NTP REPORT ON THE

TOXICITY STUDIES OF ACETONE

IN F344/N RATS AND B6C3F$_1$ MICE

(DRINKING WATER STUDIES)

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

January 1991

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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NOTE TO THE READER

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

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

The studies described in this toxicity study report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals. Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure.

Anyone who is aware of related ongoing or published studies not mentioned in this report, or of any errors in this report, is encouraged to make this information known to the NTP. Comments and questions should be directed to Dr. J.R. Bucher, NIEHS, P.O. Box 12233, Research Triangle Park NC 27709 (919-541-4532).

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TOXICITY STUDIES OF ACETONE

(CAS NO. 67-64-1)

IN F344/N RATS AND B6C3F$_1$ MICE

(DRINKING WATER STUDIES)

Dennis Dietz, Ph.D., Study Scientist

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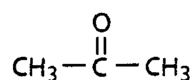

ACETONE

CAS No. 67-64-1

C_3H_6O Molecular weight 58.1

Synonyms: 2-propanone; dimethyl ketone; pyroacetic acid

ABSTRACT

Toxicity studies were conducted by administering acetone (greater than 99% pure) in drinking water to groups of F344/N rats and $B6C3F_1$ mice of each sex for 14 days or 13 weeks.

Fourteen-Day Studies: All rats and mice receiving concentrations as high as 100,000 ppm acetone in drinking water lived to the end of the 14-day studies. The mean body weights of male rats receiving 50,000 or 100,000 ppm and female rats given 100,000 ppm were lower than those of controls. Body weights of all groups of mice were similar. Kidney and liver weight to body weight ratios for exposed rats and mice were greater than those for controls. Histopathologic changes were not seen in these organs in rats or in the kidney in mice. Centrilobular hepatocellular hypertrophy was noted in male and female mice receiving 20,000 and 50,000 ppm acetone, respectively.

Thirteen-Week Studies: All rats lived to the end of the 13-week studies (drinking water concentrations as high as 50,000 ppm). The final mean body weights of rats receiving 50,000 ppm were 19% lower than that of controls for males and 7% lower for females. Water consumption by all rats that received 50,000 ppm acetone and females that received 20,000 ppm or more was notably lower than that by controls. Liver and kidney weight to body weight ratios were increased for male and female rats receiving 20,000 ppm or greater. Caudal and right epididymal weights and sperm motility were decreased for male rats given 50,000 ppm, and the percentage of abnormal sperm was increased. Leukocytosis and thrombocytopenia were observed at 20,000 ppm and above (males and females), and reticulocytopenia and erythrocytopenia were seen at 5,000 ppm and above (males). These changes, in addition to increase in erythrocyte size (MCV), are consistent with macrocytic anemia. Splenic pigmentation (hemosiderosis) noted in dosed male rats was apparently related to these changes. The increased incidence and severity of nephropathy observed in dosed male rats were considered the most prominent chemically related findings in this study.

All mice lived to the end of the 13-week studies (drinking water concentrations up to 20,000 ppm for males and up to 50,000 ppm for females). The final mean body weights of dosed and control mice were similar. Water consumption by female mice that received 50,000 ppm acetone was notably lower than that by controls. The absolute liver weight and the liver weight to body weight ratio were significantly increased for females receiving 50,000 ppm, and the absolute spleen weight and the spleen weight to body weight ratio were significantly decreased. Results from the hematologic analyses did not show any biologically significant effects. Centrilobular hepatocellular hypertrophy of minimal severity was seen in 2/10 female mice receiving 50,000 ppm. No compound-related lesions were found in male mice.

In summary, the results from these studies show that acetone is mildly toxic to rats and mice when administered in drinking water for 13 weeks. Minimal toxic doses were estimated to be 20,000 ppm acetone for male rats and male mice and 50,000 ppm acetone for female mice. No toxic effects were identified for female rats. The testis, kidney, and hematopoietic system were identified as target organs in male rats, and the liver was the target organ for male and female mice.

CONTRIBUTORS

This NTP Report on the toxicity studies of acetone is based on 14-day studies that began in August 1986 and on 13-week studies of acetone that began in November 1986 at Microbiological Associates, Inc. (Bethesda, MD).

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the Toxicity Studies on acetone on March 13, 1989, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have four major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the toxicity studies present the experimental results and conclusions fully and clearly, and (d) to judge the significance of the experimental results by scientific criteria.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICITY STUDIES OF ACETONE

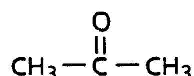
On March 13, 1989, the draft report on the toxicity studies of acetone received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. D.D. Dietz, NIEHS, introduced the short-term toxicity studies of acetone by reviewing the rationale, experimental design, and results.

Dr. Gallo, a principal reviewer, said that clinical pathology should have been done and should be the norm for these 13-week studies. He opined that lack of palatability at the higher concentrations clearly caused a depression in water consumption, resulting in the dose received being less than the intended dose. He commented on the wide ranges in temperature and relative humidity in the animal facilities. Dr. Dietz said that the temperature range was misleading, as there was just 1 day in the 13-week studies when the temperature was out of specifications.

Dr. Klaassen, a second principal reviewer, also reiterated the need for clinical chemistry measurements. He suggested that results and discussion should be separate sections.

Dr. Scala said that since there were no major objections, the Panel would accept the Report with the modifications as discussed.



ACETONE

CAS No. 67-64-1

C_3H_6O Molecular weight 58.1

Synonyms: 2-propanone; dimethyl ketone; pyroacetic acid

I. INTRODUCTION

Use and Production

Acetone is widely used as a general laboratory solvent and in industry as a solvent for fats, oils, waxes, resins, rubber, plastics, lacquers, varnishes, and rubber cements (Merck, 1983). Acetone is also a major component of nail polish remover (Arena and Drew, 1986) and is a chemical intermediate in the synthesis of a wide range of products, including chloroform, ketene, acetic acid, iodoform, mesityl oxide, tribromomethane, explosives, diacetone alcohol, airplane dope, rayon, methyl methacrylate, bisphenol A, methyl isobutyl ketone, hexylene glycol (2-methyl-2,4-pentanediol), isoprene, and isophorone (Rowe and Wolf, 1963; Nelson and Webb, 1978, 1985; Merck, 1983). A minor medicinal use for acetone is based on its bactericidal and virucidal action (Harvey, 1980). At concentrations exceeding 85% and mixed with ethanol, acetone is an effective antiseptic and keratolytic agent.

U.S. production ranged from 894 to 945 kilotons 8.1×10^8 to 8.6×10^8 kg) per year from 1983 to 1985 (Chem. Eng. News, 1985).

Exposure

Exposure to acetone may occur via inhalation or ingestion or by the dermal/ocular route, but the principal industrial health hazards are associated with the inhalation of acetone vapor at high concentrations and with repeated and prolonged extensive skin and eye contact (Rowe and Wolf, 1963; Krasavage et al., 1982; De Renzo, 1986; Plunkett, 1987). Accidental overexposure to acetone is rare. Although dermal absorption can occur (Martindale, 1982), it is minimal compared with exposure via the inhalation and oral routes (ACGIH, 1986). Acute systemic effects are possible after the percutaneous absorption of acetone but occur rarely (Martindale, 1982). Workplace exposure limits developed by the American Conference of Governmental and Industrial Hygienists are a time-weighted threshold limit value (TLV) of 750 ppm acetone and a short-term exposure limit of 1,000 ppm (ACGIH, 1986; De Renzo, 1986). A National Institute for Occupational Safety and Health criteria document for ketones recommends an exposure limit of 250 ppm acetone (NIOSH, 1978). This recommendation has not been adopted by U.S. industry, which currently operates under an Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) of 1,000 ppm acetone (Fed. Regist., 1988). The PEL set by OSHA is based on the irritant properties of acetone. The odor threshold for acetone is 100 ppm (Krasavage et al., 1982; De Renzo, 1986).

In addition to industrial exposure, concern has been expressed about the solvent-abuse potential (sniffing) of various solvents, including pure acetone and formulations containing various levels of acetone (Oliver and Watson, 1977; Geller et al., 1979a,b; Bruckner and Peterson, 1981).

Acetone has been postulated to be an intermediate in the conversion of fat to carbohydrate (Casazza et al., 1984). Trace amounts of endogenously formed acetone are detectable in normal human blood (7 µmol/liter) and urine (4-35 µmol/liter) (Rowe and Wolf, 1963; Oliver and Watson, 1977; Wigaeus et al., 1981; Krasavage et al., 1982; Pezzagno et al., 1986). Blood, alveolar air, and urine levels of acetone increase markedly during altered metabolic states associated with vitamin E deficiency, fasting, and diabetes (Garber et al., 1974; Kreisberg, 1978; Dillard and Tappel, 1979; Reichard et al., 1979; Casazza et al., 1984; Hetenyl and Ferrarotto, 1985; Gavino et al., 1987); after exposure to isopropanol (Nordmann et al., 1973; Plaa, 1980; Daniel et al., 1981; Natowicz et al., 1985); and as a result of exposure to haloethylenes, halomethanes, or haloethanes (Filser et al., 1978, 1982), acetylene (Foley, 1985), or disulfiram (DeMaster and Nagasawa, 1977). Small quantities of acetone may also be ingested as natural constituents or as additives to food (Furia and Bellanca, 1975; Martindale, 1982). During prolonged fasting or decompensated diabetes, fatty acid catabolism increases via mitochondrial β-oxidation (Lehninger, 1981) and leads to ketosis (increased blood levels of ketone bodies, including β-hydroxybutyrate, acetoacetate, and acetone) when the rate of ketone body formation exceeds the rate of removal by the peripheral tissues (Robinson and Williamson, 1980). Ketosis may also be induced by high fat diets and exercise and sometimes occurs during late pregnancy and early neonatal life. During ketosis, protein catabolism declines and there is a concomitant decreased availability of certain circulating amino acids, i.e., nitrogen conservation (Sherwin et al., 1975).

Toxicity/Carcinogenicity Studies in Humans

Repeated exposure to acetone can result in its accumulation in blood and other tissues over time and in a slow onset of toxic effects (Haggard et al., 1944; Bruckner and Peterson, 1981; Krasavage et al., 1982; Gosselin et al., 1984). Prolonged repeated exposure, however, may also induce a tolerance to many of the toxic effects (Rowe and Wolf, 1963; ACGIH, 1986). Exposure to acetone at atmospheric concentrations lower than 500 ppm is not commonly associated with any health hazards (Krasavage et al., 1982; Inoue, 1983; ACGIH, 1986). Exceptions include slight irritant effects noted in unacclimated subjects after very short exposure (3-5 minutes) (Rowe and Wolf, 1963; ACGIH, 1986), prolonged reaction times at 200 ppm (Wigaeus et al., 1981), and autonomic effects at 250 ppm (Wigaeus et al., 1981).

Although the toxic effects associated with short-term, high-level inhalation and oral exposure are similar, the adverse effects tend to be more serious after inhalation exposure to comparable doses of acetone (Rowe and Wolf, 1963; Arena and Drew, 1986). High-level inhalation exposure to acetone (greater than 1,000 ppm) affects the nervous system (central nervous system depression indicated by an initial stimulatory/excitatory restlessness phase followed by euphoria and hallucinations, narcosis, anesthesia, dyspnea, headache, vertigo, general muscular weakness including dysarthria and ataxia, and coma), gastrointestinal tract (nausea, vomiting, inflammation, and hematemesis), kidney (albuminuria, hematuria, and leukocyturia), mucous membranes (nasal, throat, and bronchial irritation and dry throat), eyes (irritation and transient corneal and conjunctival injury), and liver (hyperglycemia and increased bilirubin and urine urobilin) (Rowe and Wolf, 1963; Nelson and Webb, 1978; Geller et al., 1979a,b; Baselt, 1982; Krasavage et al., 1982; Finkel, 1983; Merck, 1983; Inoue, 1983; ACGIH, 1986; Arena and Drew, 1986; Grant, 1986). In addition, a Soviet investigator reported that hepatic lesions are associated with short-term inhalation exposure (concentration not specified) to acetone vapor (Mirchev, 1978). Although rare, short-term oral overexposure has been documented (Rowe and Wolf, 1963; Krasavage et al., 1982; De Renzo, 1986), with adverse effects similar to those associated with exposure to ethanol for equal blood levels but with greater anesthetic potency; 10-20 ml may be ingested without any untoward effects (Gosselin et al., 1984), and repeated ingestion of 25 ml acetone produces only slight drowsiness (Rowe and Wolf, 1963). The ingestion of 200 ml acetone induces hyperglycemia, restlessness, throat irritation, vomiting that progresses to hematemesis, and progressive central nervous system collapse, as

indicated by stupor and shallow respiration (Baselt, 1982; Krasavage et al., 1982; ACGIH, 1986; Arena and Drew, 1986). Mirchev (1978) found that short-term oral exposure (dose not specified) to acetone may induce hepatorenal lesions in humans. Prolonged or repeated application to the skin induces local eczematoid dermatitis and infections because of the defatting action of acetone (Rowe and Wolf, 1963; Cooper, 1974; Nelson and Webb, 1978; ACGIH, 1986; De Renzo, 1986; Plunkett, 1987). Ninety-minute topical exposure of volunteers induced mild edema and hyperemia at the exposure site; electron microscopic examination of punch biopsy specimens from the site of application showed cellular damage in the stratum corneum and stratum spinosum, including large paranuclear vacuoles (spinous cells) and edematous keratinized cells (Lupulescu and Birmingham, 1976; Krasavage et al., 1982). A single incident of prolonged contact of liquid acetone with the human eye has been reported to cause deep damage to the cornea, with permanent opacity (Grant, 1986).

There is no evidence that exposure to acetone is associated with an increased incidence of cancer in humans. Oglesby et al. (1949) reviewed the accumulated medical and acetone exposure (200-3,000 ppm) records in a cellulose acetate yarn production facility over 18 years and could not identify any harmful effects resulting from acetone exposure.

Toxicity/Carcinogenicity Studies in Animals

Numerous toxicity studies in animals show that acetone has a rather low short-term toxic potency (Smyth et al., 1962; Rowe and Wolf, 1963; Krasavage et al., 1982; NIOSH, 1985), manifested primarily as irritant and central nervous system effects. Data on mortality resulting from oral, dermal, and inhalation exposure are summarized in Table 1.

The irritant properties of acetone have been investigated by evaluating acetone-associated dermal, ocular, and respiratory changes (Rowe and Wolf, 1963; Kane et al., 1980; Krasavage et al., 1982; Rengstorff and Khafagy, 1985; NIOSH, 1985). Mild skin irritation scores (Draize test) have been reported in rabbits after skin exposure at 395-500 mg acetone (Krasavage et al., 1982; NIOSH, 1985). Mild-to-severe eye irritation in rabbits has been reported by several investigators (Rowe and Wolf, 1963; Krasavage et al., 1982; NIOSH, 1985); moderate corneal injury was also observed. The corneal changes are postulated to have been caused by dehydration of the sclera, which results in gelatinous flocculation and opacity.

Other acute toxic responses resulting from acetone exposure (via inhalation and ingestion) primarily involve the central nervous, cardiovascular, and respiratory systems (Walton et al., 1928; Haggard et al., 1944; Rowe and Wolf, 1963; Goldberg et al., 1964; Raje, 1980; Krasavage et al., 1982; Chentanez et al., 1987). The short-term effects of acetone on the central nervous system have been extensively characterized in several species, including mice, rats, guinea pigs, and baboons (Haggard et al., 1944; Rowe and Wolf, 1963; Goldberg et al., 1964; Krasavage et al., 1982). Haggard et al. (1944) showed a continuum of central nervous system deficits in rats

TABLE 1. SUMMARY OF LETHAL DOSES FOR ANIMALS IN ORAL, DERMAL, AND INHALATION STUDIES OF ACETONE

Species	LD_{50}	Species	Lethal Concentration/Duration
Oral exposure		**Inhalation exposure**	
Rats	8.5-10.7 ml/kg	Mice	46,000 ppm/1 h
Mice	0.58-9.75 g/kg	Rats	126,000 ppm/2 h
Rabbits	5.3 g/kg	Rats	16,000-42,200 ppm/4 h
Dermal exposure			
Rabbits	20 g/kg (>20 ml/kg)		

exposed at 10,415-125,000 ppm acetone for 45-480 minutes. These effects were reported to be similar to those of ethanol in terms of the acetone effect and potency, and the severity increased with both dose and duration of exposure. Continued acetone exposure at concentrations above 20,833 ppm sequentially induced a stimulation of breathing, a decrease in breathing together with depressed activity (associated acetone concentrations in blood of greater than 2,500 mg/liter), and finally a marked depression of the respiratory center (acetone blood levels greater than 6,000 mg/liter). Median concentrations of acetone in blood associated with various deficits are given in Table 2. Mice exposed to acetone vapor at 20,256 ppm for 90 minutes and at 16,880 ppm for 180 minutes showed signs of narcosis, whereas those exposed at 41,250 ppm (duration not specified) showed signs of anesthesia (Rowe and Wolf, 1963; Krasavage et al., 1982).

The short-term operant behavior of rats exposed to acetone via inhalation was examined by Goldberg et al. (1964). Female rats were trained according to an avoidance-escape paradigm. Groups were exposed by inhalation to acetone at 3,000, 6,000, 12,000, or 16,000 ppm, 4 hours per day, 5 days per week for 10 days. Body weight or growth was not affected at any dose, but escape behavior was suppressed, and ataxia was noted on day 1 in the 12,000- and 16,000-ppm groups. Avoidance behavior was inhibited in groups exposed at 6,000 ppm, 12,000 ppm, and 16,000 ppm. A tolerance developed to all the reported neurobehavioral effects.

Acetone was administered by gavage to groups of 30 Sprague Dawley rats of each sex for 13 weeks at doses of 0, 100, 500, or 2,500 mg/kg per day (Sonawane et al., 1986). There were no compound-related effects on body weight, ophthalmologic or urinalysis results, or mortality. Changes associated with acetone administration included increased hemoglobin, hematocrit, erythrocyte indices, and serum alanine aminotransferase (ALAT) activity in high dose males and females at the end of the studies; increased liver weights were noted for female rats in the 500 and 2,500 mg/kg groups. The primary findings from this study, however, were increased kidney weight in the 500 and 2,500 mg/kg groups of females, an accentuation of renal proximal tubular degeneration and intracytoplasmic hyaline droplet accumulation in the 500 and 2,500 mg/kg groups of males, and an accentuation of renal proximal tubular degeneration in the 2,500 mg/kg females. In another study, Brown and Hewitt (1984) showed that a single oral exposure to acetone (15 mmol/kg) induces degenerating apical microvilli in the S_1 and S_2 portions of male Sprague Dawley rat proximal tubules.

Sollmann (1921) exposed three rats for 18 months to 2%-5% acetone in their drinking water; feed and water consumption and weight gain were depressed. Furner et al. (1972) exposed rats to 1% acetone in drinking water for 5 days without affecting growth rates. Data from these studies are limited, however. Sollmann exposed only three rats and did not include controls, and Furner et al. evaluated a restricted

TABLE 2. CENTRAL NERVOUS SYSTEM DEFICITS INDUCED IN RATS BY INHALED ACETONE

Concentration (ppm)	Most Severe Deficit	Minimum Associated Concentration of Acetone in Blood at Which Effect Was Seen (mg/liter)	Estimated Onset of Most Severe Deficit (minutes)
2,083	None		--
10,415	Ataxia	1,500	90
20,830	Loss of righting reflex	3,000	120
41,660	Loss of corneal reflex	5,170	90
83,320	Loss of corneal reflex		50
124,980	Loss of corneal reflex		20
124,980	Respiratory failure	9,190	20

set of indices (body weight, liver weight, free fatty acids, and liver metabolism) after a limited exposure interval.

No reports are available in the literature on the long-term toxicity or carcinogenicity of acetone to animals (Kawachi et al., 1980; Soderman, 1982). Acetone has been used as a vehicle in dermal studies in mice (Hennings et al., 1983; Yuspa, 1984; Zakova et al., 1985; Ward et al., 1986). Mice generally received one or two applications per week of 0.2 ml acetone per application for 6 months to 2 years without an increased incidence of neoplasia or a toxic response. In one study, microscopic examination of the application site showed minimal-to-moderate focal acanthosis of the epidermis, slight fibrosis and occasional focal suppuration of the dermis, and ulceration of the epidermis, all attributable to local traumatization (Zakova et al., 1985). Because percutaneously applied acetone is absorbed into the systemic circulation, tissues remote from the application site may be affected, but a comprehensive evaluation of tissue sites other than the skin was not performed (Rowe and Wolf, 1963; Bruckner and Peterson, 1981; Krasavage et al., 1982; Rengstorff and Khafagy, 1985).

Ward et al. (1986) reviewed the histopathologic data from acetone controls (female SENCAR mice) dosed from 8 to 92 weeks of age. The major cause of death was glomerulonephritis and histocytic sarcomas, which appeared to originate in the uterus and to have metastasized to several tissues, including the liver, lung, and kidney. Because histopathologic data from aging mice of this strain were not available, the investigators hypothesized that these lesions were age related. Although the investigators indicated that these results were unexpected, they did not discuss any possible etiology relative to acetone exposure. Iversen et al. (1981) showed that acetone induced some preneoplastic changes in the skin of hairless mice that received topical applications (100 µl) two times per week for 10 weeks. Acetone administration was discontinued after 10 weeks, and at various intervals for the next 18 weeks, animals were given intraperitoneal injections of [^{3}H]thymidine. The results from this study showed moderate hyperplasia of the basal and suprabasal cells and a slight increase in the mitotic rate, indicating increased cell turnover at the application site.

Toxic Interactions

Perhaps the most extensively studied aspect of acetone toxicity is the ability of this common solvent to interact synergistically with and exacerbate the hepatorenal toxicity and/or mutagenicity of a wide variety of frequently encountered chemicals (nitrosamines, halogenated hydrocarbons, and acetonitrile) which are established carcinogens and/or toxicants and are often found in food and water (Pohl et al., 1977; Stevens and Anders, 1979; Haag and Sipes, 1980; Kubic and Anders, 1980; Plaa, 1980; Glatt et al., 1981; MacDonald et al., 1982; Hewitt and Plaa, 1983; Hewitt et al., 1983a; Hewitt and Brown, 1984; Lorr et al., 1984; Charbonneau et al., 1985; Freeman and Hayes, 1985; Yoo and Yang, 1985). Hewitt et al. (1986) reported that the literature is "replete" with descriptions of such interactions. Preliminary exposure to other compounds containing the carbonyl moiety and to ketogenic chemicals (those metabolized to ketones), such as isopropanol, *sec*-butanol, and *n*-hexane, and the clinical state of ketosis including diabetes will potentiate the toxic and/or mutagenic responses to many of these chemicals (Hewitt et al., 1980, 1983b; Plaa, 1980; Lorr et al., 1984; Charbonneau et al., 1985; Hewitt et al., 1986). A complete review of these interactions is beyond the scope of this report.

Metabolism and Kinetics

Acetone is readily absorbed in direct proportion to dose by all routes of administration, including inhalation, oral, and dermal (Rowe and Wolf, 1963; Krasavage et al., 1982). Once absorbed, acetone, a highly water-soluble compound, is readily taken up into the blood, is rapidly and widely distributed throughout the tissues according to their water content, and is excreted unchanged or is metabolized (Haggard et al., 1944; Rowe and Wolf, 1963; Krasavage et al., 1982; Wigaeus et al., 1982). Elimination of unmetabolized acetone is the predominant route of removal for large doses of acetone, whereas metabolism plays a larger role in removal of small doses (Haggard et al., 1944). Haggard et al. showed that rats that have blood levels of

acetone of 84 mg/liter lose 45% by elimination and 55% by metabolism, whereas rats that have blood levels of acetone of 128 mg/liter lose 64% by elimination and 36% by metabolism. The rate of acetone metabolism increases during fasting and exercise. When exposure in rats is ended, acetone in blood disappears at a rate of 5.6 mg/kg per hour. Unmetabolized acetone is excreted mainly by the lungs (40%-70% of the total), less is excreted in urine (15%-30%), and the least through the skin (10%) (Rowe and Wolf, 1963). Wigaeus et al. (1982) showed that in mice exposed to acetone at 500 ppm for 6 hours by inhalation, acetone had a tissue half-life of between 2 and 5 hours.

Although acetone has been considered to be a nonmetabolizable endogenous and exogenous compound (Morris and Cavanagh, 1987), several studies in rats and mice have shown that acetone can be metabolized by three separate gluconeogenic pathways, as illustrated in Figure 1 (Price and Rittenberg, 1950; Sakami, 1950; Mourkides et al., 1959; Casazza et al., 1984; Kosugi et al., 1986a,b). One pathway involves the conversion of acetone via methylglyoxal or methylglyoxal and D-lactate to D-glucose; two other pathways involve the conversion of acetone via L-1,2-propanediol that converts to the gluconeogenic precursors L-lactate or acetate. The ultimate fate of acetone is the conversion via glucose to ATP and carbon dioxide. Several investigators using [1,3-^{14}C]acetone or [2-^{14}C]acetone have traced radiolabeled carbon from gluconeogenic precursors and formate to alanine, aspartate, glutamate, urea, serine, methionine, choline, cholesterol, heme, arginine, and fatty acids (Price and Rittenberg, 1950; Sakami, 1950; Mourkides et al., 1959). Two initial enzymatic steps for the conversion of acetone to its gluconeogenic metabolites require oxygen and NADPH and involve a microsomal P450 fraction (Koop and Casazza, 1985; Johansson et al., 1986). This same P450 fraction also contains activity for aniline hydroxylase and NDMAd and the oxidation of aliphatic alcohols. It is inducible by fasting, experimental diabetes, and long-term ethanol or acetone exposure. Contained in this fraction are acetone monooxygenase, which catalyzes the initial enzymatic step common to all three pathways of acetone metabolism, and acetol monooxygenase, which catalyzes the next step and is unique to the first pathway. Acetone monooxygenase catalyzes the hydroxylation of the acetone methyl group to produce acetol, which is in turn oxidized at the hydroxyl position by acetol monooxygenase to yield methylglyoxal.

Another important reaction in this scheme involves the conversion of acetol to L-1,2-propanediol by an extrahepatic process that has not been characterized (Rudney, 1954; Ruddick, 1972; Casazza et al., 1984; Kosugi et al., 1986a,b). The L-1,2-propanediol formed extrahepatically returns to the liver, where it either breaks down to acetate and formate or is metabolized in sequence by alcohol dehydrogenase and aldehyde dehydrogenase to produce L-lactate.

Teratology and Reproductive Toxicity Studies

The effects of increased maternal plasma ketone levels (ketonemia) on prenatal development in both humans and experimental animals have been reviewed by Freinkel (1985). Ketosis is associated with prenatal toxicity, expressed during late pregnancy by excessive fetal growth, concurrent accelerated maturation of pancreatic islets (islet hyperplasia), and increased deposition of fat and glycogen. The interactions of maternal metabolic disturbances with fetal development are extremely complex, as evidenced by the fact that ketonemia during the embryonic period may result in retarded development of the embryo, whereas the same disturbance in late pregnancy results in excessive fetal growth (macrosomia). Ketonemia during pregnancy may also result in alterations in normal development of the central nervous system and causes such abnormalities as open neural tube, faulty neural tube-fusion and microcephaly, in addition to pericardial edema, transposition of the great vessels, and sacral dysgenesis (Gabbe, 1977).

Several in vitro studies have been conducted in an attempt to determine the teratogenic potential of acetone and have yielded negative results. No evidence of teratogenicity was found when 39 or 78 mg of acetone was injected into the yolk sacs of fertile chick eggs prior to incubation (McLaughlin et al., 1964). DiPaolo et al. (1969) added 0.2% acetone to the growth medium of

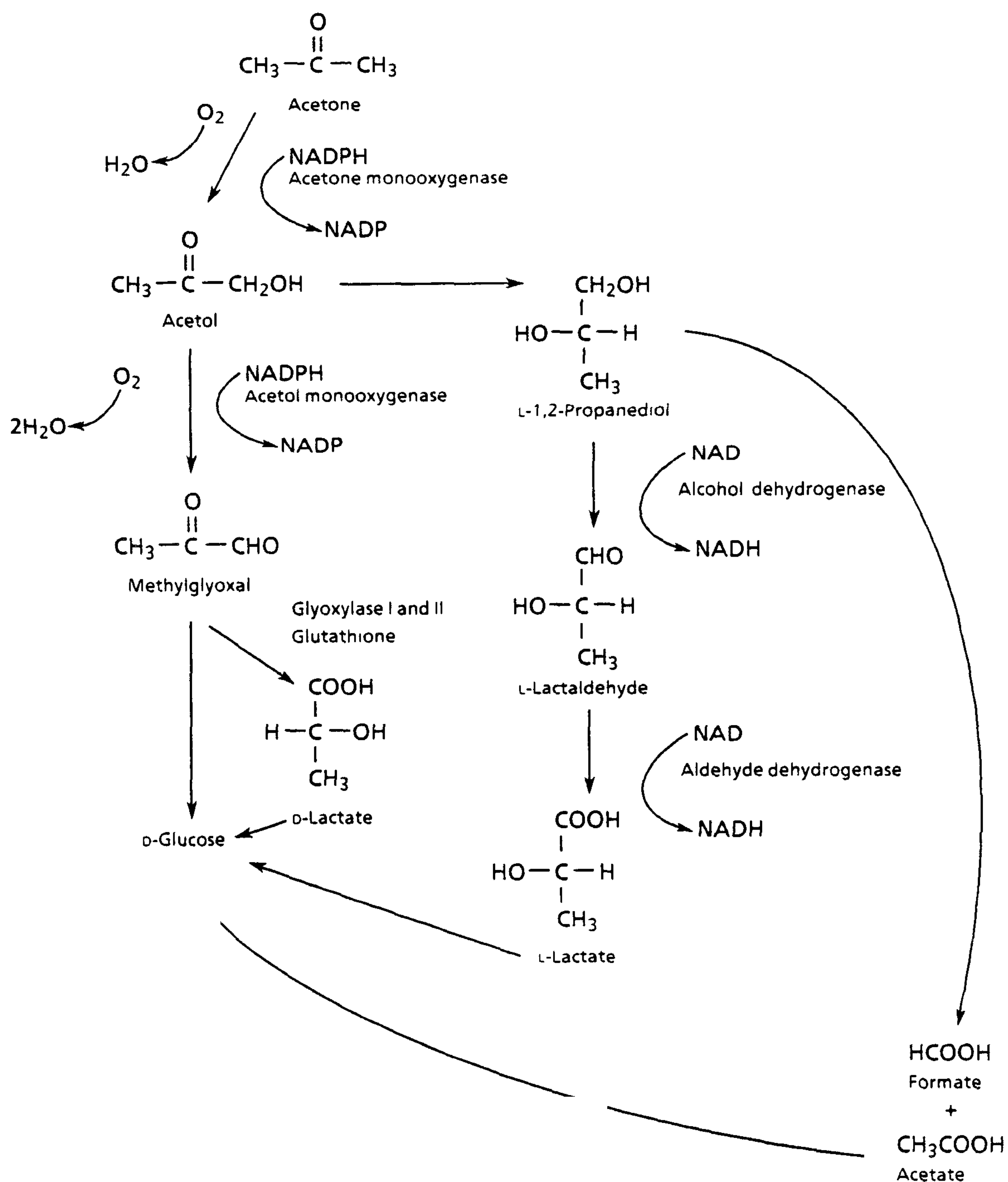


FIGURE 1. PATHWAYS OF ACETONE METABOLISM

Based on Casazza et al. (1984) and Kosugi et al. (1986b)

cultured Syrian hamster embryonic cells and detected no evidence of cellular transformation.

Guntakatta et al. (1984) tested acetone for teratogenic potential in an in vitro mouse embryo limb bud cell culture system. Acetone concentrations in culture media at concentrations as high as 100 mg/ml did not differentially affect the cellular incorporation of radiolabeled sulfate or thymidine into developing limb bud cells.

Nizyayeva (1982) studied female factory workers who received long-term exposure to acetone below the TLV, 200 mg/m^3 (85 ppm). Statistically significant increases in the incidence of complications of pregnancy were reported in these workers, including increased threat of abortion, toxicosis during the second half of pregnancy, diminished hemoglobin concentrations, and hypotension. A significant reduction in the birth weight and size of infants born to workers in chemical fiber factories relative to a control group was also reported. These complications of pregnancy were considered to be secondary to changes in general body function, notably "acidosis, disturbed carbohydrate and fat metabolism, and disturbed neuroendocrine regulation."

In an inhalation teratologic study, animals were exposed to 30 or 300 mg/m^3 acetone for either days 1-13 or 1-20 of gestation (Nizyayeva, 1982). A statistically significant, but not concentration-related, reduction in the percentage of live embryos was reported for both exposure concentrations in animals exposed on days 1-20 of gestation. The percentages of embryonal deaths for the 0, 30, and 300 mg/m^3 groups were 11%, 28%, and 23%, respectively. These findings and morphologic changes were associated with "disorders of the placental barrier."

The National Toxicology Program (NTP) recently completed teratologic studies in Sprague Dawley rats and CD®-1 mice. Female rats and mice were mated and then exposed to acetone on days 6-19 of gestation (rats) or on days 6-17 of gestation (mice). Inhalation exposure for 6 hours per day was at 0, 440, 2,200, or 11,000 ppm. A necropsy was performed on rats and mice on day 20 (rats) or day 18 (mice) of gestation for maternal and fetal evaluations. A final report on these studies is available from the NTP.

Genetic Toxicology

In unpublished NTP studies, acetone was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, or TA1537 when tested in a preincubation protocol at doses up to 10 mg/plate in both the presence and absence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9. When tested for cytogenetic effects in Chinese hamster ovary cells, acetone, at doses up to 5,000 µg/ml, did not induce sister chromatid exchanges or chromosomal aberrations in either the presence or absence of Aroclor 1254-induced male Sprague Dawley rat liver S9. There was no increase in the frequency of micronucleated normochromatic erythrocytes or polychromatic erythrocytes in the peripheral blood of male and female mice that received 5,000-20,000 ppm acetone in drinking water for 13 weeks.

Study Rationale

Acetone was nominated for study by the National Institute of Environmental Health Sciences/NTP in response to a request by the U.S. Environmental Protection Agency (EPA), which demonstrated the need for additional toxicologic data as part of an Interagency Agreement with the Agency for Toxic Substances and Disease Registry. The ultimate purpose was to generate toxicity data for chemicals found in chemical waste sites when adequate data for risk assessment are not available.

According to a review by the Mitre Co. of the National Priorities List of chemical waste sites, acetone is the 22nd most frequently found organic component of chemical waste sites. In a survey of groundwater contaminant levels conducted by the EPA in 1985, the average and maximum concentrations of acetone were 7 and 250 ppm, respectively (Yang and Rauckman, 1987). Because of its miscibility with water, it is easily carried into groundwater and surface water and, ultimately, drinking water. Very limited information concerning the oral short-term toxicity of acetone is available. A study by Sollmann (1921), who exposed rats to 25,000 ppm acetone

in drinking water, is inadequate because of the small number (three) of rats tested and the lack of concurrent controls. Furner et al. (1972) also exposed rats to acetone (10,000 ppm) in their drinking water. Their study, however, was limited by the short duration (5 days of exposure) and by the small number of relevant indices evaluated (body weight, liver weight, free fatty acids, and liver metabolism). In addition, the EPA in 1985 sponsored 13-week gavage toxicity studies of acetone in which Sprague Dawley rats were administered 0, 100, 500, or 2,500 mg/kg per day (Sonawane et al., 1986). The usefulness of the data from these studies, however, is limited because of the pharmacokinetic considerations of a bolus administration and the consequent need to more closely mimic human exposure. Dose selection for the short-term studies described in this report was based on a review of the results from the two drinking water studies mentioned herein and from other animal toxicity studies described in the literature.

II. MATERIALS AND METHODS

Procurement and Characterization of Acetone

Lot no. AN228 of acetone (99% pure by gas-liquid chromatography) used during these studies was produced by Burdick and Jackson (Muskegon, MI). Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, MO). MRI reports on the analyses performed in support of the acetone studies are on file at the National Institute of Environmental Health Sciences.

Lot no. AN228 was identified as acetone by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra (Sadtler Standard Spectra).

The purity of lot no. AN228 was determined by elemental analysis, Karl Fischer water analysis, titration with 0.01 N sodium hydroxide to the phenolphthalein endpoint to determine acetic acid content, and two gas chromatographic systems.

Results of elemental analysis for carbon and hydrogen agreed with theoretical values. The water content was 0.35%. Titration indicated 0.00022 meq acid per gram of sample (13.5 ppm as acetic acid). No peaks with areas 0.1% or greater than that of the major peak were found with either gas chromatographic system. Cumulative analytical data indicated a purity of greater than 99%.

Stability studies performed by gas chromatography, but with 0.8% (v/v) 1-propanol as an internal standard, indicated that acetone is stable as a bulk chemical for at least 2 weeks when stored protected from light at temperatures up to 50° C. In addition, stability analyses of acetone (5 mg/ml) maintained for 96 hours in inverted 500-ml rat cage drinking bottles and sipper tubes under ambient conditions (temperature and lighting) demonstrated that acetone was stable (1.5% loss). During the 13-week studies the contents of drinking bottles were sampled and analyzed for acetone concentration (weeks 1 and 7). Results from these analyses showed that samples ranged from 90.8% to 102% and from 84.0% to 95.2% of target concentrations.

Preparation and Characterization of Drinking Water Mixtures

The appropriate amounts of acetone and tap water were mixed (w/v) to give the desired concentrations. The stability of acetone in water (5 mg/ml) was determined by gas chromatographic analysis with a Porapak T column. Samples were mixed with isopropanol as an internal

standard and further diluted with methanol before analysis. Acetone in water was found to be stable for at least 21 days in the dark at room temperature. Acetone solutions were also found to be stable for at least 96 hours when stored in clear glass drinking water bottles under normal room light. During the 13-week studies, drinking water mixtures were stored for no longer than 3 weeks at room temperature.

Periodic analysis of formulated acetone/drinking water mixtures was conducted by gas chromatography at the study and analytical chemistry laboratories. One of 34 mixtures was out of specifications (±10% of the target concentration) (Table 3). The results of the analyses ranged from 87% to 106% of the target concentrations, with the mean concentration at 99.4% of the target and a standard deviation of 3.4%.

Fourteen-Day Study Design

Groups of five males and five females of each species were exposed to acetone in drinking water at concentrations of 0, 5,000, 10,000, 20,000, 50,000, or 100,000 ppm, ad libitum for 14 days. A necropsy was performed on all animals; the controls and 100,000-ppm groups received histologic examinations (Table 4).

Thirteen-Week Study Design

Groups of 10 rats of each sex and 10 female mice were exposed to drinking water containing 0, 2,500, 5,000, 10,000, 20,000, or 50,000 ppm acetone for 13 weeks. Groups of 10 male mice were exposed to acetone at 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm on the same schedule.

At the end of the studies, samples were taken from male and female rats and female mice that received 0, 2,500, 10,000, or 50,000 ppm acetone and from male mice that received 0, 1,250, 5,000, or 20,000 ppm for evaluation of sperm morphology and vaginal cytology.

Male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under strict barrier conditions at Simonsen Laboratories, Inc. Animals were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Animals were shipped to the study laboratory at 4 weeks of age. The rats were quarantined at the study laboratory for 12 days and mice for 15 days. All animals were placed on study at 6 weeks of age. A transient decrease in temperature (54° F) and relative humidity (24%) were recorded on November 14, 1986. This single incident did not induce any signs of stress; environmental conditions were within acceptable limits 1 day before and 1 day after this incident.

Details of clinical examinations and pathology procedures are outlined in Table 4. Animals found moribund and those surviving to the end of the studies were killed under carbon dioxide anesthesia. A necropsy was performed on all animals.

TABLE 3. RESULTS OF ANALYSIS OF FORMULATED DRINKING WATER MIXTURES IN THE THIRTEEN-WEEK DRINKING WATER STUDIES OF ACETONE

Target Concentration (ppm)	Number Analyzed	Determined Concentration (a) (ppm)
1,250	3	1,207 ± 23
2,500	6	2,492 ± 67
5,000	6	5,030 ± 110
10,000	7	(b) 9,877 ± 575
20,000	6	20,167 ± 561
50,000	6	49,383 ± 791

(a) Mean ± standard deviation
(b) One drinking water formulation was out of specifications and was remixed.

TABLE 4. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE DRINKING WATER STUDIES OF ACETONE

Fourteen-Day Studies	Thirteen-Week Studies
Strain and Species	
F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source	
Taconic Farms (Germantown, NY)	Simonsen Laboratories (Gilroy, CA)
Study Laboratory	
Microbiological Associates, Inc.	Microbiological Associates, Inc.
Size of Study Groups	
5 males and 5 females of each species	10 males and 10 females of each species
Doses	
0, 5,000, 10,000, 20,000, 50,000, or 100,000 ppm acetone in drinking water	Rats and female mice--0, 2,500, 5,000, 10,000, 20,000, or 50,000 ppm acetone in drinking water; male mice--0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm
Method of Animal Distribution	
Randomized for each sex on the basis of body weight into six groups per sex	Same as 14-d studies
Diet	
NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA); available ad libitum	Same as 14-d studies
Animal Room Environment	
Temp--21.1°-29.3° C; hum--37%-67%; fluorescent light 12 h/d; 12 room air changes/h	Temp--12.2°-22.2° C, hum--20%-68%, fluorescent light 12 h/d, 12 room air changes/h
Time Held Before Study	
14 d	Rats--12 d; mice--15 d
Type and Frequency of Observation	
Observed 2 × d; weighed initially and 1 × wk thereafter	Same as 14-d studies; water consumption measured 2 × wk by cage
Necropsy and Histologic Examinations	
All exposed animals received acetone until the day of the terminal kill. Necropsy performed on all animals; tissues examined histologically for control and high dose groups. Liver examined for all groups of mice except 5,000-ppm females; bone marrow examined for 50,000-ppm male rats; mandibular lymph nodes examined for all 50,000-ppm male rats and all female rats. Organ weights obtained at necropsy	All exposed animals received acetone until the day of the terminal kill. Necropsy performed on all animals; the following tissues examined histologically for control and high dose groups: adrenal glands, brain, cecum, colon, duodenum, epididymis/seminal vesicles/prostate/testes or ovaries/uterus, esophagus, eyes (if grossly abnormal), femur including marrow, gross lesions and tissue masses with regional lymph nodes, heart, ileum, jejunum, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, preputial or clitoral gland, rectum, salivary glands, skin, spinal cord and sciatic nerve (if neurologic signs present), spleen, stomach, thymus, thyroid gland, trachea, and urinary bladder. Tissues examined for lower dose groups include heart, kidneys, and spleen for 10,000- and 20,000-ppm male rats, kidneys for 5,000-ppm male rats, and bone marrow for 20,000-ppm male rats, nose examined for all groups of female rats and mandibular lymph nodes for 20,000-ppm female rats; liver examined for all female mice. Organ weights obtained and hematologic determinations performed at necropsy. Sperm morphology and vaginal cytology examinations performed

At the end of the studies, blood samples were taken from the orbital sinus under carbon dioxide anesthesia for hematologic determinations. Samples were evaluated for the following: erythrocyte, leukocyte, platelet, and differential leukocyte counts; erythrocyte, leukocyte, and platelet morphology; hemoglobin; packed cell volume (hematocrit); number of reticulocytes; mean cell volume; and mean corpuscular hemoglobin.

Organs and tissues were examined for gross lesions. Tissues were preserved in 10% neutral buffered formalin and routinely processed for preparation of histologic sections for microscopic examination. Tissues and groups examined are listed in Table 4.

Upon completion of the histologic evaluation by the laboratory pathologist, 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, and the results were reviewed and evaluated by an NTP Pathology WorkingGroup (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).

Statistical Methods

Analysis of Continuous Variables: The comparison of organ weight, hematologic, and male reproductive function data from dosed and control groups was carried out by using the nonparametric multiple comparison procedures of Dunn (1964) or Shirley (1977). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose response trends and to determine whether Dunn's or Shirley's test was more appropriate for pairwise comparisons.

III. RESULTS AND DISCUSSION

The 14-day studies were conducted to evaluate the palatability of acetone in drinking water and to characterize any gross toxicity resulting from acetone administration. The concentrations selected ranged from 100,000 ppm, the highest practical limit of exposure (10% w/v), to 5,000 ppm (0.5% w/v), a concentration lower than that previously found to be without a toxic effect (10,000 ppm or 1 0% w/v) (Furner et al., 1972). Concentrations for the 13-week studies were selected such that they would delineate between toxic and nontoxic concentrations.

Studies in Rats

Survival, body weight, and water consumption values for the short-term studies in rats are presented in Tables 5 and 6 and in Figure 2. In the 14-day and 13-week studies, water consumption was clearly depressed in all groups exposed to acetone at concentrations of 50,000 ppm or higher. The overall water consumption by female rats exposed to 20,000 ppm acetone in the 13-week studies was also depressed compared with that by controls. Despite this depressed fluid consumption, no signs of dehydration were reported. The effects of acetone on body weight gain in male rats paralleled those on water consumption. Females exposed to 50,000 ppm acetone for 14 days or 20,000 ppm acetone for 13 weeks, however, had weight gains similar to those of controls, despite moderately depressed fluid intake. Time-weighted average doses of acetone for rats in the 14-day and 13-week studies are shown in Table 7.

No changes in body weight gain or effects on feed consumption were observed in another study in male and female rats given 0, 100, 500, or 2,500 mg/kg acetone by gavage for 13 weeks (Sonawane et al., 1986). In addition, Sollmann (1921) demonstrated that acetone at 25,000 ppm

TABLE 5. SURVIVAL, MEAN BODY WEIGHTS, AND WATER CONSUMPTION OF RATS IN THE FOURTEEN-DAY DRINKING WATER STUDIES OF ACETONE

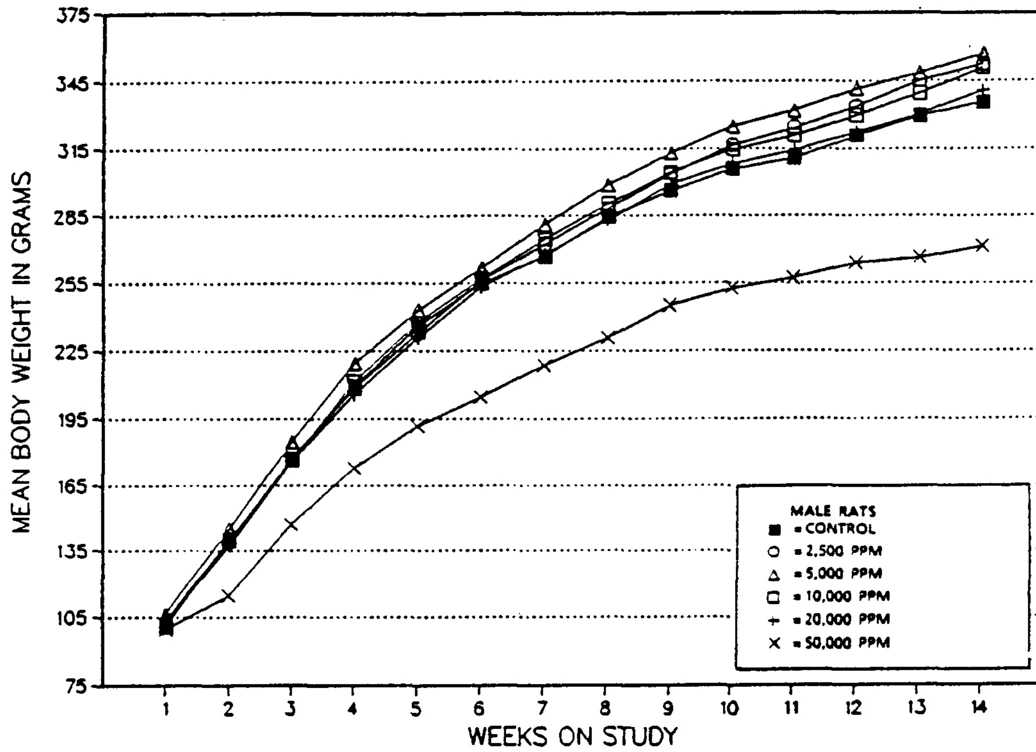
Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Necropsy	Change (c)	Necropsy Weight Relative to Controls (percent)	Water Consumption (d)
MALE						
0	5/5	126 ± 4	202 ± 8	+76 ± 4		122
5,000	5/5	129 ± 4	209 ± 8	+80 ± 4	103	123
10,000	5/5	126 ± 4	201 ± 6	+75 ± 2	100	141
20,000	5/5	125 ± 4	194 ± 3	+69 ± 3	96	113
50,000	5/5	129 ± 5	176 ± 6	+47 ± 2	87	79
100,000	5/5	127 ± 4	128 ± 11	+1 ± 9	63	74
FEMALE						
0	5/5	101 ± 2	137 ± 2	+36 ± 1		133
5,000	5/5	100 ± 1	133 ± 3	+33 ± 2	97	142
10,000	5/5	100 ± 2	138 ± 3	+38 ± 1	101	136
20,000	5/5	101 ± 2	135 ± 2	+34 ± 1	99	111
50,000	5/5	103 ± 3	134 ± 3	+31 ± 1	98	78
100,000	5/5	100 ± 2	119 ± 1	+19 ± 2	87	80

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Milliliters per kilogram per day, based on average body weight and consumption data obtained during the 2-week interval

TABLE 6. SURVIVAL, MEAN BODY WEIGHTS, AND WATER CONSUMPTION OF RATS IN THE THIRTEEN-WEEK DRINKING WATER STUDIES OF ACETONE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Water Consumption (d)
MALE						
0	10/10	101 ± 3	330 ± 5	+229 ± 4		87
2,500	10/10	103 ± 4	345 ± 9	+242 ± 7	105	79
5,000	10/10	107 ± 2	349 ± 6	+242 ± 4	106	78
10,000	10/10	101 ± 3	340 ± 9	+239 ± 7	103	80
20,000	10/10	103 ± 3	330 ± 6	+227 ± 5	100	83
50,000	10/10	100 ± 3	266 ± 7	+166 ± 4	81	65
FEMALE						
0	10/10	95 ± 3	206 ± 4	+111 ± 2		107
2,500	10/10	97 ± 3	203 ± 3	+106 ± 2	99	110
5,000	10/10	92 ± 3	196 ± 5	+104 ± 3	95	121
10,000	10/10	92 ± 2	197 ± 3	+105 ± 2	96	112
20,000	10/10	86 ± 2	191 ± 4	+105 ± 3	93	75
50,000	10/10	95 ± 2	191 ± 3	+96 ± 2	93	58

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Milliliters per kilogram per day, average for weeks 6 and 13



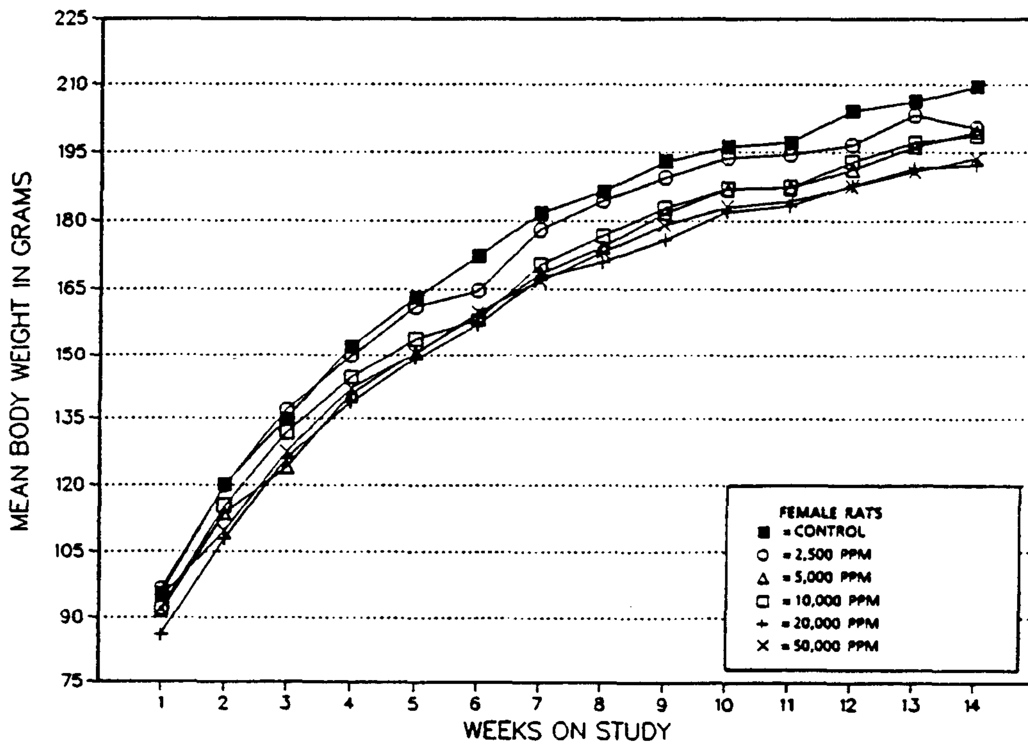


FIGURE 2. GROWTH CURVES FOR RATS ADMINISTERED ACETONE IN DRINKING WATER FOR THIRTEEN WEEKS

TABLE 7. ACETONE CONSUMPTION BY RATS IN THE FOURTEEN-DAY AND THIRTEEN-WEEK DRINKING WATER STUDIES

Concentration (ppm)	Time-Weighted Average Dose (mg/kg per day)	
	Fourteen-Day Study	Thirteen-Week Study
MALE		
0	0	0
2,500	--	200
5,000	714	400
10,000	1,616	900
20,000	2,559	1,700
50,000	4,312	3,400
100,000	6,942	--
FEMALE		
0	0	0
2,500	--	300
5,000	751	600
10,000	1,485	1,200
20,000	2,328	1,600
50,000	4,350	3,100
100,000	8,560	--

in the drinking water of rats depressed growth, fluid intake, and feed consumption.

No deaths occurred in these 14-day and 13-week studies, nor were deaths seen in a previous gavage study at doses up to 2,500 mg/kg (Sonawane et al., 1986). Relatively little overt toxicity was seen in the current studies; clinical signs were only observed for rats receiving 100,000 ppm acetone in the 14-day studies and reflected the mild debilitation associated with depressed weight gain; i.e., emaciated appearance of 6/10 rats. No ophthalmic irregularities or lesions were noted after either bolus (Sonawane et al., 1986) or continuous (current studies) exposure to acetone. Percutaneous exposure to acetone (0.5 ml per day, 5 days per week for 6 weeks) by guinea pigs, but not by rats, induces the formation of cataracts within 3 months (Rengstorff and Khafagy, 1985).

Acetone exposure induced several changes in organ weight which appeared to be independent of effects on body weight. Some absolute organ weight and relative organ weight to body weight changes observed in the current 13-week studies are listed in Table 8. The major organs affected were the kidney, liver, and testis. Minimal doses of acetone associated with these changes were: for increases in relative kidney weights, 20,000 ppm for females and 50,000 ppm for males; for increases in relative liver weights, 20,000 ppm for males and females; and for increases in relative testis weights, 50,000 ppm acetone. Similar changes at these or lower doses were noted for these organs during the 14-day studies. In addition, thymus weights of rats exposed to acetone were depressed by as much as 49.9% and 76.9% relative to controls during the 14-day studies.

The kidney weight changes were associated with nephropathy and are described later. Although the liver weight changes were not associated with acetone-mediated histopathologic effects, these changes may have been associated with the ability of acetone to induce hepatic microsomal mixed-function oxidase (Clark and Powis, 1974; Anders and Gander, 1979; Glatt et al., 1981; Bidlack and Lowery, 1982; Kitada et al., 1983; Koop et al., 1985; Ko et al., 1987).

The relative, but not the absolute, testis weight was increased at 50,000 ppm in both the 14-day and 13-week studies (Table 8). These organ weight changes are difficult to interpret because of the effect of acetone on body weight at these doses and may not be biologically significant

TABLE 8. ANALYSIS OF ORGAN WEIGHTS OF RATS IN THE THIRTEEN-WEEK DRINKING WATER STUDIES OF ACETONE (a)

Organ	Control	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	50,000 ppm
MALE						
Body weight (grams)	336 ± 4.5	353 ± 8.6	357 ± 5.7	351 ± 9.6	341 ± 6.3	**271 ± 6.8
Right kidney						
Absolute	1,264 ± 42.5	1,270 ± 38.6	1,277 ± 21.6	1,274 ± 37.6	1,315 ± 33.0	1,304 ± 44.6
Relative	3.8 ± 0.12	3.6 ± 0.08	3.6 ± 0.05	3.6 ± 0.08	3.9 ± 0.09	**4.8 ± 0.09
Liver						
Absolute	12,600 ± 250	13,410 ± 410	13,370 ± 250	13,510 ± 450	14,080 ± 470	11,650 ± 350
Relative	37.5 ± 0.54	38.1 ± 0.83	37.4 ± 0.50	38.5 ± 0.67	**41.3 ± 1.10	**43.0 ± 0.82
Lung						
Absolute	1,658 ± 60.6	1,584 ± 48.5	1,796 ± 106.5	1,863 ± 130.5	1,806 ± 67.3	1,902 ± 162.5
Relative	4.9 ± 0.18	4.5 ± 0.14	5.0 ± 0.30	5.3 ± 0.26	5.3 ± 0.25	**7.0 ± 0.53
Right testis						
Absolute	1,423 ± 18.6	1,456 ± 22.7	1,436 ± 27.3	1,457 ± 29.3	1,574 ± 118.0	1,353 ± 31.6
Relative	4.2 ± 0.05	4.1 ± 0.05	4.0 ± 0.05	4.2 ± 0.07	4.7 ± 0.40	**5.0 ± 0.07
FEMALE						
Body weight (grams)	210 ± 3.7	200 ± 3.2	200 ± 4.5	*199 ± 3.2	**192 ± 4.3	**194 ± 3.2
Right kidney						
Absolute	744 ± 22.5	760 ± 23.2	720 ± 20.9	736 ± 17.7	769 ± 18.7	*830 ± 23.4
Relative	3.5 ± 0.06	*3.8 ± 0.07	3.6 ± 0.08	3.7 ± 0.09	**4.0 ± 0.08	**4.3 ± 0.12
Liver						
Absolute	6,650 ± 105	6,137 ± 230	6,462 ± 159	6,584 ± 192	6,815 ± 322	6,437 ± 108
Relative	31.7 ± 0.37	30.6 ± 0.83	32.4 ± 0.63	33.2 ± 1.13	**35.4 ± 1.42	*33.3 ± 0.39
Lung						
Absolute	1,177 ± 40.9	1,180 ± 70.0	1,065 ± 42.2	1,183 ± 82.9	1,190 ± 59.0	1,389 ± 94.8
Relative	5.6 ± 0.15	(b) 5.9 ± 0.30	5.3 ± 0.19	6.0 ± 0.40	6.2 ± 0.33	**7.2 ± 0.51

(a) Mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified) for groups of 10 animals unless otherwise specified; P values vs. the controls by Dunn's or Shirley's test (Dunn, 1964; Shirley, 1977).
(b) Nine animals were weighed.
*P<0.05
**P<0.01

because testicular toxicants typically decrease testes weights (Amann, 1981). In the 13-week study, depressed sperm motility, caudal weight, and epididymal weight and an increased incidence of abnormal sperm were seen in high dose (50,000 ppm) male rats (Table 9). These effects are consistent with a mild toxic effect on spermatogenesis, although no effects were visible microscopically. Depressed epididymal and testes weights were also reported in ketotic rats with experimentally induced diabetes and feed restriction (Hutson et al., 1983). These changes are similar to those noted in alcoholics and in rats and mice that develop decreased testicular and accessory sex organ weights and diminished spermatogenesis (Van Thiel et al., 1979; Anderson et al., 1983; Willis et al., 1983). It is conceivable that the similar changes in male reproductive parameters resuting from exposure to ethanol or acetone could be linked to the similar metabolic fate of these two chemicals (see Introduction, Metabolism and Kinetics). The effects of ethanol do not appear to be mediated by acetaldehyde (Lahdetie, 1988).

TABLE 9. THE REPRODUCTIVE END POINTS OF MALE RATS IN THE THIRTEEN-WEEK DRINKING WATER STUDY OF ACETONE (a)

	Control	2,500 ppm	10,000 ppm	50,000 ppm
Caudal weight (grams)	0.14 ± 0.006	0.14 ± 0.006	0.15 ± 0.005	*0.10 ± 0.005
Right epididymal weight (grams)	0.44 ± 0.009	0.44 ± 0.009	0.45 ± 0.008	*0.35 ± 0.008
Abnormal sperm (percent)	0.68 ± 0.061	0.98 ± 0.156	0.92 ± 0.131	*3.42 ± 0.532
Sperm motility (percent)	75.7 ± 1.15	**71.6 ± 1.44	72.9 ± 0.80	*66.8 ± 2.69
Sperm density (10^6/g cauda)	571 ± 61.1	564 ± 19.7	570 ± 19.4	424 ± 69.5

(a) Mean ± standard error for groups of 10 animals; P values vs. the controls by Dunn's or Shirley's test (Dunn, 1964; Shirley, 1977).
*P<0.05
**P<0.01

Male rats at the two highest acetone concentrations (20,000 and 50,000 ppm) exhibited a mild but statistically significant leukocytosis, produced by an absolute increase in lymphocytes; other leukocytes were unaffected (Table 10). This effect was also noted in high dose (50,000 ppm) female rats. Epinephrine release related to fear, excitement, or exercise may result in lymphocytosis (Duncan and Prasse, 1986) and could be associated with an autonomic response mediated by acetone. The observation of Hynie et al. (1980) that acetone increases the level of cyclic AMP in human lymphocytes in vitro is interesting in this regard, since epinephrine is known to mediate many of its autonomic and metabolic effects via increased synthesis of cyclic AMP (Mayer, 1980). A mild but statistically significant depression in the erythrocyte count was observed in 20,000- and 50,000-ppm males in the current studies. Males exposed at 5,000 ppm or higher also showed depressed reticulocyte counts and minimally depressed hemoglobin levels. In addition, marginal but statistically significant increases in the mean corpuscular hemoglobin and the mean cell volume were noted (reactive anemia). Mean corpuscular hemoglobin concentrations were not affected. Overall, these results are consistent with a mild macrocytic normochromic anemia with a depressed regenerative response (reticulocytes). Uncomplicated macrocytic anemia, which is frequently produced by hemolysis, is typically accompanied by reticulocytosis. Platelet counts were marginally depressed in high dose (50,000 ppm) males and females. Macrocytic anemia associated with evidence of decreased regeneration and thrombocytopenia in humans occurs with megaloblastic anemia produced by a deficiency in folic acid or cobalamin (B_{12}) (Hillman, 1980; Rifkind et al., 1981). Anemia was not indicated in females, and the finding of selective but mild increases in the mean corpuscular hemoglobin and the mean cell volume in high dose females was inconclusive. Minimal-to-mild splenic pigmentation (hemosiderosis) was observed exclusively in the 20,000- and 50,000-ppm male rats, a finding consistent with the aforementioned hematologic findings. Peripheral blood smears from control and high dose male rats were examined to identify abnormal cells (large, oval erythrocytes or macro-ovalocytes and large hypersegmented neutrophils), which are a sign of macro-ovalocyte anemia. Although abnormal cells were not detected, histologic evaluation of bone marrow cellularity would be required to rule out this type of anemia. The hematologic effects described in the current studies were not observed in Sprague Dawley rats administered acetone (0-2,500 mg/kg per day) by gavage (Sonawane et al., 1986).

Folate deficiency in humans can be caused by long-term alcoholism (Hillman, 1980; Rifkind et al., 1981) because the daily intake of folate from food is restricted (often due to poor diet) and the enterohepatic circulation of folate is impaired by the toxic effect of alcohol on hepatic parenchymal cells. Acetone could also possibly mediate effects similar to those of ethanol to cause "folate deficiency."

Bone marrow hypoplasia during the 14-day studies was noted exclusively in 5/5 high dose (100,000 ppm) males. Since this group also

TABLE 10. HEMATOLOGIC DATA SHOWING STATISTICALLY SIGNIFICANT CHANGES FOR RATS IN THE THIRTEEN-WEEK DRINKING WATER STUDIES OF ACETONE (a)

Analysis	Control	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	50,000 ppm
MALE						
Leukocytes (10^3/microliter)	5 5 ± 0 22	6 5 ± 0 51	6 2 ± 0 36	6 4 ± 0 47	*6 9 ± 0 40	**7 3 ± 0 38
Lymphocytes (10^3/microliter)	4 4 ± 0 18	5 0 ± 0 43	4 7 ± 0 43	5 1 ± 0 33	*5 4 ± 0 33	**5 9 ± 0 33
Hematocrit (percent)	43 6 ± 0 57	43 4 ± 0 38	*42 1 ± 0 32	44 0 ± 0 61	*42 2 ± 0 49	42 7 ± 0 36
Hemoglobin (g/dl)	16 1 ± 0 21	16 3 ± 0 19	*15 6 ± 0 13	16 1 ± 0 09	*15 6 ± 0 21	*15 6 ± 0 15
Mean corpuscular hemoglobin (pg)	17 3 ± 0 11	*17 7 ± 0 13	17 3 ± 0 10	*17 8 ± 0 14	**17 7 ± 0 26	**18 6 ± 0 15
Mean cell volume (cubic microns)	46 8 ± 0 20	*47 6 ± 0 16	47 0 ± 0 21	**48 0 ± 0 21	**49 0 ± 0 42	**50 8 ± 0 25
Erythrocytes (10^6/microliter)	9 3 ± 0 14	9 1 ± 0 09	*9 0 ± 0 06	9 1 ± 0 05	**8 6 ± 0 06	**8 4 ± 0 08
Reticulocytes (10^3/microliter)	225 ± 11 0	195 ± 14 7	**171 ± 14 9	*179 ± 15 0	**168 ± 11 0	**152 ± 9 3
Platelets (10^3/microliter)	651 ± 10 7	641 ± 13 8	654 ± 13 2	617 ± 12 2	*605 ± 20 3	**501 ± 23 6
FEMALE						
Leukocytes (10^3/microliter)	4 7 ± 0 19	4 5 ± 0 38	5 0 ± 0 30	4 5 ± 0 30	5 2 ± 0 68	*5 9 ± 0 26
Lymphocytes (10^3/microliter)	3 6 ± 0 21	3 5 ± 0 36	4 0 ± 0 22	3 6 ± 0 25	3 9 ± 0 42	**4 8 ± 0 20
Hematocrit (percent)	42 8 ± 0 49	43 9 ± 0.37	43 0 ± 0 29	43 4 ± 0 37	42 3 ± 0 29	43 1 ± 0 57
Hemoglobin (g/dl)	16 0 ± 0 17	16 5 ± 0 16	16 0 ± 0 12	16 1 ± 0 14	15 7 ± 0 16	15 9 ± 0 18
Mean corpuscular hemoglobin (pg)	18 3 ± 0 29	18 8 ± 0 13	18 7 ± 0 09	18 6 ± 0 08	18 7 ± 0 08	**19 0 ± 0 08
Mean cell volume (cubic microns)	50 0 ± 0 15	50 0 ± 0 21	50 2 ± 0 13	50 3 ± 0 15	50 4 ± 0 16	**51 1 ± 0 18
Erythrocytes (10^6/microliter)	8 6 ± 0 10	8 8 ± 0 10	8 5 ± 0 07	8 7 ± 0 08	8 4 ± 0 08	8 4 ± 0 12
Reticulocytes (10^3/microliter)	145 ± 7 8	157 ± 10 3	151 ± 5 3	149 ± 6 5	139 ± 6 0	148 ± 9 0
Platelets (10^3/microliter)	684 ± 13 0	655 ± 17 4	662 ± 20 0	658 ± 15 5	*654 ± 57 2	**528 ± 17 0

(a) Mean ± standard error for groups of 10 animals. P values vs the controls by Dunn's or Shirley's test (Dunn, 1964, Shirley, 1977)
*P<0 05
**P<0 01

exhibited the most pronounced weight losses, this change probably reflects an acute toxic response to acetone. The lesion was characterized by fewer immature cells, a larger portion of mature erythrocytes and granulocytes, and more fat cells. This finding, together with the 13-week hematologic results (macrocytic anemia), is supportive of bone marrow toxicity. Bone marrow hyperplasia and abnormal morphology are expected during megaloblastic anemia, but hematoxylin- and eosin-stained sections of bone marrow from the 13-week studies did not reveal any changes. A more in-depth bone marrow analysis in future studies would be required to rule out such changes in response to short-term exposure at acetone concentrations lower than 50,000 ppm.

Aggregates of golden brown pigment (hemosiderin) were observed microscopically in the splenic red pulp of male rats; the extent and severity in all males exposed at 20,000 and 50,000 ppm were greater than in controls. These results are indicative of accelerated erythrocyte turnover and are consistent with the aforementioned hematologic results.

In the 13-week study, the incidence and severity of nephropathy in male rats increased with increased doses of acetone (Table 11). Nephropathy is a spontaneously occurring, long-term, progressive condition of the kidney seen in rats of each sex (Gray, 1977; Goldstein et al., 1988). It consists of focal or multifocal groups of regenerating cortical tubules lined with cuboidal epithelial cells containing basophilic cytoplasm. Affected tubules may be located in or adjacent to an area of interstitial fibroplasia with or without inflammatory cellular infiltration. Foci of inflammatory cellular infiltration and foci of fibroplasia may also be present as distinct entities. In addition to these changes, the mesangium and glomerular basement membranes thicken and eventually become sclerotic. The results of the current studies suggest that acetone accelerates the occurrence and severity of this condition. Other investigators have also described kidney lesions after acetone exposure (Brown

TABLE 11. NUMBER OF MALE RATS WITH SELECTED LESIONS IN THE THIRTEEN-WEEK DRINKING WATER STUDY OF ACETONE (a)

Site/Lesion	Control	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	50,000 ppm
Spleen						
Pigmentation	0	0	0	0	10	10
Kidney						
Nephropathy	6	8	8	9	10	10
Minimal severity	5	8	8	9	1	1
Mild severity	1	0	0	0	9	9

(a) Ten animals were examined.

and Hewitt, 1984; Sonawane et al., 1986). In the 13-week gavage study described by Sonawane et al. (1986), Sprague Dawley rats at 500 and 2,500 mg/kg developed more severe kidney lesions (tubular degeneration) than those naturally occurring in controls. In addition, hyaline droplet accumulation was accentuated in males at these doses. This lesion was not observed in the current studies or by any other investigators. Brown and Hewitt (1984) noted degenerative changes in the kidney (apical degeneration of proximal tubular epithelia) of male F344 rats receiving a single dose (15 mmol/kg) of acetone by gavage. The pathogenesis for acetone-induced kidney lesions has not been elucidated.

It should be noted that acetone is metabolized to formate and that formate and acidosis per se are aversive to the kidney. Accidental human exposure to formic acid reportedly results in diuresis, hematuria, anuria, and uremia (von Oettingen, 1959; Naik et al., 1980; Rajan et al., 1985), supporting the notion that the kidney is a target for formic acid toxicity. Von Oettingen (1959) indicated that formate-induced kidney injury may be associated with the ability of formate to bind to calcium, its ability to induce methemoglobin formation, or its vasoactive properties. Studies by Zitting and Savolainen (1980) which showed formic acid-induced alterations (depressed glutathione, cytochrome P450, and ethoxycoumarin deethylase) in the rat kidney further substantiate that the kidney is a target organ.

Perhaps the most universal hypothesis of chronic progressive nephropathy in aging rats is based on the relationship of this disease with high protein diets and resulting proteinuria (Gray, 1977; Goldstein et al., 1988). According to this hypothesis, elevated serum protein increases the renal blood flow and glomerular filtration rate. The resulting overburdened glomeruli become sclerotic and cease to function normally. A positive feedback stimulus for compensatory hyperperfusion further exacerbates this condition and explains the progressive nature of this lesion. The ability of acetone to depress protein catabolism (Sherwin et al., 1975) could possibly enhance the development of nephropathy.

Studies in Mice

Survival, body weight, and water consumption in the short-term studies in mice are shown in Tables 12 and 13 and in Figure 3. Water consumption was consistently depressed relative to controls in mice given drinking water containing 50,000 or 100,000 ppm acetone for 14 days. Growth retardation in males receiving 100,000 ppm was noticeably more severe than in females. In the 13-week studies, all dosed male mice showed comparable fluid intake, whereas all dosed females showed depressed fluid intake compared with that in controls. Body weight and growth in mice were not affected by acetone exposure in the 13-week studies, in contrast with the results for rats. Time-weighted average doses of acetone in the 14-day and 13-week studies in mice are shown in Table 14.

No deaths were recorded in the 14-day and 13-week studies, and no consistent clinical signs could be attributed to acetone exposure. Significant and consistent changes in organ weights (increased liver and decreased spleen weights) in

TABLE 12. SURVIVAL, MEAN BODY WEIGHTS, AND WATER CONSUMPTION OF MICE IN THE FOURTEEN-DAY DRINKING WATER STUDIES OF ACETONE

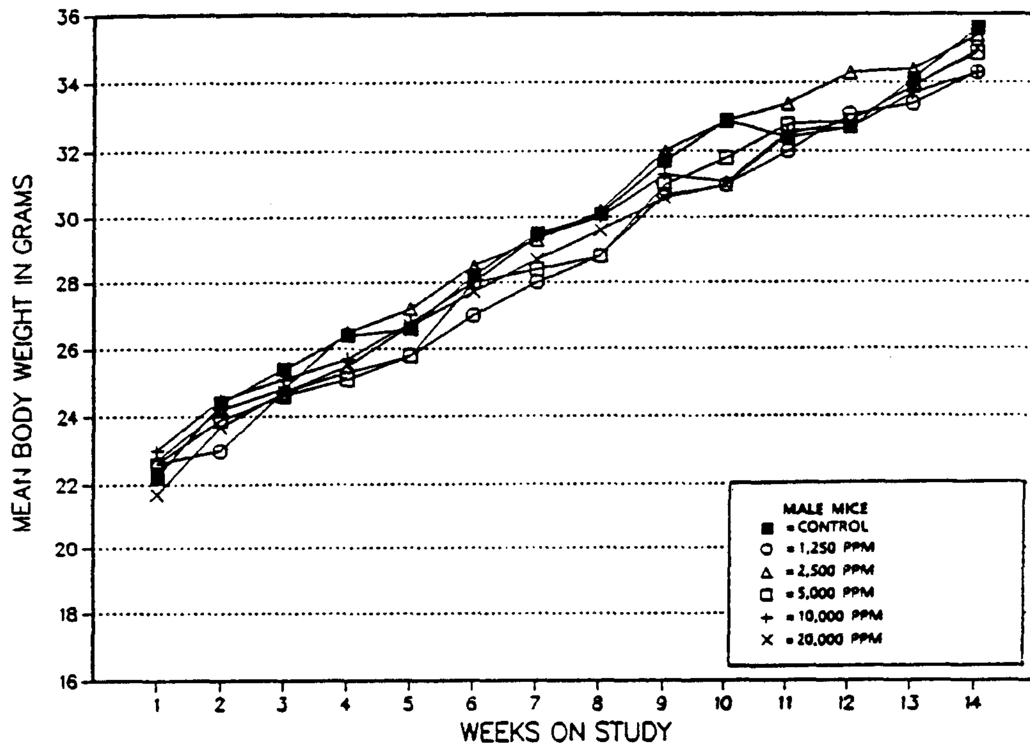
Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Necropsy	Change (c)	Necropsy Weight Relative to Controls (percent)	Water Consumption (d)
MALE						
0	5/5	22 7 ± 0 8	26 0 ± 0 3	+3 3 ± 0 7		164
5,000	5/5	23 6 ± 0 6	26 5 ± 0 8	+2 9 ± 0.4	101 9	189
10,000	5/5	22 9 ± 0 4	25 8 ± 0 8	+2 9 ± 0 6	99 2	155
20,000	5/5	23 2 ± 0 6	26 2 ± 0 5	+3 0 ± 0 5	100 8	185
50,000	5/5	23 6 ± 0 6	26 4 ± 0 6	+2 8 ± 0 1	101.5	125
100,000	5/5	24 0 ± 0 4	25 3 ± 0 4	+1 3 ± 0 3	97 3	102
FEMALE						
0	5/5	19 2 ± 0 6	21 2 ± 0 8	+2 0 ± 0 4		286
5,000	5/5	19 0 ± 0 3	20 4 ± 0 7	+1 4 ± 0 6	96 2	309
10,000	5/5	18 9 ± 0 3	21 0 ± 0 5	+2 1 ± 0 4	99 1	295
20,000	5/5	19 4 ± 0 6	21 7 ± 0 7	+2 3 ± 0 1	102 4	267
50,000	5/5	19 0 ± 0 6	21 6 ± 0 7	+2 6 ± 0 4	101 9	172
100,000	5/5	19 0 ± 0 5	20 9 ± 0 5	+1 9 ± 0 4	98 6	127

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Milliliters per kilogram per day, based on average body weight and consumption data obtained during the 2 week interval

TABLE 13 SURVIVAL, MEAN BODY WEIGHTS, AND WATER CONSUMPTION OF MICE IN THE THIRTEEN-WEEK DRINKING WATER STUDIES OF ACETONE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)	Water Consumption (d)
MALE						
0	10/10	22 2 ± 0 6	34 1 ± 1 1	+11 9 ± 0 8		224
1,250	10/10	22 6 ± 0 3	33 4 ± 0 9	+10 8 ± 0 8	97 9	278
2,500	10/10	22 7 ± 0 4	34 4 ± 0 6	+11 7 ± 0 6	100 9	204
5,000	10/10	22 6 ± 0 9	33 9 ± 0 9	+11 3 ± 0 8	99 4	259
10,000	10/10	23 0 ± 0 4	33 7 ± 1 3	+10 7 ± 1 0	98 8	208
20,000	10/10	21 7 ± 0 8	33 9 ± 0 8	+12 2 ± 0 7	99 4	196
FEMALE						
0	10/10	17 3 ± 0 6	27 0 ± 0 6	+9 7 ± 0 8		426
2,500	10/10	17 0 ± 0 5	27 7 ± 0 7	+10 7 ± 0 7	102 6	300
5,000	10/10	16 3 ± 0 5	26 7 ± 0 8	+10 4 ± 0 8	98 9	386
10,000	10/10	16 4 ± 0 6	26 4 ± 0 5	+10 0 ± 0 7	97 8	329
20,000	10/10	16 8 ± 0 3	27 1 ± 0 9	+10 3 ± 1 0	100 4	283
50,000	10/10	16 9 ± 0 4	26 8 ± 1 1	+9 9 ± 0 9	99 3	203

(a) Number surviving/number initially in group
(b) Initial group mean body weight ± standard error of the mean
(c) Mean body weight change of the group ± standard error of the mean
(d) Milliliters per kilogram per day, average for weeks 7 and 13



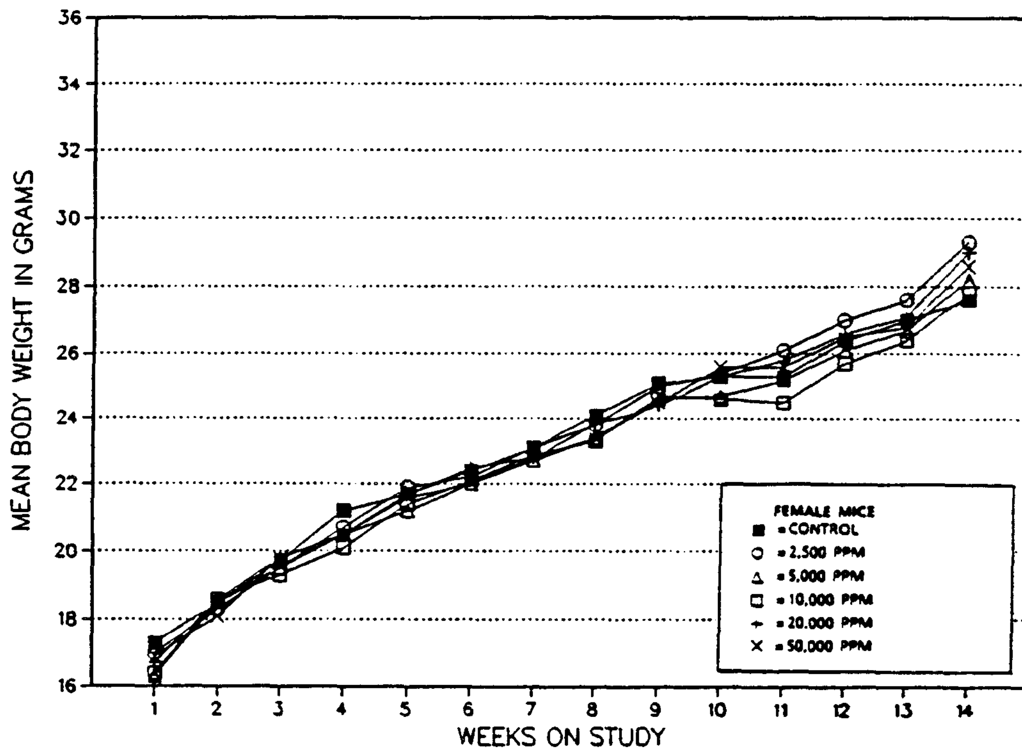


FIGURE 3. GROWTH CURVES FOR MICE ADMINISTERED ACETONE IN DRINKING WATER FOR THIRTEEN WEEKS

TABLE 14. ACETONE CONSUMPTION BY MICE IN THE FOURTEEN-DAY AND THIRTEEN-WEEK DRINKING WATER STUDIES

Concentration (ppm)	Time-Weighted Average Dose (mg/kg per day)	
	Fourteen-Day Study	Thirteen-Week Study
MALE		
0	0	0
1,250	--	380
2,500	--	611
5,000	965	1,353
10,000	1,579	2,258
20,000	3,896	4,858
50,000	6,348	--
100,000	10,314	--
FEMALE		
0	0	0
1,250	--	--
2,500	--	892
5,000	1,569	2,007
10,000	3,023	4,156
20,000	5,481	5,945
50,000	8,804	11,298
100,000	12,725	--

the 13-week studies were restricted to female mice given 50,000 ppm acetone, as shown in Table 15. Acetone exposure was associated with increased kidney and liver weights in the 14-day studies. Minimum doses for these changes were: for the kidney, 50,000 ppm acetone for male and female mice; for the liver, 5,000 and 20,000 ppm acetone for male and female mice, respectively. The increased liver weights are considered the most biologically significant acetone-mediated organ weight change because they were observed at a low dose in the 14-day studies (5,000 ppm) and because of the associated liver histopathologic changes described below.

The hematologic results for mice in the 13-week studies revealed no consistent biologic effects other than minimal increases in hematocrit and hemoglobin (primarily in females), which could be attributed to slight dehydration in groups exposed to acetone at concentrations of 5,000 ppm or higher (Table 16). Sperm morphology and vaginal cytology in male and female mice were not affected by acetone exposure.

The only histopathologic change associated with acetone exposure in both the 14-day (Table 17) and the 13-week studies was centrilobular hepatocellular hypertrophy. This lesion was characterized by cells with abundant eosinophilic cytoplasm and slightly enlarged nuclei. These changes were zonal, involving virtually all liver lobules with a minimal degree of severity, except for male mice in the 14-day study. The incidence and severity of this change in male mice in the 14-day study were positively correlated with concentration, as shown in Table 17. Minimal concentrations for this hepatic effect in males and females in the 14-day studies were 20,000 and 50,000 ppm, respectively, and this effect was seen in all 20,000-ppm males and all 100,000-ppm females. In the 13-week studies, this hepatic change only occurred with a minimal degree of severity in 2/10 50,000-ppm females. Overall, this histologic change is probably related to the ability of acetone to stimulate hepatic microsomal enzymes (Clark and Powis, 1974; Anders and Gander, 1979; Argus et al., 1980; Freeman and Hayes, 1985; Koop et al., 1985; Yoo et al., 1987). Apparently, the mild hepatic changes that occur within 14 days in male mice do not persist after 13 weeks of exposure. Such findings reflect a development of tolerance to acetone exposure, as reported in other investigations (Rowe and Wolf, 1963; Goldberg et al , 1964, ACGIH, 1986).

TABLE 15. ORGAN WEIGHTS OF FEMALE MICE IN THE THIRTEEN-WEEK DRINKING WATER STUDY OF ACETONE (a)

Organ	Control	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	50,000 ppm
Body weight (grams)	27.7 ± 0.61	29.4 ± 0.73	28.2 ± 0.87	27.8 ± 0.70	29.0 ± 1.02	28.6 ± 1.21
Liver						
Absolute	1,285 ± 30.7	1,346 ± 39.4	1,310 ± 38.9	1,334 ± 45.8	1,379 ± 35.5	*1,458 ± 57.9
Relative	46.5 ± 0.76	45.9 ± 1.03	46.6 ± 0.57	48.0 ± 0.84	47.8 ± 1.40	**51.1 ± 1.12
Spleen						
Absolute	95 ± 3.2	103 ± 7.3	93 ± 3.2	88 ± 2.4	91 ± 2.3	*85 ± 1.5
Relative	3.4 ± 0.12	3.6 ± 0.29	3.3 ± 0.11	3.2 ± 0.10	3.2 ± 0.16	*3.0 ± 0.11

(a) Mean ± standard error (absolute in milligrams, relative in milligrams per gram unless otherwise specified) for groups of 10 animals; P values vs. the controls by Dunn's or Shirley's test (Dunn, 1964; Shirley, 1977). No significant differences were observed in males.
*P<0.05
**P<0.01

TABLE 16. HEMATOLOGIC DATA SHOWING STATISTICALLY SIGNIFICANT CHANGES FOR MICE IN THE THIRTEEN-WEEK DRINKING WATER STUDIES OF ACETONE (a)

Analysis	Concentration					
MALE	*Control*	*1,250 ppm*	*2,500 ppm*	*5,000 ppm*	*10,000 ppm*	*20,000 ppm*
Hematocrit (percent)	48.9 ± 0.98	48.8 ± 0.53	48.0 ± 0.52	49.6 ± 0.79	50.1 ± 0.82	48.3 ± 0.88
Hemoglobin (g/dl)	17.2 ± 0.15	17.5 ± 0.19	17.1 ± 0.10	*17.7 ± 0.16	*17.8 ± 0.21	*17.8 ± 0.21
Mean corpuscular hemoglobin (pg)	16.6 ± 0.28	16.9 ± 0.14	16.7 ± 0.12	17.0 ± 0.16	16.7 ± 0.19	*17.5 ± 0.23
FEMALE	Control	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	50,000 ppm
Hematocrit (percent)	46.5 ± 0.73	47.0 ± 0.93	(b) 46.6 ± 0.51	46.9 ± 0.97	47.8 ± 0.63	(b) *48.4 ± 0.69
Hemoglobin (g/dl)	16.6 ± 0.11	16.7 ± 0.11	16.9 ± 0.11	16.9 ± 0.28	*17.2 ± 0.22	**17.4 ± 0.19
Mean corpuscular hemoglobin (pg)	16.8 ± 0.20	16.5 ± 0.29	17.1 ± 0.24	17.0 ± 0.24	17.2 ± 0.20	17.1 ± 0.21

(a) Mean ± standard error for groups of 10 animals unless otherwise specified. P values vs the controls by Dunn's or Shirley's test (Dunn, 1964, Shirley, 1977)
(b) Nine animals were examined
*P<0.05
**P<0.01

TABLE 17. INCIDENCES AND SEVERITY OF CENTRILOBULAR HEPATOCELLULAR HYPERTROPHY IN MICE IN THE FOURTEEN-DAY DRINKING WATER STUDIES OF ACETONE

Severity	Control	5,000 ppm	10,000 ppm	20,000 ppm	50,000 ppm	100,000 ppm
MALE						
Minimal	0/5	0/5	0/5	3/5	0/5	0/5
Mild	0/5	0/5	0/5	2/5	5/5	2/5
Moderate	0/5	0/5	0/5	0/5	0/5	3/5
FEMALE						
Minimal	0/5	--	0/5	0/5	2/5	5/5
Mild	0/5	--	0/5	0/5	0/5	0/5
Moderate	0/5	--	0/5	0/5	0/5	0/5

In summary, the results from these studies show that acetone is mildly toxic to rats and mice when administered in drinking water for 13 weeks. Minimal toxic doses were estimated to be 20,000 ppm acetone for male rats and male mice and 50,000 ppm acetone for female mice. No toxic effects were identified for female rats. Acetone doses used during the 13-week studies were equivalent to 200-3,400 mg/kg per day for rats and 380-11,298 mg/kg per day for mice. The species/sex most sensitive to acetone toxicity, based on the minimal toxic dose, is the male rat (1,700 mg/kg per day), followed by the male mouse (4,858 mg/kg per day), female mouse (11,298 mg/kg per day), and female rat (minimal toxic dose not identified). The testis, kidney, and hematopoietic system were identified as target organs for male rats, and the liver was the target organ for male and female mice.

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