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TOXICOLOGICAL REVIEW

OF

ACETONE

(CAS No. 67-64-1)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

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U.S. Environmental Protection Agency
Washington, DC

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to acetone. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of acetone.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 301-345-2870.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Director has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

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Summaries of the external peer reviewers' and public comments, and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per μg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of the hazard identification and dose-response assessment for acetone has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999),

Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988a), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d).

The initial literature search strategy employed for this compound was based on the CASRN and at least one common name. However, because the initial literature search for "acetone" yielded more than 1,500 hits, subsequent searches were limited to review articles (all years) and articles with acetone in the title. The following data bases were searched: TOXLINE (all subfiles), MEDLINE, CANCERLIT, TOXNET [HSDB, IRIS, CCRIS, EMIC (1991-present), and GENE-TOX], and RTECS. The relevant literature was reviewed through February 2003.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Chemical and physical properties of acetone are listed in Table 1. Acetone is used primarily as a chemical intermediate and as a solvent in both chemical and pharmaceutical applications. Acetone is produced endogenously and utilized in intermediary metabolism (ATSDR, 1994; WHO, 1998). As the data in Table 1 indicate, acetone is completely miscible with water and is a highly volatile organic compound.

Table 1. Physicochemical Data for Acetone

Parameter	Value	Reference
Synonyms	2-propanone; dimethyl ketone	Budavari et al., 1996
CAS registry no.	67-64-1	
Chemical formula	CH_3COCH_3	Budavari et al., 1996
Molecular weight	58.08	Budavari et al., 1996
Physical state	liquid	Budavari et al., 1996
Vapor pressure at 20°C	181.72 mm Hg	ATSDR, 1994; WHO, 1998

		(continued on the following page)
Melting point	-94°C	Budavari et al., 1996
Boiling point	56.5°C	Budavari et al., 1996
Solubility in water	miscible	Budavari et al., 1996
Log K_{ow}	-0.24	ATSDR, 1994; WHO, 1998
Henry's law constant	4.26×10^{-5} atm-m^3/mol	ATSDR, 1994; WHO, 1998
Conversion factors in air	1 ppm = 2.374 mg/m^3	ATSDR, 1994
Odor threshold in air (absolute)	13-20 ppm	ATSDR, 1994; WHO, 1998

3. TOXICOKINETICS

The toxicokinetics of acetone has been studied in part because of the role that acetone plays in normal metabolism and in disease states (ATSDR, 1994; WHO, 1998). Under starvation conditions, high-fat and low-carbohydrate diets, or uncontrolled diabetes, fat is metabolized to form acetoacetate, which in turn is converted to acetone (Wieland, 1968; Argiles, 1986). Under these conditions, a high level of acetyl CoA generated from the beta-oxidation of fatty acids coupled with a limited supply of oxaloacetate and a lack of dietary carbohydrate can lead to ketosis. Much of the research on acetone in humans relates to individuals in these states. Mean concentrations of acetone in "normal," nonexposed adult humans have been measured as 840 µg/L in blood, 842 µg/L in urine, and 715 ng/L in alveolar air (Wang et al., 1994). Stewart et al. (1975) reported blood acetone concentrations in male subjects prior to exposure to acetone as ranging from 0.73 to 1.29 mg% (mg/100 ml) and in female subjects as 2.86 mg%. Acetone blood concentrations in healthy individuals are reported to range from 0.3 to 2.0 mg/100 ml (Physicians Desk Reference, 1976). Owen et al. (1982) reported plasma acetone levels in diabetics ranging from 1.55 to 8.16 mM (9 to 47 mg%). The normal endogenous acetone turnover rate (mg/kg-day) is not known.

Overall, available data indicate that humans and rodents readily absorb acetone by inhalation, ingestion, and dermal exposure. Acetone is broadly distributed throughout the body, particularly in organs with high water content. The toxicokinetics of acetone are dose-related. Once acetone has been absorbed, it is extensively metabolized; the prevailing metabolic pathway appears to be dose-related. At low concentrations, the primary pathway appears to be through the formation of methylglyoxal. On the other hand, as the concentration of acetone increases, the propanediol pathway becomes more predominant. Although the second pathway may be involved in gluconeogenesis, it may also be used to facilitate excretion. Acetone excretion is also

dose-related. In this respect, exposure to low levels of acetone lead to small losses through expiration. Acetone appears in the urine only when exposure concentrations exceed approximately 15 ppm. The proportion of acetone lost through expiration increases at high acetone concentrations.

There is evidence of similarity between the toxicokinetic data on human subjects and rodents. In both cases, metabolism proceeds by a hepatic pathway at low concentrations and by an extrahepatic pathway followed by excretion at higher concentrations (Casazza et al., 1984; Thornalley, 1996). Both species appear to eliminate acetone from the body efficiently (Haggard et al., 1944; Sakami, 1950; Sakami and Lafaye, 1951; Stewart et al., 1975; Reichard et al., 1979; Casazza et al., 1984; Wigaeus et al., 1981; Kosugi et al., 1986a; Wang et al., 1994).

3.1. ABSORPTION

The absorption of acetone and its distribution is governed by its physicochemical parameters and related biological factors. Acetone is miscible in water and has a high vapor pressure (Table 1) and a high blood/air partition coefficient. The low K_{ow} indicates that acetone selectively partitions into an aqueous phase rather than a lipid phase; however, acetone is also slightly lipophilic, allowing for some diffusion into tissues. This suggests that although acetone is readily absorbed into the aqueous compartments of the body the lipid component may affect the rate of absorption into the body. Collectively, these factors allow for rapid absorption via the respiratory and gastrointestinal tracts, and broad distribution throughout the body, particularly into organs with high water content.

3.1.1. Oral Studies

Acetone is readily absorbed via the oral route, as indicated by early research on humans. Haggard et al. (1944) administered acetone (50 mg/kg acetone diluted in water; final concentration and total volume not provided) to male subjects and estimated that between 65 and 93% of the acetone was metabolized while the residual material was excreted from the body over a period of 2 hours. Both the level of metabolism and excretion through the lungs and urine, and the short period of time in which these occur indicate that acetone is rapidly absorbed in humans.

Anecdotal information concerning the oral absorption of acetone in humans is provided in case studies. Studies involving the accidental ingestion of acetone, indicate that acetone is readily absorbed through the gastrointestinal tract. In one case study (Herman et al., 1997) a 17-

month-old girl was accidently given approximately 4.88 mL/kg of acetone through her gastronomy tube and was found gagging, nonresponsive, and diaphoretic with dilated sluggish pupils. Clinical chemistry analyses demonstrated elevated levels of serum ketones. A second case study (Ramu et al., 1978) involved the accidental ingestion of nail polish remover. The subject became listless and lethargic with a shortened attention span. While both studies indirectly demonstrate that acetone is readily absorbed via the gastrointestinal tract a quantitative assessment of absorption cannot be determined.

Rapid absorption of acetone in rodents was demonstrated by Price and Rittenberg (1950). When rats were administered 0.22 mg of ^{14}C-acetone (1.2 mg/kg) in water, 47% of the acetone was expelled as $^{14}CO_2$ within 13.5 hours following administration (29% released within the first 2.5 hours). In a second experiment in which rats were administered pulses of ^{14}C-acetone on a daily basis over a 7.5-day period, 67 to 76% of the administered acetone was expelled as $^{14}CO_2$ over the ensuing 24-hour period. The measurements started with short intervals extending to 6 hours during the first day of the study, with 56% of the administered radiolabel appearing during the initial 6 hours. On subsequent days, measurements were made at 6-hour intervals. The testing scheme does not support comparison with the initial study, which demonstrated high levels of absorption and rapid metabolism with successive applications of the chemical on the rate of absorption. The expiration of acetone was determined following the administration of ^{14}C-acetone (only 7% of the administered acetone was expelled in the breath, with peak levels achieved 2 hours following administration). Overall, the data indicate that acetone is rapidly absorbed into the body upon ingestion. The acetone that is expelled in the breath is in the unmetabolized form or CO_2.

3.1.2. Inhalation Studies

The most important physicochemical parameters relating to exposure to acetone are the vapor pressure and Henry's constant. Acetone has a high blood-air partition coefficient ($K_{B/A}$) of 301 (Dills et al., 1994; Wigaeus et al., 1981; ATSDR, 1994; WHO, 1998). However, a range of partition coefficients has been reported, including 245 by Sato and Nakijima (1979) and 210 by Hallier et al. (1981). The high $K_{B/A}$ indicates that acetone is rapidly absorbed into the body via the inhalation route. During inspiration, acetone passes through the epithelial cells in the nasal cavity and is dissolved into the bloodstream where it is transported. The fraction of acetone remaining in the nasal tissue evaporates during expiration into the environment. The differences between the concentration of acetone during inhalation and exhalation accounts for the portion that is absorbed and dissolved into the bloodstream (Dahl et al., 1991).

Hallier et al. (1981) conducted studies using rats in desiccators to achieve maximum saturation for determining the partition coefficients. Rats were placed in a desiccator that was saturated with acetone. Measurements of acetone in the head space were made over time and the pharmacokinetic parameters were determined by loss of acetone. The equilibrium constants (K_{eq}) were determined to be 330, 210, and 220 for urine/air, blood/air, and whole animal/air ratios, respectively. The K_{eq} for oil/air ratio was 125. By measuring the amount of acetone taken up by the rats in the desiccator, it was determined that the maximum amount of acetone absorbed slightly exceeded the water pool of the rodents. The authors proposed that the limited lipophilicity of acetone contributes to a minor amount of nonmetabolized acetone that moves into the lipid fraction of tissues.

Biological conditions such as the rate of respiration and the blood/air partition coefficient have also been shown to be key factors in the uptake of acetone via the inhalation route. Wigaeus et al. (1981) exposed male volunteers to 1,300 mg/m^3 (547.6 ppm) acetone at rest or 700 mg/m^3 (294.9 ppm) with exercise for 2 hours. The total amount taken up increased as ventilation increased with exercise, but the relative uptake ranged from 39-52% for both exposures. Total uptake was 0.6 and 1.2 g, respectively, for the 1,300 mg/m^3 (resting) and 700 mg/m^3 (active) exposures. Acetone concentrations in alveolar air were 30-40% of those in inspiratory air. This fraction did not change with exposure time or workload. In contrast, acetone concentrations in blood increased continuously during exposure, with no sign of equilibrium between concentrations of inspired air and blood. The constant rate of absorption by inhalation, in contrast to the continuous increase of acetone in the blood, most likely reflects the high $K_{B/A}$ in conjunction with its movement from the nasal cavity and distribution throughout the body. Given the high $K_{B/A}$ of acetone, it is surprising that only about 40% of the administered acetone is absorbed into the body. This may be due to the relatively low lipid solubility of acetone, which provides resistance to the transfer of acetone from the air through the nasal tissue and into the bloodstream. At the end of the 1,300 and 700 mg/m^3 exposures, acetone concentrations in arterial blood were 15 and 75 mg/kg, respectively. Acetone concentrations in alveolar air dropped rapidly during the first 5 minutes following exposure. Half-times for acetone in alveolar air, arterial blood, and venous blood averaged 4.3, 3.9, and 6.1 hours, respectively. Elimination of acetone via the lungs was about 20% of total uptake, whereas only about 1% of uptake was excreted via the urine.

Other studies support the findings of Wigaeus et al. (1981) for the uptake and elimination of acetone in humans. Mean relative acetone uptake averaged 53% in volunteers exposed to 21-211 ppm acetone for up to 4 hours at rest or 2 hours with intermittent exercise (Pezzagno et al.,

1986). Acetone concentrations in the capillary blood of male volunteers were shown to increase steadily during a 2-hour exposure to 231 ppm acetone followed by a monoexponential postexposure decay curve with a calculated half-life of 4.3 hours (Ernstgård et al., 1999).

Although there are only a few studies specifically measuring the uptake of acetone by inhalation, a number of studies demonstrate indirectly that acetone is rapidly absorbed via inhalation. For example, Wigaeus et al. (1982) measured the distribution of acetone in mice following inhalation exposure to 1,200 mg/m^3 (500 ppm) 2-^{14}C-acetone. The study did not involve a quantitative measure of the total amount of distributed acetone, but did measure the distribution of acetone in individual organs. The study demonstrated a continuous increase in the tissue concentration of acetone and total radioactivity during the first 6 hours of exposure. This study is discussed in greater detail in Section 3.2.

Similar results have been found in dogs and rats. Acetone uptake by the respiratory tract of the dog was 42-54% for exposure concentrations of 0.36-0.80 µg/mL (150-340 ppm) for an unspecified duration (Egle, 1973). Blood concentrations of acetone in rats exposed to 150 ppm increased gradually over a 2-hour period, then reached a plateau at steady state for the remainder of the 4-hour exposure (Geller et al., 1979a). Plasma acetone levels in female rats corresponded with increasing exposure concentrations, but were not related to pregnancy status or gestation time (Mast et al., 1988).

3.1.3. Dermal Studies

Dermal absorption of acetone has been shown to occur rapidly in humans. In a Japanese study (Fukabori et al., 1990) translated and described by ATSDR (1994) and WHO (1998), cotton soaked with acetone was applied to the skin of volunteers for 2 hours/day for 4 days (dose information not provided). Resulting levels of acetone were 5-12 mg/L in blood, 5-12 ppm in alveolar air, and 8-14 mg/L in urine. When the daily exposure was increased to 4 hours, the body burden more than doubled. Absorption was immediate, with peak levels occurring at the end of each application. Concomitant inhalation exposure is expected from dermal exposure. From the alveolar air and urine concentrations, the study authors calculated that 2- and 4-hour dermal exposures were equivalent to 2-hour inhalation exposures of 50-150 ppm and 250-500 ppm, respectively.

Studies concerning the level of dermal absorption of acetone in rodents are limited, although acetone is frequently used as a vector in dermal studies of other chemicals (NTP, 1991,

1995, 1997). However, no available studies included an acetone control and a naive control. Together, these controls could be used to directly measure acetone uptake into the body via the dermal route.

3.2. DISTRIBUTION

Tissue distribution of 2-^{14}C-acetone following inhalation exposure to 1,200 mg/m^3 (500 ppm) was studied in mice (Wigaeus et al., 1982). Acetone in blood, lung, kidney, brain, pancreas, spleen, thymus, heart, testis, vas deferens, muscle, and subcutaneous and intraperitoneal white adipose tissue reached steady state concentrations within 6 hours and showed little or no accumulation when exposures were prolonged up to 24 hours or repeated 6 hours/day for 5 days. In contrast, radioactivity in liver and brown adipose tissue continued to increase during a 24-hour exposure and increased in adipose tissue with repeated exposures. Of all studied tissues, the liver contained the highest level of radioactivity and the adipose tissues the lowest. Only about 10% of the radioactivity in the liver was unchanged acetone. Half-times for acetone elimination were 2-3 hours for blood, kidney, lung, brain, and muscle, and greater than 5 hours for subcutaneous adipose tissue. By 24 hours after exposure, acetone concentrations had returned to endogenous levels in all tissues. The study supports older data (Haggard et al., 1944) that acetone distributes evenly in body water and does not accumulate with repeated exposure. The accumulation of radioactivity in liver and brown adipose tissue may reflect high metabolic turnover in these tissues.

Scholl and Iba (1997) measured the distribution of acetone in rats following acetone inhalation. Male Sprague-Dawley rats were exposed to 1,000 ppm (2,400 mg/m^3) of acetone for 3 hr/day for 10 days. Tissue concentrations of acetone were determined 1 h following the final exposure. Mean concentrations of acetone in the plasma, liver, lung, and kidney were 35.3, 13.2, 11.4, and 21.8 μg/g, respectively. The concentration of acetone in plasma relative to the three organs approximates the octanol-water partition coefficient (log $K_{o/w}$ = -0.24) and reflects the greater solubility in water, which is the major constituent of plasma, compared with lipid solubility.

3.3. METABOLISM

Acetone has been broadly studied as a metabolic intermediate that is naturally formed in humans and rodents under normal metabolic conditions and at higher concentrations under conditions of fasting, ingestion of high-fat, low-carbohydrate diets, and uncontrolled diabetes. The proposed metabolic pathways for acetone are shown in Figure 1. Reichard et al. (1979) studied the metabolism of acetone in 15 human subjects undergoing starvation ketosis to evaluate the role of acetone in starvation-induced ketonemia in humans. Acetone radiolabeled (^{14}C) on the carbonyl carbon was administered to measure the amount of acetone expired in the breath, excreted in the urine, and metabolized to CO_2. The study used human subjects of both sexes ranging from 22 to 52 years of age. The subjects were divided by weight into obese and nonobese categories where the nonobese individuals were -7 to +16% and obese individuals were +38 to +155% of the ideal body weight (based on Metropolitan Life Insurance tables) at the start of the study. The obese individuals were starved for either 3 (n=6) or 21 (n=3) days and the nonobese were starved for 3 days (n=3). Following administration, periodic measurements were taken of the subjects' expired air and the amount of $^{14}CO_2$ was determined. On the day ^{14}C-acetone was administered, hourly measurements were taken over a six-hour period for total glucose, acetoacetate, β-hydroxybutyrate, acetone in plasma and urine. The amount of radiolabel in each constituent was used to determine the metabolism of acetone.

Of the studied components, the radiolabel was found in $^{14}CO_2$, acetone and glucose. While the overall concentration of plasma acetone remained constant over the six-hour period, the amount (specific activity) of ^{14}C acetone decreased and the amount (specific activity) of ^{14}C glucose and $^{14}CO_2$ increased indicating that acetone is metabolized into glucose which is subsequently metabolized to CO_2. No radiolabel was found in β-hydroxybutyrate, acetoacetate, or plasma free fatty acids indicating that while ketoacids are metabolized to acetone, there is no reverse reaction that accounts for an increase in ketoacids and an exacerbated ketotic state. The absence of radiolabel in fatty acids indicates that starvation conditions are not conducive to lipogenesis. The study also found that the ratio of acetone in expired air and urine is proportional to the plasma concentration in humans. The principal findings in these studies were that, depending upon the plasma acetone concentration, excretion of acetone from the body in breath and urine accounts for about 2 to 30% of the endogenous acetone under fasting conditions. Conversion of acetone to other biological compounds is the primary mode of acetone elimination. The authors estimate that 50-70% of the acetone that was eliminated in 3-day fasted subjects was lost through metabolism. On the basis of the specific activity data, approximately 4-11% of plasma glucose production could theoretically be derived from acetone.

Owen et al. (1982) evaluated the metabolism of ketone bodies and the toxicokinetics of acetone in diabetics. The study consisted of selecting nine patients that the study authors characterized as "acutely ill" with acidemia, hyperglycemia, and hyperketonemia. The patients were dosed with 0.75-1.56 μmol of 2-^{14}C-acetone intravenously. Blood samples were taken every 1-2 hours for up to 10 hours. Breath and blood samples were taken simultaneously. During the study, insulin therapy was dictated by the patients' clinical situation. The mean time between acetone injection and insulin administration was 3 hours with a range of 1-6 hours. Analyses were conducted for acetone, β-hydroxybutyrate and acetoacetate in plasma, breath and urine, and for glucose in plasma.

A mean plasma acetone concentration of 4.96 mM with a range of 1.55-8.91 mM was determined at the start of the study. The higher acetone concentrations in this cohort are consistent with patients exhibiting severe ketoacidosis while the lower levels are similar to values seen in healthy, fasting individuals. The data demonstrates a relationship between the plasma acetone levels and levels of acetone in the breath ($r=0.95$, $p<0.001$) and, to a lesser extent urine ($r=0.68$, $p<0.05$). In one subject ^{14}C-acetone declined at a linear rate while the specific activity of $^{14}CO_2$ increased, reaching peak levels at 6 hours following dosing and declining thereafter. However, overall ^{14}C radiolabel was detected in the protein of all patients and in the lipids of six of the nine subjects, albeit at low levels. The radiolabel was found in glucose in only 3/9 subjects; these were the individuals with the lowest levels of plasma acetone at the start of the study. Although the authors determined an average acetone turnover rate of 265±47 μmol/min/1.73 m^2, there was no relationship between the individual acetone turnover rate and plasma acetone concentration as has been observed in fasting individuals.

Plasma concentrations of acetoacetate and β-hydroxybutyrate fluctuated considerably while the concentration of acetone remained constant reflecting a steady state condition. This is apparently achieved through a combination of metabolism and excretion. A positive linear relationship between the blood concentration of acetone and the amount lost in the breath was noted, and a negative relationship with *in vivo* metabolism. For example, at "low" (1-2.5 mM) plasma acetone concentrations ~20% of the acetone was lost through expired air and ~75% was lost through metabolism, while at "high" (8-9 mM) concentrations ~80% was lost through expired air and ~20% was lost through metabolism. Urinary excretion of acetone accounted for about 7% of the acetone excreted, independent of plasma acetone concentration. These data show that the percentage of acetone exhaled in breath increases and the percentage metabolized diminishes as acetone plasma concentration increases. The ^{14}C radiolabel on the carbonyl carbon

of acetone was detected in glucose, protein and lipids while no label was detected in β-hydroxybutyrate, acetoacetate or free fatty acids.

The same authors conducted a follow-up study (Reichard et al., 1986) to elucidate the pathway involved in the metabolism of acetone to glucose. Seven patients with diabetic ketoacidosis received an infusion of 2-^{14}C-acetone over a 6-hour period. Patients were not maintained on insulin therapy during the study. The authors monitored the incorporation of the radiolabel into acetol and 1,2-propanediol, and subsequently into glucose. The data demonstrated a linear relationship between acetol and propanediol formation and plasma acetone. In subjects with lower plasma acetone concentrations the acetol concentration was higher than propanediol, while at higher acetone concentrations, the acetol level was lower than propanediol. The study demonstrates the incorporation of the label into glucose. Interestingly, in six of the seven patients the label was incorporated predominantly into the 1, 2, 5, and 6 carbons of glucose while in one patient the label was found predominantly in the 3 and 4 carbons. The data suggest two separate metabolic pathways for converting acetone to glucose. The patient in which the resulting glucose was predominantly labeled in the 3 and 4 positions also had the highest level of endogenously produced acetone at the start of the study. The relative amount of intermediates (acetol and propanediol) may suggest that the predominant pathway is via the methylglyoxal pathway at low acetone concentrations, while the propanediol pathway predominates at higher levels. The study does not report changes in glucose levels with the infusion of acetone.

The average plasma acetone turnover rate was 533 μmol/min/1.73m^2, a value twice that obtained in the Owen et al. (1982) study via the single injection technique. In addition, plasma acetone turnover rates were directly related to the plasma acetone concentrations, unlike the results of the Owen et al. (1982) study. The authors state that the single injection technique may have resulted in inadequate mixing of the labeled acetone in the body pools of acetone and may have been responsible for the failure to show a linear relationship between plasma acetone concentrations and acetone turnover rates.

Sakami (1950) conducted a series of rat studies to determine the metabolism of exogenously administered acetone. The first study involved starving the rats for 2 days followed by gavaging with ^{14}C-labeled acetone and measuring the generation of $^{14}CO_2$. The rats were dosed and placed in metabolic chambers where the gas was sampled on an hourly basis. The animals received additional dosing of ^{14}C acetone at 3-, 6-, and 10-hour samplings. Radioactivity trapped by the bicarbonate was $^{14}CO_2$ indicating acetone is metabolized to CO_2.

While the study does not describe the method for trapping CO_2, it is possible that the radioactivity that was analyzed was nonmetabolized expired acetone.

In the same study, rats were sacrificed following incubation and necropsied, and the liver homogenized. Serine, glycogen, methionine, and choline fractions were recovered and assayed for radioactivity. Of the fractions tested, ^{14}C was predominantly found in the glycogen that was recovered from the liver, indicating a metabolic process that involves the conversion of acetone to glucose. Extracts from the viscera of the rats contained radioactivity in serine, methionine, and choline indicating that formate contributes to their synthesis at the same positions within the molecule as those found in the labeled constituents. Hence, the authors propose that acetone is converted to serine, methionine, and choline through the oxidation and decarboxylation of acetone to acetate and formate. The authors do not speculate on a pathway. Although they may be correct in stating that the ^{14}C label is incorporated into the amino acids, the authors do not provide support for the contention that formate is an intermediate in the process. It should be noted that methionine is an essential amino acid in humans.

As a follow-up to their initial work, Sakami and Lafaye (1951) conducted a study to elucidate the pathway for acetone metabolism. The authors proposed a method for elucidating the metabolic pathway based on the locations of ^{14}C in glucose that is synthesized from acetone. If the metabolism of 2-^{14}C-acetone proceeded through the cleavage of acetone to acetate and formate, the acetate would form acetyl CoA, which would lead to higher labeling at the 3- and 4-positions relative to the 2- and 5-positions. Six male rats were fasted for 24 hours and gavaged with glucose and 2-^{14}C-acetone and placed in a metabolic cage for 4 hours. The amount of $^{14}CO_2$ generated from acetone increased with time over the course of the incubation period. At the end of 4 hours the animals were sacrificed, livers were removed, and glycogen was extracted. The glycogen was digested to glucose and subjected to microbial degradation to identify sites of ^{14}C labeling on the glucose molecule. The ^{14}C label was found in all positions in the glucose molecule, although with higher labeling in the 2- and 5-positions than the 1- and 6-positions. The authors indicate that the level of labeling in the 1- and 6- labeled molecules may be an artifact of the degradation process.

Although the data from Sakami (1950) and Sakami and Lafaye (1951) provide strong evidence that exogenous acetone is readily absorbed via the gastrointestinal tract and converted to CO_2, glucose/glycogen, and other metabolites, the studies fail to provide adequate support to define a metabolic pathway.

On the basis of findings that acetone is converted to glucose in the body, Casazza et al. (1984) set out to describe the metabolic pathway(s) for the conversion of acetone to glucose or other cellular constituents. They conducted studies that employed a combination of whole animals, hepatocyte cultures, and microsomes from rat livers to develop a composite pathway for the metabolism of acetone in the rodent. Pretreatments included rats that received either nontreated drinking water or drinking water with 1% acetone up to 14 hours prior to sampling, when the acetone-treated water was replaced with nontreated water. Rats from both groups received either an intraperitoneal treatment of saline or saline with 5 μmol/g of acetone. Rats that had been pretreated with 1% acetone in drinking water up to 14 hours prior to sampling and had received an intraperitoneal injection of acetone produced detectable levels of 1,2-propanediol and 2,3-butanediol. Rats that received no intraperitoneal injection or received the intraperitoneal injection with no pretreatment with acetone failed to produce detectable levels of either of the two diols, indicating the role of enzyme induction in determining a particular pathway in the metabolism of acetone.

In the same study, elevated levels of lactate were found in rats that received pretreatment of acetone compared with rats receiving drinking water in the nontreated and treated groups. To determine the metabolic pathway leading to lactate, acetone was incubated *in vitro* in a microsomal suspension and acetol was recovered. When acetol was added to the microsomal suspension in the presence of glyoxalase I and glutathione, D-lactate was recovered, but in the absence of glyoxalase I and glutathione, methylglyoxal was recovered. Incubation of rat hepatocytes with acetol or methylglyoxal produced D-lactate and glucose. However, when rats were pulsed with ^{14}C-acetone and supplemented with D-lactate the incorporation of ^{14}C into glucose was not reduced, indicating that the predominant route of gluconeogenesis is not via the formation of D-lactate, but directly from methylglyoxal through glyoxalase(s). By analyzing the kinetics of the conversion of acetone or acetol to glucose and D-lactate, the rate of utilization of acetol could not be accounted for by the formation of glucose and D-lactate. These findings suggest the presence of an alternate pathway for the conversion of acetone to glucose.

Further studies (Casazza et al., 1984) were performed using hepatocytes from a rat starved for two days given 1% acetone in drinking water. The hepatocytes were incubated with L-lactate and L-1,2-propanediol with and without specific inhibitors of aldehyde dehydrogenase (cyanamide) or alcohol dehydrogenase (pentylpyrazole). The rate of glucose formation from L-lactate was reduced 12 and 50% in the presence of pentylpyrazole or cyanamide, respectively, compared with the control. The rate of glucose formation from L-1,2-propanediol in the

presence of pentylpyrazole or cyanamide was comparable to the rate of glucose formation in the controls (without L-1,2-propanediol).

A comparison of the kinetics for L-1,2-propanediol production from hepatocytes with those observed in the whole rat indicates that the enzymatic activity in the liver could not account for the total amount of L-1,2-propanediol formed in the whole animal. Additionally, 1,2-propanediol concentrations were comparable in the hepatic artery and vein of perfused liver containing acetol. The data suggest that most of the metabolism occurring via the formation of 1,2-propanediol is extrahepatic and that 1,2-propanediol production provides a second pathway for the formation of glucose from acetone.

In summary, Casazza et al. (1984) provide evidence that there are two pathways (hepatic and extrahepatic) involved in the metabolism of acetone to glucose. The hepatic route involves the conversion of acetone to acetol mediated by acetone monooxygenase, which has been shown to be CYP2E1. Acetol in turn may be converted to methylglyoxal via the CYP2E1 enzyme system or converted to 1,2-propanediol. Methylglyoxal is converted either directly to glucose or to D-lactate, which is converted to glucose. The data support the direct pathway of methylglyoxal to glucose as opposed to the D-lactate route. From the data presented in this study, it is not possible to determine which of the two pathways, i.e., the 1,2-propanediol or methylglyoxal pathway, predominates.

Although the studies are comprehensive, they have notable limitations. Treatments for most of the assays were not replicated, and, therefore, provide no measure of variability. In addition, the authors note that formate and acetate production has been proposed as a pathway for the metabolism of acetone; however, this issue is not addressed in their paper. It is not clear whether the authors analyzed for an "active" form of acetate or formate and failed to find it.

Kosugi et al. (1986a) demonstrated that the predominant pathway for the metabolism of acetone is dose-dependent, with the methylglyoxal pathway predominating at lower concentrations and the 1,2-propanediol pathway predominating at higher concentrations. These studies support the human studies in diabetics (Reichard et al., 1986). Rats were fasted for 20-24 hours (except for two controls) and then received either "trace" amounts or 1.6 mmol of 2-^{14}C-acetone through a tail-vein catheter. ^{13}C-lactate was administered via the same route. "Trace" amount was defined as the specific activity of the undiluted 2-^{14}C acetone as it was received from the distributor. The infusion of acetone took approximately four hours to complete. Following the infusion of radiolabeled acetone, a sample of blood was drawn, the animals were

sacrificed, and the disposition of the ^{14}C- label was determined. Rats were either necropsied and the livers removed to recover the glycogen that was digested to glucose, or they were frozen and powdered and the glucose recovered. For all samples, the glucose was degraded and analyzed for the placement of ^{14}C in the glucose molecule. Regardless of whether the rats were fasted or infused with glucose and acetone, the infusion of trace amounts of 2-^{14}C acetone into the rat resulted in 5-10% of the radiolabel in the 3 or 4 positions of the glucose molecule. In rats that received the 1.6 mmol acetone solution, 23-40% of the ^{14}C label was in either the 3 or 4 positions on the resulting glucose. Differences in the location of ^{14}C indicate that there is more than one metabolic pathway and that the pathway selection is dose-dependent.

Kosugi et al. (1986b) conducted a second study comparing the metabolism of radiolabeled 2-^{14}C-lactate with that of 2-^{14}C-acetone. The study authors proposed that if acetone is metabolized prior to any randomization of the carbons in which lactate serves as an intermediate, as postulated by Casazza et al. (1984), the resultant glucose molecule should be the same as that seen with lactate, with most of the ^{14}C in carbons 1, 2, 5, and 6 of the glucose molecule. Therefore, differences in ^{14}C distribution in the glucose molecule between the two levels of acetone administration indicate that in low concentrations acetone is metabolized through a pathway that involves lactate as an intermediate, but at high levels acetone is metabolized through a pathway that does not involve lactate as an intermediate or byproduct. The authors postulate that the catabolism of glucose most likely proceeds through the formation of acetate as proposed by Sakami (1950).

Gavino et al. (1987) conducted studies to clarify the proposed pathways by which acetone is utilized at millimolar concentrations. Sprague-Dawley rats were starved for 48 hours prior to the start of the study. Livers were perfused with glucose and bovine serum albumin for 30 minutes, after which 2-^{14}C-acetone was added to the perfusate. The data demonstrate that as the acetone concentration decreased, the concentration of acetoacetate increased. The perfusate was analyzed for the incorporation of ^{14}C into potential metabolites including citrate, lactate, β-hydroxybutyrate, acetate, 1,2-propanediol, and acetol. The ^{14}C radiolabel was found in glucose, CO_2 and volatiles, but not in β-hydroxybutyrate indicating that acetone is not carboxylated. Although the authors acknowledge that considering the proximity of elution there might be some incorporation of ^{14}C into 1,2-propanediol, most of the ^{14}C that was introduced into the perfusate as 2-^{14}C-acetone was found in acetate with the radiolabel in the 1 position.

Casazza et al. (1984) proposed two main pathways of acetone metabolism: one proceeding via methylglyoxal and a second through 1,2-propanediol. Kosugi et al. (1986a)

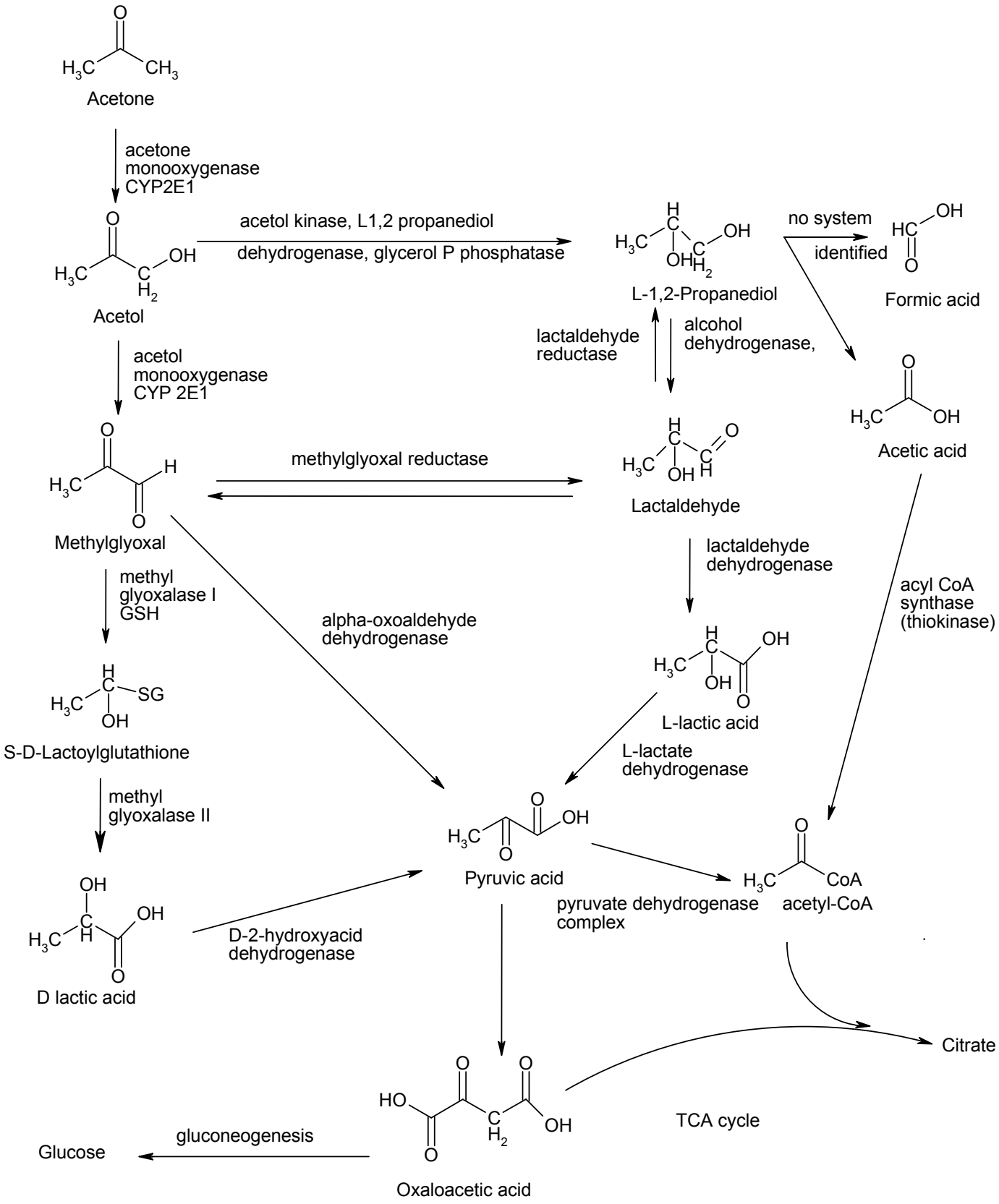
provide evidence that the route of metabolism differs depending on the amount of acetone. For example, at lower concentrations metabolism proceeds mostly through the methylglyoxal pathway, but as the concentration of acetone increases catabolism proceeds to a correspondingly greater degree via the 1,2-propanediol pathway. Results of the Gavino et al. (1987) study indicate that the catabolism of acetone proceeds via a pathway that generates acetate but not via 1,2-propanediol. However, a significant difference between the two studies is that Kosugi et al. (1986a) administered acetone via a tail cannula, which subjected the acetone to systemic metabolism. Gavino et al. (1987), on the other hand, conducted a hepatic perfusion study. The differing results are consistent with the mechanism proposed by Casazza et al. (1984), who stated that the methylglyoxal pathway was largely a hepatic pathway and the 1,2-propanediol pathway was largely extrahepatic.

Therefore, the metabolic pathway(s) for acetone is proposed to have three possible routes, all of which start with the conversion of acetone to acetol. From acetol the pathway diverges to the formation of either methylglyoxal or 1,2-propanediol. A third pathway that has been proposed involves the production of formic acid which is the metabolite that is mostly responsible for the toxic effects resulting from consumption of methanol. Following ingestion, methanol is readily converted to formaldehyde and subsequently to formic acid. Under conditions of methanol poisoning, formic acid inhibits the formation of cytochrome c oxidase, which causes histotoxic hypoxia leading to several adverse effects. The most sensitive effect is amblyopia and amaurosis, a condition of the optic nerve causing impaired vision or blindness. At higher levels, formic acid affects other organs, particularly those with high oxygen consumption rates such as the brain, heart, and kidneys. At high enough levels, methanol poisoning will cause death, presumably due to the formation of formic acid (Liesivuori and Savolainen, 1991).

Data supporting the formation of formic acid as a product of acetone metabolism are sparse. Hallier et al. (1981) found that rats placed in a desiccator saturated with acetone demonstrated low (4.7% of the absorbed acetone) amounts of formate in the urine. In a recent review of the literature concerning the metabolism of acetone, Kalapos (1999) indicates that enzyme systems have not been identified to mediate the formation of formate from acetone, and that the toxic effects attributed to acetone are inconsistent with those of formic acid.

Methylglyoxal is an α-oxoaldehyde formed from the metabolism of acetone, and has been shown in rodents to be catabolized to lactic acid through glyoxalase I and II and glutathione, or metabolized directly into a gluconeogenesis route by an undetermined pathway.

Methylglyoxal has been shown to have potentially adverse effects at levels higher than normally seen in the body, including: genotoxicity (Barnett and Munoz, 1998), depletion of glutathione (Ankrah and Appiah-Opong, 1999), and the induction of apoptosis (Thornalley, 1998). Methylglyoxal has also been shown to induce sex-linked recessive lethal responses in *Drosophila melanogaster* germinal cells, although only at high concentrations. Depletion of glutathione resulting from the catabolism of methylglyoxal to lactate may be significant. Ankrah and Appiah-Opong (1999) demonstrated that perinatal exposure to methylglyoxal altered the tolerance of mice to glucose, while postnatal blood analyses demonstrated a decreased amount of glutathione-S-transferase and a decreased ability of red blood cells to tolerate oxidative stress. Finally, methylglyoxal has been shown to arrest growth and induce apoptosis in human leukemia cells (Kang et al., 1996).



(after Kalapos, 1999)

Figure 1. Pathway for the metabolism of acetone.

Bondoc et al. (1999) demonstrated the role of CYP2E1 in the metabolism of endogenously-produced acetone under ketogenic conditions using a knockout mouse model lacking the ability to express CYP2E1. When mice were fed ad libitum, plasma acetone levels in strain-matched CYP2E1-competent mice, and CYP2E1-null mice had comparable levels of plasma acetone. After fasting for 48 hours, the competent strain had a 2.5- to 4.4-fold increase in plasma acetone while the CYP2E1-null mice had a 48-fold increase. This study provides strong evidence for the role of CYP2E1 in the catabolism of acetone.

In brief, based on human and animal studies and *in vitro* studies, the metabolism of acetone may occur via at least two routes (Figure 1). The principal metabolic pathways are dependent on the site of metabolism and on the concentration of acetone. The metabolites are incorporated into glucose and other substrates of intermediary metabolism that ultimately produce CO_2. In the first metabolic step, common to all potential pathways, acetone is oxidized to acetol by acetone monooxygenase, an activity associated with CYP2E1. This step requires O_2 and NADPH (Casazza et al., 1984). In the first pathway, acetol is converted to methylglyoxal, which in turn is metabolized to glucose through a lactate intermediate. The conversion of acetone via the methylglyoxal pathway is mediated by acetone monooxygenase (CYP2E1) and acetol monooxygenase (CYP2E1) to form methylglyoxal. The conversion of methylglyoxal to lactate is mediated by glyoxylase I and II and glutathione-S-transferase. This pathway is primarily a hepatic pathway. In the second pathway, the acetol intermediate is converted to L-1,2-propanediol by an extrahepatic mechanism that has not been fully characterized. The metabolism of acetone via the 1,2-propanediol pathway to lactate is mediated by alcohol dehydrogenase and aldehyde dehydrogenase (Dietz et al., 1991). Gluconeogenesis may proceed through the formation of an active form of acetate. 1,2-Propanediol may be converted to glucose through a series of intermediates including lactate. As noted above, Sakami (1950) proposed a pathway by which acetone is converted to formate and acetate.

The data also demonstrates that the pathways for acetone metabolism are concentration-dependant. At lower concentrations, acetone is metabolized in the liver through the methylglyoxal pathway similar to biological conditions of fasting or exertion where the acetone is formed from fatty acids to produce glucose. Thus, at low plasma concentrations acetone serves as a gluconeogenic substrate. At higher concentrations an alternate pathway predominates and mediates the conversion of acetone to 1,2-propanediol. Although some studies indicate that 1,2-propanediol serves as an intermediate in the production of glucose, it is conceivable that the conversion from acetone to the diol diverts acetone from gluconeogenesis and facilitates the loss of acetone via urine.

Enzymes involved in the metabolism of acetone are inducible. The metabolism of acetone through the methylglyoxal route is mediated largely by CYP2E1, which can be induced by fasting, experimental diabetes, or exposure to ethanol or acetone; therefore, acetone induces its own metabolism (ATSDR, 1994; Mandl et al., 1995; WHO, 1998). Inhibition of CYP2E1 activity resulted in an increase in endogenous acetone levels in rats (Chen et al., 1994). Acetone significantly increased both the microsomal protein content and the activity of CYP2E1 in rat liver 18 hours after a single oral dose of 15 mmol/kg body weight (Brady et al., 1989) and in mouse liver 24 hours after a single oral dose or administration of 1% in the drinking water for eight days (Forkert et al., 1994). Treatment with acetone or starvation conditions leads to increases in protein content and enzyme activity in the rat kidney (Ronis et al., 1998). Acetone inhalation exposure has also been shown to potentiate enzyme induction by the solvents toluene and xylene (Nedelcheva, 1996).

3.4. EXCRETION

In a previously described study, Wigaeus et al. (1981) exposed male subjects to two concentrations of acetone, with and without exercise. Individuals at rest were exposed to 1,300 mg/m^3 and on a second occasion to 700 mg/m^3 either with light exercise or with increasingly strenuous exercise for two hours. As noted earlier, inhalation uptake increased with exertion. The exposure treatment was followed by a 4-hour monitored period of elimination consisting of intermittent walking and rest during which expiratory samples and urine were collected and analyzed for nonmetabolized acetone. The highest concentration of acetone in urine was found between 3 and 3.5 hours following exposure. Absorbed acetone was lost through the lungs in the form of nonmetabolized acetone (16, 20 and 27% for resting, light exercise, and more strenuous exercise, respectively). For all three exposure scenarios, approximately 1% of the absorbed acetone was lost through the kidneys, the remainder was presumed to be metabolized. The higher concentration of acetone that was lost through the lungs corresponds to a greater amount of acetone absorbed by the body, suggesting saturation of acetone metabolism.

In male volunteers given oral acetone doses of 40-80 mg/kg, an estimated 65-93% of the dose was metabolized, with the remainder being eliminated in the urine and expired air in about two hours, indicating rapid and extensive absorption by the gastrointestinal tract (Haggard et al., 1944).

Wang et al. (1994) found that workers with a mean occupational exposure to acetone of 141.8 ppm had blood and urine concentrations at the end of a shift of 23 mg/L and 22 mg/L,

respectively; acetone concentrations remained slightly elevated 16 hours after the end of the shift and the blood half-life was calculated to be 5.8 hours. A positive linear correlation has been shown between acetone concentrations in the breathing zone of workers and urinary (Kawai et al., 1992), blood (Wang et al., 1994), and alveolar concentrations (Wang et al., 1994). Breath decay curves from experimentally-exposed individuals have been shown to be highly reproducible, and the narrow range of acetone in the breath at a specific postexposure time indicates that breath analysis could be a reliable method to estimate the magnitude of recent acetone exposure by inhalation (Stewart et al., 1975). Urine concentrations, on the other hand, were shown to increase only when workers were exposed to acetone concentrations greater than 15 ppm (Kawai et al., 1992). Differences between the findings of Wang et al. (1994) and Wigaeus et al. (1981) may reflect dose-related differences, given that the subjects in the Wigaeus et al. (1981) study were exposed to acetone concentrations that were roughly five-fold higher than those in the Wang et al. (1994) study.

Haggard et al. (1944) injected 11 rats intraperitoneal and followed the fate of acetone over a 4- to 6-hour period. Blood acetone levels and the amount of acetone lost through the lungs and urine were compared with that lost by metabolism. The data indicate that the percent of acetone lost by elimination in the urine and expiration is directly proportional and that lost via metabolism is inversely proportional to the blood acetone content. At higher blood concentrations acetone is predominantly lost via elimination, whereas at low concentrations it is metabolized. Based on their data, the critical point is approximately 100 mg acetone/L blood.

A principal finding in these studies is that the mode of acetone excretion appears to be dose-related. At low concentrations acetone appears to be excreted primarily through expiration. At concentrations above 15 ppm acetone appears in the urine at about 1% of the exposure level. Levels of acetone lost through expiration increase disproportionately at higher concentrations.

3.5. PBPK MODELING

Information on the toxicokinetics of acetone and isopropanol, whose major metabolite is acetone (Nordmann et al., 1973), has been used to develop physiologically-based, pharmacokinetic models to compare the uptake, distribution and metabolism of the chemicals in rats and humans by different routes of exposure (Clewell et al., 2001; Gentry, et al., 2002; Kumagai and Matsunaga, 1995). The tissues described in the models include those associated with uptake (lungs and skin), metabolism (liver) and fat storage with slowly- and rapidly-perfused compartments. The models have been validated for human exposure for the inhalation pathway, but not the oral pathway.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS — EPIDEMIOLOGY AND CASE REPORTS

The available data on the human health effects of acetone exposures are limited. Most of the available studies involve exposures in occupational settings.

4.1.1. Cancer Studies

Ott et al. (1983a,b) conducted a retrospective cohort study to evaluate the effects of methylene chloride on the hematologic and circulatory systems of the workers at a plant that manufactured cellulose diacetate and cellulose triacetate. The study monitored several causes of death, including deaths from malignant neoplasms. The control cohort selected for the study was a second plant that also manufactured cellulose triacetate fibers but used acetone as a solvent. The two plants were in close proximity to each other and were operated by the same company. The study involved production employees who worked in areas of high exposure to either methylene chloride and acetone (as the exposed cohort) or just acetone (for the control cohort) between January 1, 1954, and January 1, 1977. Employment ranged from three months to 23 years with time-weighted-average acetone concentrations of 380 to 1,070 ppm depending on the job category. In the study, 948 acetone-exposed workers were the reference cohort for comparison to workers exposed to acetone plus methylene chloride; comparisons to unexposed controls were not made. For the acetone-exposed workers, the total number of deaths observed from all causes was 24 and 3 for men and women, respectively, compared with the total expected of 53.8 and 6.7 for men and women, respectively. There were no deaths among the 107 nonwhite women in the exposed cohort. Among the acetone-exposed workers the incidence of "malignant

neoplasms" was 5 and 2 compared with an expected incidence of 10 and 2.3 for men and women, respectively.

This study has several shortcomings that limit its use for assessing health effects in humans. The focus was on deaths resulting from cardiovascular effects from exposure to methylene chloride; other health effects related to acetone exposure were secondary. Workers exposed only to acetone were selected as the referent cohort. The incidence of death was compared with expected deaths calculated from U.S. death rates for white men, nonwhite men, and white women. Also, the acetone-exposed cohort is smaller than the methylene chloride cohort. Finally, the study lists deaths by malignant neoplasms without noting the cell type or target organ.

4.1.2. Noncancer Studies

4.1.2.1. *Controlled Studies*

Groups of four men were exposed to 0, 200, 1,000, or 1,250 ppm acetone for 3 or 7.5 hours/day for 4 consecutive days on 4 successive weeks with one week of fluctuating concentrations (750-1,250 ppm) for 3 days (Stewart et al., 1975). Two groups of 2-4 women were exposed to 0 or 1,000 ppm for 3 or 7.5 hours/day on 4 consecutive days over the course of one week. During the first week and on the first day of each week all subjects were exposed to ambient air and measurements of relevant parameters were determined to establish baseline conditions and concentrations, followed by four subsequent days of exposure to acetone at the designated concentration. Male subjects were exposed to concentrations of 200, 1,000 or 1,250 ppm and fluctuating concentrations (750-1,250 ppm for 3 days) during weeks 2, 3, 4, and 6, respectively. Female subjects were exposed to 1,000 ppm during week 5.

The concentration of acetone in the breath, blood, and urine was directly related to exposure concentration, but decreased steadily postexposure. Odor intensity increased with exposure concentration, but no exposure-related adverse subjective signs were reported. Clinical chemistry analyses, hematological analyses, urinalyses, electroencephalograms, electrocardiograms, and cognitive and pulmonary function tests remained normal and did not vary from preexposure levels. No neurological abnormalities occurred and the modified Romberg test and the heel-to-toe test remained normal. The visual evoked response measurements were taken following exposures on the second and fourth days of exposure during each week of exposure. Three of four males exposed to 1,250 ppm had a statistically significant

increase in total visual evoked response amplitude on individual days, but no consistent pattern was observed. An early menstrual period was reported by three of four women after four days of exposure to 1,000 ppm for 7.5 hours. The significance of a premature menstrual period was not clear, and the study authors stated that additional research was needed. This study is characterized as a six-week study by ATSDR (1994) and is used to derive a minimum risk level for intermediate and chronic exposure. As presented in its protocols, this study was conducted over a six-week period; however, the subjects were only exposed to acetone for 4 days/week for four weeks (males) and four days for 1 week (females). The visual evoked response measurements were made after the second and fourth days of exposure which constitutes a relatively short exposure period.

A group of 11 male and 11 female volunteers were tested for neurobehavioral performance before, during, and after a 4-hour exposure to 250 ppm acetone (Dick et al., 1988, 1989). Initial analyses of all behavioral tests indicated that sex differences did not exist, with the exception of the profile of mood states (POMS). Dual task performance measurements showed mild but statistically significant increased response times and false alarm percent both during and postexposure. Measurements of postural sway were also slightly increased from acetone exposure, but statistical significance was not reached. Only men had a statistically significant drop on the anger hostility scale of the POMS test. No effects were found on visual vigilance, choice reaction time, and memory scanning.

4.1.2.2. *Cohort Studies*

Satoh et al. (1996) examined the neurotoxic effects of acetone in 110 male workers at an acetate fiber plant. A total of 67 nonexposed male workers at the same plant served as controls. Mean worker age and length of acetone exposure were 37.6 and 14.9 years, respectively. Tests used to assess narcosis included finger tapping, simple reaction time, and choice reaction time. Memory tests consisted of the Benton visual retention test and forward and backward digit span. Workers were classified into highly exposed (>500 ppm), moderately exposed (250-500 ppm), and less exposed (<250 ppm) groups as determined by the acetone level in the breathing zone. Acetone levels in alveolar air, urine, and blood were directly correlated with exposure levels, indicating that an equilibrium is reached under continuous exposure resulting in absorption of acetone into the cardiovascular system. During or after work symptoms of eye irritation, tearing, acetone odor, and nausea were reported by 13.7-45.1% of exposed workers vs. 3.9-23.5% of unexposed controls. Over the previous six months, heavy or faint feelings in the head, nausea, and weight loss were reported by 23.6-25.8% of exposed workers vs. 2.9-9.8% of controls. The

symptoms showed a dose-response relationship. The authors speculated that they were probably the result of peak exposure during a single day. No differences between exposed workers and controls were observed on the Manifest Anxiety and Self-rating Depression scales or for electrocardiogram, phagocytic activity, hematology, and clinical chemistry. A statistically significant decrease in simple reaction time and digit span activity was observed among exposed workers aged 30-44 years, but not in workers aged <30 or ≥45 years. The study authors questioned whether the differences in only one age group were real or chance findings.

In a similar study, 71 factory workers with a mean age and length of exposure of 36 and 14 years, respectively, were evaluated for both central and peripheral nervous system effects (Mitran et al., 1997). Exposure concentrations over an 8-hour shift ranged from 416 to 890 ppm acetone. Mood disorders; irritability; memory difficulties; sleep disturbances; headache; numbness of the hands or feet; eye and/or nose irritation; bone, joint, and/or muscle pain; nausea; and abdominal pains were reported slightly more frequently in exposed workers as compared with controls. However, the time during the work shift when the symptoms occurred or were reported was not stated. Results of motor nerve conduction tests on the median, ulnar, and peroneal nerves indicated statistically significant reductions in latency, amplitude, and/or duration of both proximal and distal responses, but no consistent pattern of effect.
A statistically significantly reduction in the nerve conduction velocity of all three nerves was observed in exposed workers as compared with controls. Statistically significant delayed reaction times for the visual test and a lower mean distributive attention score were identified in exposed workers when compared with the controls.

Although the results of the Mitran et al. (1997) study indicate a possible neurotoxic effect from acetone exposure, the study has several shortcomings. For example, there is too little information for a critical appraisal of the study design such as selection of controls, parameters used for matching for age and other variables, experimental procedures, i.e., blind versus non-blind determinations of neurotoxicity, and temperature control during neurological performance procedures. Age-matching of the subjects and consistent temperature control are critical parameters in nerve conduction velocity measurements. Some information regarding the selection of matched controls was reported by Mitran (2000) in a reply to an analysis of the Mitran et al. (1997) study made by Graham (2000). Additional confounding factors include: (1) no dose-response relationship was established; and (2) it is not possible to rule out co-exposure to other toxicants as the factory was a coin and metal plant and exposure to metals and other contaminants may be likely. Some of these issues are discussed in an EPA memorandum by Boyes and Herr (2002).

Eight occupationally-exposed and eight nonexposed workers were studied for subjective symptoms on nine shift days (during work) and during leisure at 8 hours following termination of exposure (Kiesswetter et al., 1994). The authors recorded the feeling of well-being at the start, middle and end of each shift, and at eight hours following termination of exposure for nine separate days. Personal measurements indicated that on average the workers were exposed to 2,730 mg/m^3 (1,150 ppm) during the first half of the shift and to 1,720 mg/m^3 (725 ppm) during the second half of the shift. For each determination of well-being measurement the subjects reported on tension, tiredness, complaints, and annoyance. The only endpoint demonstrating a relationship between ambient acetone concentrations and ratings of well-being was in the "annoyance" category which tended to occur during the middle and at the end of the shift.

Semen parameters were evaluated for 25 workers at a reinforced plastic production plant (Jelnes, 1988). Breathing zone measurements indicated mean acetone concentrations of 69.6 (range of 21.9-246.4) ppm, 162 (55.7-562.9) ppm, and 94.5 (28.3-189.9) ppm approximately 28, 15, and 10 weeks, respectively, prior to semen analyses. Concurrent exposures to slightly higher levels of styrene also occurred. Semen from the reference group was collected from samples deposited at a fertility clinic. Semen volume, sperm concentration, and serum concentrations of follicle stimulating and luteinizing hormones were not different from age-matched controls. The percent of live sperm was significantly higher ($p \leq 0.01$; 80% vs. 68%) whereas the percent of immobile sperm was significantly lower ($p \leq 0.01$; 30% vs. 40%) in the exposed group as compared with controls. The exposed men also had a decrease in the percent of normal sperm (47 vs. 60%) due to increased percentages of amorphous and pyriform sperm head shapes. This study is limited by several factors, including the fact that the semen samples used in the control group were collected from a fertility clinic, and subjects were concurrently exposed to styrene.

4.1.2.3. *Case Reports*

Clinical signs and symptoms of toxicity were reported for workers at three manufacturing plants where acetone was used along with other solvents (Parmeggiani and Sassi, 1954; as reviewed in OECD, 1998). Drowsiness, eye and throat irritation, dizziness, inebriation, and headache were complaints from six employees exposed to 309-918 ppm for up to three hours over a 7-15 year period. At the second plant where acetone concentrations ranged from 84 to 147 ppm, four workers reported nausea, abdominal pain, headache, vertigo, loss of appetite, vomiting, and other debilitating symptoms. At the third site, eye, nose, throat, and bronchi irritation, along with central nervous system disturbances were documented for 11 workers exposed to 13-86 ppm acetone in conjunction with "high" concentrations of carbon disulfide.

Filter press operators were evaluated for clinical signs and symptoms of toxicity over a two year period (Raleigh and McGee, 1972). During the process of cleaning presses, filter cloths saturated with cellulose acetate dissolved in acetone were removed and replaced, which caused short-term (about 2-3 hours) exposure to much higher acetone concentrations than normally present in the work area. During the first year of the study, average acetone concentrations in the breathing zone of workers while pulling filters was 2,300 ppm (326-5,548 ppm) and while dressing presses was 300 ppm (69-904 ppm). In contrast, acetone concentrations in the general air were 110 ppm (29-286 ppm). Similar concentrations were measured during the second year of the study. Of the nine workers monitored, seven complained of eye irritation, four of throat irritation, and two of nasal irritation, three reported headaches, and three noted lightheadedness. Generally, the symptoms were transient, intermittent, and occurred when the concentrations of acetone "greatly" exceeded 1,000 ppm. No indications of central nervous system effects were found as assessed by gait, the finger-to-nose test, and Romberg sign (loss of joint position sense).

Effects from oral exposure to acetone are limited to case reports. In one case report, a 17-month-old girl was given approximately 4.88 mL/kg (3,850 mg/kg bolus dose) of acetone through her gastronomy tube (Herman et al., 1997). The child was found gagging, unresponsive, and diaphoretic with dilated sluggish pupils, right arm tonic-clonic activity, and left eye deviation, and she was unresponsive to verbal or painful stimuli. Serum ketones were still present at a 1:32 dilution and the abdomen was distended and firm. Following intubation and supportive therapy, the child recovered fully. Another case report described a 53-year-old woman admitted to the hospital after ingestion of nail polish remover (Ramu et al., 1978). Vital signs were generally normal, but neurological examination showed that even though she was oriented, the patient was lethargic but arousable and had a shortened attention span. Her blood acetone concentration was 0.25 g/dL. The woman was admitted for observation and her condition gradually improved as blood acetone levels declined.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS

4.2.1. Prechronic Studies

4.2.1.1. *Oral Studies*

Groups of five male and five female F344/N rats were administered acetone in drinking water at concentrations of 0, 5,000, 10,000, 20,000, 50,000, or 100,000 ppm for 14 days (NTP, 1991; Dietz et al., 1991). Time-weighted average doses for males were 0, 700, 1,600, 2,600, 4,300, and 6,900 mg/kg-day, respectively, and for females 0, 750, 1,500, 2,300, 4,350, and 8,600 mg/kg-day, respectively. All animals survived to termination. Water consumption was reduced in both sexes at 50,000 and 100,000 ppm. Final body weights of males given 50,000 and 100,000 ppm were 87 and 63%, respectively, of controls, and final body weights of females given 100,000 ppm were 87% of controls. Increases in relative liver and kidney weights were observed in both sexes at ≥20,000 ppm, relative testes weights were increased at ≥50,000 ppm, and thymus weights were decreased in "exposed" animals; further details of organ weight data were not included. Bone marrow hypoplasia was noted in all high-dose males, but in none of the controls. More detailed hematology and clinical chemistry studies were not included.

Groups of five male and five female B6C3F1 mice were administered acetone in drinking water at concentrations of 0, 5,000, 10,000, 20,000, 50,000, or 100,000 ppm for 14 days (NTP, 1991; Dietz et al., 1991). Time-weighted average doses for males were 0, 960, 1,600, 3,900, 6,300, and 10,000 mg/kg-day, respectively, and for females 0, 1,600, 3,000, 5,500, 8,800, and 13,000 mg/kg-day, respectively. Water consumption was reduced in both sexes at 50,000 and 100,000 ppm and final body weights of high-dose males and females were slightly less than the controls. All animals survived to termination. Kidney weights were increased at ≥50,000 ppm for males and females, and liver weights were increased at ≥5,000 ppm in males and ≥20,000 ppm in females. Dose-related increases in the incidence and severity of centrilobular hepatocellular hypertrophy were observed in males at ≥20,000 ppm and in females at ≥50,000 ppm. Hematology and clinical chemistry evaluations were not conducted.

Groups of 10 male and 10 female F344/N rats were administered acetone in the drinking water at concentrations of 0, 2,500, 5,000, 10,000, 20,000, or 50,000 ppm for 13 weeks (NTP, 1991; Dietz et al., 1991). Time-weighted average doses for males were 0, 200, 400, 900, 1,700, and 3,400 mg/kg-day, respectively, and for females 0, 300, 600, 1,200, 1,600, and 3,100 mg/kg-day, respectively. No deaths occurred in any group. Water consumption was decreased in high-

dose males and in females given 20,000 and 50,000 ppm acetone. Mean final body weight of the high-dose males was 81% of the controls; body weights of the females were unaffected by treatment. No clinical signs of toxicity or ophthalmic abnormalities were observed in any group. At necropsy, statistically significant ($p \leq 0.01$ or 0.05) increases in the following organ weights were noted: relative kidney weights were 114% of controls for 20,000 ppm females and 126 and 123% of controls for 50,000 ppm males and females, respectively; relative liver weights were 110 and 112% of controls for 20,000 ppm males and females, respectively, and 115 and 105% of controls for 50,000 ppm males and females, respectively; and relative testis weights were 119% of controls at 50,000 ppm. Liver weight changes were not associated with microscopic lesions and were thought to result from enzyme induction. In high-dose males, depressed sperm motility, caudal weight, epididymal weight and an increased incidence of abnormal sperm were seen (data for testicular effects were given only for the 0, 2,500, 10,000, and 50,000 ppm groups; see also Section 4.3.1.1). Males given the two highest concentrations of acetone had increases in the incidence and severity of nephropathy, indicating early onset and enhanced progression of the disease. In males given 0, 2,500, 5,000, 10,000, 20,000, or 50,000 ppm acetone, nephropathy was observed in all treatment groups including the controls. As such, the incidence of nephropathy rated as mild was taken as the indicator of toxicity. For the 0, 2,500, 5,000, 10,000, 20,000, and 50,000 ppm doses the incidence of minimal nephropathy was 5, 8, 8, 9, 1, and 1 (out of 10 animals) and for mild nephropathy 1, 0, 0, 0, 9 and 9 (out of 10 animals), respectively. The authors of the study identify the kidney effects as the most prominent chemically-related effect. Nephropathy was not observed in females. Pigment deposition in the spleen was observed in 10/10 males in the 20,000 and 50,000 ppm groups compared with 0/10 controls.

Other endpoints were noted at 20,000 and 50,000 ppm doses of acetone, including statistically significant ($p \leq 0.01$ or 0.05) changes in hematology in males. For the 20,000 and 50,000 ppm groups, leukocytes were 125 and 133% of controls, erythrocyte counts 92% and 90% of controls, reticulocyte counts 75 and 68% of controls, hemoglobin levels 97% of controls in both groups, mean corpuscular hemoglobin was 102 and 108% of controls, and mean cell volume was 105 and 109% of controls, respectively. Changes in red blood cell parameters of 20,000 and 50,000 ppm males were consistent with mild macrocytic normochromic anemia with a depressed regenerative response. Mild leukocytosis was also observed in high-dose females, but this single difference was not considered biologically significant. Clinical chemistry parameters were not measured. In summary, the testis, kidney, and hematologic system were identified by the study authors as target organs for male rats, with a LOAEL of 1,700 mg/kg-day and a NOAEL of 900 mg/kg-day. A LOAEL for female rats was not identified.

Groups of 10 male and 10 female B6C3F1 mice were administered acetone in the drinking water at concentrations of 0, 1,250 (males only), 2,500, 5,000, 10,000, 20,000, or 50,000 (females only) ppm for 13 weeks (NTP, 1991; Dietz et al., 1991). Time-weighted average doses for males were 0, 380, 600, 1,400, 2,300, and 4,900 mg/kg-day, respectively, and for females 0, 900, 2,000, 4,200, 5,900, and 11,000 mg/kg-day, respectively. No deaths occurred and no clinical signs of toxicity were observed in any group. Water consumption was not affected in males; however, dose-related decreases in water consumption were seen in all treated females. Body weight and growth of the treated animals were not affected in either sex. Hematology parameters, sperm morphology, and vaginal cytology were not affected by acetone treatment. Organ weights from the treated males were similar to the controls. However, statistically significant ($p \leq 0.01$ or 0.05) increases in high-dose female absolute and relative liver weights were 113 and 110% of controls, respectively. Statistically significant ($p \leq 0.05$) decreases in absolute and relative spleen weights were 89 and 88% of controls, respectively. The only microscopic lesion seen in mice was centrilobular hepatocellular hypertrophy, observed in two high-dose females and considered due to enzyme induction. Mild hepatic changes were observed in males exposed to ≥20,000 ppm for 14 days (see above) but did not persist after 13 weeks of exposure, suggesting the development of tolerance toward acetone. In summary, the liver was identified as the target organ in male and female mice. The reference to this effect as an adverse effect is uncertain because the morphological changes may reflect induction of enzymes rather than an untoward effect on the liver. Effects that were noted in the rat, particularly males, were not evident with the mice. The LOAELs for males and females were 4,900 and 11,000 mg/kg-day, respectively, and the NOAELs were 2,300 and 5,900 mg/kg-day, respectively. It should be noted that the LOAEL for male mice was selected by the study authors on the basis of the transient findings in the 14-day study.

Groups of 30 male and female Sprague-Dawley rats were administered acetone by oral gavage at doses of 0, 100, 500, or 2,500 mg/kg-day for 90 days; 10 animals/sex/group were designated for interim sacrifice at 46-47 days (American Biogenics Corp., 1986). Survival, body weights, food consumption, ophthalmology examinations, and gross necropsy findings were similar between the treated and control groups. Clear salivation was observed between day 27 and study termination in a total of 21 males and 24 females at the high dose. Red blood cell parameters (hemoglobin, hematocrit, mean cell volume, and/or mean cell hemoglobin) in the high-dose groups increased in a statistically significant ($p \leq 0.01$ or 0.05) manner for males at interim sacrifice and for males and females at final sacrifice. However, the study author did not consider the magnitude of the increases to be biologically significant. One animal in the control group, one in the low dose group, and four in the high dose group died prematurely; the deaths

were attributed to dosing errors. Differences in clinical chemistry parameters were not dose-related and were not consistent over time or between sexes. Statistically significant ($p \leq 0.01$ or 0.05) increases in the absolute and/or relative liver and kidney weights were observed in the mid-dose females and in the high-dose males and females when compared with their respective controls. Relative (to brain and/or body weights) liver and kidney weights of the high-dose males were 111-117% of the controls. Absolute kidney weights of mid-dose females were 110-112% of controls and absolute and relative kidney weights of the high-dose females were 114-118% and 111-123%, respectively, of control levels. Absolute and relative liver weights of mid-dose females were 115 and 113%, respectively, and of high-dose females were 121 and 115-125%, respectively, of the controls. Although nephropathy incidence rates were similar between the treated and control groups, an increase in the severity of tubular degeneration of the kidneys in mid- and high-dose males and females, and hyaline droplet accumulation in mid- and high-dose males was observed. Statistical comparisons were not conducted for the increased severity of the kidney effect. However, the nephropathy exhibited a dose-response with respect to the numbers of animals affected. The numbers of male rats exhibiting tubular degeneration characterized as mild or moderate (in comparison with minimal) were 0, 1, 9, and 17 out of 30 animals for the 0, 100, 500, and 2,500 mg/kg-day group, respectively. Based on organ weight changes and kidney lesions in males and females, the LOAEL for this study is 500 mg/kg-day and the NOAEL is 100 mg/kg-day.

4.2.1.2. *Inhalation Studies*

Male Sprague-Dawley rats were exposed to 19,000 ppm (45,106 mg/m^3) acetone for 3 hours/day, 5 days/week, for 8 weeks (Bruckner and Peterson, 1981a). Although body weight gains of the treated animals were slightly less than air-exposed controls, statistical significance was not reached at any time. There were statistically significant ($p \leq 0.01$) decreases in the kidney weights of treated animals when compared with the controls after 4 weeks of exposure, but which returned to control levels after 8 weeks. Serum SGOT activities were not markedly altered in treated animals at weeks 2, 4, and 8; however, LDH activity, and BUN and liver triglyceride levels were not affected during the study. No microscopic lesions were observed in the liver, brain, heart, and kidneys of the acetone-exposed animals. Females were not included and no other concentrations of acetone were tested.

4.2.2. Chronic Studies

No studies on the chronic toxicity of acetone to laboratory animals are available.

4.2.3. Cancer Studies

No studies are available on the carcinogenicity of acetone to animals. Acetone has been extensively used as a vehicle to dissolve test chemicals in dermal studies in mice (NTP, 1991, 1995, 1997; Ward et al., 1986; Zakova et al., 1985). Generally mice received one to two applications per week for up to two years without an increase in neoplasia or any other toxic response. However, no studies contained a naive control to provide a basis for comparison.

4.3. REPRODUCTIVE AND DEVELOPMENTAL STUDIES

4.3.1. Reproductive Studies

4.3.1.1. *Oral Studies*

Ten male rats (Mol/Wis., SPF) were administered 0.5% acetone (5,000 ppm) in the drinking water for six weeks and then bred to untreated females of the same strain (Larsen et al., 1991). An additional group of 10 males was treated for six weeks followed by a 10-week recovery period prior to breeding. Doses to the animals were not calculated. No effects were seen in either group for number of pregnancies, number of fetuses/litter, testis weights, or testis histopathology. However, when combined with 0.13-0.5% 2,5-hexanedione (2,5-HD), acetone potentiated the testicular atrophy induced by 2,5-HD. The single concentration used in this study prevents identification of a LOAEL or NOAEL.

In a previously discussed study, groups of 10 male rats were administered 0, 2,500, 5,000, 10,000, 20,000, or 50,000 ppm acetone in the drinking water for 13 weeks (NTP, 1991; Dietz et al., 1991 [see Section 4.2.1.1]). Data for testicular effects were given only for the 0, 2,500, 10,000, and 50,000 ppm groups. The high-dose group (3,400 mg/kg-day) had: increased relative testis weights (119% of controls), decreased caudal and right epididymal weights (71-80% of controls, $P<0.05$ for both), depressed sperm motility (66.8% vs. 75.7% for controls, $P<0.05$), and an increased incidence of abnormal sperm (3.42% vs. 0.68% for controls, $P<0.05$). Data on the relative testis weights are difficult to interpret. Normally, reproductive toxicants generate a decrease in testis weights. However, the change in relative weights of the testis may be a result of overall decrease in body weight. There were no dose-related statistically

significant differences in absolute testis weight. The NTP (1991) study authors state that the decrease in sperm motility at the highest dose was consistent with mild reproductive effects. These effects
are characterized as consistent with mild toxic effects on spermatogenesis. The authors also noted that there were no morphological or histological effects seen microscopically.

Data on the toxicity of acetone on male reproductive organs suggest that at high doses there is a mild testicular effect, as indicated by diminished sperm motility and malformed sperm. Larsen et al. (1991) indicate that at drinking water doses of 5,000 ppm there is no effect on male reproductive capacity. At comparable doses in the NTP (1991) and Dietz et al., (1991) studies, there were no detectable reproductive effects. It is unknown if the reduced sperm motility and higher percentage with malformations noted at 50,000 ppm translates into impaired reproductive ability.

4.3.1.2. *Inhalation Studies*

Reproductive toxicity studies following the inhalation route are not available.

4.3.2. Developmental Studies

4.3.2.1. *Oral Studies*

Developmental toxicity studies following the oral route of exposure are not available.

4.3.2.2. *Inhalation Studies*

Presumed pregnant Sprague-Dawley rats (26-29/group) were exposed to 0, 440, 2,200, or 11,000 ppm acetone for 6 hours/day on gestation days 6-19 (Mast et al., 1988). Maternal body weights of the high-concentration dams were statistically significantly ($p \leq 0.05$) less than controls on gestation days 14, 17, and 20 and cumulative weight gain was reduced from gestation days 14 until termination. No clinical signs of toxicity or maternal deaths were observed. Treatment did not affect maternal liver and kidney weights, number of implantations, mean percent of live pups/litter, mean percent of resorptions/litter, and fetal sex ratios (Table 2). Statistically significant ($p \leq 0.05$; 84%-86% of control) reductions in fetal body weights from the high-concentration group were observed when compared with controls (Table 3). Since the study does not report food consumption, the reduction in maternal and fetal weight as a function of reduced food consumption is not known. The incidence of fetal malformations was not

statistically significantly increased in any exposed group. However, the percent of litters containing at least one pup with a malformation was 11.5% in the 11,000-ppm group (3/26 litters) compared with 3.8% for the control group (1/26 litters). Fetal malformations occurring at a single incidence in the high-concentration group varied and included cleft sternum, ectopic heart, major vessel malformation, edema, arrhinia, and microstomia; in addition, two fetuses from one litter had a missing tail. Although there are several types of developmental effects noted, there is no effect that predominates. Maternal plasma acetone levels measured 30 minutes postexposure were increased: by 17 hours postexposure, acetone levels in the dams exposed to 440 and 2,200 ppm were similar to control levels, but remained elevated in the dams exposed to 11,000 ppm.

Table 2. Rat body, uterine, and extragestational weight gain of pregnant dams

Parameter	**Acetone Concentration (ppm)**			
	0 ppm	**440 ppm**	**2,200 ppm**	**11,000 ppm**
Number of Dams	26	27	29	26
Gestational Day	**Maternal Weight (grams)**			
0	272.6±17.7	273.6±15.7	273.1±18.6	274.5±19.9
6	295.4±22.3	290.5±28.5	298.5±19.2	299.0±23.9
10	312.5±18.8	311.2±20.0	314.7±21.1	304.4±24.9
14	337.6±19.8	333.8±21.5	334.8±22.1	322.1±28.9
17	362.8±21.5	362.0±23.8	359.8±24.5	348.7±26.1
20	401.2±29.5	398.5±27.9	390.8±28.1	371.3±29.1*
Uterine Weight (grams)	83.2±19.0	79.7±14.5	74.5±16.3	67.1±13.9*
Extra-gestational Weight Gain (grams)	45.3±16.7	45.1±13.2	43.2±15.2	29.7±14.4*

*P<0.05

Table 3. Average fetal weights in rats

Parameter	Acetone Concentration (ppm)			
	0 ppm	**440 ppm**	**2,200 ppm**	**11,000 ppm**
Litters Examined	26	27	29	26
Fetal Weight (M&F)	3.6±0.4	3.7±0.4	3.5±0.3	3.1±0.3*
Male Weight (grams)	3.7±0.4	3.8±0.2	3.6±0.3	3.1±0.3*
Female Weight (grams)	3.5±0.3	3.6±0.2	3.4±0.3	3.0±0.3*
Percent Male Fetuses	51.6±13.7	49.9±14.4	51.9±15.5	49.5±13.2

* $P<0.05$

Presumed pregnant Swiss (CD-1) mice (26-31/group) were exposed to 0, 440, 2,200, or 6,600 ppm acetone for 6 hours/day on gestation days 6-17 (Mast et al., 1988). No clinical signs of toxicity, maternal deaths, or effects on maternal body weights were observed (Table 4). Fetal weights in the 6,600 ppm exposure group were about 8% lower than controls (1.3 g±0.1 and 1.2 g ±0.1, for control and 6,600 ppm groups, respectively, $p \leq 0.05$) (Table 5). Statistically significant increases in the absolute and relative liver weights of the 6,600 ppm group were observed when compared with the controls, which may be indicative of enzyme induction. Maternal kidney weights, number of implantations, mean percent of live pups/litter, percent of total intrauterine deaths, and fetal sex ratios were not affected by treatment. A statistically significant increase in the percent of late resorptions/litter was observed in the high-concentration group as compared with the controls (7.8% vs. 3.2%, respectively). However, this increase was not sufficient to result in a decrease in the number of live fetuses/litter. The incidence of fetal malformations and variations in mice was not affected by exposure at any level with the exception of a statistically significant increase in the percent of fetuses (on a litter basis) with reduced ossification of the sternebrae in the high dose group (1.5 % ±4.0 and 9.9 % ±17.6 for controls and 6,600 ppm groups, respectively) (Table 6). The authors stated that this effect might not be biologically significant since the incidence was <10%. No differences in the percent of live fetuses per litter with at least one malformation were seen between the control group (28.2% ±28.6) and the 6,600 ppm group (25.4% ±27.7) when all malformations were combined.

Table 4. Mean body, uterine, and extragestational body weight gain of pregnant mice

Parameter	Acetone Concentration (ppm)			
	0 ppm	**440 ppm**	**2,200 ppm**	**6,600 ppm**
Number of Dams	26	28	29	31
Gestation Day	**Maternal Body Weight (grams)**			
0	27.7±2.6	27.3±2.7	27.5±3.3	27.5±2.5
6	29.9±2.6	29.7±2.7	30.0±3.8	30.0±2.7
9	31.5±2.7	illegible	32.2±3.5	32.2±2.7
12	37.1±3.1	illegible	37.5±4.1	37.6±3.3
15	44.6±3.9	44.7±4.2	45.1±5.0	45.5±3.7
18	52.5±5.0	53.0±5.9	52.1±8.4	53.0±6.6
Uterine Weight (grams)	18.7±3.4	18.4±3.8	17.9±3.5	17.6±2.7
Extra-gestational Weight Gain (grams)	6.1±2.1	7.3±2.8	6.7±4.9	7.7±3.0

Table 5. Average fetal mouse weight and percent male fetuses

Parameter	Acetone Concentration (ppm)			
	0 ppm	**440 ppm**	**2,200 ppm**	**6,600 ppm**
Litters Examined	26	28	29	31
Fetuses Examined	292	307	318	344
Fetal Weight (grams)	1.3±0.1	1.4±0.1	1.3±0.1	1.2±0.1*
Male Fetuses (grams)	1.4±0.1	1.4±0.1	1.3±0.1	1.2±0.1*
Female Fetuses (grams)	1.3±0.1	1.3±0.1	1.3±0.1	1.2±0.1*

* $P<0.05$

Table 6. Mean percent of live fetal mice per litter with malformations and variations

Parameters	Acetone Concentrations (ppm)			
	0 ppm	440 ppm	2,200 ppm	6,600 ppm
Malformation				
Folded Retina	0.6±3.3	0	0	0.5±3.0
Exencephaly	0	0	0	0.4±2.2
Limb Flexure	1.3±3.9	0.6±3.4	0.5±2.7	0.2±1.4
Fused Ribs	0	0	0.3±1.9	0
Fused Sternebrae	0	0	0.3±1.5	0
Kinked Tail	0	0.3±1.5	0	0
Percent Fetuses/Litter with ≥1 malformation	1.6±4.1	0.9±3.7	1.1±3.5	0.9±2.9
Other Variations				
Supernumerary Ribs	23.3±27.3	29.5±28.0	20.7±21.8	12.9±17.1
Misaligned Sternebrae	5.1±10.0	8.2±12.2	5.0±7.7	9.4±14.5
Extra Sternebral Ossification Sites	0.4±2.0	3.2±16.8	0.2±1.3	0
Dilated Ureter	0	0	0	1.2±4.6
Reduced Sternebral Ossifications	1.5±4.0	2.4±4.9	2.7±6.6	9.9±17.6*
% Fetuses/Litter with ≥1 Variation or Reduced Ossification	28.2±28.6	38.1±29.4	26.5±24.3	25.4±27.7

* $P<0.05$

4.4. OTHER STUDIES

4.4.1. Neurotoxicity Studies

4.4.1.1. *Oral Studies*

Ladefoged et al. (1989) compared the effects of exposure to ethanol, acetone, and 2,5-hexanedione alone or in combination for six weeks. From the third week on, mice were monitored for nerve conduction velocity and rotarod performance. No effects on nerve conduction velocity or on balance time with the rotarod test were observed in male Wistar rats administered 0.5% acetone in the drinking water for six weeks. Peripheral distal axonopathy

was not observed in Sprague-Dawley rats (sex not specified) given 0.5% acetone for eight weeks followed by 1% acetone for four weeks in the drinking water (Spencer et al., 1978). While water consumption was reported in graphical form by Ladefoged et al. (1989), it was not reported by Spencer et al. (1978) and the animal doses are unknown.

4.4.1.2. *Inhalation Studies*

In a subchronic schedule-controlled operant behavior (SCOB) study-rats were trained to press a lever to obtain food in standard behavioral test chambers (CMA, 1997). Operant sessions were conducted 5 days/week for 9 weeks prior to acetone exposures. The operant set included fixed ratio response rate, fixed ratio pause duration, fixed interval response rate, and fixed interval index of curvature. Forty male Crl:CDBR rats (10 per group) were exposed to concentrations of 0, 1,000, 2,000 or 4,000 ppm acetone for 6 hours/day, 5 days/week for 13 weeks. Of the four measures, only the mean fixed ratio pause duration demonstrated a response at the two higher levels, starting with week eight and continuing through weeks 13 and 15 for the two highest levels, respectively. The NOAEL and LOAEL for the mean fixed ratio pause duration measure are 1,000 and 2,000 ppm, respectively. The author stated that the differences observed were the result of unusual performance by the control group and were not treatment-related. The control group exhibited an unexplained increase in the pause duration between week seven and the end of the study. The other SCOB measures failed to demonstrate exposure-related effects.

Acetone was evaluated for effects on a delayed match-to-sample task in male juvenile baboons (Geller et al., 1979b). Animals were continuously exposed in inhalation chambers to 500 ppm for 7 days. Even though accuracy was not affected by exposure, two of four animals showed marked changes in the number of extra responses made during exposure compared with their own baseline; for one animal, extra responses increased at all test times, whereas for the other, extra responses increased for the first two days of exposure and consistently decreased thereafter.

Male rats were exposed by inhalation to 19,000 or 25,300 ppm acetone for 3 hours and subjected to a series of performance/reflex tests both during and after exposure. Concentration-dependent central nervous system depression was evident during exposure as measured by a battery of simple tests of unconditioned performance and reflexes. Recovery was apparent 9 hours after exposure to 19,000 ppm and 21 hours after exposure to 25,300 ppm (Bruckner and Peterson, 1981b).

Female CFE rats were exposed by inhalation to 3,000, 6,000, 12,000, or 16,000 ppm acetone for 4 hours/day, 5 days/week, for 10 exposures (Goldberg et al., 1964). Growth rate was not affected during the study. The two highest concentrations produced ataxia in several animals after the first exposure, however, tolerance developed and this was not observed on subsequent days. Avoidance behavior was inhibited at concentrations of 6,000 ppm and higher.

Male Sprague-Dawley rats, trained on a fixed ratio-fixed interval (FR-FI) schedule of reinforcement, were exposed to 150 ppm acetone for 0.5, 1, 2, or 4 hours. No effects were observed during the 0.5-hour exposure. Both FR and FI rates increased during the 1-hour exposure and decreased during the 2-hour exposure as compared with controls. During the 4-hour exposure, FI responses approximated control levels for two rats and were above the control level for the third animal, whereas FR rates were below controls for two of the three animals (Geller et al., 1979a).

4.4.2. Isopropanol Studies

Considering that isopropanol is metabolized mainly to acetone (Brugnone et al., 1983; Slauter et al., 1994; Nordmann et al., 1973), toxicity studies on isopropanol may provide additional information on the toxicity of acetone. The kinetics of acetone formation from isopropanol varies among the available studies and depends on the species and concentration of parent compound. The study descriptions for isopropanol, as indicated below, are not intended to be an exhaustive review of the scientific literature but may inform toxicological issues raised by data limitations for acetone.

4.4.2.1. *Oral Studies*

Bevan et al. (1995) conducted a two-generation reproduction toxicity study on rats with isopropanol. Thirty male and thirty female Sprague-Dawley rats (designated as the P1 generation) were dosed by oral gavage with 0, 100, 500, and 1,000 mg/kg-day for at least 10 weeks prior to mating. The females were dosed during mating, gestation and lactation while males were dosed during mating and delivery. The second generation parental (P2) were selected from the offspring of the first generation (F1) and were dosed for 10-13 weeks before mating to produce a single litter.

Findings in the P1 group include increased liver weight in the P1 males and females, and P2 females. The authors reported that no adverse effects of treatment were evident from the

gross postmortem examination. Histopathology revealed kidney effects in the mid- and high-dose P1 males and in all treated groups of the P2 male rats. The effects were characterized by an increased number of hyaline droplets in the epithelial cells of the proximal convoluted tubules, increased incidence and severity of epithelial degeneration and hyperplasia, increased incidence of proteinaceous casts in the renal tubules, and increased mononuclear infiltration. With the exception of six male rats (out of 26) that exhibited centrilobular hypertrophy of the liver, there were no other treatment-related microscopic changes observed in the reproductive organs, liver, or kidneys, nor were gross abnormalities evident.

The authors reported that the F1 and F2 offspring that survived to scheduled termination had no treatment-related abnormalities. There was a statistically significant decrease in survival of the F1 offspring in the high dose group from postnatal day 0-4 for the high dose group and on postnatal day 4 only for the 500 mg/kg-day group. A statistically significant decrease in survival index was also seen in the F2 offspring in the mid and high dose groups on postnatal days 1 and 7. Statistically significant decreases in F1 male body weights from the high dose group were found, when compared with controls on PND 0 and 1 and in male and female offspring in the F2 generation at PND 0, 1 and 4 compared with the controls. The lower fetal body weight may reflect the larger litter sizes seen with the treatment (F1 12.4 pups/litter for controls vs. 14.4 in the high dose group; F2 13.2 vs. 14.4 in the controls and high dose group, respectively). There was an increased incidence of postweaning mortality in the high dose group of the F1 generation which coincided with the onset of gavaging with isopropanol. The authors speculate that this may be due to an immature metabolic system that is incapable of effectively metabolizing isopropanol.

The only reproductive parameter that appeared to be affected by isopropanol treatment was a statistically significant decrease in male mating index in the P2 high dose group (73.1% vs. 93.3% for controls $P<0.05$). This is "slightly" below the historical control values for P2 male mating index (range 80-96.6%, mean 91%) (study authors characterization). In interpreting this study the authors questioned the significance of this finding: they noted no similar effect in the P1 generation, no treatment-related effect in the P2 female fecundity index or fertility index, the absence of adverse effects on litter size, and the lack of histopathological findings in the male reproductive organs. The authors also note that all of the females that mated became pregnant.

Tyl et al. (1994) conducted developmental studies on rats and rabbits. In the rat study, 25 dams were dosed by gavage at levels of 0, 400, 800, and 1,200 mg/kg-day from gestation days 6 through 15. One dam in the mid-dose and two dams in the high-dose group died during

treatment. Maternal body weights were statistically equivalent across treatments but maternal weight change was statistically reduced at the high dose group for the gestational period PND 0-20 (141.8 vs. 157.8 for controls). The authors speculate that the difference may be due to the reduced uterine weight. The fetal weights were reduced at the mid- (97.3, 94.7, and 94.3%) and high- (92.1, 91.9, and 95.4%) dose group for all fetuses, male fetuses, and female fetuses, respectively. There were no late fetal deaths observed in this study and there was no evidence of treatment-related external, visceral, skeletal, or organ malformations or variations in the offspring. The study authors reported that isopropanol, when administered by gavage to rats, is maternally toxic at 800 and 1,200 mg/kg-day including lethality. The study authors note that there is no evidence of teratogenicity at doses that are maternally toxic by the gavage route of administration.

New Zealand white rabbits (15 per group) were dosed by gavage at doses of 0, 120, 240, and 480 mg/kg-day from gestation day 6 through 18. Four does in the high dose group died during the dosing period following dosing. The mortality was considered treatment-related. Mean maternal body weight was lower in the high-dose group compared with those in the other groups from the onset of dosing through delivery. A statistically significant reduction in maternal body weight change was observed in the high dose group compared with other groups. This coincided with lower food consumption in that group. The study authors reported that dams in the high dose group exhibited flushed or warm ears early in the dosing. They noted that this is indicative of typical adult mammalian alcohol intoxication.

There was a statistically significant reduction in mean fetal body weight per litter in the high dose group for all fetuses (93.5%), male fetuses (92.7%), and female fetuses (86.0%) compared with controls. There were no other statistically significant, treatment-related effects on any gestational parameters. There were no treatment-related changes in the incidence of external, visceral, skeletal, or total malformations or variations in any dose group. The authors established maternal and developmental NOAELs of 240 and 480 mg/kg-day, respectively, for the rabbits. Overall, the authors concluded that the only expression of toxicity is the reduction of fetal weight and there were no signs of teratogenicity in either rats or rabbits. The study authors concluded that isopropanol was not teratogenic.

Bates et al. (1994) conducted a developmental neurotoxicity study on Sprague-Dawley rats. Four groups of 64 mated dams were administered isopropanol by gavage at doses of 0, 200, 700, and 1,200 mg/kg-day from gestation day 6 through postnatal day (PND) 21. On PND 4 the litters were culled to 4:4 sex ratio, when possible, or 5:3. One male and female set of pups from

each litter was tested for motor activity using a figure-eight maze (PND 13, 17, 21, 47 and 58); a second set was tested for auditory startle (PND 22 and 60); and a third set for learning and memory in an active avoidance test (5 days at PND 60-64). One dam in the high dose group died on PND 15. No other exposure-related clinical signs were observed in the dams. There were no treatment-related effects on maternal or pup weight, sex ratio, pup developmental landmarks or pup survival. None of the neurodevelopmental assays revealed any treatment-related developmental effects in the offspring. The total motor activity increased to a maximum on day 47 for all groups (including the control) which decreased for all groups in day 58 compared with day 47. The study authors state that this is a normal occurrence and, therefore, does not constitute a treatment-related effect. Maximum startle amplitude and latency to maximum amplitude were unaffected by isopropanol treatment. Similarly, examination of learning and memory performance, as indicated by the active avoidance test, revealed no treatment-related effects. The pathology assessment of the brains revealed no treatment related abnormality in either the brain weights or histology. The authors note that a consistent finding in both the developmental and neurodevelopmental studies (both studies were conducted at the same facility) was the almost complete lack of clinical signs in the pups even at maternally toxic (lethal) doses.

4.4.2.2. *Inhalation Studies*

Burleigh-Flayer et al. (1994) conducted an inhalation neurotoxicity study on CD-1 mice and Fischer rats. Five groups of rats or mice (10/sex) were exposed to isopropanol at concentrations of 0, 100, 500, 1,500 and 5,000 ppm for 6 hours/day, 5 days/week for 13 weeks. The study authors report that during exposure, some animals demonstrated narcosis, ataxia, and hypoactivity. This effect was not seen in rats following the first week of the study. Female rats demonstrated a 57% increase in motor activity in the 5,000 ppm group at weeks nine and 13. Otherwise, there were no changes in any of the parameters of the functional observational battery. No effects were seen with males. The authors report an increase in body weight and body weight gain in female rats in the 5000 ppm group which corresponded with increased food and/or water consumption.

In addition to neurotoxicity, the authors conducted hematological and histological examinations of the animals. Hematological effects in male and female rats in the form of reduced erythrocyte counts that were observed at six weeks in the high-exposure group were no longer present at 13 weeks. In female mice at the high exposure, similar blood effects were evident at 13 weeks. No blood effects were evident in male mice. The only organs affected at

the end of the 13-week exposure were the liver and kidney. The liver weights of male and female rats were increased 5 and 8% in the high exposure group, respectively, compared with controls. Ar necropsy, there were no gross lesions determined to be exposure related. The only microscopic change observed was hyaline droplets within the kidneys of all male rats (including controls). The size and frequency of the hyaline droplets were increased for the isopropanol exposure groups compared to the control group. These differences were not clearly concentration-related, although the changes were most pronounced in the high dose group. Neuropathologic examination revealed no exposure-related lesions in the central or peripheral nervous system of exposed rats. Thus, neurological effects were noted at the highest dose and kidney changes were noted at all doses.

In a subchronic study, Burleigh-Flayer et al. (1998) conducted neurotoxicity evaluations on CD-1 mice and Fisher F344 rats. The animals were exposed to concentrations of 0, 100, 500, 1,500, or 5,000 ppm of isopropanol for 6 hours/day and 5 days/week for 13 weeks. The study authors reported ataxia, narcosis, hypoactivity, and/or a lack of a startle response following exposure in some rats or mice in the 5,000 ppm group and in some mice in the 1,500 ppm group. In the functional observational battery, none of the males exhibited statistically significant effects compared with the control group while motor activity increased in the female group following either 9- or 13-week exposures in the 5,000 ppm group. In a follow-up study, two groups of 30 female Sprague-Dawley rats were exposed to concentrations of either 0 or 5,000 ppm of isopropanol. The two groups were divided into either 9- or 13-week sessions, and spontaneous motor activity was assessed after 4, 7, 9, 11, and 13 weeks of exposure.
A statistically significant increase in motor activity was observed after 4 weeks of exposure compared with the control group. The increased activity was not evident 2 days after cessation of the 9-week exposure or 2 weeks after cessation of the 13-week exposure.

Nelson et al. (1988) conducted a developmental study on groups of 15 pregnant SD rats exposed to isopropanol at concentrations of 0, 3,500, 7,000, or 10,000 ppm for 7 hours/day on gestation days 1-19. The study authors report that at the two highest exposures, dams exhibited signs of narcosis, an unsteady gait after exposure, a statistically significant reduction in food consumption and reduced weight gain. With repeated exposure, the symptoms were less evident indicating adaptation to isopropanol. Animals at the lowest exposure exhibited no abnormal symptoms. Animals in the mid- and high-exposure group exhibited reduced food intake and reduced weight gain during the first two weeks of the study at 7,000 ppm and for the entire study at 10,000 ppm. Of the 15 dams in the high exposure group 6 animals were not pregnant, 4 had total resorptions of the fetuses, and the remaining 5 dams had an increased number of resorptions

compared with controls. Offspring in the 7,000 and 10,000 ppm group had an increased incidence of rudimentary cervical ribs. Overall, the incidence of litters with skeletal or visceral malformations were 4/15, 1/14, 12/13, and 5/5 for the 0, 3,500, 7,000, and 10,000 ppm groups, respectively. The malformations and variations seen at the higher concentrations in this study were reported at maternally-toxic exposures. The only concentration at which maternal toxicity was not observed was 3,500 ppm. At this exposure level, the only effect observed was a 3.8% decrease in fetal weight in females (3.00 g ±0.38 vs. 3.12 g ±0.29 for controls) and a 4.3% decrease in males (3.13 g ±0.36 vs. 3.27 g ±0.27 for controls).

4.4.3. Genotoxicity Studies

The genotoxicity of acetone has been well studied in *in vitro* assays, with the results almost entirely negative (ATSDR, 1994; OECD, 1998; U.S. EPA, 1988b; WHO, 1998). All studies cited in the GENE-TOX data base were negative, with the exception of one study for which no conclusion was drawn.

Neither sister chromatid exchange (SCE) nor chromosome aberrations were induced in Chinese hamster ovary cells by acetone at a concentration not exceeding 1% in the culture flask with or without metabolic activation (Loveday et al., 1990). Acetone was also negative for inducing sister chromatid exchanges in human (Tucker et al., 1993) and nonhuman (Latt et al., 1981) cell types in the absence of metabolic activation. Acetone did not induce chromosome aberrations *in vitro* (Preston et al., 1981).

Concentrations of acetone up to 0.6% did not change the background DNA synthesis rate, i.e., induce unscheduled DNA synthesis, in cultured human epithelial cells. Higher concentrations (up to 10%) inhibited background synthesis in a concentration-related manner (Lake et al., 1978). The chemical was negative for reverse mutations at concentrations up to 10 mg/plate in the Ames reversion test with five strains of *Salmonella typhimurium* in the presence or absence of a metabolic activation system (NTP, 1991; Kier et al., 1986; De Flora et al., 1984). Cell transformation was not seen in Syrian hamster embryo cells at acetone concentrations up to 8% (Heidelberger et al., 1983). Acetone was not mutagenic to *Arabidopsis* at concentrations up to 500 mM (Rédei, 1982). Male and female hamsters did not show an increase in micronuclei in polychromatic erythrocytes in the bone marrow following injection with 865 mg/kg (Basler, 1986).

In contrast to the above reports, acetone, at concentrations of 6.98-7.83%, produced aneuploidy in an inconsistent manner, but did not induce recombination or point mutation in *Saccharomyces cerevisiae*. However, overnight storage on ice of cells in growth medium containing acetone resulted in strong induction of aneuploidy (Zimmermann et al., 1985). The significance of this study is unknown.

As an α-ketoaldehyde, methylglyoxal, a potential metabolite of acetone, is chemically reactive in Schiff-base type reactions with amines or thiol groups. It has tested positive in some, but not all, genotoxicity studies including *S. typhimurium* and *E. coli* reverse mutation assays (Mitchell, 1993; Kranendonk et al., 1996), Chinese hamster lung cell HGPRT assays (Cajelli et al., 1987), and SCE induction assays (Tucker et al., 1989; Nishi et al., 1989; Migliore et al., 1990). It should be noted that no genotoxicity studies for acetone have included enzyme activation systems specific for the production of methylglyoxal.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION — ORAL AND INHALATION

Potential exposure to acetone occurs through normal endogenous production and from potential exogenous sources. Following the ingestion of food, glucose, starches, and other carbohydrates are used to generate essential cellular components, energy, glycogen, or fatty acids that may ultimately be stored as fat. Under normal conditions, the body uses glucose from consumed foods and glycogen from the liver to provide energy. In a study designed to measure the levels of acetone in the blood, urine and breath of occupationally-exposed and nonexposed workers, Wang et al. (1994) reported a mean value of 840 μg/L of acetone in the blood of the nonexposed cohort. Stewart et al. (1975) reported blood acetone concentrations in male subjects ranging from 0.73 to 1.32 mg% and in females ranging from 2.86 to 3.58 mg% prior to initial acetone exposure. Acetone blood concentrations in the range of 0.3 to 2.0 mg/100 ml are reported to be normal in healthy individuals (Physicians Desk Reference, 1976). However, under conditions of stress such as fasting and exertion, the metabolism of fat can result in higher levels of acetone in the body. In particular, the brain and reticulocytes, which are incapable of using fat as an energy source, rely on the regeneration of glucose, where acetone serves as an intermediate in the metabolic process, as the energy source. As such, acetone is a normal cellular constituent that the body is capable of metabolizing at low concentrations. Acetone also occurs endogenously at elevated levels under some disease conditions, most prominently with diabetes. The normal endogenous turnover rate (mg/kg-day) for acetone is not known.

Several studies in humans and animals suggest that acetone has a short persistence in the bloodstream. Following exposure, acetone is rapidly absorbed and distributed throughout the body and is rapidly lost. The ability to compensate for fluctuations in the concentration of acetone is a function of both the chemical's physicochemical properties and the body's biological capabilities, including: the absorption, metabolism, and excretion components of toxicokinetics. Therefore, the body appears capable, under normal circumstances, of controlling the concentration of acetone in the blood and tissues. At low concentrations, such as those generated endogenously under normal conditions, acetone is largely retained and serves as an intermediate in gluconeogenesis. Under high-level exposure conditions, excess acetone is eliminated. Acetone has a high $K_{B/A}$ (blood-air partition coefficient), thereby suggesting a high capacity for absorbing exogenous acetone vapors during inhalation while contributing to the retention of acetone in the bloodstream. However, under high levels of exposure only about 45% of the inhaled acetone is actually dissolved in the bloodstream, a level considerably lower than what is predicted based solely on the $K_{B/A}$. The limited number of studies on the absorption of acetone in humans via the gastrointestinal tract suggest that most ingested acetone is absorbed into the body (Haggard et al., 1944; Price and Rittenberg, 1950).

Metabolism affords another mechanism for compensating fluctuating acetone levels in the body. As proposed by Casazza et al. (1984) and based on several rodent studies, two possible pathways for the metabolism of acetone have been characterized. The methylglyoxal pathway leads to the formation of glucose and is mediated largely by enzymes from the hepatic microsomes. A second pathway appears to be extrahepatic and proceeds through the formation of 1,2-propanediol. Studies by Kosugi et al. (1986a,b) indicate that the methylglyoxal pathway predominates at low acetone concentrations and serves as a mechanism for gluconeogenesis. At higher concentrations acetone is also metabolized via the extrahepatic 1,2-propanediol pathway, which is believed to occur largely in the kidney. This pathway may contribute to the production of glucose, but could also be used as a means to readily eliminate acetone from the body.

A third mechanism that allows the body to adjust for elevated exposure to acetone is excretion. The fate of acetone in the body appears to be dose- or concentration-dependent. At low levels, human data indicate that acetone is mostly lost through metabolism to other cellular constituents, whereas at higher levels, nonmetabolized acetone is detected in the expired air (Wigaeus et al., 1981). Exposure to acetone at ambient air concentrations below 15 ppm produces no detectable acetone in the urine (Kawai et al., 1992), whereas at higher concentrations acetone is detected in urine at approximately 1% of the blood plasma concentration (Wang et al., 1994; Wigaeus et al., 1981). At significantly higher concentrations, some studies have found that the relative amount of acetone excreted increased relative to the

amount ingested, thereby increasing the rate at which acetone is lost from the body. As such, the body maintains feedback systems that both actively and passively control the amount of acetone taken up and retained.

Similarly, Reichard et al. (1979) working with fasting individuals, found that following intravenous administration of ^{14}C-acetone, the specific activity of ^{14}C-acetone decreased while the plasma concentration of acetone remained stable. Overall, the data indicate that excess acetone may be readily lost from the body.

Based on laboratory studies and human data, there are primarily five toxicological endpoints associated with exposure to acetone through inhalation or ingestion in humans and/or rodents, including: eye and respiratory irritation, nephrotoxicity, hematological effects, neurotoxicity, and male reproductive effects. Effects in humans via inhalation exposure have been studied and generally appear during exposure to concentrations of 500 ppm or above (Dick et al., 1988, 1989; Satoh et al., 1996; Mitran et al., 1997). Some of the effects are largely subjective, including: eye and nose irritation, mood swings and, at higher levels, nausea. The irritating effects of acetone may be due to its lipid solvent properties. Considering that the effects appear during exposure and dissipate following cessation of exposure indicates that they are caused by the parent material rather than a metabolite. Data on the neurotoxic effects of acetone are limited to inhalation exposure. The neurotoxic effects noted in humans are reported to be both mild and transient. Mitran et al. (1997) reported statistically significant reductions in nerve conduction velocity in workers. Animal data are generally consistent with the effects noted in humans. Other studies demonstrate transient impaired learning or response following inhalation exposure in rodents (CMA, 1997). This is consistent with the relatively nonpolar, lipophilic properties of acetone, which enhances its ability to cross the blood-brain barrier. As acetone is lost from the system, either by excretion or metabolism, irritation and other neurotoxic effects subside.

The most extensive animal studies conducted on acetone are drinking water and gavage studies. Both rats and mice in the Dietz et al. (1991) and NTP (1991) studies had higher levels of hemoglobin, lower red blood cell count (male rats only), and higher mean cell volume at the highest concentrations. The conditions are symptomatic of megaloblastic anemia. In humans, the condition is associated with folic acid and vitamin B_{12} deficiencies. In the 14-day study at the highest concentration (100,000 ppm) the study authors (Dietz et al., 1991) reported bone marrow hypoplasia which supports the concern that bone marrow could be a target organ for acetone at high doses. A second possible explanation is toxicity associated with depletion of GSH from elevated levels of methylglyoxal. Ankrah and Appiah-Opong (1999), in a mouse

drinking water study, found that perinatal exposure to methylglyoxal resulted in depletion of GSH stores in red blood cells and lower ability to refract oxidative stress.

Nephrotoxicity was noted in both the gavage and drinking water studies. In both cases the effects were mild; however, the effect also occurred at a lower dose and was more pronounced in the gavage study. Also in the gavage study, American Biogenics Corp. (1986) noted tubular degeneration, an effect that was not found in the drinking water study. A possible explanation is related to the metabolism of the chemical. At lower concentrations acetone is metabolized mainly through the hepatic pathway; at higher concentrations the extrahepatic pathway becomes more significant. Bolus administration of acetone in the gavage study can be expected to lead to an acute increase in the amount of acetone in the blood. At higher concentrations, acetone can be expected to reach target organs including the kidney, causing the noted effect at lower concentrations than that seen following administration of acetone via drinking water. This is consistent with the interpretation offered by the authors of the NTP (1991) study, who state that a possible mechanism of action for chronic progressive nephropathy is based on the effect of higher concentrations of protein in the serum resulting in higher blood flow and glomerular filtration rate, leading to the burdened glomeruli becoming sclerotic (NTP, 1991). This effect could be exacerbated by lower water consumption that was seen at the higher acetone concentrations.

Male reproductive effects were also identified in the rodent studies, including: testicular higher relative testis weight, reduced sperm motility, and more abnormal sperm at the higher doses compared with controls (NTP, 1991; Dietz, 1991). The study authors note that the data are difficult to interpret because testicular toxicants typically decrease testes weights and therefore, the relative increase in weight may be more indicative of the reduced body weights in the treated animal studies. Other statistically significant reproductive effects indicate that the effects are minimal. An increase in abnormal sperm from <1 to 3.5%, and decrease in sperm motility from 75.7 to 66.8% were seen at the highest doses. Although studies concerning the distribution of acetone indicate that radiolabeled material reaches the testis (Wigaeus, 1982), there is no evidence demonstrating that the radiolabel present in the testis is due to acetone or a metabolite. The one study reporting reproductive effects in humans is inconclusive (Jelnes, 1988). While there are no two-generation reproductive toxicity studies for acetone, data from isopropanol, which is metabolized primarily to acetone, may provide useful information. A two-generational gavage study (Bevan et al., 1995) in rats indicates that the only reproductive effect was a statistically significant reduction in the P2, but not P1, male mating index in the high dose group (1,000 mg/kg-day) which the study authors characterized as slightly below historical controls.

Data on developmental effects resulting from oral exposure to acetone are not available but may be informed by the results of an inhalation study on acetone and from oral exposure data on isopropanol. Inhalation studies by Mast et al. (1988) reported an increased incidence in skeletal malformations in rats and delayed skeletal development in mice at high exposures, although the types of malformations are inconsistent. The most consistent finding with exposure to acetone was decreased fetal weight at high exposure (86-92% of control at 6,600 ppm for mice, and 84-86% of control at 11,000 ppm for rats). The developmental effects of isopropanol have been studied by the oral route of exposure. Bevan et al. (1995) showed there was a decrease in the survival of F1 offspring at high doses of isopropanol (1,000 mg/kg-day), a decrease in body weight in high dose F1 male rats and F2 male and female rats, and no treatment-related abnormalities. Tyl et al. (1994) showed maternal toxicity at 800 and 1,200 mg/kg-day in rats and no evidence of developmental effects in offspring. The authors also reported decreases in fetal body weight at 480 mg/kg-day in rabbits with no other developmental effects noted. Gavage studies (Tyl et al., 1994) reported NOAELs of 400 and 480 mg/kg-day for rats and rabbits, respectively, based on reduced fetal weight. No effects were observed at doses up to 1,200 mg/kg-day on postimplantation loss, litter size, sex ratio, or external, visceral, or skeletal abnormalities for either model. Similarly, Bates et al. (1994) found no evidence of neurobehavioral or morphological abnormalities from the prenatal administration of isopropanol by gavage to pregnant rats.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

In accordance with the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999) *data are inadequate for an assessment of the human carcinogenic potential* of acetone. This weight-of-evidence determination is based on the availability of one human study of limited utility, no chronic animal studies, and no additional information on structural analogues with known carcinogenic potential. Acetone has tested negative in almost all genotoxicity studies. The previous IRIS entry included a weight of evidence classification of Group D - not classifiable as to human carcinogenicity - based on the Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1986a).

Acetone has a long history of industrial use as a solvent. To date there are no epidemiological studies demonstrating an association between exposure to acetone and increased risk of cancer. The only human study available is an epidemiological study of workers in a cellulose acetate plant where the workers were exposed to acetone concentrations of 380-1,070 ppm (time-weighted average) (Ott et al., 1983a,b). In this study, 948 workers served as the reference cohort for a comparison to workers exposed to a mixture of methylene chloride and

acetone. For the acetone-exposed workers, the total number of deaths observed from all causes was 24 and 3 for men and women, respectively, compared with the total expected of 53.8 for men and 6.7 for women. Among the acetone-exposed workers the incidence of "malignant neoplasms" was 5 and 2 compared with an expected incidence of 10 and 2.3 for men and women, respectively. Limitations of this study are discussed in Section 4.1.1. Although a chronic bioassay has not been conducted for oral or inhalation exposure routes, acetone has frequently been used as a solvent or vehicle control to dissolve test chemicals in dermal studies in animals (NTP, 1991, 1995, 1997) with no evidence of increased tumor incidence. Nevertheless, without a naive control the ability to determine the background incidence of cancer following these exposures is limited. Chronic and less than lifetime studies on methylglyoxal, a potential metabolite of acetone, reported no signs of cancer in rats, although both studies are limited by methodology and reporting (Fujita et al., 1986; Takahashi et al., 1989). In addition, a cancer bioassay (Burleigh-Flayer et al., 1997) in F344 rats for isopropanol (which is metabolized primarily to acetone) by the inhalation route of exposure indicated an increase in interstitial cell adenomas of the testes in male rats. The adenomas were found at an unusually low incidence in control rats in this study which may confound the results. There were no increased frequencies of neoplastic lesions noted for female rats of male or female mice in any exposure group.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

Little information is available on the potential for increased susceptibility to acetone exposure in children. Acute oral LD_{50} values for 14-day-old rats (16-50 g), young adult rats (80-160 g), and older adult rats (300-470 g) were 5.6, 9.1, and 8.5 ml/kg, respectively, indicating little age-related variability (Kimura et al., 1971).

There are no available developmental studies on acetone by the oral route of exposure. However, two-generational studies on isopropanol, which is mainly metabolized to acetone, may provide some information on the potential effects of acetone [Section 4.4.2.]. The administration of isopropanol caused a reduced survival index in both F1 and F2 generations in the high-dose groups and decreased fetal body weight in F1 males and F2 males and females through gestation day 4 (Bevan et al., 1995). Lehman et al. (1945) conducted a drinking water study on isopropanol at doses up to 2,500 ppm which also reported retarded weight in the F1 generation during the first week with the effect diminishing over time. The authors reported no other remarkable findings.

Developmental studies by the inhalation route of exposure indicate that reduced fetal body weight in high-dose exposure groups is the most consistent effect seen in rats and mice (Mast et al., 1988) [Section 4.3.2.2.].

No other studies are available which examine age-related differences in effects of acetone exposure. In addition, the endogenous production rate of acetone in children under normal (or any other) conditions is not known.

4.7.2. Possible Gender Differences

No studies indicating differences in the uptake or retention of acetone between men and women are available. In a measure of subjective effects, Dick et al. (1988, 1989) reported that of volunteers exposed to 250 ppm acetone vapor for 4 hours, only males exhibited a statistically significant reduction in the anger hostility scale of the profile of mood states. In animal studies, male rats exposed to 20,000 and 50,000 ppm acetone in drinking water exhibited an increase in severity of progressive nephropathy compared with controls. A similar effect was not seen in females (Dietz et al., 1991; NTP, 1991). In addition, male rats were more sensitive than female rats to hematological, hepatic and renal effects (Dietz et al., 1991; NTP, 1991, American Biogenics Corp., 1986).

4.7.3. Other Possible Susceptible Populations

The role of acetone in fasting individuals and diabetics is complicated and not well understood (Reichard et al., 1979, 1986; Owens et al., 1982). Acetone is produced endogenously; higher concentrations are produced during fasting due to an increase in fat catabolism, which can result in ketosis (Reichard et al., 1979). This implies that individuals on fasting diets who are also exposed to exogenous acetone will have a higher body burden of acetone than nondieters exposed to the same amount of exogenous acetone, perhaps making them more susceptible to any adverse effects. Individuals with impaired kidney function, most notably diabetics, may also constitute a susceptible population. Data from Dietz et al. (1991) and NTP (1991) indicate kidney effects in male rats in which members of all dosing groups, including the control group, exhibited mild progressive nephropathy. The possibility that acetone exposure may accelerate this condition cannot be ignored. In addition, diabetics may be more susceptible to acetone exposure due to the production of the methylglyoxal metabolite which may contribute to the formation of glycated reaction products that are a complication of long-term diabetes. Methylglyoxal has also been shown to affect glucose tolerance indicating potential concerns for individuals with diabetes (Ankrah and Appiah-Opong, 1999).

To the degree that acetone exposure may contribute to hematological or kidney effects, individuals on high protein/low carbohydrate diets as well as diabetics may also comprise a susceptible population due to an already elevated endogenous acetone level.

One of the most studied effects of acetone is the induction of microsomal enzymes, particularly CYP2E1. Acetone thereby induces its own metabolism, and potentiates the toxicity of numerous other chemicals (e.g., 1,1-dichloroethylene, 1,2-dichlorobenzene, carbon tetrachloride) that are metabolized by CYP2E1 by enhancing their metabolism to reactive intermediates (Morgott, 2001; Traiger and Plaa, 1974; Hewitt and Plaa, 1983; Brondeau et al., 1989). The induction of CYP2E1 by acetone poses a potential concern for humans exposed to acetone and those chemicals whose toxicity may be augmented by acetone.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

There are no human studies or chronic animal studies available for the derivation of an RfD. Two shorter duration studies are available, including a short-term exposure (14 days) study using 5 animals per dose group (NTP, 1991; Dietz et al., 1991) that is not considered suitable for the derivation of an RfD due to the acute nature of the dosing regimen. The principal study identified for derivation of the oral RfD is a subchronic drinking water study (NTP, 1991; Dietz et al., 1991). Acetone was administered in the drinking water (e.g., 0, 200, 400, 900, 1,700, and 3,400 mg/kg-day for male rats) of mice and rats for 13 weeks. Male rats appeared to be the most sensitive species, with the kidney, hematologic system, and testes identified as target organs. Enhanced progression of mild nephropathy and effects consistent with macrocytic normochromic anemia with a depressed regenerative response were found at a high-dose of 1,700 mg/kg-day. In addition, depressed sperm motility, caudal and epididymal weights, and an increased incidence of abnormal sperm occurred at 3,400 mg/kg-day. A LOAEL of 1,700 mg/kg-day and a NOAEL of 900 mg/kg-day were identified for mild nephropathy.

One additional 90-day study is available (American Biogenics Corp., 1986) in which rats were administered 0, 100, 500, or 2,500 mg/kg-day acetone by gavage. Organ weight changes and kidney lesions were identified at a dose of 500 mg/kg-day. While the gavage study included clinical chemistry analyses, the data failed to show dose-related effects consistent with the nephropathy noted in the histology, thereby raising questions about the significance of the effect. Differences in the observed effect level in the drinking water study versus the gavage study may

relate to the method of administration. As noted earlier, acetone is readily absorbed through the gastrointestinal tract (Section 3.1). Under conditions of short-term elevated exposure levels such as those produced in gavage or bolus experiments, more acetone appears to be shunted to the kidney, producing higher concentrations in the urine and higher rates of metabolism through the propanediol pathway compared with the more gradual administration through drinking water. This could account for differences in the nephropathy severity levels observed with drinking water compared with gavage administration. For this reason, the gavage study was not chosen as the principal study.

Mild nephropathy was chosen as the critical effect, and was seen in male rats only in the Dietz et al. (1991) and NTP (1991) study. The choice of critical effect is supported by the report of tubular degeneration of the kidneys in male and female rats and hyaline droplet accumulation in males at 500 and 2,500 mg/kg-day in the American Biogenics Corp. (1986) study. An oral gavage study on isopropanol (Bevan et al., 1995), which is metabolized primarily to acetone, also supports nephropathy as the critical effect. In this two-generation reproductive toxicity study, kidney effects (including an increased number of hyaline droplets in epithelial cells of the proximal tubules and an increase in severity of epithelial degeneration) were noted at 500 and 1000 mg/kg-day P1 male rats and also at 100 mg/kg-day in P2 male rats. Changes in hematological parameters (erythrocyte and leukocyte counts and hemoglobin levels) in male rats, but not mice, were noted in the Dietz et al. (1991) study. Red blood cell parameters only were significantly affected at 2,500 mg/kg-day in male rats in the American Biogenic Corp. (1986) study.

EPA has examined general issues related to hyaline droplet formation and concluded that a mode of action for renal pathology involving the formation of hyaline droplets containing α_{2u}-globulin is probably unique to male rats in certain instances and may not be relevant to human risk assessment if certain criteria can be met (U.S. EPA, 1991b). In brief, these criteria include: (1) an increase in the number and size of hyaline (protein) droplets in kidney proximal tubule cells of treated male rats; (2) immunohistochemical evidence of α_{2u}-globulin accumulating protein in the hyaline drops; and (3) histopathological evidence of kidney lesions associated with α_{2u}-globulin nephropathology. In the NTP (1991) and Dietz et al.(1991) study in F344 rats, the nephropathy is characterized by proximal tubule degeneration and intracytoplasmic droplets of granules (hyaline droplets) in the proximal tubular epithelium with the severity of these lesions showing a dose-related increase in males only. However, tubular degeneration of the kidneys in mid- and high-dose males and females, and hyaline droplet accumulation in mid- and high-dose males was observed in the American Biogenics (1986) study in SD rats. Furthermore, although immunohistochemical analysis of hyaline droplet protein has not been performed following

acetone exposure, hyaline droplet histochemistry following inhalation exposure to isopropanol, which is primarily metabolized to acetone, has been examined (Fowler and Martin, 1994). In this study kidney sections were examined by immunohistochemical staining with α_{2u}-globulin following nine day inhalation exposure in F344 rats to 0, 5,000 and 10,000 ppm isopropanol. There were no substantive differences between the exposure groups in intensity or distribution of α_{2u}-globulin. Appropriate studies to ascertain the issue of human relevance related to the observed kidney nephropathy of acetone have not been performed. Thus, the above criteria have not been met for acetone and the relevance of the observed kidney nephropathy remains unknown. The renal lesions seen in both the drinking water (NTP, 1991; Dietz et al., 1991) and gavage (American Biogenics, 1986) studies may represent an enhancement by acetone of the chronic progressive nephropathy commonly seen in aging rats (Baylis and Corman, 1998).

5.1.2. Methods of Analysis

The data were analyzed using the NOAEL/LOAEL approach. Based on the findings of NTP (1991) and Dietz et al. (1991), the critical effect is nephropathy. The LOAEL is 1,700 mg/kg-day (20,000 ppm dose group) and the NOAEL is 900 mg/kg-day (10,000 ppm dose group) based on an increased incidence of mild nephropathy. The values were calculated as time-weighted average doses based on body weights, water consumption, and nominal drinking water concentrations. The available PBPK models for oral exposure were not used for the derivation of the human equivalent dose because they have not been validated against human data.

Minimal or mild nephropathy was present in all groups. The authors characterized mild nephropathy as an adverse effect due to a progression of the condition with treatment. The incidence and severity of nephropathy are provided in Table 7. One out of ten animals in the control group was rated with a mild degree of nephropathy. No animals in groups dosed at intermediate levels of 10,000 ppm or lower were rated with mild nephropathy. In contrast, nine out of ten animals dosed at both 20,000 and 50,000 ppm were rated with mild nephropathy. This type of response is not amenable to benchmark dose modeling, since a graded dose-response curve is lacking (a response of 1/10 animals for controls compared with a response of 9/10 animals at the lowest response level) and the lowest response is much higher than what might be considered a low, e.g., 10%, benchmark response. For this reason, the NOAEL/LOAEL approach was used.

Table 7. Incidence and severity of nephropathy in F344 male rats following drinking water administration of acetone

Severity of Nephropathy	Control	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	50,000 ppm
Minimal	5/10	8/10	8/10	9/10	1/10	1/10
Mild	1/10	0/10	0/10	0/10	9/10	9/10

5.1.3. RfD Derivation - Including Application of Uncertainty Factors (UFs)

The NOAEL used for RfD derivation is 900 mg/kg-day. The following UFs are applied to the effect level: 10 for consideration of intraspecies variation (UF_H; human variability), 3 ($10^{1/2}$) for interspecies differences (UF_A; animal to human), 3 to account for extrapolation from subchronic studies to chronic exposure conditions (UFs; subchronic to chronic), and 10 to account for a deficient data base (UF_D). The total UF = $10 \times 10^{1/2} \times 10^{1/2} \times 10 = 1000$.

An UF of 10 was applied for intraspecies uncertainty to account for susceptible subpopulations. This factor accounts for humans who may be more susceptible to acetone exposure than the general population but for whom data are not available. This may include individuals who have elevated levels of endogenous acetone due to high-fat low-carbohydrate diets, fasting conditions, or uncontrolled diabetes. In addition, the production of glycated endproducts may be increased due to acetone exposure. These endproducts have been shown to be responsible for many of the complications associated with diabetes.

An UF of 3 was used to account for laboratory animal-to-human interspecies differences. This UF accounts for differences in the toxicokinetics and toxicodynamics between the model species and humans. The data indicate that the toxicokinetics in the rat and humans are similar and that both species eliminate acetone from the body efficiently (Haggard et al., 1944; Sakami, 1950; Sakami and Lafaye, 1951; Stewart et al., 1975; Reichard et al., 1979; Casazza et al., 1984; Wigaeus et al., 1981; Kosugi et al., 1986a; Wang et al., 1994). In both humans and rodents metabolism proceeds by a hepatic pathway at low concentrations and by an extrahepatic pathway followed by excretion at higher concentrations but qualitative toxicokinetic comparisons between rats and humans are not available (Haggard et al., 1944; Casazza et al., 1984; Wigaeus et al, 1981; Kosugi et al., 1986b; Gavino et al., 1987; Kawai et al., 1992). Thus, the toxicokinetic component of the UF for interspecies extrapolation is 3. The critical effects identified from the principal study are kidney-related (Dietz et al., 1991; NTP, 1991). Male rats given the two highest doses of acetone had increases in the incidence and severity of nephropathy; the severity rating increased from minimal at low doses to mild at high doses. It is not known with certainty whether the observed nephropathy is a result of male rat-specific

hyaline droplet formation due to α_{2u}-globulin accumulating protein (see Section 5.1.1). However, nephropathy was observed in all treatment groups including controls. The authors report that the effects are morphologically similar to the spontaneously occurring and long-term progression of nephropathy (chronic progressive nephropathy) found among aging rats. Acetone exposure may serve to enhance this effect. However, the nephropathy was increased only from a severity rating of "1, minimal" to "2, mild" on a scale of 1 to 5. For these reasons, the toxicodynamic component of the UF for interspecies extrapolation is 1 indicating that humans are not anticipated to be more susceptible than animals to the nephrotoxic effects of acetone exposure.

An UF of 3 was used to account for extrapolation from subchronic studies to chronic exposure conditions. The principal study is a subchronic study. No chronic studies are available. However, acetone is endogenously produced in the human body. Several reports note the presence of acetone in normal nonfasting individuals indicating that humans are routinely exposed to acetone (Stewart et al., 1975; Physicians Desk Reference, 1976; Wang et al., 1994). Toxicokinetic studies in both humans and animals indicate that acetone elimination occurs through excretion, exhalation and metabolism by various routes.

In addition, acetone does not accumulate in the body nor are its metabolites considered significantly toxic. Acetone is metabolized to acetol which in turn, is metabolized via two potential pathways to glucose. Intermediates include methylglyoxal, 1,2-propanediol, lactaldehyde, and lactate, none which have been demonstrated to be overtly toxic.

An UF of 10 was used to account for data base uncertainty. The available data base for acetone includes subchronic gavage and drinking water studies in mice and rats, including measurements of several reproductive parameters. There is one neurotoxicity study in rats which evaluated effects on nerve conduction velocity and rotarod performance and one reproductive toxicity study with a single dose regimen. The data base lacks a multigenerational study and adequate studies of the oral neurotoxicity, developmental and developmental neurotoxicity of acetone.

The only available neurotoxicity study in mice demonstrates no effects on nerve conductivity and rotarod performance. Nevertheless, human inhalation studies on acetone, while inadequate, indicate potential neurotoxic effects. This raises concern for neurodevelopmental effects from oral exposure because the nervous system undergoes developmental processes unique to early life stages.

There are no developmental toxicity studies for acetone by the oral route of exposure; however, information on this endpoint is possibly informed by inhalation studies and studies on isopropanol. Inhalation studies on acetone reported a slight increase in the incidence of skeletal malformations in rats, although the types of malformations did not demonstrate a consistent effect. The most consistent finding was decreased fetal weight that was not associated with any other observable adverse effect (Mast et al., 1988). Developmental toxicity studies on isopropanol following gavage administration to rats and rabbits indicated reduced fetal weight at doses of 800 and 1200 mg/kg-day but no other effects at any dose (Tyl et al., 1994). In addition, a two-generation gavage study on isopropanol in rats (Bevan et al., 1995) indicated a statistically significant reduction in the P2, but not P1, male mating index at 1,000 mg/kg-day that the study authors characterized as slightly below historical controls. On the other hand, a developmental neurotoxicity study (Bates et al., 1994) on isopropanol in rats indicated no toxicity at doses as high as 1,200 mg/kg-day. It is difficult to draw firm conclusions on the potential developmental effects from oral exposure to acetone based on inhalation studies following acetone exposure and oral studies using isopropanol. However, evaluated collectively, the data may indicate potential developmental and reproductive effects resulting from ingestion of acetone.

The RfD is based on a NOAEL which obviates the need for an UF due to LOAEL to NOAEL extrapolation.

A modifying factor was not needed.

$$RfD = NOAEL \div UF$$
$$900 \text{ mg/kg-day} \div 1000 = 0.9 \text{ mg/kg-day}$$

The RfD is applied to ingested acetone only and is in addition to the acetone formed endogenously by catabolism of fat. The turnover rate (mg/kg-day) for the endogenous production of acetone under normal conditions is not known. The RfD generated in this assessment differs from the previous RfD (0.1 mg/kg-day). This difference is accounted for, in part, by a change in the principal study. The previous RfD is based on the gavage study conducted by American Biogenics Corp. (1986). The administered doses were 0, 100, 500, or 2,500 mg/kg-day. The critical effect noted was kidney pathology, and the NOAEL was 100 mg/kg-day. The RfD invoked uncertainty values of 10 for intraspecies and interspecies extrapolation, and 10 for extrapolation from a subchronic to a chronic exposure scenario. Although the point of departure noted in the gavage study is lower, the drinking water route more closely mimics potential long-term human exposure scenarios and the study is considered more thorough.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

Several controlled and cohort studies from inhalation exposure to acetone are available. Dick et al. (1988) reported effects from short-term exposure to 250 ppm acetone including a mild statistically significant increase in dual task performance measurements and in the identification of false alarms during and immediately following exposure. Other neurological measurements demonstrated no treatment-related effects. In a cohort study of workers occupationally exposed to acetone at levels exceeding 500 ppm, Satoh et al. (1996) found an increase in reports of eye irritation and nausea compared with a nonexposed control cohort. The authors speculate that the reports may be the result of peak exposures during the course of the day. They found no differences between the exposed and nonexposed cohort for the Manifest Anxiety scale or the Self-rating Depression scale. The only reported neurological effect was a statistically significant decrease in simple reaction time and digit span among workers aged 30-44. No differences were reported in workers in younger and older age groups. The study authors questioned whether the differences in only one age group were chance findings. Kiesswetter et al. (1994) report in a study of workers exposed to acetone concentrations of 725 and 1,150 ppm during the morning and afternoon shifts, respectively, that the only category of well-being related to exposure of acetone was in the "annoyance" category.

In a cohort study, 71 factory workers with mean age and length of exposure of 36 and 14 years, respectively, were evaluated for both central and peripheral nervous system effects (Mitran et al., 1997). Exposure concentrations over an 8-hour shift ranged from 416 to 890 ppm acetone. Mood disorders, irritability, memory difficulties, sleep disturbances, headache, numbness of the
hands or feet, eye and/or nose irritation, bone, joint and/or muscle pain, nausea, and abdominal pain were reported slightly more frequently in exposed workers as compared with controls. The time during the work shift when the symptoms occurred or were reported was not stated. Although the results of motor nerve conduction tests on the median, ulnar and peroneal nerves indicated statistically significant reductions in latency, amplitude and/or duration of both proximal and distal responses, no consistent pattern of effect was observed. Statistically significant reductions in nerve conduction velocity in all nerves studied was reported in exposed workers as compared with controls. For the exposed workers, statistically significant delays in reaction time were observed for the visual test and a lower mean distributive attention score when compared with the controls.

The Mitran et al. (1997) study presents minimal study information which confounds a meaningful appraisal of the study design. This includes a lack of information regarding the selection of controls, parameters used for age-matching and other variables, experimental procedures, i.e., blind versus nonblind determinations, and temperature control. Age-matching and consistent temperature control are known critical parameters in nerve conduction velocity

measurements. Additional potential confounding issues associated with this study include no establishment of a dose-response relationship, and an inability to rule out coexposure to other toxins as the factory was a coin and metal plant where exposure to other toxins might be considered likely. Some of these issues are discussed in Boyes and Herr (2002). For these reasons, the study is not considered appropriate for the establishment of an RfC for acetone.

Stewart et al. (1975) exposed 20 adults of both sexes to acetone vapor concentrations of 0, 200, 1,000, 1,250 ppm or varying concentrations (males) and 0 or 1,000 ppm (females) for either 3½ or 7 hours for 4 days/week on successive weeks. One of four subjects exposed to 1,000 ppm and two of four subjects exposed to 1,250 ppm for 7 hours demonstrated a statistically significant effect on the visual evoked response. The effect was reported following either two or four successive days of exposure. The exposure regime indicates that the exposure duration at each concentration did not extend beyond 4 days for each dose with a 3 day interim period without dosing. While this study may be appropriate for establishing exposure limits for short-term exposure, it is not suitable for deriving a reference value for chronic exposure.

Mast et al. (1988) conducted developmental studies in rats and mice by the inhalation route of exposure. Statistically significant decreases in maternal weight and weight gain in rats were observed at the highest (11,000 ppm) exposure when compared with the controls. The effect was not observed in mice. The authors reported that there were no overt signs of developmental toxicity in either rats or mice. There were single incidences of fetal abnormalities in the high-exposure rats. Statistically significant changes in the incidence of fetal malformations in mice were not observed following exposure at any level with the exception of an increase in the percent of fetuses (on a litter basis) with reduced ossification of the sternebrae. The authors stated that this might not be biologically significant since the incidence was <10%. The offspring of rats and mice had a small, but statistically significant increase in late term resorptions compared with controls but the increase was not considered to be enough to account for a reduction in live fetuses. Similarly, there was a small but statistically significant decrease in fetal weight of offspring in both rats and mice as described by the study authors. The significance of the fetal body weight effect is questioned in light of the minimal severity of the effect, and the negative findings of other parameters including resorptions, number of live births, and number of births per litter, which were comparable to controls.

Overall, the most pronounced effect of acetone reported in human inhalation studies is irritation of the eyes and respiratory tract. Additionally, human data indicate that exposure to acetone may produce neurobehavioral effects. Studies that report responses over time note that the most pronounced effects occur during initial exposure and dissipate over time. Similarly,

animal data also indicate that neurological effects from less than lifetime inhalation exposure are mild and transient. Although the available data base may be sufficient to support concerns for short-term exposure (based largely on irritation) extrapolation to chronic exposure is not recommended. Available human and animal studies on acetone exposure provide insufficient information for the generation of an RfC. The available PBPK models are not amenable for route-to-route extrapolation from the oral route to the inhalation route of exposure since the oral exposure models have not been validated.

More research is needed on the toxicity of acetone following inhalation exposure. It should be noted that the previous IRIS assessment for acetone did not derive an RfC. Additional information can be found in Sections 4.1 and 4.2.1.2 of this review.

5.3. CANCER ASSESSMENT

Studies necessary to support a quantitative assessment of the carcinogenicity of acetone in humans are not available. One epidemiological study published in the literature considers the incidence of cancer in occupationally exposed individuals (Ott et al., 1983a,b; and as reviewed in ATSDR, 1994); however, this study has several limitations: acetone-exposed individuals served as the referent cohort and not the study cohort, the study was limited in size, and cancer was not the principal focus of the study. There are no available lifetime cancer studies on animals via either the inhalation or ingestion route. Acetone has been used as a vehicle for dissolving test chemicals for several dermal studies in animals with no observed tumor incidences (NTP 1991, 1995, 1997); however, in the absence of a naive control the data are not adequate to evaluate the potential for the induction of cancer. There are no cancer studies in which low molecular weight saturated ketones that are similar in structure to acetone are shown to be carcinogenic. *In vitro* genotoxicity studies with acetone are almost uniformly negative.

Neither IARC nor NTP have a human cancer classification for acetone.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HAZARD POTENTIAL

Acetone is used industrially as a solvent and feedstock; biologically it serves a role in human metabolism during fasting/starvation. Commercial acetone is used in the production of high-volume chemicals including methacrylates, bisphenol A and other ketones, and as a solvent. Small amounts are used in the pharmaceutical industry. In humans, acetone is formed

endogenously under conditions of starvation, uncontrolled diabetes, or with high fat/low carbohydrate diets. Under conditions where glucose stores are depleted acetone provides a means of supplying glucose to tissues that are incapable of metabolizing fatty acids.

Exogenous acetone is readily absorbed via inhalation, ingestion, and dermal exposures. The water solubility of acetone allows for broad distribution to the water compartments of the body. At relatively low levels of exposure acetone may be lost through expired air and metabolized through the methylglyoxal pathway. At higher levels a second pathway producing 1,2-propanediol becomes more active and nonmetabolized acetone is lost through the urine. Earlier work on acetone metabolism proposed a metabolic pathway that produced an "active" form of acetate and formate. Although the active form of acetate may be acetyl CoA, the evidence to support the production of formate from acetone is sparse.

No human studies following oral exposure to acetone are available. Studies on rodent exposure to orally-administered acetone have identified several treatment-related health effects. Subchronic oral exposure resulted in kidney, testis, and hematologic system effects; however, the effects were characterized as mild. Although the nephrotoxic effects noted in rodents have been identified as the most critical effects, they tend to occur in male rats only and at high levels of exposure (20,000 and 50,000 ppm in drinking water).

Inhalation studies in humans have been conducted on both volunteers and occupationally-exposed individuals (Dick et al., 1988,1989; Kiesswetter et al., 1994; Stewart et al., 1975). These studies have examined, almost exclusively, either the toxicokinetics or neurological effects of acetone. The effects reported in these studies appear to be mild and transient. Clinical studies and case reports suggest slight neurological effects, mostly of the subjective type, in individuals exposed to varying concentrations of acetone. In most studies the subjects report discomfort, irritation of the eyes and respiratory passages, mood swings, and nausea following exposure to acetone vapor at concentrations of 500 ppm or higher. The fact that the effects subside following termination of exposure indicates that acetone may be the active compound, rather than a metabolite. Clinical chemistry analyses conducted in several studies demonstrated no exposure-related effects. Data on nerve conductivity are inconclusive. Case reports of accidental poisoning also indicate that the effects (e.g., lethargy and drowsiness) are short-lived. Transient effects are seen in male rats exposed to acetone by inhalation. Inhalation exposure to pregnant rats and mice did not cause statistically significant malformations in the offspring, but did result in lower fetal body weights in both species.

There is one human epidemiological study for cancer incidence available that has several

limitations. There are no chronic animal studies that address the issue of the human carcinogenic potential of acetone.

6.2. DOSE RESPONSE

Quantitative estimates of human risk as a result of oral exposure to acetone are based on laboratory animal exposures because no human data are available. The reference dose for chronic oral exposure is 0.9 mg/kg-day. The RfD is based on a subchronic oral study in the rat (NTP, 1991; Dietz et al., 1991) with a NOAEL of 900 mg/kg-day and a composite UF of 1,000. Although complete histopathological analyses were performed (both sexes were used, and both mice and rats were studied) confidence in the study is medium because the study is a subchronic rather than a chronic study. Confidence in the overall data base is medium because there are two well-conducted subchronic studies and considerable supporting data on humans, but no chronic or multigenerational oral studies on acetone are available. Additional information on the toxicity of acetone is provided by studies on isopropanol, a chemical that is metabolized primarily to acetone.

A reference concentration (RfC) has not been determined. There are no studies demonstrating conclusive effects either in humans or animals arising from chronic exposure. Confidence in the overall data base is low because little supporting data and no chronic or reproductive inhalation studies are available.

Lack of sufficient evaluation of reproductive toxicity by either oral or inhalation exposure is considered a data base deficiency. Changes in testicular weight were observed in male rats following oral exposure and a premature menstrual period occurred in 3 of 4 women acutely exposed by inhalation. The significance of these endpoints of reproductive toxicity in men and women is unknown at this time.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The support document and IRIS summary for acetone have undergone both internal peer review by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. Comments were also received from the public. A summary of significant comments made by the external reviewers and the public and EPA's response to these comments follows.

Comments from External Peer Review

1. Overall quality of the assessment.

Comment: The reviewers stated that overall the assessment is well conducted and well written and that an adequate effort was made to integrate the findings of different toxicology studies. One reviewer indicated that the animal studies were better characterized than the human studies.

Response: The human studies have been revised and more extensively characterized in Section 4.1.

2. The use of secondary data in the assessment.

Comment: The reviewers stated that the assessment appropriately uses secondary data and that the studies are well characterized. Several reviewers recommended the inclusion of several omitted studies and indicated that PBPK models for isopropanol and acetone (Gentry et al., 2002; the abstract for this publication was cited by the reviewer) are available.

Response: The recommended studies for inclusion in the assessment were considered. The available PBPK models are now included in the text in Section 3.5.

3. RfD derivation (selection of the principal study, critical effect, and generation of the dose-response).

The external review draft assessment identified the Dietz et al. (1991) and NTP (1991) subchronic drinking water study as the principal study. Kidney, hematological and testicular effects were identified as the critical effects and a NOAEL/LOAEL approach was used for the point of departure. The draft assessment used a NOAEL of 900 mg/kg-day (based on the time-weighted average) for nephrotoxicity and applied a total UF of 3000. The data were determined to not be amenable to benchmark dose analysis due to poor model fit. The individual UFs in the draft assessment were applied as follows: intraspecies UF=10, interspecies UF=3, subchronic to chronic UF=10, data base UF=10.

(A) Comment: All reviewers indicated the drinking water study is the best choice for the principal study. All reviewers agreed that nephrotoxicity is the best available chemically-related response to use as the critical effect although all stated that the significance of the effect and its relevance to humans is questionable in that the effect was male rat-specific and may be due to α_{2u}-globulin-induced hyaline droplet formation. In addition, the change was similar to a naturally-occurring nephropathy seen in aging F344 rats. Thus, at best this effect might represent an earlier onset of a condition that would have occurred anyway. One reviewer recommended a stronger rationale for the decision to apply a NOAEL/LOAEL point of departure in lieu of a benchmark dose based on modeling. One reviewer indicated that toxicity information on acetone may be available from studies on isopropanol, which when metabolized produces acetone.

Response: The renal effects chosen for the critical effect have been re-examined and significant changes to the text have been made. The study descriptions in Section 4.2.1.1 have been augmented with more detailed information on the observed effects. In addition, significant changes have been made in Section 5.1.1 to justify and clarify the use of nephropathy as the

critical effect, particularly in reference to EPA Guidelines on $Alpha_{2u}$-globulin: Association with Chemically-Induced Renal Toxicity and Neoplasms in the Male Rat (U.S. EPA, 1991b). The rationale for using a NOAEL/LOAEL approach in lieu of benchmark dose modeling has been augmented in Section 5.1.2. In brief, the type of response observed is not amenable to benchmark dose modeling since a graded dose response curve is lacking (a response of 1/10 animals for controls compared with a response of 9/10 animals at the lowest response level). References to attempts at benchmark dose modeling have been omitted. A review of the data base for isopropanol toxicity for oral and inhalation exposures has been included in Section 4.4.2. The data base has been taken into consideration in the acetone assessment, primarily with respect to the application of UFs as indicated in Section 5.1.3.

(B) Comment: Two reviewers commented on the choice of UFs for the derivation of the RfD. The comments were mainly directed at the use of a 10-fold UF for extrapolation from a subchronic to chronic exposure duration. One reviewer questioned whether environmental exposures to acetone at the level of the proposed RfD (0.3 mg/kg-day in the external peer review draft) would present a negligible increase in the body burden compared with the acetone that a healthy individual generates on a daily basis. This reviewer indicated that humans may have an ability to equilibrate and metabolize excess acetone. Along these same lines, another reviewer indicated that the pharmacokinetic behavior of acetone indicates that it does not accumulate and is eliminated efficiently through metabolism and via partitioning into expired air and urine. This reviewer stated that accumulation would not occur even with chronic daily exposure to high levels. At physiological levels of acetone, the prevalent elimination pathway would be through intermediary metabolism as indicated in Figure 1 of the Toxicological Review. In addition, the statement was made that there appeared to be little difference in acetone doses that were adverse in the two week study versus the 13 week study by NTP (1991). This reviewer also questioned the use of a 10-fold UF for data base uncertainty given that inhalation studies indicated little or no developmental hazard. The reviewer stated that the UF for data base insufficiency could be reduced by supplementing the data base with the developmental studies available in the inhalation data base.

Response: The UF for subchronic to chronic extrapolation has been changed from a 10 to a 3. The text in Section 5.1.3 has been significantly augmented to reflect the rationale for this change. The change is primarily a result of the comments of the external peer reviewers.

An UF of 10 has been retained to account for data base uncertainty. The available data base for acetone includes subchronic gavage and drinking water studies in mice and rats, including measurements of several reproductive parameters. There is one neurotoxicity study in rats which

evaluated effects on nerve conduction velocity and rotarod performance and one reproductive toxicity study with a single dose regimen. The data base lacks a multigenerational study and adequate studies of the oral neurotoxicity, developmental and developmental neurotoxicity of acetone. The only available neurotoxicity study in mice demonstrates no effects on nerve conductivity and rotarod performance. Nevertheless, human inhalation studies on acetone, while inadequate, indicate potential neurotoxic effects. This raises concern for neurodevelopmental effects from oral exposure because the nervous system undergoes developmental processes unique to early life stages. It is difficult to draw firm conclusions on the potential developmental effects from oral exposure to acetone based on inhalation studies following acetone exposure and oral studies using isopropanol. The available inhalation studies on acetone and the oral and inhalation data base for isopropanol (which is metabolized primarily to acetone) has been included in the text and taken into consideration to inform the oral data base for acetone.

4. The decision to not derive an RfC.

The draft IRIS assessment of acetone does not derive an RfC.

Comment: All reviewers agreed with the decision to not derive an RfC. One reviewer indicated it would be useful to augment the discussion of why the available studies are not adequate to support an RfC. One reviewer stated that there may be enough toxicokinetic data available to do a route-to-route extrapolation for oral to inhalation exposure. Another reviewer indicated that an examination of potential route-to-route extrapolation should be discussed but that this endeavor would still be insufficient to derive an RfC.

Response: Additional text has been added to Sections 4.2.1.2, 4.3.1.2, 4.3.2.2, 4.4.1.2 and 5.2 to better describe the results of the available inhalation studies and the rationale for not deriving an RfC. In addition, text has been added (Section 3.5) to indicate that the available PBPK models have not been validated in humans for oral exposure making route-to-route extrapolation tenable.

5. Cancer weight of evidence determination.

The cancer weight of evidence determination in the external review draft assessment was based on the Guidelines for Carcinogen Risk Assessment (1986) and the Proposed Guidelines for Carcinogen Risk Assessment (1996). Since the release of the external review draft assessment, EPA has begun to use the Draft Revised Guidelines for Carcinogen Risk Assessment (1999). The 1996 and 1999 guidelines apply different descriptors for weight of evidence. The external review draft classifies acetone as category D - *not classifiable as to human carcinogenicity* and

uses the weight of evidence descriptor that human carcinogenic potential *cannot be determined* based on the existing (1986) and proposed (1996) guidelines, respectively.

Comment: The reviewers agreed with the weight of evidence classification and descriptor. One reviewer recommended including classifications from other agencies, e.g., IARC, NTP.

Response: The weight of evidence classification according to the 1986 guidelines that was included in the external review draft has been removed. Under the Draft Revised Guidelines for Carcinogen Risk Assessment (1999), the weight of evidence descriptor for acetone has been changed to indicate that "data are inadequate for an assessment of human carcinogenic potential."
The assessment has been revised (see Section 5.3) to reflect that IARC and NTP have not assessed the human carcinogenicity of acetone.

6. Additional comments.

(A) Comment: All reviewers made editorial comments.

Response: Editorial comments have been taken into consideration and edits to the text have been made in numerous instances.

(B) Comment: One reviewer noted that information from other agencies such as ATSDR, NIOSH and WHO should be included in the assessment.

Response: Information used in the assessment of acetone by various agencies has now been taken into account. The studies that were used in the evaluation by ATSDR, NIOSH and WHO have been discussed in the text of the Toxicological Review. For example, the studies used by ATSDR in their quantitation of inhalation toxicity (e.g., Stewart et al., 1975) have been described in detail. The shortcomings of this study for the purposes of deriving a chronic RfC have been described in Sections 4.1.2.1 and 5.2. WHO (1998) derived an RfD for acetone based on the same principal study and point of departure (900 mg/kg-day) as that used in this assessment. The WHO applied an UF of 100 (no explanation of individual uncertainty factors) for an RfD of 9 mg/kg-day.

<u>Comments from the Public</u>

(A) Comment: One reviewer indicated there is very little discussion in the draft document of normal levels of acetone found in healthy individuals as a result of endogenous production. The

reviewer pointed out that the document emphasized endogenous production of acetone only under conditions of stress (i.e., diabetes or fasting conditions). The comparison of endogenous levels under normal conditions with the RfD was stated to be an important consideration. The reviewer indicated that a published study (Reichard et al., 1979) suggests that in normal healthy adults the rate of acetone production is in the range of 41 mg/kg-day which can be doubled by merely fasting. The reviewer indicated that the Reichard et al. (1979) study did not derive this value directly but that it had been derived in Patty's Toxicology (cited as Morgott (2001) in this document). This background production would be more than 135-fold the RfD (0.3 mg/kg-day in the external review draft). The reviewer stated that this information needs to be considered and the apparent discrepancy between the wide range of normal endogenous production in healthy adults and the proposed RfD needs to be reconciled.

Response: It is agreed that if the endogenous production of acetone (i.e., turnover rate expressed as mg/kg-day) under normal conditions were known, it would be useful to provide comparisons between this value and the RfD. However, the proposal to extrapolate this value from the Reichard et al. (1979) study does not seem feasible. In this study acetone turnover was monitored in lean and obese subjects under conditions of starvation ketosis (3 and 21 day starvation periods). Nonfasting individuals were not monitored and acetone turnover rates under normal conditions were not determined. The proposed derivation (Morgott, 2001) from the Reichard et al. (1979) data utilizes the y-intercept of a graphical representation showing the relationship between acetone turnover rates, expressed as micromoles per square meter per minute, and the plasma acetone concentration from the data corresponding to individuals undergoing starvation ketosis. The y-intercept (20 μmol/min-m^2) is then converted to the turnover rate of 41 mg/kg-day by utilizing the conversion factors of 70 kg body weight and 1.73 m^2 surface area. It is not evident that extrapolation from data on individuals in starvation ketosis would yield the acetone turnover rate in normal individuals. No other studies were located in which the acetone turnover rate could be derived.

(B) Comment: One reviewer indicated the 10-fold UF for subchronic to chronic extrapolation was not necessary. The reviewer stated that acetone exhibits low subchronic toxicity and that there is evidence of acetone's ready metabolism and elimination from the body. This reviewer also stated that the 10-fold UF for data base insufficiencies also needs to be re-examined in light of the existence of a negative oral one-generation reproduction study on acetone and the fact that only minimal effects on selected male reproductive organs in other subchronic studies have been reported. In addition, inhalation developmental toxicity studies in two species have been conducted and resulted in high NOAELs.

Response: The UF for subchronic to chronic extrapolation has been changed from a 10 to a 3. The text in Section 5.1.3 has been significantly augmented to reflect the rationale for this change. The UF for insufficiencies in the data base remains at 10 (see response to Comment 3B above).

(C) Comment: One reviewer contended that EPA should state expressly that acetone is unlikely to cause cancer in humans. The reviewer stressed that available information on acetone supports a weight of evidence classification of "E" (evidence of noncarcinogenicity for humans) by applying the Guidelines for Carcinogen Risk Assessment (1986) and "not likely to be carcinogenic to humans" according to the Proposed Guidelines for Carcinogen Risk Assessment (1996).

Response: The cancer weight of evidence determination is based on the lack of formal studies that specifically address the potential for human cancer from exposure to acetone. In addition, in determining the weight of evidence descriptor for acetone, EPA has considered such factors as the absence of data on low molecular weight ketones causing cancer in humans or laboratory animals; no identified mode of action data to support concern for the carcinogenicity of acetone; routine use of acetone as a vehicle in dermal cancer studies (albeit in the absence of naive controls); and the genotoxicity data which are largely negative. A search of the scientific literature identified one human study that assessed cancer incidence and no chronic animal studies. The limitations of the available studies are discussed in the Toxicological Review. The absence of studies limits the ability to reach an affirmative determination of evidence of noncarcinogenicity for humans.

Since the release of the external draft Toxicological Review and IRIS Summary for acetone, EPA has begun to apply the Draft Revised Guidelines for Carcinogen Risk Assessment (1999) in lieu of the Proposed Guidelines for Carcinogen Risk Assessment (1996) and the 1986 Cancer Guidelines. Section 2.6.2 of the 1999 guidelines identify criteria for use of descriptors for weight of evidence. The guidance states that application of the descriptor "not likely to be carcinogenic to humans . . . is used when the available data are considered robust for deciding that there is no basis for human hazard concern." Examples provided in the guidelines concerning the strength of data include extensive human experience that demonstrates a lack of carcinogenic effect, or animal evidence that demonstrates lack of carcinogenic effect in at least two well-designed, well-conducted studies. For these reasons, data are inadequate for an assessment of the human carcinogenic potential of acetone using the Draft Revised Guidelines for Carcinogenic Risk Assessment (1999).