4 ± 0.7	50.5 ± 0.4	50.6 ± 0.3	51.9 ± 0.5*	45.9 ± 2.2
Hematocrit (manual) (%)						
Day 3	43.5 ± 0.4	42.4 ± 0.9	43.0 ± 0.5	42.5 ± 0.6	43.0 ± 0.4	44.0 ± 0.4
Day 23	51.4 ± 0.6	48.4 ± 0.4*	49.0 ± 0.3	51.6 ± 1.3	49.9 ± 0.4	51.9 ± 1.3
Week 13	48.2 ± 0.6	49.4 ± 0.5	48.7 ± 0.5	48.8 ± 0.4	49.9 ± 0.6	43.3 ± 2.1
Hemoglobin (g/dL)						
Day 3	14.3 ± 0.1	13.9 ± 0.3	14.1 ± 0.2	13.8 ± 0.2	13.9 ± 0.1	14.6 ± 0.1
Day 23	17.2 ± 0.2	15.8 ± 0.1**	16.0 ± 0.2**	17.0 ± 0.4	16.4 ± 0.1	17.2 ± 0.4
Week 13	15.8 ± 0.2	16.6 ± 0.2*	16.2 ± 0.1	16.1 ± 0.1	16.6 ± 0.2*	14.2 ± 0.8
Erythrocytes (10^6/µL)						
Day 3	7.27 ± 0.08	7.12 ± 0.17	7.24 ± 0.10	6.97 ± 0.08	7.14 ± 0.09	7.45 ± 0.09
Day 23	8.91 ± 0.08	8.15 ± 0.06**	8.37 ± 0.10	8.97 ± 0.24	8.67 ± 0.07	9.14 ± 0.24
Week 13	9.47 ± 0.11	9.99 ± 0.15*	9.73 ± 0.10	9.70 ± 0.07	9.83 ± 0.10	8.25 ± 0.47
Reticulocytes (10^6/µL)						
Day 3	0.47 ± 0.02	0.46 ± 0.02	0.45 ± 0.03	0.44 ± 0.02	0.44 ± 0.02	0.48 ± 0.02
Day 23	0.29 ± 0.02	0.26 ± 0.01	0.27 ± 0.02	0.27 ± 0.01	0.21 ± 0.02**	0.20 ± 0.02**
Week 13	0.29 ± 0.01	0.28 ± 0.01	0.26 ± 0.02	0.24 ± 0.01	0.27 ± 0.02	0.36 ± 0.03
Nucleated erythrocytes (10^3/µL)						
Day 3	0.04 ± 0.02	0.06 ± 0.02	0.03 ± 0.01	0.02 ± 0.02	0.05 ± 0.02	0.04 ± 0.02
Day 23	0.04 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.04 ± 0.01
Week 13	0.03 ± 0.02	0.05 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.49 ± 0.19**
Mean cell volume (fL)						
Day 3	63.4 ± 0.3	62.7 ± 0.3	62.9 ± 0.4	63.1 ± 0.4	62.8 ± 0.4	63.0 ± 0.3
Day 23	60.4 ± 0.3	60.7 ± 0.4	60.4 ± 0.4	59.4 ± 0.4	59.7 ± 0.3	59.0 ± 0.4*
Week 13	52.6 ± 0.2	51.6 ± 0.2	52.0 ± 0.2	52.2 ± 0.2	52.8 ± 0.2	55.8 ± 0.6*
Mean cell hemoglobin (pg)						
Day 3	19.7 ± 0.1	19.5 ± 0.1	19.5 ± 0.1	19.8 ± 0.1	19.5 ± 0.2	19.6 ± 0.1
Day 23	19.3 ± 0.1	19.3 ± 0.2	19.2 ± 0.1	19.0 ± 0.2	19.0 ± 0.1*	18.9 ± 0.1**
Week 13	16.7 ± 0.1	16.6 ± 0.1	16.6 ± 0.1	16.6 ± 0.1	16.9 ± 0.1	17.2 ± 0.1*
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.0 ± 0.2	31.1 ± 0.1	31.1 ± 0.2	31.5 ± 0.4	31.2 ± 0.2	31.1 ± 0.2
Day 23	32.0 ± 0.1	31.9 ± 0.1	31.8 ± 0.1	32.0 ± 0.2	31.8 ± 0.1	31.9 ± 0.2
Week 13	31.8 ± 0.2	32.2 ± 0.1	32.0 ± 0.1	31.9 ± 0.2	32.0 ± 0.1	30.8 ± 0.4
Platelets (10^3/µL)						
Day 3	1,057 ± 22	1,012 ± 22	1,037 ± 35	1,071 ± 32	1,037 ± 29	1,043 ± 17
Day 23	972.0 ± 18.6	948.9 ± 17.2	928.1 ± 15.0	959.9 ± 15.0	949.8 ± 39.5	852.7 ± 37.4**
Week 13	755.4 ± 18.7	806.7 ± 12.7	812.6 ± 22.8	854.8 ± 24.3*	884.3 ± 16.0**	550.3 ± 49.8
Leukocytes (10^3/µL)						
Day 3	8.25 ± 0.22	8.79 ± 0.36	8.86 ± 0.50	8.64 ± 0.56	9.32 ± 0.58	8.62 ± 0.50
Day 23	9.34 ± 0.39	9.43 ± 0.27	9.20 ± 0.33	7.53 ± 0.37**	6.60 ± 0.29**	4.46 ± 0.21**
Week 13	10.40 ± 0.33	10.51 ± 0.30	8.66 ± 0.61*	7.51 ± 0.50**	7.13 ± 0.36**	4.62 ± 0.32**

TABLE D1 Hematology Data for F344/N Rats in the 13-Week Study of Urethane in Drinking Water (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE (continued)						
Segmented neutrophils (10^3/µL)						
Day 3	1.20 ± 0.12	1.50 ± 0.21	1.07 ± 0.11	1.38 ± 0.21	1.55 ± 0.20	1.35 ± 0.21
Day 23	1.18 ± 0.12	1.28 ± 0.10	1.36 ± 0.14	1.08 ± 0.10	1.37 ± 0.15	1.01 ± 0.11
Week 13	1.75 ± 0.13	2.09 ± 0.17	1.66 ± 0.24	1.50 ± 0.11	1.46 ± 0.08	1.24 ± 0.29
Lymphocytes (10^3/µL)						
Day 3	6.95 ± 0.21	7.18 ± 0.34	7.71 ± 0.56	7.20 ± 0.62	7.64 ± 0.43	7.19 ± 0.32
Day 23	8.05 ± 0.35	8.11 ± 0.25	7.72 ± 0.30	6.35 ± 0.30**	5.14 ± 0.25**	3.42 ± 0.24**
Week 13	8.50 ± 0.29	8.20 ± 0.35	6.84 ± 0.49*	5.89 ± 0.43**	5.51 ± 0.32**	3.27 ± 0.12**
Monocytes (10^3/µL)						
Day 3	0.10 ± 0.03	0.09 ± 0.03	0.07 ± 0.02	0.05 ± 0.02	0.11 ± 0.04	0.05 ± 0.02
Day 23	0.05 ± 0.02	0.02 ± 0.01	0.06 ± 0.02	0.07 ± 0.02	0.05 ± 0.02	0.03 ± 0.01
Week 13	0.11 ± 0.04	0.13 ± 0.05	0.10 ± 0.02	0.08 ± 0.03	0.12 ± 0.03	0.05 ± 0.02
Eosinophils (10^3/µL)						
Day 3	0.01 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
Day 23	0.06 ± 0.02	0.02 ± 0.01	0.06 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.01 ± 0.01
Week 13	0.04 ± 0.02	0.08 ± 0.04	0.06 ± 0.03	0.03 ± 0.01	0.04 ± 0.03	0.06 ± 0.01
FEMALE						
n	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 3	44.7 ± 0.4	44.6 ± 0.3	44.8 ± 0.4	45.2 ± 0.2	44.6 ± 0.4	46.9 ± 0.5**
Day 23	53.5 ± 1.9	48.2 ± 0.4	48.0 ± 0.5	48.4 ± 0.3	52.5 ± 1.2	56.1 ± 0.7
Week 13	47.1 ± 0.5	47.0 ± 0.4	48.2 ± 0.4	48.8 ± 0.5	51.3 ± 1.3**	50.4 ± 1.2*
Hematocrit (manual) (%)						
Day 3	43.7 ± 0.5	42.8 ± 0.3	42.9 ± 0.4	43.0 ± 0.2	42.4 ± 0.3	44.4 ± 0.4
Day 23	50.5 ± 1.5	46.2 ± 0.4	45.9 ± 0.3	46.4 ± 0.3	49.9 ± 1.1	52.9 ± 0.6
Week 13	46.3 ± 0.5	45.5 ± 0.6	46.9 ± 0.3	47.6 ± 0.7	50.0 ± 1.3*	48.0 ± 1.2
Hemoglobin (g/dL)						
Day 3	14.3 ± 0.2	14.2 ± 0.1	14.3 ± 0.1	14.4 ± 0.1	14.2 ± 0.1	15.1 ± 0.2**
Day 23	17.3 ± 0.6	15.6 ± 0.1	15.6 ± 0.2	15.5 ± 0.1	16.8 ± 0.4	17.9 ± 0.2
Week 13	15.5 ± 0.1	15.4 ± 0.1	15.8 ± 0.1	16.0 ± 0.2*	16.5 ± 0.4*	15.6 ± 0.4
Erythrocytes (10^6/µL)						
Day 3	7.31 ± 0.09	7.26 ± 0.05	7.33 ± 0.08	7.36 ± 0.06	7.29 ± 0.09	7.68 ± 0.10*
Day 23	8.77 ± 0.31	7.82 ± 0.06	7.93 ± 0.08	7.90 ± 0.07	8.60 ± 0.20	9.38 ± 0.12
Week 13	8.48 ± 0.13	8.59 ± 0.08	8.78 ± 0.07	8.83 ± 0.09	8.99 ± 0.25	8.40 ± 0.26
Reticulocytes (10^6/µL)						
Day 3	0.41 ± 0.02	0.41 ± 0.02	0.47 ± 0.02	0.45 ± 0.02	0.43 ± 0.02	0.41 ± 0.01
Day 23	0.26 ± 0.01	0.24 ± 0.02	0.23 ± 0.01	0.22 ± 0.01	0.24 ± 0.02	0.22 ± 0.01
Week 13	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	1.09 ± 0.23**
Nucleated erythrocytes (10^3/µL)						
Day 3	0.03 ± 0.02	0.09 ± 0.03	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.03 ± 0.01
Day 23	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.07 ± 0.01*
Week 13	0.01 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.03 ± 0.01	0.07 ± 0.02*	0.22 ± 0.06**
Mean cell volume (fL)						
Day 3	61.3 ± 0.4	61.6 ± 0.3	61.2 ± 0.3	61.4 ± 0.4	61.2 ± 0.3	61.1 ± 0.3
Day 23	60.9 ± 0.4	61.8 ± 0.4	60.7 ± 0.4	61.3 ± 0.4	61.1 ± 0.2	59.9 ± 0.4
Week 13	55.6 ± 0.4	54.8 ± 0.2	55.0 ± 0.2	55.3 ± 0.2	57.0 ± 0.3**	60.2 ± 1.0**

TABLE D1 Hematology Data for F344/N Rats in the 13-Week Study of Urethane in Drinking Water (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
FEMALE (continued)						
Mean cell hemoglobin (pg)						
Day 3	19.6 ± 0.1	19.5 ± 0.1	19.5 ± 0.1	19.5 ± 0.1	19.5 ± 0.1	19.7 ± 0.1
Day 23	19.8 ± 0.1	20.0 ± 0.1	19.7 ± 0.0	19.7 ± 0.1	19.5 ± 0.1*	19.1 ± 0.1**
Week 13	18.3 ± 0.3	18.0 ± 0.1	18.1 ± 0.1	18.1 ± 0.1	18.3 ± 0.1*	18.6 ± 0.2*
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.0 ± 0.1	31.8 ± 0.1	31.8 ± 0.1	31.7 ± 0.1	31.9 ± 0.1	32.2 ± 0.1
Day 23	32.5 ± 0.1	32.5 ± 0.2	32.6 ± 0.2	32.1 ± 0.1	32.0 ± 0.1*	31.9 ± 0.1*
Week 13	32.9 ± 0.2	32.8 ± 0.1	32.9 ± 0.2	32.8 ± 0.1	32.2 ± 0.2*	30.9 ± 0.3**
Platelets (10^3/µL)						
Day 3	973.0 ± 31.1	974.6 ± 27.7	990.5 ± 20.9	976.9 ± 24.7	975.9 ± 23.6	1,000.7 ± 24.9
Day 23	854.2 ± 16.4	813.3 ± 20.4	829.5 ± 16.1	969.0 ± 26.7	854.0 ± 34.0	677.5 ± 18.3**
Week 13	772.0 ± 39.4	859.7 ± 20.1	882.1 ± 25.6	939.0 ± 39.3	750.1 ± 26.8	555.5 ± 36.9*
Leukocytes (10^3/µL)						
Day 3	9.97 ± 0.28	9.34 ± 0.17	9.09 ± 0.25	10.01 ± 0.35	8.84 ± 0.26	9.15 ± 0.40
Day 23	8.64 ± 0.45	8.41 ± 0.28	7.05 ± 0.24**	6.77 ± 0.19**	5.79 ± 0.19**	4.94 ± 0.27**
Week 13	9.43 ± 0.44	7.32 ± 0.30**	6.69 ± 0.24**	6.78 ± 0.28**	5.07 ± 0.19**	4.17 ± 0.19**
Segmented neutrophils (10^3/µL)						
Day 3	1.90 ± 0.20	1.68 ± 0.11	1.46 ± 0.20	1.79 ± 0.25	1.46 ± 0.19	1.84 ± 0.23
Day 23	1.19 ± 0.13	1.25 ± 0.13	1.02 ± 0.08	1.13 ± 0.12	1.19 ± 0.12	1.38 ± 0.10
Week 13	1.46 ± 0.16	1.16 ± 0.17	1.22 ± 0.13	1.47 ± 0.15	0.90 ± 0.06**	0.84 ± 0.10**
Lymphocytes (10^3/µL)						
Day 3	8.00 ± 0.25	7.57 ± 0.17	7.55 ± 0.23	8.09 ± 0.24	7.27 ± 0.17*	7.23 ± 0.36*
Day 23	7.36 ± 0.38	7.06 ± 0.29	5.97 ± 0.26*	5.60 ± 0.27**	4.55 ± 0.15**	3.50 ± 0.21**
Week 13	7.88 ± 0.41	6.01 ± 0.22**	5.34 ± 0.26**	5.17 ± 0.26**	4.10 ± 0.18**	3.23 ± 0.20**
Monocytes (10^3/µL)						
Day 3	0.06 ± 0.02	0.03 ± 0.01	0.06 ± 0.03	0.11 ± 0.03	0.07 ± 0.02	0.08 ± 0.03
Day 23	0.03 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
Week 13	0.06 ± 0.02	0.10 ± 0.02	0.10 ± 0.03	0.09 ± 0.03	0.05 ± 0.01	0.04 ± 0.02
Eosinophils (10^3/µL)						
Day 3	0.01 ± 0.01	0.06 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.04 ± 0.02	0.01 ± 0.01
Day 23	0.06 ± 0.02	0.09 ± 0.03	0.06 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
Week 13	0.04 ± 0.02	0.05 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.06 ± 0.02

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

TABLE D2 Hematology Data for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE						
n						
Day 3	10	10	10	10	10	10
Day 16	0	0	0	0	0	10
Day 23	10	10	10	10	10	0
Week 13	10	10	10	10	10	8
Hematocrit (automated) (%)						
Day 3	45.7 ± 0.4	45.0 ± 0.3	44.7 ± 0.5	45.6 ± 0.5	45.8 ± 0.2	49.6 ± 0.4**
Day 16	)	)	)	)	)	54.1 ± 0.5
Day 23	52.6 ± 0.8	50.9 ± 0.7	50.1 ± 0.3*	50.1 ± 0.4*	49.8 ± 0.3**	)
Week 13	49.5 ± 0.6	51.5 ± 0.5	51.0 ± 0.5	49.3 ± 0.6	50.4 ± 0.5	50.3 ± 0.5
Hematocrit (manual) (%)						
Day 3	44.4 ± 0.5	43.6 ± 0.4	44.0 ± 0.7	44.4 ± 0.6	43.7 ± 0.2	46.8 ± 0.3**
Day 16	)	)	)	)	)	51.5 ± 0.5
Day 23	49.9 ± 0.7	49.0 ± 0.7	48.4 ± 0.3	48.6 ± 0.4	48.4 ± 0.3	)
Week 13	48.2 ± 0.5	49.3 ± 0.4	49.8 ± 0.5	48.2 ± 0.6	48.9 ± 0.5	48.8 ± 0.6
Hemoglobin (g/dL)						
Day 3	14.4 ± 0.2	14.1 ± 0.1	14.0 ± 0.2	14.3 ± 0.2	14.3 ± 0.1	15.3 ± 0.1**
Day 16	)	)	)	)	)	17.7 ± 0.1
Day 23	16.5 ± 0.3	16.1 ± 0.3	15.9 ± 0.2	15.9 ± 0.1*	15.9 ± 0.1*	)
Week 13	15.8 ± 0.1	16.4 ± 0.2*	16.2 ± 0.1	15.9 ± 0.2	16.1 ± 0.2	15.9 ± 0.2
Erythrocytes (10^6/µL)						
Day 3	7.32 ± 0.07	7.14 ± 0.07	7.13 ± 0.09	7.30 ± 0.09	7.29 ± 0.04	7.87 ± 0.09**
Day 16	)	)	)	)	)	9.54 ± 0.10
Day 23	8.74 ± 0.12	8.38 ± 0.17	8.29 ± 0.10*	8.37 ± 0.08*	8.41 ± 0.08*	)
Week 13	9.43 ± 0.09	9.86 ± 0.09	9.77 ± 0.08	9.58 ± 0.11	9.61 ± 0.09	9.16 ± 0.10
Reticulocytes (10^6/µL)						
Day 3	0.49 ± 0.02	0.47 ± 0.01	0.48 ± 0.01	0.46 ± 0.03	0.47 ± 0.02	0.56 ± 0.01**
Day 16	)	)	)	)	)	0.09 ± 0.01
Day 23	0.25 ± 0.01	0.25 ± 0.02	0.21 ± 0.01*	0.21 ± 0.01	0.21 ± 0.01	)
Week 13	0.21 ± 0.01	0.27 ± 0.01**	0.23 ± 0.01*	0.27 ± 0.02**	0.25 ± 0.02*	0.46 ± 0.05**
Nucleated erythrocytes (10^3/µL)						
Day 3	0.08 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.02 ± 0.01*
Day 16	)	)	)	)	)	0.01 ± 0.01
Day 23	0.00 ± 0.00	0.03 ± 0.02	0.01 ± 0.01	0.05 ± 0.02*	0.03 ± 0.01*	)
Week 13	0.02 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	0.12 ± 0.07
Mean cell volume (fL)						
Day 3	62.5 ± 0.3	63.0 ± 0.4	62.8 ± 0.4	62.7 ± 0.3	62.9 ± 0.3	63.0 ± 0.4
Day 16	)	)	)	)	)	56.8 ± 0.3
Day 23	60.3 ± 0.2	61.0 ± 0.6	60.5 ± 0.5	59.9 ± 0.4	59.3 ± 0.4	)
Week 13	52.6 ± 0.3	52.2 ± 0.2	52.4 ± 0.3	51.7 ± 0.3	52.6 ± 0.4	55.0 ± 0.5**
Mean cell hemoglobin (pg)						
Day 3	19.7 ± 0.1	19.7 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.4 ± 0.1
Day 16	)	)	)	)	)	18.6 ± 0.1
Day 23	18.9 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.0 ± 0.1	18.9 ± 0.1	)
Week 13	16.7 ± 0.1	16.6 ± 0.1	16.6 ± 0.1	16.6 ± 0.0	16.8 ± 0.1	17.4 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.5 ± 0.1	31.2 ± 0.2	31.2 ± 0.1	31.3 ± 0.1	31.2 ± 0.1	30.8 ± 0.1**
Day 16	)	)	)	)	)	32.7 ± 0.2
Day 23	31.4 ± 0.1	31.6 ± 0.1	31.7 ± 0.1	31.7 ± 0.1	31.8 ± 0.2	)
Week 13	31.9 ± 0.2	31.8 ± 0.2	31.9 ± 0.2	32.3 ± 0.2	32.0 ± 0.3	31.7 ± 0.3

TABLE D2 Hematology Data for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE (continued)						
Platelets (10^3/µL)						
Day 3	1,040 ± 16	1,063 ± 29	1,089 ± 17	1,068 ± 23	1,022 ± 27	1,156 ± 30**
Day 16	)	)	)	)	)	652.9 ± 40.8
Day 23	853.6 ± 12.9	913.9 ± 9.2	835.1 ± 14.8	865.9 ± 24.5	811.3 ± 19.9	)
Week 13	723.3 ± 15.1	752.0 ± 15.7	801.4 ± 18.6*	754.7 ± 20.6	760.4 ± 20.9	665.5 ± 38.5
Leukocytes (10^3/µL)						
Day 3	9.32 ± 0.39	8.16 ± 0.25	8.55 ± 0.50	8.93 ± 0.62	10.27 ± 0.44	9.15 ± 0.40
Day 16	)	)	)	)	)	6.81 ± 0.56
Day 23	10.19 ± 0.32	10.39 ± 0.39	8.79 ± 0.31**	8.09 ± 0.46**	5.88 ± 0.22**	)
Week 13	10.60 ± 0.43	10.19 ± 0.31	9.59 ± 0.46	8.71 ± 0.33**	6.94 ± 0.17**	4.95 ± 0.75**
Segmented neutrophils (10^3/µL)						
Day 3	1.74 ± 0.19	1.29 ± 0.13	1.64 ± 0.10	1.57 ± 0.28	2.23 ± 0.15	1.98 ± 0.15
Day 16	)	)	)	)	)	1.06 ± 0.10
Day 23	1.34 ± 0.12	1.64 ± 0.16	1.26 ± 0.10	1.54 ± 0.19	0.91 ± 0.07*	)
Week 13	1.65 ± 0.15	1.74 ± 0.13	1.79 ± 0.11	1.65 ± 0.17	1.56 ± 0.10	1.26 ± 0.16
Lymphocytes (10^3/µL)						
Day 3	7.50 ± 0.37	6.79 ± 0.30	6.85 ± 0.43	7.28 ± 0.41	7.94 ± 0.34	7.11 ± 0.34
Day 16	)	)	)	)	)	5.70 ± 0.51
Day 23	8.80 ± 0.31	8.64 ± 0.39	7.47 ± 0.34*	6.52 ± 0.33**	4.91 ± 0.20**	)
Week 13	8.86 ± 0.37	8.26 ± 0.24	7.59 ± 0.37*	6.87 ± 0.27**	5.33 ± 0.21**	3.60 ± 0.59**
Monocytes (10^3/µL)						
Day 3	0.07 ± 0.03	0.08 ± 0.03	0.06 ± 0.02	0.04 ± 0.01	0.05 ± 0.03	0.04 ± 0.02
Day 16	)	)	)	)	)	0.04 ± 0.02
Day 23	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	)
Week 13	0.03 ± 0.02	0.15 ± 0.04	0.09 ± 0.04	0.11 ± 0.03	0.05 ± 0.02	0.07 ± 0.03
Eosinophils (10^3/µL)						
Day 3	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.02 ± 0.01
Day 16	)	)	)	)	)	0.01 ± 0.01
Day 23	0.02 ± 0.02	0.07 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	)
Week 13	0.07 ± 0.02	0.04 ± 0.02	0.13 ± 0.03	0.08 ± 0.03	0.06 ± 0.02	0.03 ± 0.01
FEMALE						
n						
Day 3	10	10	10	10	10	10
Day 15	0	0	0	0	0	10
Day 23	10	10	10	10	10	0
Week 13	9	10	10	10	10	0
Hematocrit (automated) (%)						
Day 3	46.6 ± 0.4	46.5 ± 0.5	46.9 ± 0.3	46.4 ± 0.6	47.5 ± 0.5	50.8 ± 0.5**
Day 15	)	)	)	)	)	54.4 ± 1.0
Day 23	50.1 ± 0.4	53.9 ± 0.5	49.7 ± 0.6	49.4 ± 0.5	50.1 ± 0.7	)
Week 13	47.6 ± 0.6	46.3 ± 0.4	47.1 ± 0.4	48.4 ± 0.4	50.8 ± 0.5**	)
Hematocrit (manual) (%)						
Day 3	44.5 ± 0.3	44.6 ± 0.3	45.2 ± 0.4	44.7 ± 0.7	45.6 ± 0.6	47.5 ± 0.5**
Day 15	)	)	)	)	)	50.6 ± 0.7
Day 23	47.9 ± 0.3	50.7 ± 0.6	47.5 ± 0.4	47.0 ± 0.3	47.8 ± 0.6	)
Week 13	46.4 ± 0.6	45.5 ± 0.4	46.2 ± 0.3	47.2 ± 0.3	49.4 ± 0.5**	)

TABLE D2 Hematology Data for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
FEMALE (continued)						
Hemoglobin (g/dL)						
Day 3	14.7 ± 0.1	14.7 ± 0.2	14.9 ± 0.1	14.6 ± 0.2	15.0 ± 0.1	16.0 ± 0.2**
Day 15	)	)	)	)	)	17.6 ± 0.3
Day 23	16.1 ± 0.2	17.3 ± 0.2	15.9 ± 0.2	15.7 ± 0.2	15.9 ± 0.3	)
Week 13	15.9 ± 0.2	15.4 ± 0.1	15.4 ± 0.1	15.6 ± 0.1	16.5 ± 0.2	)
Erythrocytes (10^6/µL)						
Day 3	7.61 ± 0.07	7.64 ± 0.09	7.69 ± 0.07	7.58 ± 0.12	7.74 ± 0.08	8.29 ± 0.09**
Day 15	)	)	)	)	)	9.24 ± 0.17
Day 23	8.16 ± 0.07	8.81 ± 0.09**	8.11 ± 0.09	8.02 ± 0.11	8.27 ± 0.14	)
Week 13	8.61 ± 0.10	8.26 ± 0.13	8.41 ± 0.06	8.61 ± 0.06	8.92 ± 0.09	)
Reticulocytes (10^6/µL)						
Day 3	0.39 ± 0.01	0.40 ± 0.02	0.39 ± 0.02	0.37 ± 0.02	0.38 ± 0.01	0.41 ± 0.01
Day 15	)	)	)	)	)	0.08 ± 0.01
Day 23	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.20 ± 0.02	)
Week 13	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.25 ± 0.01*	)
Nucleated erythrocytes (10^3/µL)						
Day 3	0.06 ± 0.03	0.07 ± 0.02	0.08 ± 0.02	0.03 ± 0.02	0.07 ± 0.03	0.02 ± 0.01
Day 15	)	)	)	)	)	0.01 ± 0.01
Day 23	0.05 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.05 ± 0.01	)
Week 13	0.03 ± 0.02	0.01 ± 0.01	0.11 ± 0.03	0.03 ± 0.01	0.04 ± 0.02	)
Mean cell volume (fL)						
Day 3	61.2 ± 0.3	61.0 ± 0.5	61.0 ± 0.4	61.6 ± 0.3	61.4 ± 0.4	61.4 ± 0.3
Day 15	)	)	)	)	)	59.1 ± 0.3
Day 23	61.4 ± 0.3	61.2 ± 0.3	61.5 ± 0.3	61.6 ± 0.4	60.6 ± 0.3	)
Week 13	55.2 ± 0.4	56.3 ± 0.6	56.0 ± 0.2	56.1 ± 0.1*	57.0 ± 0.2**	)
Mean cell hemoglobin (pg)						
Day 3	19.3 ± 0.1	19.2 ± 0.1	19.4 ± 0.1	19.3 ± 0.1	19.4 ± 0.1	19.3 ± 0.0
Day 15	)	)	)	)	)	19.0 ± 0.1
Day 23	19.7 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.2 ± 0.1**	)
Week 13	18.5 ± 0.1	18.7 ± 0.3	18.3 ± 0.1	18.2 ± 0.1*	18.5 ± 0.1	)
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.6 ± 0.1	31.5 ± 0.1	31.8 ± 0.2	31.4 ± 0.1	31.6 ± 0.1	31.5 ± 0.1
Day 15	)	)	)	)	)	32.3 ± 0.1
Day 23	32.0 ± 0.1	32.1 ± 0.1	32.0 ± 0.1	31.9 ± 0.1	31.7 ± 0.1	)
Week 13	33.5 ± 0.3	33.3 ± 0.3	32.7 ± 0.1*	32.4 ± 0.1**	32.4 ± 0.1**	)
Platelets (10^3/µL)						
Day 3	1,015.5 ± 25.6	1,048.5 ± 20.2	1,045.8 ± 24.7	986.0 ± 22.8	1,048.3 ± 20.1	1,088.5 ± 17.6
Day 15	)	)	)	)	)	512.5 ± 35.3
Day 23	865.0 ± 24.6	858.7 ± 18.1	887.1 ± 31.0	881.5 ± 15.7	941.4 ± 27.8*	)
Week 13	767.0 ± 27.1	734.3 ± 49.0	805.4 ± 21.1	828.4 ± 22.0	778.4 ± 31.3	)
Leukocytes (10^3/µL)						
Day 3	9.51 ± 0.31	9.11 ± 0.31	9.49 ± 0.40	10.17 ± 0.49	10.09 ± 0.33	9.74 ± 0.18
Day 15	)	)	)	)	)	5.82 ± 0.31
Day 23	9.68 ± 0.33	8.92 ± 0.34	8.72 ± 0.54	6.84 ± 0.23**	6.16 ± 0.28**	)
Week 13	8.44 ± 0.37	6.21 ± 0.28**	6.88 ± 0.32**	6.85 ± 0.24**	6.48 ± 0.49**	)
Segmented neutrophils (10^3/µL)						
Day 3	1.33 ± 0.14	1.62 ± 0.14	1.70 ± 0.19	1.72 ± 0.20	2.08 ± 0.17**	1.86 ± 0.14**
Day 15	)	)	)	)	)	1.26 ± 0.14
Day 23	1.37 ± 0.16	1.24 ± 0.09	1.58 ± 0.20	1.13 ± 0.15	0.99 ± 0.13	)
Week 13	1.23 ± 0.13	1.06 ± 0.18	1.25 ± 0.11	1.62 ± 0.18	1.59 ± 0.25	)

TABLE D2 Hematology Data for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
FEMALE (continued)						
Lymphocytes (10^3/µL)						
Day 3	8.04 ± 0.30	7.33 ± 0.27	7.70 ± 0.28	8.41 ± 0.45	7.91 ± 0.32	7.81 ± 0.11
Day 15	)	)	)	)	)	4.46 ± 0.24
Day 23	8.22 ± 0.38	7.64 ± 0.38	7.02 ± 0.45*	5.64 ± 0.18**	5.06 ± 0.19**	)
Week 13	7.04 ± 0.39	5.04 ± 0.24**	5.49 ± 0.31**	5.09 ± 0.26**	4.76 ± 0.29**	)
Monocytes (10^3/µL)						
Day 3	0.07 ± 0.03	0.11 ± 0.03	0.03 ± 0.01	0.03 ± 0.02	0.07 ± 0.03	0.04 ± 0.02
Day 15	)	)	)	)	)	0.06 ± 0.02
Day 23	0.06 ± 0.02	0.03 ± 0.01	0.07 ± 0.03	0.02 ± 0.02	0.07 ± 0.02	)
Week 13	0.11 ± 0.03	0.07 ± 0.03	0.10 ± 0.03	0.12 ± 0.03	0.08 ± 0.01	)
Eosinophils (10^3/µL)						
Day 3	0.08 ± 0.03	0.05 ± 0.03	0.07 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.03 ± 0.02
Day 15	)	)	)	)	)	0.04 ± 0.02
Day 23	0.04 ± 0.02	0.02 ± 0.01	0.05 ± 0.03	0.05 ± 0.02	0.04 ± 0.01	)
Week 13	0.07 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.06 ± 0.02	)

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data; no statistical tests were performed on data collected on Day 16 (males) or Day 15 (females).

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

TABLE D3 Clinical Chemistry Data for F344/N Rats in the 13-Week Study of Urethane in Drinking Water[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	10
Week 13	10	10	10	10	10	7
Urea nitrogen (mg/dL)						
Day 3	17.3 ± 0.4	17.1 ± 0.6	17.4 ± 0.8	16.7 ± 0.7	16.5 ± 0.5	18.5 ± 0.5
Day 23	24.1 ± 1.0	20.0 ± 0.5**	20.3 ± 0.3**	21.3 ± 0.6	22.3 ± 0.4	24.0 ± 1.4
Week 13	17.2 ± 0.3	18.3 ± 0.8	20.5 ± 0.6**	20.3 ± 0.5**	19.3 ± 0.5**	21.1 ± 0.9**
Creatinine (mg/dL)						
Day 3	0.49 ± 0.02	0.50 ± 0.00	0.50 ± 0.02	0.48 ± 0.03	0.44 ± 0.02	0.43 ± 0.02*
Day 23	0.59 ± 0.03	0.63 ± 0.02	0.60 ± 0.03	0.61 ± 0.04	0.53 ± 0.02	0.61 ± 0.03
Week 13	0.59 ± 0.02	0.58 ± 0.01	0.58 ± 0.02	0.58 ± 0.01	0.56 ± 0.03	0.53 ± 0.04
Total protein (g/dL)						
Day 3	5.9 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.8 ± 0.1
Day 23	6.9 ± 0.1	6.5 ± 0.1*	6.6 ± 0.1	6.9 ± 0.1	6.6 ± 0.1	6.5 ± 0.1*
Week 13	6.5 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	5.8 ± 0.3
Albumin (g/dL)						
Day 3	3.3 ± 0.1	3.2 ± 0.1	3.2 ± 0.0	3.1 ± 0.0*	3.2 ± 0.0	3.2 ± 0.1
Day 23	3.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	3.8 ± 0.1
Week 13	3.5 ± 0.1	3.7 ± 0.1	3.8 ± 0.1*	3.9 ± 0.1*	3.7 ± 0.1	3.2 ± 0.2
Alanine aminotransferase (IU/L)						
Day 3	40 ± 2	43 ± 3	42 ± 2	41 ± 1	40 ± 1	39 ± 2
Day 23	41 ± 1	47 ± 2	45 ± 1	40 ± 2	39 ± 1	50 ± 2*
Week 13	50 ± 2	49 ± 2	48 ± 2	39 ± 1**	44 ± 1**	57 ± 9*
Alkaline phosphatase (IU/L)						
Day 3	645 ± 16	651 ± 14	654 ± 14	626 ± 10	592 ± 10**	568 ± 11**
Day 23	372 ± 16	474 ± 8	435 ± 14	382 ± 16	361 ± 10	339 ± 24
Week 13	229 ± 5	218 ± 11	234 ± 14	220 ± 7	255 ± 6	198 ± 35
Creatine kinase (IU/L)						
Day 3	519 ± 89	598 ± 119	440 ± 54	524 ± 134	503 ± 49	683 ± 168
Day 23	413 ± 37	402 ± 42	437 ± 64	511 ± 56	409 ± 45	728 ± 165
Week 13	294 ± 74	182 ± 24	310 ± 80	225 ± 36	198 ± 25	219 ± 62
Sorbitol dehydrogenase (IU/L)						
Day 3	6 ± 0	6 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 1*
Day 23	6 ± 0	6 ± 0	7 ± 0*	7 ± 1*	7 ± 0**	7 ± 0*
Week 13	7 ± 0	8 ± 0	7 ± 1	6 ± 0	8 ± 1	11 ± 1*
Bile acids (µmol/L)						
Day 3	36.5 ± 3.1	37.6 ± 4.9	42.7 ± 4.6	47.4 ± 2.9	46.0 ± 5.9	39.0 ± 2.3
Day 23	28.5 ± 3.0	28.6 ± 2.8	28.3 ± 3.6	26.1 ± 3.9	27.0 ± 3.0	39.1 ± 2.7
Week 13	16.7 ± 0.8	16.9 ± 1.4	20.1 ± 2.2	19.9 ± 1.2	28.5 ± 2.5**	32.6 ± 7.4*

TABLE D3 Clinical Chemistry Data for F344/N Rats in the 13-Week Study of Urethane in Drinking Water (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
FEMALE						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	21.4 ± 0.9	20.8 ± 0.8	18.7 ± 0.9	20.1 ± 0.9	18.7 ± 0.7	21.9 ± 0.7
Day 23	18.6 ± 1.1	21.5 ± 0.6*	22.0 ± 0.6*	20.1 ± 0.7	20.8 ± 0.7	24.7 ± 0.9**
Week 13	19.8 ± 0.7	20.7 ± 0.5	20.2 ± 0.5	17.8 ± 1.2	18.4 ± 0.5	26.0 ± 3.6
Creatinine (mg/dL)						
Day 3	0.52 ± 0.01	0.52 ± 0.02	0.50 ± 0.00	0.51 ± 0.01	0.45 ± 0.02**	0.48 ± 0.01*
Day 23	0.58 ± 0.03	0.59 ± 0.02	0.58 ± 0.03	0.56 ± 0.02	0.52 ± 0.01	0.48 ± 0.01**
Week 13	0.54 ± 0.02	0.51 ± 0.01	0.54 ± 0.02	0.51 ± 0.01	0.49 ± 0.04	0.53 ± 0.02
Total protein (g/dL)						
Day 3	5.5 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.0	5.6 ± 0.1
Day 23	6.3 ± 0.1	5.9 ± 0.1*	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	5.9 ± 0.1
Week 13	6.8 ± 0.1	6.8 ± 0.1	7.0 ± 0.1	6.6 ± 0.1	6.3 ± 0.1*	5.6 ± 0.1**
Albumin (g/dL)						
Day 3	3.2 ± 0.0	3.3 ± 0.0	3.2 ± 0.0	3.2 ± 0.1	3.1 ± 0.0	3.3 ± 0.0
Day 23	3.8 ± 0.1	3.4 ± 0.1**	3.7 ± 0.0	3.6 ± 0.1	3.5 ± 0.0*	3.6 ± 0.1
Week 13	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.7 ± 0.1	3.4 ± 0.1**	3.2 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 3	35 ± 1	38 ± 1	36 ± 1	37 ± 1	33 ± 1	41 ± 3
Day 23	29 ± 2	37 ± 1**	38 ± 1**	40 ± 1**	37 ± 1**	47 ± 3**
Week 13	37 ± 1	38 ± 1	36 ± 1	34 ± 1	35 ± 2	43 ± 2
Alkaline phosphatase (IU/L)						
Day 3	555 ± 15	553 ± 13	532 ± 11	553 ± 17	452 ± 12**	429 ± 10**
Day 23	327 ± 21	398 ± 11	404 ± 4*	403 ± 15	391 ± 11	317 ± 15
Week 13	192 ± 6	230 ± 7**	205 ± 9*	215 ± 12*	234 ± 9**	235 ± 18**
Creatine kinase (IU/L)						
Day 3	424 ± 34	452 ± 63	521 ± 79	500 ± 37	475 ± 70	374 ± 63
Day 23	522 ± 93	509 ± 66	415 ± 93	452 ± 69	327 ± 75*	396 ± 98
Week 13	204 ± 30	190 ± 37	215 ± 70	220 ± 38	478 ± 181	307 ± 102
Sorbitol dehydrogenase (IU/L)						
Day 3	6 ± 0	6 ± 0	5 ± 0	6 ± 0	7 ± 0	10 ± 1**
Day 23	7 ± 1	6 ± 0	7 ± 1	7 ± 0	7 ± 0	8 ± 0
Week 13	5 ± 1	5 ± 0	6 ± 1	6 ± 0	5 ± 0	8 ± 0**
Bile acids (µmol/L)						
Day 3	23.3 ± 4.5	29.1 ± 2.1	47.8 ± 4.7**	38.1 ± 6.0**	39.2 ± 3.2**	51.0 ± 5.6**
Day 23	25.5 ± 2.8	45.3 ± 4.4**[2]	48.5 ± 5.1**	42.0 ± 2.8**	33.4 ± 3.0	31.3 ± 2.2
Week 13	22.2 ± 2.7[2]	34.5 ± 3.5	22.1 ± 4.3	27.3 ± 4.4	19.8 ± 2.7	23.5 ± 2.6

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

TABLE D4 Clinical Chemistry Data for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol[1]

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
MALE						
n						
Day 3	10	10	10	10	10	10
Day 16	0	0	0	0	0	10
Day 23	10	10	10	10	10	0
Week 13	10	10	10	10	10	8
Urea nitrogen (mg/dL)						
Day 3	17.8 ± 0.7	17.6 ± 0.4	17.5 ± 0.4	18.1 ± 0.4	18.3 ± 0.6	20.7 ± 0.6**
Day 16	)	)	)	)	)	22.0 ± 0.6
Day 23	19.7 ± 1.1	22.3 ± 1.2	20.3 ± 0.5	21.1 ± 0.8	23.3 ± 0.4**	)
Week 13	16.9 ± 0.4	16.4 ± 0.4	17.2 ± 0.5	17.3 ± 0.3	16.0 ± 0.3	20.9 ± 1.8**
Creatinine (mg/dL)						
Day 3	0.52 ± 0.04	0.52 ± 0.03	0.51 ± 0.02	0.53 ± 0.03	0.47 ± 0.02	0.56 ± 0.03
Day 16	)	)	)	)	)	0.65 ± 0.03
Day 23	0.59 ± 0.03	0.61 ± 0.01	0.56 ± 0.02	0.60 ± 0.02	0.58 ± 0.03	)
Week 13	0.59 ± 0.01	0.60 ± 0.03	0.60 ± 0.02	0.58 ± 0.01	0.61 ± 0.02	0.55 ± 0.02
Total protein (g/dL)						
Day 3	5.8 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	6.6 ± 0.1**
Day 16	)	)	)	)	)	5.7 ± 0.1
Day 23	6.4 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	)
Week 13	6.9 ± 0.1	7.1 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.0 ± 0.1	6.5 ± 0.1*
Albumin (g/dL)						
Day 3	3.2 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	3.8 ± 0.1**
Day 16	)	)	)	)	)	3.4 ± 0.1
Day 23	3.7 ± 0.1	3.7 ± 0.1	3.7 ± 0.0	3.7 ± 0.1	3.8 ± 0.0	)
Week 13	3.6 ± 0.1	3.7 ± 0.0	3.7 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.4 ± 0.1*
Alanine aminotransferase (IU/L)						
Day 3	43 ± 2	41 ± 1	39 ± 1	39 ± 1	37 ± 1**	33 ± 1**
Day 16	)	)	)	)	)	201 ± 24
Day 23	46 ± 1	44 ± 2	44 ± 1	46 ± 3	42 ± 2	)
Week 13	49 ± 1	50 ± 1	45 ± 2	43 ± 1*	42 ± 1**	47 ± 2
Alkaline phosphatase (IU/L)						
Day 3	628 ± 12	641 ± 13	639 ± 20	604 ± 15	612 ± 13	570 ± 15*
Day 16	)	)	)	)	)	267 ± 9
Day 23	424 ± 20	378 ± 12*	415 ± 9	438 ± 7	425 ± 10	)
Week 13	224 ± 7	215 ± 10	203 ± 4	218 ± 7	234 ± 7	266 ± 11*
Creatine kinase (IU/L)						
Day 3	490 ± 71	661 ± 174	475 ± 76	460 ± 57	446 ± 23	533 ± 106
Day 16	)	)	)	)	)	677 ± 157
Day 23	408 ± 80	303 ± 43	352 ± 87	352 ± 49	470 ± 90	)
Week 13	200 ± 24	341 ± 46	277 ± 36	271 ± 34	274 ± 48	288 ± 93
Sorbitol dehydrogenase (IU/L)						
Day 3	6 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 0	7 ± 1
Day 16	)	)	)	)	)	11 ± 1
Day 23	7 ± 0	7 ± 0	7 ± 0	8 ± 1	7 ± 0	)
Week 13	6 ± 0[2]	8 ± 1	6 ± 0	8 ± 1	7 ± 1	8 ± 0*
Bile acids (µmol/L)						
Day 3	44.9 ± 3.8	40.8 ± 3.7	40.3 ± 4.2	41.4 ± 6.5	56.1 ± 4.0	30.4 ± 4.5
Day 16	)	)	)	)	)	42.9 ± 5.9
Day 23	27.0 ± 3.5	36.6 ± 6.3	20.8 ± 1.7	35.0 ± 7.4	27.6 ± 2.1	)
Week 13	22.2 ± 2.0	21.0 ± 1.4	20.5 ± 1.7	21.2 ± 1.5	23.1 ± 1.7	27.3 ± 2.4

TABLE D4 Clinical Chemistry Data for F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol (continued)

	0 ppm	110 ppm	330 ppm	1,100 ppm	3,300 ppm	10,000 ppm
FEMALE						
n						
Day 3	10	10	10	10	10	10
Day 15	0	0	0	0	0	10
Day 23	10	10	9	10	10	0
Week 13	9	10	10	10	10	0
Urea nitrogen (mg/dL)						
Day 3	18.1 ± 0.4	20.3 ± 0.6**	18.9 ± 0.7	18.9 ± 0.5	21.2 ± 0.6**	19.5 ± 0.9*
Day 15	)	)	)	)	)	23.3 ± 1.4
Day 23	21.4 ± 0.7	21.1 ± 0.9	20.7 ± 0.8	22.8 ± 0.7	24.5 ± 0.7*	)
Week 13	18.7 ± 0.3	21.8 ± 0.5	18.2 ± 0.6	18.4 ± 0.5	18.2 ± 0.4	)
Creatinine (mg/dL)						
Day 3	0.48 ± 0.03	0.47 ± 0.02	0.50 ± 0.03	0.52 ± 0.03	0.49 ± 0.02	0.49 ± 0.01
Day 15	)	)	)	)	)	0.50 ± 0.02
Day 23	0.57 ± 0.02	0.56 ± 0.02	0.59 ± 0.02	0.57 ± 0.02	0.56 ± 0.02	)
Week 13	0.51 ± 0.02	0.55 ± 0.02	0.52 ± 0.01	0.50 ± 0.00	0.50 ± 0.02	)
Total protein (g/dL)						
Day 3	5.7 ± 0.0	5.6 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	6.0 ± 0.0**	6.2 ± 0.1**
Day 15	)	)	)	)	)	5.5 ± 0.1
Day 23	6.1 ± 0.1	6.4 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1	)
Week 13	6.8 ± 0.1	6.8 ± 0.1	7.0 ± 0.1	6.5 ± 0.1*	6.4 ± 0.1**	)
Albumin (g/dL)						
Day 3	3.2 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	3.3 ± 0.0	3.5 ± 0.1**	3.7 ± 0.1**
Day 15	)	)	)	)	)	3.5 ± 0.1
Day 23	3.6 ± 0.0	3.8 ± 0.1*	3.6 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	)
Week 13	3.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	)
Alanine aminotransferase (IU/L)						
Day 3	32 ± 1	33 ± 1	35 ± 1	31 ± 1	30 ± 2	30 ± 2
Day 15	)	)	)	)	)	121 ± 25
Day 23	29 ± 1	25 ± 1	30 ± 1	30 ± 1	33 ± 1**	)
Week 13	33 ± 1	37 ± 2	32 ± 1	31 ± 1	30 ± 1	)
Alkaline phosphatase (IU/L)						
Day 3	471 ± 10	509 ± 12	490 ± 10	471 ± 8	469 ± 11	446 ± 10
Day 15	)	)	)	)	)	271 ± 12
Day 23	348 ± 6	309 ± 5	340 ± 9	354 ± 4	380 ± 15	)
Week 13	191 ± 6	214 ± 10	179 ± 8	180 ± 6	199 ± 9	)
Creatine kinase (IU/L)						
Day 3	512 ± 71	378 ± 47	632 ± 125	657 ± 128	636 ± 51	429 ± 40
Day 15	)	)	)	)	)	678 ± 145
Day 23	216 ± 42	269 ± 32	257 ± 24	322 ± 61	269 ± 21	)
Week 13	186 ± 22	387 ± 127	159 ± 21	135 ± 17	177 ± 45	)
Sorbitol dehydrogenase (IU/L)						
Day 3	6 ± 0	7 ± 1	6 ± 0	6 ± 0	6 ± 0	7 ± 0
Day 15	)	)	)	)	)	10 ± 1
Day 23	8 ± 0	8 ± 1	8 ± 0	7 ± 1	7 ± 0*	)
Week 13	5 ± 0	5 ± 0	6 ± 0	6 ± 0*	7 ± 1**	)
Bile acids (µmol/L)						
Day 3	34.2 ± 2.2	23.8 ± 2.9*	49.9 ± 5.0	51.3 ± 8.7	29.4 ± 3.1	20.5 ± 2.2**
Day 15	)	)	)	)	)	33.4 ± 3.5
Day 23	36.8 ± 4.3	13.0 ± 1.1**	37.2 ± 5.1	29.8 ± 5.2	19.9 ± 3.2*	)
Week 13	41.2 ± 5.1	29.8 ± 6.2	25.9 ± 3.5	26.5 ± 3.6	20.6 ± 3.8**	)

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data; no statistical tests were performed on data collected on Day 16 (males) or Day 15 (females).

[2] n=9.

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

APPENDIX E

Reproductive Tissue Evaluations and Estrous Cycle Characterization

TABLE E1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Study of Urethane in Drinking Water[1]

Study Parameters	0 ppm	330 ppm	1,100 ppm	3,300 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	326 ± 5	310 ± 4*	313 ± 5	296 ± 6**
Left epididymis	0.431 ± 0.008	0.424 ± 0.005	0.425 ± 0.005	0.441 ± 0.008
Left cauda epididymis	0.183 ± 0.005	0.181 ± 0.003	0.182 ± 0.004	0.181 ± 0.004
Left testis	1.49 ± 0.02	1.46 ± 0.01	1.49 ± 0.02	1.49 ± 0.03
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.00 ± 0.28	8.95 ± 0.26	8.74 ± 0.23	8.99 ± 0.30
Spermatid heads (10^7/testis)	13.40 ± 0.41	13.11 ± 0.37	12.96 ± 0.32	13.39 ± 0.45
Spermatid count (mean/10^{-4} mL suspension)	67.00 ± 2.06	65.53 ± 1.85	64.80 ± 1.58	66.93 ± 2.27
Epididymal spermatozoal measurements				
Motility (%)	98.73 ± 0.17	98.44 ± 0.16	98.31 ± 0.19*	98.26 ± 0.18**
Concentration (10^6/g cauda epididymal tissue)	697 ± 22	702 ± 14	615 ± 16**	600 ± 24**

[1] Data are presented as mean ± standard error. Differences from the control group for left epididymal, cauda epididymal, and testis weights and spermatid measurements are not significant by Dunn's test.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE E2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Study of Urethane in Drinking Water[1]

Study Parameters	0 ppm	1,100 ppm	3,300 ppm	10,000 ppm
n	10	10	8	10
Necropsy body weight (g)	190 ± 3	178 ± 3*	158 ± 3**[2]	130 ± 5**
Estrous cycle length (days)	4.60 ± 0.31	4.90 ± 0.42	4.69 ± 0.23[3]	5.20 ± 0.33
Estrous stages (% of cycle)				
Diestrus	30.8	40.0	37.5	40.0
Proestrus	14.2	15.0	17.5	17.5
Estrus	35.8	25.0	26.7	27.5
Metestrus	17.5	20.0	15.8	14.2
Uncertain diagnoses	1.7	0.0	2.5	0.8

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
[2] n=10.
[3] Estrous cycle longer than 12 days or unclear in 2 of 10 rats.
* Significantly different ($P \leq 0.01$) from the control group by Shirley's test.
** Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

TABLE E3 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol[1]

Study Parameters	0 ppm	330 ppm	1,100 ppm	3,300 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	312 ± 5	307 ± 6	302 ± 5	296 ± 4*
Left epididymis	0.433 ± 0.007	0.440 ± 0.004	0.413 ± 0.005	0.420 ± 0.003
Left cauda epididymis	0.182 ± 0.007	0.185 ± 0.004	0.179 ± 0.003	0.183 ± 0.003
Left testis	1.44 ± 0.07	1.52 ± 0.02	1.44 ± 0.02	1.47 ± 0.01
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.67 ± 0.74	9.96 ± 0.31	9.87 ± 0.27	10.06 ± 0.30
Spermatid heads (10^7/testis)	14.34 ± 1.34	15.10 ± 0.43	14.21 ± 0.34	14.77 ± 0.44
Spermatid count (mean/10^{-4} mL suspension)	71.70 ± 6.70	75.48 ± 2.14	71.05 ± 1.69	73.83 ± 2.21
Epididymal spermatozoal measurements				
Motility (%)	99.07 ± 0.11	98.79 ± 0.16	97.55 ± 0.32**	98.01 ± 0.28**
Concentration (10^6/g cauda epididymal tissue)	645 ± 30	633 ± 27	613 ± 14	540 ± 16**

[1] Data are presented as mean ± standard error. Differences from the control group for left epididymal, cauda epididymal, and testis weights and spermatid measurements are not significant by Dunn's or Shirley's test.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE E4 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Study of Urethane in 5% Ethanol[1]

Study Parameters	0 ppm	330 ppm	1,100 ppm	3,300 ppm
n	10	10	10	9
Necropsy body weight (g)	186 ± 2	189 ± 2	186 ± 3	168 ± 2**[2]
Estrous cycle length (days)	5.40 ± 0.19	5.65 ± 0.37	5.70 ± 0.42	6.94 ± 0.61*[3]
Estrous stages (% of cycle)				
Diestrus	39.2	39.2	40.8	39.2
Proestrus	15.8	14.2	16.7	12.5
Estrus	27.5	32.5	26.7	32.5
Metestrus	17.5	14.2	15.8	15.0
Uncertain diagnoses	0.0	0.0	0.0	0.8

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
[2] n=10.
[3] Estrous cycle longer than 12 days or unclear in 1 of 10 rats.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE E5 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water[1]

Study Parameters	0 ppm	110 ppm	330 ppm	1,100 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	39.1 ± 0.7	39.3 ± 0.9	40.0 ± 0.8	29.4 ± 0.4**
Left epididymis	0.048 ± 0.002	0.046 ± 0.001	0.046 ± 0.002	0.044 ± 0.002
Left cauda epididymis	0.016 ± 0.001	0.017 ± 0.001	0.016 ± 0.001	0.014 ± 0.001
Left testis	0.118 ± 0.002	0.118 ± 0.003	0.118 ± 0.002	0.116 ± 0.001
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.15 ± 1.09	19.64 ± 0.78	19.23 ± 0.59	19.74 ± 0.64
Spermatid heads (10^7/testis)	2.25 ± 0.13	2.30 ± 0.10	2.27 ± 0.07	2.29 ± 0.08
Spermatid count (mean/10^{-4} mL suspension)	70.48 ± 3.96	72.05 ± 3.19	70.85 ± 2.27	71.65 ± 2.45
Epididymal spermatozoal measurements				
Motility (%)	98.00 ± 0.13	97.72 ± 0.11	97.66 ± 0.12	96.89 ± 0.25**
Concentration (10^6/g cauda epididymal tissue)	1,621 ± 82	1,254 ± 86*	1,385 ± 104	1,226 ± 83**

[1] Data are presented as mean ± standard error. Differences from the control group for left epididymal, cauda epididymal, and testis weights and spermatid measurements are not significant by Dunn's or Shirley's test.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE E6 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water[1]

Study Parameters	0 ppm	110 ppm	330 ppm	1,100 ppm
n	9	8	9	1
Necropsy body weight[2] (g)	32.7 ± 0.8	32.5 ± 0.7	32.0 ± 0.5	21.2 ± 0.7**
Estrous cycle length (days)	4.67 ± 0.22[3]	4.31 ± 0.09[4]	4.83 ± 0.42[3]	4.50[5]
Estrous stages[6] (% of cycle)				
Diestrus	33.0	42.5	31.7	80.0
Proestrus	16.0	14.2	17.5	3.3
Estrus	34.0	31.7	31.7	10.8
Metestrus	17.0	11.7	19.2	5.8

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test.
[2] n=10 for all groups.
[3] Estrous cycle longer than 12 days or unclear in 1 of 10 mice.
[4] Estrous cycle longer than 12 days or unclear in 2 of 10 mice.
[5] Estrous cycle longer than 12 days or unclear in 9 of 10 mice.
[6] Evidence suggests that females in the 1,100 ppm group differ significantly ($P \leq 0.05$, Wilk's Criterion) from the control females in the relative length of time spent in estrous stages. Females in this group spent more time in diestrus and less time in the other estrous stages than control females.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE E7 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol[1]

Study Parameters	0 ppm	330 ppm	1,100 ppm	3,300 ppm
n	10	9	10	9
Weights (g)				
Necropsy body weight	41.6 ± 0.8	41.1 ± 0.5	34.3 ± 0.4**	28.2 ± 0.4**
Left epididymis	0.045 ± 0.001	0.049 ± 0.001	0.044 ± 0.001	0.040 ± 0.001*
Left cauda epididymis	0.016 ± 0.001	0.017 ± 0.001	0.016 ± 0.001	0.013 ± 0.001*
Left testis	0.119 ± 0.003	0.124 ± 0.002	0.110 ± 0.006	0.110 ± 0.002*
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.20 ± 0.71	18.83 ± 0.78	18.03 ± 1.30	20.52 ± 0.71
Spermatid heads (10^7/testis)	2.29 ± 0.10	2.33 ± 0.10	2.02 ± 0.19	2.25 ± 0.07
Spermatid count (mean/10^{-4} mL suspension)	71.50 ± 3.19	72.75 ± 3.19	63.15 ± 5.98	70.36 ± 2.23
Epididymal spermatozoal measurements				
Motility (%)	98.29 ± 0.25	96.70 ± 0.24**	97.13 ± 0.63*	35.33 ± 11.67**
Concentration (10^6/g cauda epididymal tissue)	1,168 ± 100	987 ± 62	976 ± 34	1,053 ± 65

[1] Data are presented as mean ± standard error. Differences from the control group for spermatid measurements and epididymal spermatozoal concentration are not significant by Dunn's test.
* Significantly different ($P \le 0.05$) from the control group by Shirley's test.
** Significantly different ($P \le 0.01$) from the control group by Shirley's test.

TABLE E8 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol[1]

Study Parameters	0 ppm	330 ppm	1,100 ppm	3,300 ppm
n	9	8	8	0
Necropsy body weight[2] (g)	32.3 ± 0.5	30.6 ± 0.9	26.3 ± 0.3**	19.4 ± 1.1**
Estrous cycle length (days)	4.78 ± 0.19[3]	4.56 ± 0.15[3]	5.56 ± 0.58[4]	)[5]
Estrous stages[6] (% of cycle)				
Diestrus	36.1	39.8	47.5	92.4
Proestrus	19.4	18.5	15.0	2.2
Estrus	28.7	29.6	26.7	5.4
Metestrus	15.7	12.0	10.8	0.0

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test.
[2] For the control and 1,100 ppm groups, n=10; for the 330 and 3,300 ppm groups, n=9.
[3] Estrous cycle longer than 12 days or unclear in 1 of 10 mice (control group) or 1 of 9 mice (330 ppm group).
[4] Estrous cycle longer than 12 days or unclear in 2 of 10 mice.
[5] Estrous cycle longer than 12 days or unclear in 9 of 9 mice.
[6] Evidence suggests that females in the 3,300 ppm group differ significantly ($P \le 0.05$, Wilk's Criterion) from the control females in the relative length of time spent in estrous stages. Females in this group spent more time in diestrus and less time in the other estrous stages than control females.
** Significantly different ($P \le 0.01$) from the control group by Shirley's test.

APPENDIX F

Genetic Toxicology

TABLE F1 Mutagenicity of Urethane in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9	+hamster S9 5%	+hamster S9 10%	+hamster S9 30%	+rat S9 5%	+rat S9 10%	+rat S9 30%
STUDY 1								
TA100	0	128 ± 13.2	165 ± 4.2	156 ± 8.7	172 ± 1.2	152 ± 1.0	169 ± 11.3	166 ± 8.4
	100	148 ± 5.9	172 ± 5.8	167 ± 6.1	195 ± 15.0	160 ± 7.2	155 ± 10.5	170 ± 5.5
	333	137 ± 6.7	156 ± 13.0	179 ± 4.2	194 ± 19.7	165 ± 4.4	166 ± 10.4	201 ± 9.1
	1,000	152 ± 7.8	159 ± 11.8	183 ± 12.2	179 ± 10.8	173 ± 5.8	147 ± 3.3	178 ± 15.5
	3,333	136 ± 3.8	163 ± 10.4	176 ± 7.5	193 ± 3.9	166 ± 6.0	154 ± 8.5	151 ± 7.5
	10,000	126 ± 7.2	158 ± 6.6	182 ± 4.4	209 ± 11.9	160 ± 11.1	155 ± 5.2	172 ± 6.4
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative	Negative
Positive control[3]		431 ± 4.7	1,080 ± 43.1	704 ± 27.2	627 ± 6.9	686 ± 28.3	443 ± 13.7	380 ± 11.0
TA1535	0	26 ± 4.2			18 ± 2.6			14 ± 1.5
	100	21 ± 2.3						14 ± 0.7
	333	19 ± 3.0						10 ± 3.1
	1,000	16 ± 1.5			14 ± 1.3			14 ± 0.9
	3,333	18 ± 4.8			29 ± 2.6			12 ± 2.0
	6,666				32 ± 0.9			
	10,000	17 ± 2.9			51 ± 3.0			14 ± 1.2
	15,000				56 ± 2.7			
Trial summary		Negative			Positive			Negative
Positive control		355 ± 1.7			467 ± 25.7			110 ± 12.6
TA1537	0	15 ± 0.9			7 ± 0.9			11 ± 1.5
	100	6 ± 2.0			8 ± 1.5			15 ± 1.8
	333	11 ± 2.3			8 ± 1.2			10 ± 2.0
	1,000	15 ± 2.1			7 ± 1.2			8 ± 1.8
	3,333	7 ± 3.0			8 ± 0.3			10 ± 0.7
	10,000	9 ± 2.3			7 ± 0.7			10 ± 1.3
Trial summary		Negative			Negative			Negative
Positive control		554 ± 97.4			53 ± 6.7			45 ± 2.3
TA97	0	152 ± 3.7			172 ± 6.5			198 ± 7.3
	100	119 ± 8.2			208 ± 11.9			211 ± 1.9
	333	106 ± 7.4			190 ± 23.7			219 ± 0.9
	1,000	136 ± 4.1			214 ± 5.7			232 ± 9.0
	3,333	142 ± 8.4			213 ± 7.0			213 ± 6.1
	10,000	139 ± 8.7			195 ± 13.2			212 ± 11.5
Trial summary		Negative			Negative			Negative
Positive control		580 ± 46.4			445 ± 35.0			391 ± 13.1
TA98	0	21 ± 2.8			35 ± 2.1			55 ± 3.1
	100	21 ± 3.7			30 ± 1.5			41 ± 4.2
	333	23 ± 3.5			38 ± 2.9			38 ± 3.2
	1,000	17 ± 0.6			34 ± 3.4			39 ± 1.8
	3,333	24 ± 4.1			35 ± 4.7			31 ± 4.0
	10,000	18 ± 1.2			36 ± 4.7			32 ± 2.2
Trial summary		Negative			Negative			Negative
Positive control		552 ± 20.5			401 ± 5.5			109 ± 2.3

TABLE F1 Mutagenicity of Urethane in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9	Revertants/plate +30% hamster S9	Revertants/plate +30% rat S9
STUDY 2				
TA100	0	122 ± 12.3	150 ± 18.9	176 ± 8.5
	100	116 ± 6.0	169 ± 17.7	169 ± 5.7
	333	129 ± 5.0	164 ± 4.2	165 ± 9.2
	1,000	123 ± 10.8	155 ± 8.1	158 ± 5.2
	3,333	121 ± 6.7	139 ± 15.6	138 ± 8.1
	10,000	126 ± 9.1	162 ± 10.5	158 ± 10.4
Trial summary		Negative	Negative	Negative
Positive control		1,036 ± 10.2	832 ± 34.1	568 ± 22.5
TA97	0	167 ± 3.5	168 ± 10.5	198 ± 15.5
	100	157 ± 4.5	167 ± 3.0	139 ± 6.9
	333	186 ± 8.1	182 ± 8.2	161 ± 5.9
	1,000	197 ± 6.2	183 ± 7.1	190 ± 11.8
	3,333	187 ± 11.3	182 ± 7.2	214 ± 4.7
	10,000	181 ± 4.4	193 ± 6.2	204 ± 8.7
Trial summary		Negative	Negative	Negative
Positive control		335 ± 25.2	549 ± 47.2	360 ± 10.0
TA98	0	37 ± 4.0	39 ± 4.6	20 ± 0.3
	100	39 ± 3.9	40 ± 1.7	23 ± 3.3
	333	30 ± 3.0	31 ± 1.2	23 ± 4.4
	1,000	28 ± 2.0	24 ± 2.4	19 ± 0.6
	3,333	26 ± 5.2	30 ± 1.9	17 ± 2.3
	10,000	24 ± 3.5	33 ± 1.5	22 ± 3.2
Trial summary		Negative	Negative	Negative
Positive control		432 ± 19.0	488 ± 24.9	155 ± 14.3

TABLE F1 Mutagenicity of Urethane in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9	+hamster S9 5%	+hamster S9 5%	+hamster S9 10%	+hamster S9 10%
STUDY 2 (continued)						
TA1535	0	14 ± 0.3	17 ± 2.6	11 ± 0.3	13 ± 1.0	13 ± 1.3
	100	11 ± 2.3		12 ± 1.3		12 ± 2.6
	333	12 ± 1.3	32 ± 1.3	10 ± 0.3	25 ± 1.8	10 ± 0.3
	1,000	11 ± 1.2	35 ± 7.3	13 ± 0.7	27 ± 1.8	9 ± 1.5
	3,333	11 ± 3.6	33 ± 3.2	13 ± 1.8	38 ± 1.0	9 ± 1.5
	6,666		37 ± 3.3		35 ± 0.9	
	10,000	9 ± 1.2	28 ± 3.8	10 ± 1.0	30 ± 4.4	12 ± 1.3
Trial summary		Negative	Weakly Positive	Negative	Positive	Negative
Positive control		601 ± 67.2	257 ± 20.7	144 ± 25.2	108 ± 10.5	74 ± 7.8

TA1535 (continued)

Dose (µg/plate)	+hamster S9 (continued) 30%	30%	30%	30%	+rat S9 5%	10%	30%	30%
0	11 ± 1.8	30 ± 2.0	14 ± 2.9	13 ± 0.3	13 ± 0.3	15 ± 1.0	14 ± 3.1	18 ± 3.2
100	11 ± 3.4		12 ± 1.5	10 ± 2.8			14 ± 1.9	
333	12 ± 0.6	39 ± 5.2	12 ± 1.5	14 ± 2.0	11 ± 0.3	9 ± 1.0	16 ± 2.6	14 ± 3.7
1,000	18 ± 3.5	43 ± 3.7	16 ± 3.9	14 ± 3.5	11 ± 3.2	9 ± 1.2	14 ± 2.8	10 ± 1.5
1,666				15 ± 1.5				
3,333	17 ± 1.2	42 ± 3.2	23 ± 2.1	21 ± 1.5	9 ± 0.6	10 ± 1.7	12 ± 2.1	14 ± 1.2
6,666		45 ± 2.6		21 ± 2.7	7 ± 1.9	10 ± 1.2		13 ± 1.5
10,000	31 ± 4.9	43 ± 8.7	21 ± 2.0	24 ± 2.2	8 ± 0.6	9 ± 2.3	11 ± 1.2	15 ± 1.9
16,666				27 ± 2.5	13 ± 1.3	9 ± 1.5		15 ± 2.3
Trial summary	Equivocal	Negative	Negative	Weakly Positive	Negative	Negative	Negative	Negative
Positive control	299 ± 49.9	410 ± 36.0	266 ± 27.8	303 ± 21.7	135 ± 6.5	88 ± 6.1	83 ± 11.1	112 ± 7.8

[1] Studies were performed at SRI, International. The detailed protocol and these data are presented in Zeiger *et al.* (1992); 0 µg/plate is the solvent control.

[2] Revertants are presented as the mean ± standard error from three plates.

[3] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537 and TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for trials with metabolic activation with all strains was 2-aminoanthracene.

TABLE F2 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Urethane[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[2] (%)
-S9								
Trial 1								
Summary: Negative								
Medium		50	1,046	417	0.39	8.3	26.5	
Mitomycin-C	0.0005	50	1,046	545	0.52	10.9	26.5	30.70
	0.0050	10	210	327	1.55	32.7	26.5	290.59
Urethane	160	50	1,050	451	0.42	9.0	26.5	7.74
	500	50	1,046	395	0.37	7.9	26.5	−5.28
	1,600	50	1,047	433	0.41	8.7	26.5	3.74
	5,000	50	1,051	500	0.47	10.0	26.5	19.33
					P=0.014[3]			
Trial 2								
Summary: Positive								
Medium		50	1,051	474	0.45	9.5	26.0	
Mitomycin-C	0.0005	50	1,045	600	0.57	12.0	26.0	27.31
	0.0050	10	211	333	1.57	33.3	26.0	249.94
Urethane	1,000	50	1,049	477	0.45	9.5	26.0	0.82
	1,600	50	1,045	580	0.55	11.6	26.0	23.07*
	3,000	50	1,050	576	0.54	11.5	26.0	21.64*
	5,000	21[4]	437	278	0.63	13.2	26.0	41.06*
					P<0.001			
+S9								
Summary: Positive								
Medium		50	1,050	446	0.42	8.9	26.0	
Cyclophosphamide	0.1	50	1,048	588	0.56	11.8	26.0	32.09
	0.6	10	210	217	1.03	21.7	26.0	143.27
Urethane	160	50	1,050	435	0.41	8.7	26.0	−2.47
	500	50	1,049	542	0.51	10.8	26.0	21.64*
	1,600	50	1,049	431	0.41	8.6	26.0	−3.27
	5,000	50	1,048	565	0.53	11.3	26.0	26.92*
					P<0.001			

[1] Study was performed at Environmental Health Research and Testing, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the protocol is presented in Galloway *et al.* (1987).
[2] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells.
[3] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose.
[4] Due to the cytostatic action of urethane, only 21 cells could be evaluated.
* Positive (>20% increase over solvent control).

TABLE F3 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Urethane[1]

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Harvest time: 13.0 hours Summary: Negative					Harvest time: 13.0 hours Summary: Negative				
Medium					Medium				
	200	1	0.01	0.5		200	4	0.02	2.0
Mitomycin-C					Cyclophosphamide				
0.0625	200	60	0.30	23.5	2.5	200	33	0.17	15.5
0.2500	50	40	0.80	48.0	7.5	50	22	0.44	34.0
Urethane					Urethane				
1,600	200	6	0.03	3.0	1,600	200	3	0.02	1.5
3,000	200	6	0.03	3.0	3,000	200	6	0.03	3.0
5,000	200	2	0.01	1.0	5,000	200	8	0.04	3.5
				P=0.345[2]					P=0.111

[1] Study was performed at Environmental Health Research and Testing, Inc. Abs = aberrations. A detailed description of the protocol is presented in Galloway *et al.* (1987).

[2] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose.

TABLE F4 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Urethane[1]

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested			
				Mating 1	Mating 2	Mating 3	Total[2]
Feeding	12,000	3	0	55/861	46/991	16/345	117/2,197 (5.33%)
	0			0/863	0/1,122	0/865	0/2,850 (0.00%)
							P<0.001[3]

[1] Study was performed at Brown University. A detailed description of the protocol and these data are presented in Foureman *et al.* (1994).
[2] Total number of lethal mutations/total number of X chromosomes tested for three mating trials.
[3] Significance of total number of lethal mutations/total number of X chromosomes tested by a normal approximation to the binomial test (Margolin *et al.*, 1983).

TABLE F5 Induction of Reciprocal Translocations in *Drosophila melanogaster* by Urethane[1]

Route of Exposure	Dose (ppm)	Transfers Translocations/Total F_1 Tested				Total No. of Tests	Total No. of Translocations	Total Translocations (%)
		1	2	3	4			
Feeding	11,000	3/1,598	1/1,479	0/401	0/0	3,478	4	0.12
Historical control	0					116,163	2	0.00
								P<0.001[2]

[1] Study was performed at Brown University. A detailed description of the protocol and these data are presented in Foureman *et al.* (1994).
[2] Significance of percent translocations tested by the conditional binomial response test (Kastenbaum and Bowman, 1970).

TABLE F6 Frequency of Micronuclei in Peripheral Blood and Bone Marrow Erythrocytes of B6C3F$_1$ Mice Following Treatment with Urethane in Drinking Water[1]

Concentration (mg/mL)[2]	Micronucleated NCEs/1,000 Cells[3]	Micronucleated PCEs/1,000 Cells[3]
PERIPHERAL BLOOD ANALYSES		
Day 45		
Male		
0.00	1.77 ± 0.16	2.39 ± 0.26
0.05	2.80 ± 0.26	4.05 ± 0.72
0.20	4.52 ± 0.42*	5.03 ± 1.01*
0.75	9.20 ± 0.76*	7.95 ± 1.07*
2.00	19.03 ± 2.15*	17.63 ± 2.39*
	P<0.001[4]	P<0.001[5]
Female		
0.00	1.31 ± 0.08	2.33 ± 0.29
0.05	1.85 ± 0.19	1.81 ± 0.21
0.20	4.18 ± 0.22*	4.24 ± 0.50
0.75	9.27 ± 0.50*	10.73 ± 1.56*
2.00	17.32 ± 1.09*	21.08 ± 2.60*
	P<0.001	P<0.001
Week 13		
Male		
0.00	2.05 ± 0.10	3.23 ± 0.44
0.05	2.80 ± 0.28	4.13 ± 0.60
0.20	4.09 ± 0.23*	4.58 ± 0.88
0.75	10.22 ± 1.42*	9.96 ± 1.91*
2.00	22.19 ± 2.16*	22.75 ± 2.74*
	P<0.001	P<0.001
Female		
0.00	1.57 ± 0.09	1.90 ± 0.33
0.05	2.01 ± 0.15	2.38 ± 0.38
0.20	4.26 ± 0.39*	3.49 ± 0.65
0.75	9.95 ± 0.57*	9.13 ± 0.95*
2.00	22.75 ± 1.28*	22.84 ± 1.76*
	P<0.001	P<0.001

TABLE F6 Frequency of Micronuclei in Peripheral Blood and Bone Marrow Erythrocytes of B6C3F$_1$ Mice Following Treatment with Urethane in Drinking Water (continued)

Concentration (mg/mL)	Micronucleated NCEs/1,000 Cells	Micronucleated PCEs/1,000 Cells
BONE MARROW ANALYSES		
Male		
0.00	)[6]	2.83 ± 0.46
0.05	)	3.13 ± 0.44
0.20	)	4.38 ± 0.50
0.75	)	10.75 ± 1.13*
2.00	)	27.50 ± 3.94*
		P<0.001
Female		
0.00	)	2.00 ± 0.39
0.05	)	2.88 ± 0.35
0.20	)	4.50 ± 0.60*
0.75	)	10.88 ± 0.81*
2.00	)	23.75 ± 1.87*
		P<0.001

[1] Study was performed by the United States Department of Agriculture (Western Regional Center, CA). NCEs = normochromatic erythrocytes; PCEs = polychromatic erythrocytes. Data are presented as mean ± standard error.
[2] 0.05 mg/mL = 50 ppm; 0.20 mg/mL = 200 ppm; 0.75 mg/mL = 750 ppm; 2.00 mg/mL = 2,000 ppm.
[3] Ten thousand normochromatic erythrocytes and 2,000 polychromatic erythrocytes were scored at each time period for each of 12 control and 8 exposed mice per group.
[4] Significance of micronucleated NCEs/1,000 NCEs tested by analysis of variance.
[5] Significance of micronucleated PCEs/1,000 PCEs tested by Cochran-Armitage trend test.
[6] Not applicable.
* Positive (P<0.006) by pairwise comparison to the control group with Student's *t*-test (NCEs/1,000 NCEs) or Kastenbaum-Bowman's binomial test (PCEs/1,000 PCEs).

TABLE F7 Frequency of Micronuclei in Peripheral Blood Erythrocytes of B6C3F$_1$ Mice in the 13-Week Study of Urethane in Drinking Water[1]

Concentration (ppm)	Number Examined	Micronucleated NCEs/1,000 NCEs[2]
MALE		
0	5	2.8 ± 0.5
110	5	4.0 ± 0.2
330	5	7.3 ± 0.3*
1,100	5	13.9 ± 0.4*
		P<0.001[3]
FEMALE		
0	5	1.9 ± 0.2
110	5	5.0 ± 0.8*
330	5	5.8 ± 0.3*
1,100	5	18.6 ± 1.0*
		P<0.001

[1] Study was performed by Environmental Health Research and Testing, Inc. NCEs = normochromatic erythrocytes. Data are presented as mean ± standard error.
[2] Two thousand normochromatic erythrocytes were scored per animal.
[3] Significance of micronucleated NCEs/1,000 NCEs tested by a one-tailed trend test.
* Positive (P<0.008) by pairwise comparison to the control group with a *t*-test.

TABLE F8 Frequency of Micronuclei in Peripheral Blood Erythrocytes of B6C3F$_1$ Mice in the 13-Week Study of Urethane in 5% Ethanol[1]

Concentration (ppm)	Number Examined	Micronucleated NCEs/1,000 NCEs[2]
MALE		
0	5	3.3 ± 0.5
110	5	5.4 ± 1.3
330	5	7.1 ± 0.5*
1,100	5	14.3 ± 1.7*
3,300	5	15.5 ± 1.7*
		P<0.001[3]
FEMALE		
0	5	3.1 ± 0.3
110	5	4.1 ± 0.5
330	5	5.6 ± 0.5
1,100	5	10.8 ± 2.2*
3,300	5	18.0 ± 2.1*
		P<0.001

[1] Study was performed by Environmental Health Research and Testing, Inc. NCEs = normochromatic erythrocytes. Data are presented as mean ± standard error.
[2] Two thousand normochromatic erythrocytes were scored per animal.
[3] Significance of micronucleated NCEs/1,000 NCEs tested by a one-tailed trend test.
* Positive (P<0.006) by pairwise comparison to the control group with a *t*-test.

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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	*n*-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	*o*-Cresol *m*-Cresol *p*-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	*p*-Chloro-α,α,α-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	*t*-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
18	Methyl Ethyl Ketone Peroxide	Dermal	92-3341
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N, N-Dimethylformamide	Inhalation	93-3345
23	*o*-Nitrotoluene *m*-Nitrotoluene *p*-Nitrotoluene	Dosed Feed	92-3346
24	1,6-Hexanediamine	Inhalation	93-3347
25	Glutaraldehyde	Inhalation	93-3348
26	Ethylene Glycol Ethers	Drinking Water	93-3349
27	Riddelliine	Gavage	94-3350
28	Tetrachlorophthalic Anhydride	Gavage	93-3351
29	Cupric Sulfate	Drinking Water, Dosed Feed	93-3352
30	Dibutyl Phthalate	Feed	95-3353
31	Isoprene	Inhalation	95-3354

NTP TECHNICAL REPORTS ON TOXICITY STUDIES
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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
32	Methylene Bis(thiocyanate)	Gavage	94-3381
33	2-Chloronitrobenzene 4-Chloronitrobenzene	Inhalation	93-3382
35	Chemical Mixture of 25 Groundwater Contaminants	Drinking Water	93-3384
36	Pesticide/Fertilizer Mixtures	Drinking Water	93-3385
37	Sodium Cyanide	Drinking Water	94-3386
38	Sodium Selenate Sodium Selenite	Drinking Water	94-3387
39	Cadmium Oxide	Inhalation	95-3388
40	β-Bromo-β-nitrostyrene	Gavage	94-3389
42	1,3-Diphenylguanidine	Feed	95-3933