TOXICOLOGICAL REVIEW

OF

ACETONE

(CAS No. 67-64-1)

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

August 2001

NOTICE

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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 of other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

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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 system 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 these hazard identification and dose-response assessments 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: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1987a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a), and *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996b); *Recommendation 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 Concentration and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b); and

memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

The initial literature search strategy employed for this compound was based on the CASRN and at least one common name. However, because the initial search for acetone yielded more than 1,500 hits, subsequent searches limited to review articles (all years) and for acetone as a word in the title (1993-present) were conducted. The following data bases were searched: TOXLINE (all subfiles), MEDLINE, CANCERLIT, TOXNET [HSDB, IRIS, CCRIS, EMIC (1991-present), and GENE-TOX], and RTECS.

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 also an endogenous compound utilized in intermediary metabolism (ATSDR, 1994; WHO, 1998). As the data below indicate, acetone is completely miscible with water and highly volatile.

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 mmHg	ATSDR, 1994; WHO, 1998
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/TOXICODYNAMICS RELEVANT TO ASSESSMENTS

The pharmacokinetics of acetone has been well studied in large part because of the role that acetone plays in normal metabolism and in disease states (ATSDR, 1994; WHO, 1998). Under starvation conditions, high-fat diet, or uncontrolled diabetes, fat is metabolized to form acetoacetate, which in turn is metabolized to acetone (Wieland, 1968). Much of the research on acetone in humans is the result of research on individuals in these states. Mean concentrations of acetone in "normal," nonexposed 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).

3.1. ABSORPTION

The absorption of acetone into and its distribution throughout the body is governed by its physicochemical parameters and related biological factors. Acetone is miscible in water and has a high vapor pressure and a high blood/air partition coefficient. The low K_{ow} indicates that acetone would selectively partition into aqueous phase rather than a lipid phase; however, acetone is slightly lipophilic, which allows 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 in organs with high water content.

3.1.1. Inhalation

The most important physicochemical parameters accounting for the potentially high level of 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 absorption values has been reported, including 245 by Satoh 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 dissolves in epithelial cells, becomes entrained in the bloodstream, and is transported from the nasal cavity. The fraction of the acetone remaining in the nasal tissue evaporates during expiration into the environment. The difference between the concentrations of acetone during the inhalation and the exhalation accounts for the portion that is entrained into the bloodstream and the amount that is metabolized immediately upon absorption (which is negligible) (Dahl et al., 1991). There are no studies reporting differences in uptake and distribution between men and women or between adults and children.

Hallier et al. (1981) conducted studies using rats in desiccators to achieve maximum saturation for determining the partition coefficients. Rats were placed in a 6.4 L desiccator that was saturated with acetone. Measurements of acetone in the headspace were made over time and the pharmokinetic 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. Acetone is miscible in water. By measuring the amount of acetone taken up by the rats in the desiccator, the authors determined that the maximum amount of acetone absorbed slightly exceeded the water pool of the rodents. The authors propose that the limited lipophilicity of acetone contributes to a minor amount of nonmetabolized acetone that moves into the lipid fraction of organ tissues.

Biological conditions have also been shown to be key factors in the uptake of acetone via the inhalation route. The uptake of volatile solvents is a function of both the rate of respiration and the blood/air partition coefficient. Male volunteers were exposed for 2 hours to either 1,300 mg/m^3 (547.6 ppm) at rest or to 700 mg/m^3 (294.9 ppm) with exercise (Wigaeus et al., 1981). The total amount taken up increased as ventilation increased with exercise, but the relative uptakes ranged from 39%-52% for both exposures. Total uptake for the two exposures was 0.6 g and 1.2 g, respectively, for the 1,300 mg/m^3 (resting) and 700 mg/m^3 (active). 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. The authors also note that given the high $K_{B/A}$ of acetone, it is surprising that only about 40% of the amount of acetone administered is absorbed into the body. This may be because of the relatively low lipid solubility of acetone, which provides resistance to the transfer of acetone from the air through the nasal tissue 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 uptake and elimination of acetone in humans. Mean relative acetone uptake averaged 53% in volunteers exposed to 21-211 ppm 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 and follow a monoexponential decay curve postexposure 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, there are a number of studies that demonstrate indirectly that acetone is rapidly absorbed via inhalation. For example, Wigaeus et al. (1982) conducted a study designed to measure the distribution of acetone in mice following inhalation exposure to 1,200 mg/m^3 2-^{14}C-acetone. The study did not involve a quantitative measure of the total amount of acetone taken up, but did measure the distribution of the acetone in individual tissues and organs. The study demonstrated a continuous increase in the tissue concentration of acetone and total radioactivity during the first 6 hours of exposure.

Similar results have been found in dogs and rats. Acetone uptake by the respiratory tract of the dog was 42%-54.2% for exposure concentrations of 0.36-0.80 µg/mL (151.6-337 ppm) for an unspecified duration (Egle, 1973). Blood concentrations of acetone in rats exposed to 150 ppm increased gradually over 2 hours, then plateaued 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 time of gestation (Mast et al., 1988).

3.1.2. Oral

Acetone is readily absorbed via the oral route, as indicated by early research on humans. Haggard et al. (1944) administered acetone 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 on the absorption of acetone in humans is provided in the form of case studies. These studies, involving accidental ingestion of acetone, indicate that acetone is readily absorbed through the gastrointestinal tract. In one case 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 chemistries demonstrated elevated levels of serum ketones (Herman et al., 1997). A second case study involved the accidental ingestion of nail polish remover (Ramu et al., 1978). The subject became listless and lethargic with a shortened attention span. These case studies indirectly demonstrate that acetone is readily absorbed via the gastrointestinal tract, but a quantitative assessment of absorption cannot be derived.

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, the authors report that 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 for the first day 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. Such a testing scheme does not allow for comparison with the initial study, which demonstrated high levels of absorption and rapid metabolism with effects of successive applications of the chemical on the rate of absorption. The authors also tracked the expiration of acetone following administration of the ^{14}C-acetone. They found that only 7% of the administered acetone was expelled in the breath, with peak levels achieved 2 hours following administration. Overall, the data indicate that upon ingestion, acetone is rapidly absorbed into the body and expelled either in the unmetabolized form or as CO_2.

Indirect evidence of absorption of acetone is available from studies in which acetone was administered in conjunction with other treatments, resulting in differential effects. Kenyon et al. (1998)

conducted studies to compare the metabolism of benzene with and without pretreatment of acetone. As noted below, acetone has been shown to be an inducer of cytochrome P-450 IIEI, which is involved in the metabolism of benzene. In their study, Kenyon et al. (1998) administered acetone as a 1% solution in the drinking water for 8 days prior to benzene treatment. The data demonstrated that pretreatment with acetone resulted in significantly higher levels of benzene metabolites compared with mice receiving no pretreatment. Although these studies are not amenable to quantitative determination of uptake of acetone, they demonstrate through these effects that acetone is absorbed via the gastrointestinal tract.

3.1.3. Dermal

Dermal absorption of acetone has also been shown to occur fairly rapidly in humans. In a Japanese study 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. 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 concentrations 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.

Actual studies on the level of dermal absorption of acetone in rodents are limited, although acetone is frequently used as a vector for dermal studies of other chemicals and hence serves as a control treatment (NTP, 1991, 1995, 1997). However, a search of NTP and other databases failed to identify dermal studies using both an acetone control and a naive control, which could be used to either directly measure uptake into the body via the dermal route or indirectly through observed effects.

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 concentrations in blood, lung, kidney, brain, pancreas, spleen, thymus, heart, testis, vas deferens, muscle, and subcutaneous and intraperitoneal white adipose tissues reached steady state 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 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, kidneys, lungs, brain, and muscles, but were slightly greater than 5 hours for subcutaneous adipose tissue. By 24 hours after exposure, acetone concentrations had returned to endogenous levels in all tissues (Wigaeus et al., 1982). These data confirm 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 reflects 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 of acetone for 3 h per 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 the plasma relative to the three organs approximates the octanol-water partition coefficient (log $K_{o/w}$ = -0.24), reflecting the greater solubility in the 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 conditions of fasting, ingestion of high-fat diets, and uncontrolled diabetes. Reichard et al. (1979) studied the metabolism of acetone in 15 human subjects undergoing starvation ketosis. The study measured 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, who ranged from 22 to 52 years of age. The subjects were categorized by weight into obese and nonobese categories where the nonobese individuals were -7% to +16% and obese 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 days (n=6) or 21 days (n=3) and the nonobese were starved for 3 days (n=3). The study found that in humans, the ratio of acetone in expired air was proportional to the plasma concentration. The principal findings in these studies were that, depending upon the plasma acetone concentration, excretion of acetone from the body in breath and urine accounted for about 2% to 30% of the endogenous acetone. In this study, the authors found that 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. Another significant finding occurred following the administration of ^{14}C-labeled acetone to fasting individuals. Within 6 hours of administration, ^{14}C was detected in serum glucose and expired CO_2. These data suggest that under conditions that lead to the production of ketone bodies in the blood, acetone is converted to glucose, which is subsequently metabolized to CO_2.

Sakami (1950) conducted a series of studies on the rat to determine the metabolism of exogenous 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. The authors purport that radioactivity trapped by the bicarbonate is $^{14}CO_2$ and state that this study demonstrates that acetone is metabolized to CO_2. The study does not describe in depth the method for trapping CO_2. It is therefore possible that the radioactivity that was analyzed was nonmetabolized expired acetone.

In the same study, animals were killed following incubation and necropsied, and the liver was 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 conversion of acetone bodies to glucose.

Extracts from the viscera of the rats contained radioactivity in serine, methionine, and choline. The authors contend that formate contributes to the synthesis of these amino acids at the same positions of 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 (Sakami, 1950). 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 the biosynthesis of methionine involves oxaloacetate and the biosynthesis of serine is from phosphoglycerate, neither of which is inconsistent with the metabolic pathways proposed by Cassazza et al. (1984).

As a follow-up to their initial work, Sakami and Lafaye (1951) conducted a study to elucidate the pathway for metabolism of acetone. The authors propose a method for elucidating the metabolic pathway based on the locations of ^{14}C in glucose that is synthesized from acetone. The authors speculate that 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 3,4 positions relative to the 2,5 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 incubation. At the end of 4 hours the animals were killed, livers were removed, and glycogen was extracted. The glycogen was digested to glucose and subjected to microbial degradation to identify sites of ^{14}C on the glucose molecule. The ^{14}C label was found in all positions in the glucose molecule, although with higher labeling in the 2,5 positions than the 1,6 positions. The authors indicate that the level of label in the 1,6 molecule may be an artifact of the degradation process.

The two studies by Sakami and colleague (Sakami, 1950; Sakami and Lafaye, 1951) are limited by the state of technology. This point is indicated by the interpretation of the results from their second study (Sakami and Lafaye, 1951), in which the authors attribute anomalous findings to possible artifacts. They also state that the findings may suggest a metabolic pathway that had not previously been considered. Although the data on the two studies by Sakami and colleague provide strong evidence that exogenous acetone is readily absorbed via the gastrointestinal tract and converted to CO_2, glucose/glycogen, and other metabolites, these 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. The 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 IP 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 IP injection of acetone produced detectable levels of 1,2-

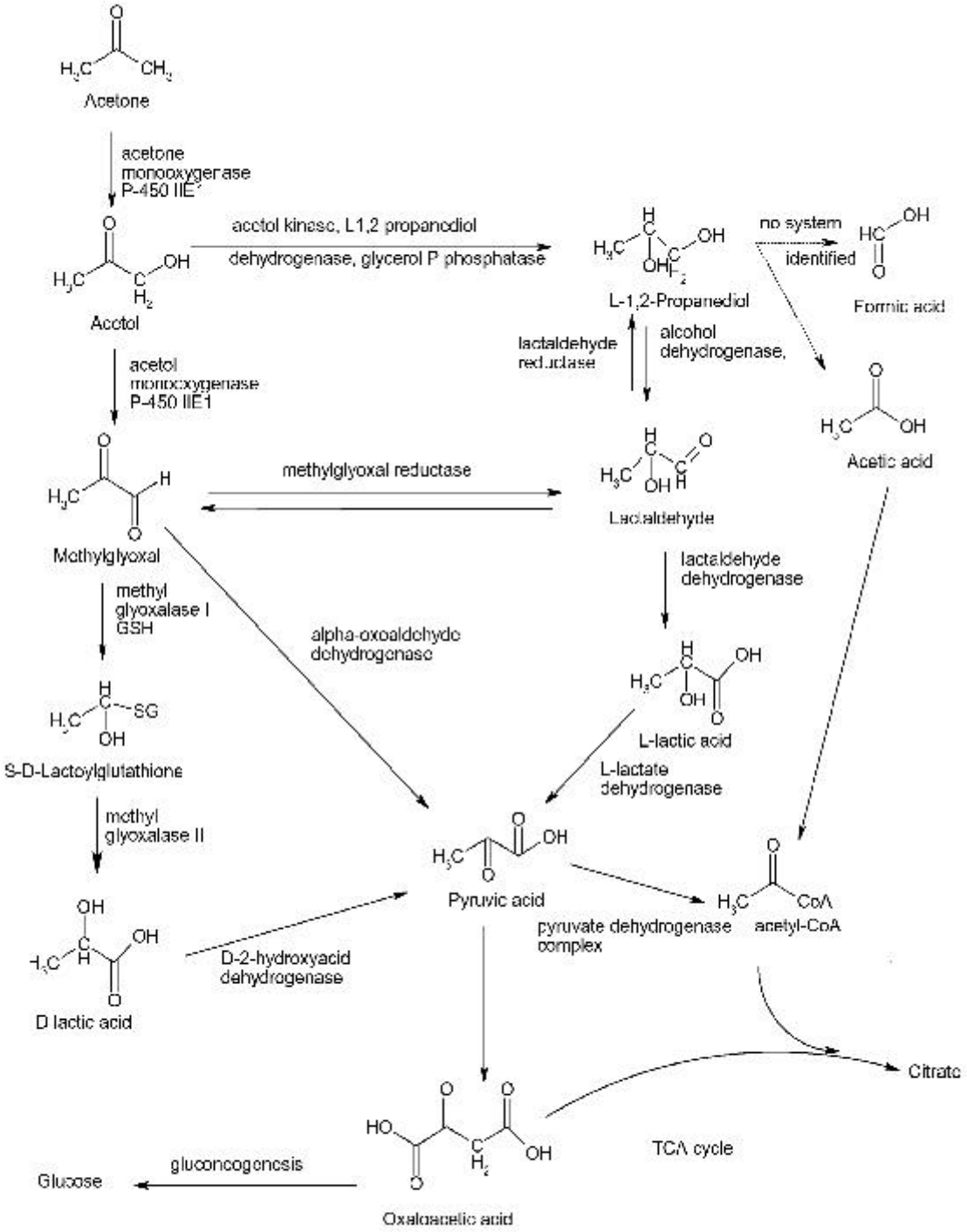
propanediol and 2,3-butanediol, whereas animals that received no acetone IP injection or received the IP injection with no pretreatment with acetone failed to produce detectable levels of either of the two diols, indicating the role of induction in determining a particular pathway in the metabolism of acetone.

In the same study the authors found elevated levels of lactate in rats that received pretreatment of acetone compared with rats receiving drinking water for both the nontreated and treated groups. They found the site of activity to be microsomal. Working with rat liver microsomes and relying on enzyme-specific inhibitors, the authors incubated acetone in a microsomal suspension and recovered acetol. When acetol was added to the microsomal suspension in the presence of glyoxylase 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, 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 I and II. By analyzing kinetics of the conversion of acetone or acetol to glucose and D-lactate, the authors concluded that the rate of utilization of acetol is not accounted for by the formation of glucose and D-lactate, suggesting the presence of an alternate pathway for the conversion of acetone to glucose (Casazza et al., 1984).

Hepatocytes from a rat starved for 2 days given drinking water with 1% acetone were incubated with 5 mM L-lactate and L-1,2-propanediol with and without specific inhibitors of aldehyde dehydrogenase (cyanide) or alcohol dehydrogenase (pentylpyrazole). The rate of formation of glucose from L-lactate was reduced 12% and 50% in the presence of pentylpyrazole and cyanide, respectively, compared with the control. The rate of formation of glucose from L-1,2-propanediol in the presence of pentylpyrazole and cyanide, respectively, is comparable to the rate of glucose formation in the controls (without the 1,2-propanediol) (Casazza et al., 1984).

1,2-Propanediol production provides a second pathway for the formation of glucose from acetone. Casazza et al. (1984) compared the derived kinetics for the production of 1,2-propanediol from hepatocytes with those observed in the whole rat and concluded that the enzymatic activity in the liver could not account for the total amount of 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. These data suggest that most of the metabolism via the formation of 1,2-propanediol is extrahepatic.

Casazza et al. (1984) provide evidence that there are two pathways involved in the metabolism of acetone to glucose, one hepatic and the the other extrahepatic (Figure 1). The hepatic route involves the conversion of acetone to acetol mediated by acetone monooxygenase, which has been shown to be cytochrome P-450IIEI. Acetol in turn may be converted to methylglyoxal via P-450IIEI enzyme system or converted to 1,2-propanediol. Methylglyoxal is converted either directly to glucose or to D-lactate, which feeds into glucose. Data provided by Casazza et al. (1984) support the direct pathway of methylglyoxal to glucose as opposed to the D-lactate route. From the data presented in this study, the authors note that they are unable to determine which of the two pathways, 1,2-propanediol or methylglyoxal, predominates.



(after Kalapos, 1999)

Figure 1. Pathway for the metabolism of acetone.

The paper by Casazza et al. (1984) is comprehensive in its approach but has its limitations. Treatments for most of the assays were not replicated, and therefore provide no measure of variability. A second issue is that the authors note in the introduction that formate and acetate production has been proposed as a pathway for the metabolism of acetone; however, that is not addressed in their paper. Based on the publication 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 the lower concentrations and the 1,2-propanediol pathway predominating at higher concentrations. The rats used in their study 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. Additionally, ^{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 about 4 hours to complete. Following the infusion of radiolabeled acetone, a sample
of blood was drawn, the animals were killed, 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 the rats were frozen and powdered and the glucose recovered. For all samples, the glucose was degraded and analyzed for placement of ^{14}C in the glucose molecule. Regardless of whether the rats fasted or were infused with glucose along with 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, the 23%-40% of the ^{14}C label was in either the 3 or 4 positions on the resulting glucose. Differences in location of the ^{14}C in the glucose indicate that there is more than one pathway at work and that selection of the pathway is dose dependent.

Kosugi et al. (1986b) conducted a second study comparing radiolabeling of 2-^{14}C-lactate with that of 2-^{14}C-acetone. They proposed that if acetone is metabolized prior to any randomization of the carbons in a pathway in which lactate serves as an intermediate, as postulated by Casazza et al. (1984), the labeling of 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 actone 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 a byproduct (Kosugi et al., 1986a). The authors postulate that their data indicate 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. They starved Sprague-Dawley rats for 48 hours prior to the start of the study. The 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 incorporation of ^{14}C into potential metabolites including citrate, lactate, â-hydroxybutryate, acetate,

1,2-propanediol, and acetol. 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: one proceeding via methylglyoxal and a second through 1,2-propanediol (Figure 1). Kosugi et al. (1986a) provide evidence that the route of metabolism differs depending on the amount of acetone; at lower concentrations metabolism proceeds mostly through the methylglyoxal pathway, but as the concentration of acetone increases the catabolism proceeds to a correspondingly greater degree via the 1,2-propanediol pathway. Gavino et al. (1987) tested the pathways with a perfusion study and found evidence that catabolism of acetone proceeded via a pathway that generated acetate and 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) conducted a hepatic perfusion study. The different results in metabolic pathway are consistent with the mechanism proposed by Casazza et al. (1984), who stated that the methylglyoxal pathway was largely a hepatic reaction and the 1,2-propanediol pathway was largely extrahepatic.

Sakami (1950) proposed a pathway that involved the formation of acetate and formate. As noted above, there are studies that support the presence of acetate (possibly in the form of acetyl coenzyme A); there is a single study in which the authors report the presence of formate as a metabolite of acetone, albeit at low levels (Hallier et al., 1981).

Bondoc et al. (1999) demonstrated the role of P-450IIEI in the metabolism of endogenously produced acetone under ketogenic conditions using a knockout mouse lacking the ability to express CYPIIE1. When mice were fed ad libitum, plasma acetone levels in strain matched CYPIIEI-competent mice, and CYPIIEI-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 CYPIIEI-null mouse had a 48-fold increase. This study provides strong evidence for the role of CYPIIE1 in the catabolism of acetone.

In brief, based on whole animal and in vitro studies, acetone metabolism appears to be composed of at least two routes (Figure 1). The principal metabolic pathways of metabolism appear to be dependent on the site of metabolism and on the amount of exogenous acetone. The fragments are incorporated into glucose and other substrates of intermediary metabolism that ultimately produce CO_2. In the first step, common to all three pathways, acetone is oxidized to acetol by acetone monooxygenase, an activity associated with cytochrome P-450IIE1. This step requires O_2 and NADPH (Casazza et al., 1984). Acetol is converted to methylglyoxal, which in turn is metabolized to glucose through a lactate intermediate. In the second pathway, the acetol intermediate is converted to L-1,2-propanediol by an extrahepatic mechanism that has not been fully characterized. 1,2-Propanediol is converted to glucose through a series of intermediates including lactate. As noted above, Sakami (1950) proposes a pathway whereby acetone is converted to formate and acetate. The production of acetate has already been presented; however, there are no studies to date that support the production of formate as a metabolite of acetone.

The metabolism of acetone involves some of the same enzyme systems as those involved in the metabolism of ethanol. The conversion of acetone via the methylglyoxal pathway is mediated by acetone monooxygenase cytochrome P-450IIEI and acetol monooxygenase cytochrome P-450IIEI to form methylglyoxal. The conversion of methylglyoxal to lactate is mediated by glyoxalyase I and II with glutathione-s-transferase. The metabolism of acetone via the 1,2-propanediol pathway to lactate is mediated by alcohol dehydrogenase and aldehyde dehydrogenase (Dietz et al., 1991). The subsequent metabolism of lactate and methylglyoxal to glucose is poorly characterized, but the data presented above indicate that gluconeogenesis proceeds through the formation of an active form of acetate.

Enzymes involved in metabolism of acetone are inducible. The metabolism of acetone through the methylglyoxal route is mediated largely by cytochrome P-450IIE1, 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). Acetone significantly increased both the microsomal protein content and the activity of cytochrome P-450IIE1 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 of 5 mL/kg or administration of 1% in the drinking water for 8 days (Forkert et al., 1994). Treatment with acetone or starvation conditions leads to increases in both 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). In contrast, inhibition of cytochrome P-450IIE1 activity results in an increase in endogenous acetone levels (Chen et al., 1994).

The data demonstrate two defined pathways for metabolism of acetone. 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. At higher concentrations an alternate pathway kicks in to mediate 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 also conceivable that the conversion to the diol diverts acetone from gluconeogenesis and facilitates loss of acetone via the urine.

3.4. EXCRETION

In a previously described study, Wigaeus et al. (1981) exposed male subjects to two concentrations of acetone with and without exercise. The 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 2 hours. As noted earlier, inhalation uptake increased with exertion. The exposure treatment was followed by a 4-hour monitored period of elimination in the laboratory that consisted of intermittent walking and rest during which the authors collected and analyzed expiratory samples and urine for nonmetabolized acetone. The highest concentration of acetone in the urine was found between 3 and 3.5 hours following exposure. The authors found 16%, 20%, and 27% of the absorbed acetone was lost through the lungs in the form of nonmetabolized acetone for the resting, light exercise, and more strenuous exercise, respectively, thereby demonstrating a complementary response but reflecting similar properties as those applied to absorption via the inhalation route. For all three exposure scenarios, about 1% of the absorbed acetone was lost through the kidneys. The remainder is presumed

to be metabolized. The higher concentration of acetone lost through the lungs corresponding to greater amounts of acetone collected by the body suggests a saturation of the metabolism of acetone.

In workers with a mean occupational exposure to acetone of 141.8 ppm, blood (source not stated) and urine concentrations at the end of the shift were 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 (Wang et al., 1994). 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 were highly reproducible, and the narrow range of acetone in the breath at a specific time post-exposure indicated that breath analysis could be a reliable method to estimate the magnitude of recent acetone exposure by inhalation (Stewart et al., 1975). However, urinary concentrations 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 fivefold higher than those in the Wang et al. (1994) study.

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 2 hours, indicating rapid and extensive absorption by the gastrointestinal tract (Haggard et al., 1944).

There is strong agreement between the data on human subjects and those on rodents. Scholl and Iba (1997) measured the effect of the interaction of acetone and pyridine, two chemicals known to be inducers of P-450 IIE1, on the persistence of the chemicals in plasma. Although the objective of the study was to evaluate the interaction of the two chemicals, the use of exposure data for the chemicals individually allows for assessment of the persistence of acetone in the plasma. Rats were exposed to acetone via inhalation or interperitoneal injection. For inhalation exposures male Sprague-Dawley rats were placed in an exposure chamber and subjected to 1,000 ppm of acetone vapor for 8 hours per day for 3 days. With intraperitoneal exposure the rats were administered 400 mg/mL at a dose of 400 mg/kg. For both routes of exposure plasma samples were taken over a 20-hour period following exposure and analyzed by gas chromatography. The concentration of acetone was fit to a single first-order equation and plasma half-lives of acetone were determined from the resulting equation. The authors determined the half-life of acetone in the plasma to be 4.5 and 4.1 hours for inhalation and interperitoneal injection, respectively. The authors noted that acetone exhibited a short half-life in plasma independent of route of exposure; however, the methods employed by the authors only allow for detection of acetone and do not provide information on its fate.

A principal finding in these studies is that the mode of excretion of acetone 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.

Haggard et al. (1944) injected 11 rats intraperitoneally and followed the fate of acetone over a 4- to 6-hour period. The authors measured the blood acetone levels and the amounts of acetone lost by elimination through the lungs and urine compared with those lost by metabolism. The data indicate that the percentage of acetone lost by elimination in the urine and expiration is directly, 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 is metabolized. Based on their data, the breakeven point is about 100 mg acetone/L blood.

Overall, available data indicate that acetone is rapidly absorbed by inhalation, ingestion, and dermally, and distributed throughout the body, particularly in organs and tissues with high water content. Many of the processes that involve the pharmacokinetics of acetone appear to be dose-related. Once it has been absorbed, acetone is extensively metabolized, but selection of the metabolic pathway appears to be dose-related. At low concentrations, the primarly pathway appears to be via formation of methylglyoxal. As the concentration of acetone increases, the propanediol pathway becomes increasingly involved. Although the second pathway appears to be involved in gluconeogenesis, it may also be used to facilitate excretion. The levels of excretion also appear to act in a dose-related fashion. Exposure to low levels of actone lead to small losses through the breath. Acetone appears in the urine only at concentrations of 15 ppm or higher. The proportion of acetone lost through the breath increases at high acetone concentrations. These data indicate that the body invokes a number of mechanisms to control the level of acetone.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS — OCCUPATIONAL, VOLUNTEER STUDIES, AND CASE REPORTS

4.1.1. Cancer Effects

Ott et al. (1983a,b) conducted a retrospective cohort study to evaluate the effects of methylene chloride on workers at a plant that manufactured cellulose diacetate and cellulose triacetate. Nonetheless, 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 operated by the same company. The focus of the study was the effect of exposure to methylene chloride on the hematopoietic and circulatory systems of the workers. 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 3 months to 23 years with time-weighted-average acetone concentrations of 380 to 1,070 ppm depending on job category (Ott et al., 1983a,b; and as reviewed in ATSDR, 1994). In this study, the 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.

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 exposure to acetone were secondary. The 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. Findings of this study should be taken in the context of these limitations.

4.1.2. Noncancer Effects

4.1.2.1. *Clinical Study*

Groups of four men were exposed to 0, 200, 1,000, or 1,250 ppm acetone for 3 or 7.5 hours/day on 5 consecutive days; groups of 2-4 women were exposed to 0 or 1,000 ppm for 3 or 7.5 hours/day on 4 consecutive days (Stewart et al., 1975). 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. The health of the individuals was unaffected by exposure. Clinical chemistries, hematologies, 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. Three of four males exposed to 1,250 ppm had a 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 4 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.

Six male students were exposed to 250 and 500 ppm acetone on 6 consecutive days for 6 hours/day with a 45-minute lunch break (Matsushita et al., 1969; OECD, 1998). An additional group also exposed to 250 ppm exercised to double their metabolic rate. Response times to a visual stimulus were increased on all 6 days in the 500-ppm group and on 2 of 6 days in the 250-ppm group. Subjective symptoms of uncomfortable odor and eye, nose, and throat irritation were noted at 500 ppm, but were only slight at 250 ppm regardless of workload. General weakness persisted the next morning in all exposure groups.

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 significantly increased response times and false alarm percents 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 significant drop on the anger hostility scale of the POMS test. No effects were found on visual vigilance, choice reaction time, and memory scanning (results uninterpretable because of learning effects).

Volunteers exposed to 1,000 ppm acetone, 400 ppm of ethyl acetate, or acetone/ethyl acetate mixture (500/200 ppm) for 4 or 8 hours. Although those exposed to ethyl acetate alone or the mixture demonstrated higher levels of annoyance and complaints, the group exposed to acetone showed no change in the subjective symptoms of tension, tiredness, complaints, or annoyance (Seeber et al., 1992).

4.1.2.2. *Cohort Study*

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; tests of memory were 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 the environmental levels, which indicates that under continuous exposure to acetone vapors an equilibrium is reached that results 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 last 6 months, heavy or faint feeling in the head, nausea, and weight loss were reported by 23.6%-25.8% of exposed workers vs. 2.9%-9.8% of controls. These symptoms showed a dose-response relationship and were probably the result of peak exposure during a single day. No differences between exposed workers and controls were observed for Manifest Anxiety scale, Self-rating Depression scale, electrocardiogram, phagocytic activity, hematology, and clinical chemistry. Simple reaction time and digit span were significantly lower only 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 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. 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, when during the work shift 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. Nerve conduction velocity of all three nerves was significantly reduced in exposed workers as compared with

controls. Exposed workers also had significantly delayed reaction time for the visual test and a lower mean distributive attention score compared with controls.

Although the results of the Mitran et al. (1997) study seem to indicate a possible neuropathy from acetone exposure, the study has been criticized on several points (Boyes and Herr, 1999). Experimental and methodological details were incomplete, making it impossible to determine what, or how, tests were done. An adequate description of the "matched" control group was not provided. The study authors did not state whether temperature was controlled in nerve conduction studies. Results of the nerve conduction tests were inconsistent and did not indicate a clear neuropathy. Subjective symptoms, such as numbness of the hands and feet, were not corroborated by objective evaluation of sensory perception. Finally, acetone has not previously been associated with neuropathy in exposed workers or laboratory animals (Sections 4.1 and 4.4.1).

Semen parameters were evaluated in 25 workers at a reinforced plastic production plant (Jelnes, 1988). Breathing zone measurements indicated acetone concentrations of 69.6 (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 percentage of live sperm was significantly higher (p 0.01; 80% vs. 68%) whereas the percentage of immobile sperm was significantly lower (p 0.01; 30% vs. 40%) in the exposed group as compared with controls. The exposed men also had a decrease in the percentage of normal sperm (47% vs. 60%) due to increased percentages of amorphous and pyriform sperm head shapes. This study is limited by several factors, not the least of which is that the semen samples used for the control group were collected from a fertility clinic. The lower levels of live sperm and higher levels of immotile sperm in the control group are not unexpected with individuals experiencing reproductive problems. However, the higher levels of malformed heads generally indicate that the chemical to which the study cohort was exposed reached the testis and may be indicative of germ cell mutagenicity (U.S. EPA, 1996b). It should be noted, however, that subjects in the study cohort were also exposed to styrene. Given the universally negative results of acetone in genotoxicity studies (Section 4.4.2), the malformations may be related to coincidental styrene exposure.

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 et al., 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 3 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 final 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 period of 2 years (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, these 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 were limited to case reports. In one case report, a 17-month-old girl was given approximately 4.88 mL/kg of acetone through her gastrostomy 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.

Case reports also described the effects of ingestion of formic acid (formate), a metabolite of acetone (Naik et al., 1980; Rajan et al., 1985). In patients hospitalized after ingestion of domestic products containing approximately 40% formic acid, one of the most critical effects was acute renal failure. Renal biopsy and postmortem examination showed histopathological evidence of tubular necrosis (Naik et al., 1980; Rajan et al., 1985).

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS

4.2.1. Prechronic Studies

4.2.1.1. *Oral Studies*

Several subchronic oral studies were conducted in both mice and rats. In male rats, the testis, kidney, and hematopoietic system were identified as target organs. The only effects noted in female rats and in male and female mice were in the liver and were indicative of enzyme induction. These studies are described below.

Groups of five male and five female F344/N rats were administered acetone in the 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, 714, 1,616, 2,559, 4,312, and 6,942 mg/kg/day, respectively, and for females were 0, 751, 1,485, 2,328, 4,350, and 8,560 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 $B6C3F_1$ mice were administered acetone in the 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, 965, 1,579, 3,896, 6,348, and 10,314 mg/kg/day, respectively, and for females were 0, 1,569, 3,023, 5,481, 8,804, and 12,725 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 controls. All animals survived to termination. Kidney weights were increased at 50,000 ppm for males and females, and liver weights were increased at 5000 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 were 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. 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, significant (p 0.05 or 0.01) 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 be a result of enzyme induction. In high-dose males, depressed sperm motility, caudal weight, and epididymal weight and an increased incidence of abnormal sperm were seen (data for testicular lesions 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 both 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, nephropathy was observed in 6/10, 8/10, 8/10, 9/10, 10/10, and 10/10, respectively, with severity ratings of 1.2, 1.0, 1.0, 1.0, 1.9, and 1.9, respectively (1 = minimum, 2 = mild, 3 = moderate, 4 = severe). 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.

Also at 20,000 and 50,000 ppm of acetone, males had significant (p 0.05 or 0.01) changes in hematology. For the 20,000- and 50,000-ppm groups, leukocytes were 125% and 133% of controls, erythrocyte counts were 92% and 90% of controls, reticulocyte counts were 75% and 68% of controls, hemoglobin levels were 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. 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. A 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 hematopoietic 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 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; Sonawane et al., 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 cell parameters (hemoglobin, hematocrit, mean cell volume, and/or mean cell hemoglobin) in the high-dose groups were significantly (p 0.05 or 0.01) increased for males at interim sacrifice and for males and females at final sacrifice; however, the author considered that the magnitude of the increases was not biologically significant. Differences in clinical chemistry parameters were not dose-related and were not consistent over time or between sexes. Absolute and/or relative liver and kidney weights were significantly (p 0.05 or 0.01) increased in the mid-dose females and in the high-dose males and females as 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 incidence rates were similar between the treated and control groups, an increase in 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. 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.

Although Sonawane et al. (1986) noted similar kidney effects as the NTP study, the gavage study included clinical chemistries. These data, however, failed to show dose-related effects consistent with the nephropathy noted in the histology, thereby raising questions about the significance of the effect. Additionally, the presence of hyaline droplets in the kidneys raises questions about the relevance

of this study to humans. Differences in observed effect level may have to do with the method of administration. As noted earlier, acetone is readily absorbed through the gastrointestinal tract (Section 3.1). At elevated levels, more acetone appears to be shunted to the kidney, producing higher concentrations in the urine and higher levels of metabolism through the propanediol pathway compared with the more gradual administration through the drinking water. This would account for differences in the severity of the nephropathy levels observed with drinking water compared with gavage administration. Additionally, the increase in kidney weights was more produced in the gavage study than in the drinking water study.

Groups of 10 male and 10 female $B6C3F_1$ 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, 611, 1,353, 2,258, and 4,858 mg/kg/day, respectively, and for females were 0, 892, 2,007, 4,156, 5,945, and 11,298 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. In high-dose females, absolute and relative liver weights were significantly (p 0.05 or 0.01) increased to 113% and 110% of controls, respectively, and absolute and relative spleen weights were significantly (p 0.05) decreased to 89% and 88% of controls, respectively. The only microscopic lesion 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, indicating a development of tolerance to acetone. In summary, the liver was identified as the target organ in male and female mice. The reference to this as an adverse effect is questionable because the morphological changes may reflect induction of enzymes rather than an adverse effect on the liver. Effects that were noted in the rat, particularly male rat, were not evident with the mice. The LOAELs for males and females were 4,858 and 11,298 mg/kg/day, respectively, and the NOAELs were 2,258 and 5,945 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. The fact that effects noted in the 14-day studies were not evident in longer studies is consistent with the pharmacokinetics of acetone.

4.2.1.2. *Inhalation Studies*

Male Sprague-Dawley rats were exposed to 19,000 ppm acetone (45,106 mg/m^3) for 3 hours/day, 5 days/week, for 8 weeks (Bruckner and Peterson, 1981a). Body weight gains of the treated animals were slightly less than air-exposed controls; however, statistical significance was not reached at any time. Kidney weights of the treated animals were significantly (p 0.01) less than the controls after 4 weeks of exposure, but were similar to controls after 8 weeks. Serum SGOT activities were slightly elevated (not significant) in treated animals at weeks 2, 4, and 8; however, LDH activity and BUN and liver triglyceride levels were not affected at any time during the study. No microscopic

lesions were observed in the liver, brain, heart, and kidneys of 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 were found in the available literature.

4.2.3. Cancer Studies

No studies have been found on the carcinogenicity of acetone to animals. Acetone has been extensively used as a vehicle 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 2 years without an increase in neoplasia or any other toxic response. However, neither these studies nor other studies found in a review of the literature contained a naive control in addition to an acetone vehicle control.

4.3. REPRODUCTION AND DEVELOPMENTAL STUDIES

4.3.1. Reproduction Studies

4.3.1.1. *Oral Studies*

Ten male rats (Mol/Wis., SPF) were administered 0.5% acetone (equivalent to 5,000 ppm) in the drinking water for 6 weeks and then bred to untreated females of the same strain. An additional group of 10 males was treated for 6 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 (Larsen et al., 1991). 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]). 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), depressed sperm motility (66.8% vs. 75.7% for controls), and an increased incidence of abnormal sperm (3.42% vs. 0.68% for controls). Data for testicular lesions were given only for the 0, 2,500, 10,000, and 50,000-ppm groups. Relative testis weights data are difficult to interpret. Normally reproductive toxicants generate a decrease in testis weights. However, this may be a result of overall decrease in body weight. There were no dose-related statistically significant differences in absolute testis weight. The NTP study authors state that the decrease in sperm motility at the highest dose was consistent with mild reproductive effects.

Data on the toxicity of acetone on male reproductive organs indicate that at high doses there is a mild testicular effect, as indicated by diminished sperm motility and malformed sperm. Whether these effects translate into diminished reproductive ability at this level is not clear. 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 study, there were no detectable reproductive effects. Whether the reduced sperm motility and higher percentage with malformations noted at 50,000 ppm translates into impaired reproductive ability is not known.

4.3.1.2. *Inhalation Studies*

Reproductive toxicity studies following the inhalation route of exposure were not found.

4.3.2. Developmental Toxicity Studies

4.3.2.1. *Oral Studies*

Developmental toxicity studies following the oral route of exposure were not found.

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 (GD) 6-19 (Mast et al., 1988). Maternal body weights of the high-concentration dams were significantly (p 0.05) less than controls on GD 14, 17, and 20 and cumulative weight gain was reduced from GD 14 until termination. No clinical signs of toxicity or maternal deaths were observed. Maternal liver and kidney weights, number of implantations, mean percent of live pups/litter, mean percent of resorptions/litter, and fetal sex ratios were not affected by treatment. Fetal body weights from the high-concentration group were significantly (p 0.05; 84%-86% of control) reduced as compared with controls. The incidence of fetal malformations was not 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 consistent effect that predominates. Maternal plasma acetone levels measured 30 minutes postexposure increased in relation to dose. 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. The concentration of plasma acetone did not increase over gestation for any group. A complete assessment of the maternal respiratory tract was not made. Based on decreased body weights of the dams and fetuses, the maternal and developmental toxicity LOAEL is 11,000 ppm and the NOAEL is 2,200 ppm.

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 GD 6-17 (Mast et al., 1988). No clinical signs of toxicity,

maternal deaths, or effects on maternal body weights were observed. Absolute and relative liver weights were significantly increased in the 6,600-ppm group as compared with the controls, 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 slight but 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. Fetal body weights from the high-concentration group were significantly (p 0.05; 86%-92% of control) reduced as compared with the controls. The incidence of fetal malformations was not significantly increased in any exposed group. A maternal toxicity LOAEL was not identified, but a complete assessment of the respiratory tract was not made. The developmental toxicity LOAEL was 6,600 ppm based on reduced fetal body weights and the developmental toxicity NOAEL was 2,200 ppm.

The incidence of rat litters with malformations at 11,000 ppm makes up a small increase compared with the control group. The most pronounced effect was the reduced body weight of the offspring at the high dose. However, this effect may reflect the decreased body weight of the dams. The fetal body weight effect may be discounted in light of the negative findings of other parameters including resorptions, number of live births, and number of births per litter, which were comparable to the controls. The mice also had reduced fetal body weight gains at the high dose (6,600 ppm). As with the rat studies, there was no indication that the lower fetal body weight was related to other developmental effects.

4.4. OTHER STUDIES

4.4.1. Neurotoxicity

4.4.1.1. *Oral Studies*

Ladefoged et al. (1989) compared the effects of exposure to ethanol, acetone, and 2,5-hexanedione in combination for 6 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 6 weeks (Ladefoged et al., 1989). Peripheral distal axonopathy was not observed in Sprague-Dawley rats (sex not specified) given 0.5% acetone for 8 weeks followed by 1% acetone for 4 weeks in the drinking water (Spencer et al., 1978). Water consumption was reported in graphical form by Ladefoged et al. (1989) and not at all by Spencer et al. (1978). Therefore, doses to the animals are not known.

4.4.1.2. *Inhalation Studies*

In a subchronic schedule-controlled operant behavior (SCOB) study commissioned by the Chemical Manufacturers Association (CMA) and conducted by scientists at Dupont's Haskell Laboratory under a consent agreement with EPA, rats were trained to press a lever to obtain food in

standard behavioral test chambers (CMA, 1997). Operant sessions were conducted 5 days per 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 animals (10 per dose) were exposed to doses of 0, 1,000, 2,000 or 4,000 ppm acetone for 6 hours per day, 5 days per 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 8 and continuing through weeks 13 and 15 for the two highest doses, respectively. The NOAEL and LOAEL for the mean fixed ratio pause duration measure were 1,000 and 2,000 ppm, respectively. The submitter states that the differences observed were the result of unusual performance by the control group and not a treatment-related effect. The authors of the study state that the control group exhibited an unexplained increase in the pause duration between week 7 and the end of the study. The other SCOB measures failed to demonstrate exposure-related effect.

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 6000 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 Genotoxicity

The genotoxicity of acetone has been well studied and reviewed, with the results almost entirely negative (ATSDR, 1994; OECD, 1998; U.S. EPA, 1988b; WHO, 1998). All studies evaluated by the GENETOX panel and cited in the GENETOX database were negative, with the exception of one study for which no conclusion was drawn (GENETOX, 1999).

Neither sister chromatid exchange 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 cell types (Latt et al., 1981) 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% actually inhibited background synthesis in a concentration-related manner (Lake et al., 1978). The chemical was negative at concentrations up to 10 mg/plate in the Ames reversion test with five strains of *S. 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* 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.

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

Acetone's effects in humans are of interest as a result of the role that it plays in energy generation, particularly under conditions of stress, and because of exposure from a variety of sources. Nutritionally, following the ingestion of food, glucose, starches, and other carbohydrates are used to generate essential cellular components, energy, glycogen, or fatty acids that are stored as fat. Under "normal" conditions, the body uses glucose from consumed foods and glycogen from the liver to satisfy the need for energy. Under conditions of stress, including starvation or exertion, the body resorts to the metabolism of fat as its energy source. However, some tissues, including the brain and hematopoietic system, are incapable of using fat as an energy source. To compensate, the body resorts to a process of regenerating glucose through a pathway in which acetone serves as an intermediate. As such, acetone is a normal cellular constituent that the body is capable of metabolizing at low concentrations.

Acetone appears endogenously at elevated levels under disease conditions, most prominently with diabetes.

The ability of the body to buffer the concentration of acetone is a function of both the chemical's physicochemical properties and the body's biological capabilities. These include the absorption, metabolism, and excretion components of pharmacokinetics. The target organs are the kidney, liver, and blood. Several studies in humans and animals indicate that acetone has short persistence in the bloodstream. Following exposure, acetone is rapidly absorbed and distributed throughout the body and is rapidly lost.

The pharmacokinetics of acetone are interesting in that 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 during periods of stress, the body retains acetone, as it serves as an essential component for gluconeogenesis, whereas under conditions of high levels of exposure the body is capable of ridding itself of excess acetone. This is evident in the absorption, metabolism, and elimination kinetics of acetone. Acetone has a high $K_{B/A}$, which would indicate a high capacity for absorbing exogenous acetone vapors during inhalation and contributing to the retention of acetone in the blood stream. However, under high levels of exposure only about 45% of the inhaled acetone is actually entrained 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 almost all of the ingested acetone is absorbed into the body (Haggard et al., 1944; Price and Rittenberg, 1950).

Metabolism affords another mechanism for buffering the amount of acetone in the body. As proposed by Casazza et al. (1984), and based on studies in rodents, two metabolic pathways for 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 is extrahepatic and proceeds through the formation of 1,2-propanediol. Studies by Kosugi (1986a,b) indicate that the methylglyoxal pathway predominates at low acetone concentrations and serves as an efficient mechanism for gluconeogenesis. At higher concentrations more of the acetone appears to be 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 of more readily removing acetone from the body.

In other assessments of acetone (ATSDR, 1994; WHO, 1998) the metabolic pathway for acetone catabolism is proposed to have three 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 production of formic acid. Formic acid is the chemical that is most 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 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 that leads to 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 (Liesivuori and Savolainen, 1991).

Data supporting the formation of formic acid as a product of acetone metablism are sparse. The only mention of formic acid appearing in urine following treatment is reported by Hallier et al. (1981), who found that rats placed in a desiccator that was saturated with acetone demonstrated a small (4.7% of the absorbed acetone) but detectable amount of formate in the urine. However, rodents are more efficient than humans at metabolizing formate. Therefore, it would be more difficult to isolate formate in a rodent study than in a human study. One would also be less likely to see adverse toxic effects from exposure to formate in rodents than in humans (Liesivuori and Savolainen, 1991). In a recent review of the literature on the metabolism of acetone, Kalapos (1999) indicates that to date there is no identified enzyme system that mediates the formation of formate from acetone. Furthermore, toxic effects noted in humans and laboratory animals are inconsistent with those of formic acid, in which the most sensitive endpoint is impaired vision. Effects noted from exposure to acetone are not consistent with those noted with formic acid or with methanol, which is metabolized to formic acid.

Methylglyoxal is á-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 gluconeogenesis by an undetermined route. Methylglyoxal has been shown to have cytotoxic effects at levels higher than normally seen in the body. These include genotoxicity (Barnett and Munoz, 1998), depletion of glutathione (Ankrah and Appiah-Opong, 1999), and the induction of apoptosis (Thornalley, 1998). Methylglyoxal has been shown to induce sex-linked recessive lethal responses in *Drosophila melanogaster* germinal cells, but 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. 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 in human leukemia cells and induce apoptosis (Kang et al., 1996). This activity has led to the testing of methylglyoxal as an anticancer drug (Thornalley, 1996).

A third mechanism that buffers the concentration of acetone in the body occurs through excretion. The fate of acetone in the body appears to be dose related. Data on humans indicate that acetone at low levels is largely retained within the system, whereas at higher levels nonmetabolized acetone is detected in the expired air. Exposure to acetone at ambient air concentrations below 15 ppm produces no detectable acetone in the urine, whereas at higher concentrations acetone is found at about 1% of the blood plasma concentrations. At significantly higher concentrations some studies have found that relative amounts 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 by the body.

Based on laboratory studies and human data, there are four endpoints that have been found in association with exposure to acetone either by inhalation or ingestion: neurotoxicity, hematology, nephrotoxicity, and male reproductive effects. The neurotoxic effects appear to be both mild and transient at the levels to which the human subjects were exposed or animals were tested. The effects noted in humans are largely subjective and include eye and nose irritation, mood swings and, at higher levels, nausea. Effects in humans generally appear during exposure to concentrations at 500 ppm (Matsushita et al., 1969; Dick et al., 1988, 1989; Satoh et al., 1996). The fact that the effects appear during exposure and dissipate following cessation of exposure indicates that the effects are the result of the parent material and not a metabolite. Animal data are consistent with the effects noted in humans. Although there are some studies that indicate impaired learning or response following inhalation exposure in rodents (CMA, 1997), overall the effects appear to be transient. Data on the neurotoxic effects of acetone are limited to inhalation exposure.

The most extensive studies conducted on acetone are a drinking water study and a gavage study. The effects noted in these studies include hematology, nephrology, and reproductive effects. Both rats and mice in the NTP (1991) study had higher levels of hemaglobin, lower red blood cell count (male rats only), and higher mean cell volume at the highest concentrations. These conditions are symptomatic of megaloblastic anemia. When this condition is seen in humans, it is associated with folic acid and vitamin B_{12} deficiencies. The authors suggest that comparable effects may reflect parallel metabolism. The authors state that hematological effects are of questionable biological significance.

Nephrotoxicity was noted in both the gavage and drinking water studies. In both cases the effects are considered mild. However, the effect occurred at a lower dose and was more pronounced in the gavage study. Also in the gavage study, Sonawane et al. (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 divert a larger concentration of acetone to the kidney, thereby causing the noted effect at lower concentrations. This is not inconsistent with the interpretation offered by the authors of the NTP 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, in turn leading to the burdened glomeruli becoming sclerotic (NTP, 1991). This effect could also be excerbated by lower water consumption than was seen with the higher acetone concentrations.

Male reproductive effects were also identified in the rodent studies. These included testicular lesions, higher relative testis weight, reduced sperm motility, and more abnormal sperm at the higher doses compared with controls. Studies on the distribution of acetone indicate that radiolabeled material reaches the testis (Wigaeus, 1982), but there is no evidence whether this effect is related to acetone or a metabolite. It is also interesting to note that most reproductive toxicants decrease the size of the testis. Increase in the relative weight of the testis in the high-dose treatment may be an artifact of the decreased weight of the whole animal. In general, malformed sperm may indicate that a particular chemical is genotoxic; however, acetone has been shown to be almost uniformly negative in all

genotoxicity studies. As noted above, methylglyoxal has been shown to be cytotoxic and genotoxic. Nonetheless, despite these findings, breeding studies in rats have not demonstrated differences in the ability of exposed males to breed or in effects to the offspring. The one study reporting reproductive effects in humans is poorly conducted and inconclusive.

It is interesting to note that the temporal and distributional aspects of effects noted in the various studies seem to track the distribution, retention, and metabolism of acetone. Neurotoxic effects noted in several human and animal studies are minor and short lived, which indicates that the parent compound, and not a metabolite, is the causative agent. 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. In turn, at high levels of ingestion, systemic effects were found in the liver, kidney, blood, and testis, with related male reproductive effects in rodents.

Casazza et al. (1984) proposes that a significant amount of acetone metabolism occurs in the liver. Among the liver effects is hypertrophy, which is consistent with induction of liver enzyme systems. Similar effects are noted with chronic alcoholism and are believed to reflect an adaptive response to chronic ethanol exposure. Casazza et al.(1984) noted that a second metabolic pathway, which the work of Gavino et al. (1987) indicates is secondary, occurs via the formation of 1,2-propanediol. The metabolism of acetone via this pathway appears to occur largely in the kidney. Adverse effects at high levels include nephrotoxic effects on the glomeruli. The authors of the study note that nephropathy is a long-term progressive condition that arises spontaneously in rats of both genders, but administration of acetone may accelerate this condition. Authors of the NTP study attribute this to the formation of formate as a metabolite of acetone (NTP, 1991). However, although kidney effects have been noted with formic acid administration (Naik et al., 1980; Rajan et al., 1985) the most sensitive endpoint attributed to formate production as a metabolite is impaired vision, which is seen with methanol consumption. To date, there are no studies that identify amblyopia or other ocular effects as arising from acetone exposure.

In comparison with the drinking water study, the effects that are evident occur at lower concentrations and are more pronounced following gavaging than with a more temperate drinking water application. The bolus effect that comes with gavage administration, particularly with the speed of acetone absorption, would be expected to result in a greater burden on the kidneys, both on the metabolism of acetone and the excretion of both nonmetabolized acetone and its metabolites. In humans with uncontrolled diabetes mellitus, renal disease is one of the more serious effects. However, the mechanism behind this pathology is unknown.

In inhalation studies, Bruckner and Peterson (1981a) found that male Sprague-Dawley rats exposed to 19,000 ppm of acetone vapor for 8 weeks had an increase in kidney weight from treated animals compared with controls at 4 weeks but not at 8 weeks. The authors observed no microscopic lesions on the kidneys, liver, heart, or brain of the acetone-treated animals. The differences in kidney weight observed at 4 weeks are consistent with studies on oral administration. However, the absence of effects at 8 weeks suggests an adaptive response to acetone, which is also consistent with oral

studies. Also, in comparison with the NTP (1991) study, the Bruckner and Peterson (1981a) study suggests that inhalation exposure may provide an improved mechanism for controlling the effects of acetone exposure, thereby avoiding the kidney lesions that were seen with drinking water (NTP, 1991; Dietz, 1991) and gavage (Sonawane et al., 1986) studies. This would be consistent with the lower level of absorption of acetone via the inhalation route compared with ingestion.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

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. In an epidemiological study of workers in a cellulose acetate plant where the workers were exposed to acetone concentrations of 380-1070 ppm (time-weighted average) depending on job category (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. Although a chronic bioassay has not been conducted utilizing oral or inhalation exposure routes, the chemical has been used as a solvent/vehicle control in dermal studies in animals (NTP, 1991, 1995, 1997); however, without a naive control the ability to determine background incidence of cancer is limited. Genotoxicity studies are almost uniformly negative. A review of data on cancer studies for small, saturated ketones has failed to identify any chemicals of a structure analogous to acetone that have been shown to be carcinogenic to humans or rodents by any route of administration, or that could be used to demonstrate a mechanism of action for carcinogenicity.

Under the current Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1987a), the weight of evidence for carcinogenicity from animal and human studies are classified as Group D-*not classifiable as to human carcinogenicity.* This is based on the dearth of studies necessary for evaluating the potential carcinogenicity of acetone. There are no chronic animal studies on acetone, and only one epidemiological study which, as noted above, has limitations. When the totality of available data are considered as in the narrative above, according to the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), acetone's potential for human carcinogenicity may best be described as "*cannot be determined*" on the basis of inadequate data to perform an assessment. This weight-of-evidence determination is based on the availability of one human study of limited utility, no chronic animal studies, and limited availability of additional information such as structural analogues with known carcinogenic potential. Acetone has tested negative in almost all genotoxicity studies.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

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). On the basis of this study alone no conclusions based on the susceptibility of different age groups can be drawn.

4.7.2. Possible Gender Differences

No differences in uptake or retention have been found between men and women (ATSDR, 1994; WHO, 1998). Experimental studies have not reported differences between men and women; however, in animal studies, male rats were more susceptible than females. The significance of this to humans is questionable.

4.7.3. Possible Sensitive Populations

People with conditions that compromise the ability to buffer the amount of acetone in the body may be particularly sensitive to acetone exposure. These would include individuals with diabetes mellitus or chronic alcoholism.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

The key study identified for derivation of the oral RfD is the subchronic drinking water study (NTP, 1991; Dietz et al., 1991). Acetone was administered in the drinking water of mice and rats for 13 weeks. Male rats appeared to be the most sensitive species, with the testes, kidney, and hematopoietic system identified as target organs. Critical effects at 1,700 mg/kg/day included early-onset and enhanced progression of nephropathy and effects consistent with macrocytic normochromic anemia with a depressed regenerative response. 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.

In another subchronic study, acetone was administered by gavage to male and female rats (American Biogenics Corp., 1986; Sonawane et al., 1986). Based on organ weight changes and kidney lesions in both sexes, the LOAEL was 500 mg/kg/day and the NOAEL was 100 mg/kg/day. No changes in clinical chemistry parameters indicative of kidney damage were found in this study, but these endpoints were not measured in the drinking water study (NTP, 1991; Dietz et al., 1991). The approximately threefold lower values for the gavage study as compared to the drinking water study are probably due to differences manifested from bolus dosing versus more continuous exposure. However, the gavage study supports the drinking water study in that the kidney was a target organ in both studies.

The current RfD (0.1 mg/kg/d) is based on the gavage study by Sonawane et al. (1986). The administered doses were 0, 100, 500, or 2,500 mg/kg/d. The critical effect noted was kidney

pathology, and the NOAEL was 100 mg/kg/d. The RfD invoked uncertainty values of 10 for intraspecific and interspecific extrapolation, and for extrapolation from a subchronic to a chronic exposure scenario. Although the effect level noted in the gavage study is more conservative, the drinking water route more closely mimics potential long-term human exposure scenarios.

5.1.2. Methods of Analysis

The data were analyzed using the NOAEL/LOAEL approach. Standard uncertainty and modifying factors were applied. Based on the NTP study (NTP, 1991; Dietz et al., 1991), the critical effects are kidney and testes lesions and changes in hematology. The LOAEL is 1,700 mg/kg/day and the NOAEL is 900 mg/kg/day. These values were calculated as time-weighted average doses based on body weights, water consumption, and nominal drinking water concentrations.

NTP in its summary determined that the most sensitive animal among those tested was the male rat and that the critical effects were to the kidney, the blood, and testis. A BMD_{10} was not used for the following reasons. First was the gradation of the effect. The basis for the determination was the rapid progression of the nephropathy to the mild stage, which was negative or minimal for doses below 1,700 mg/kg/d and positive for 9 of 10 rats at and above 1,700, the next higher dose. Second, with these data the chi-square analysis for the BMD indicated a poor fit of the model to the data ($p < 0.008$). Third, an analysis of a BMD_{10} provided a value that fell below the NOAEL. Accordingly, the data from the principal study are not amenable to BMD analysis.

5.1.3. Oral Reference Dose Derivation

A reference dose was estimated from the NOAEL of 900 mg/kg/day, an uncertainty factor (UF) of 3,000 and a modifying factor (MF) of 1 (U.S. EPA, 1993). Uncertainty factors of 10 each were applied for intraspecific extrapolation to account for sensitive members of the population, for a subchronic to a chronic exposure extrapolation, and to account for database insufficiencies. The intraspecific extrapolation was applied to account for members of the population who might be particularly sensitive to acetone. This might apply to individuals who are producing elevated levels of endogenous acetone because of stress or uncontrolled diabetes that might increase the body burden. The uncertainty factor for a subchronic to a chronic study extrapolation addresses certain effects that appear in the subchronic study that might be more pronounced with prolonged exposure. The uncertainty factor applied for database insufficiencies addresses the absence of chronic, reproductive, and developmental studies. A partial UF of 3 ($10^{½}$) was used for interspecific extrapolation because the lesions were transient (liver), of questionable biological significance to humans (kidney), not severe enough to adversely affect the health of the animals clinically (kidney, hematopoietic, testicular), and/or not supported by clinical chemistry abnormalities (kidney). The RfD is based on a NOAEL, which obviates the need for an additional UF:

$$RfD = NOAEL \div UF$$
$$900 \text{ mg/kg/day} \div 3000 = 0.3 \text{ mg/kg/day}$$

The value generated in this assessment is slightly higher than the previous RfD (0.1 mg/kg/d). This difference is accounted for by a change in the principal study and the inclusion of an additional uncertainty factor that was not applied in the previous assessment for database insufficiency.

The RfD is based on the NTP study (1991; Dietz et al., 1991), in which male and female rats and mice were exposed to acetone in the drinking water for 13 weeks. The previous IRIS assessment was based on a study in which male and female rats were administered acetone by gavage for 90 days (American Biogenics Corp., 1986; Sonawane et al., 1986).

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

No principal study was identified for inhalation exposure.

5.2.2. Methods of Analysis

Not applicable.

5.2.3. Inhalation Reference Concentration Derivation

Not applicable.

5.3. CANCER ASSESSMENT

Studies necessary to support an assessment of the carcinogenicity of acetone in humans are not available. There is one epidemiological study published in the literature that 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 published lifetime cancer studies on animals via either the inhalation or ingestion route. Acetone has been used as a control/vehicle for several dermal studies in animals (NTP 1991, 1995, 1997); however, in the absence of a naive control the data are not adequate to evaluate the potential for the generation of cancer. Anecdotal evidence suggests that the potential for carcinogenicity of acetone may be low. There are no cancer studies in which the low-molecular-weight saturated ketones are shown to be carcinogenic (based on searches of NTP and IARC databases conducted in 2000). Genotoxicity studies with acetone are almost uniformly negative.

6. MAJOR CONCLUSIONS IN CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HAZARD POTENTIAL

Acetone is used industrially as a solvent and feedstock; biologically it serves a vital role in human metabolism. 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 the human body acetone is formed endogenously under conditions of starvation, uncontrolled diabetes, or exertion as a means of supplying glucose to tissues that are incapable of metabolizing fatty acids. Acetone is miscible in water and highly volatile, which potentially contributes to both inhalation and ingestion exposure.

Exogenous acetone is readily absorbed via inhalation, ingestion, and dermally. 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 becomes more active and nonmetabolized acetone is lost through the urine. Early work in 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.

Data on the toxicity of acetone are limited. Human studies have been conducted on both volunteers and occupationally exposed individuals. These studies have examined, almost exclusively, either the pharmacokinetics or neurological effects of acetone. Although studies on humans indicate that acetone is readily absorbed via the lungs and gastrointestinal tract, the effects appear to be mild and transient. Clinical studies and case reports suggest slight neurological effects, mostly of the subjective type, on individuals exposed to varying concentrations of acetone. In most studies the subjects report discomfort, irritation, mood swings, and nausea following exposure to acetone vapor at concentrations of 500 ppm or higher. The fact that these effects subside following termination of exposure indicates that acetone, and not a metabolite, is the active compound. Clinical chemistries conducted in several studies demonstrated no exposure-related effects. Data on nerve conductivity are inconclusive. Case reports of accidental poisoning also indicate that effects, which include lethargy and drowsiness, are short-lived.

Treatment of rodents with acetone have identified no significant health effects. The male rat appears to be the most sensitive laboratory animal to the effects of orally administered acetone. Subchronic oral exposure resulted in lesions of the kidney, testis, and hematopoietic system; however, these effects were mild. Transient effects were seen in male rats exposed to acetone by inhalation. Inhalation exposure to pregnant rats and mice did not cause significant malformations in the offspring, but did result in lower fetal body weights in both species. The effects noted in rodents tended to occur in male rats and at high levels of exposure (20,000 and 50,000 ppm in drinking water), although in the absence of clinical chemistries and other indicators, the significance of these effects is questionable. Those studies that included measures of clinical chemistries in animals and humans found no treatment-related effects. Therefore, the relevance of effects seen in rat studies to humans is uncertain.

Effects noted in the drinking water and gavage studies were not evident in a subchronic inhalation study. Bruckner and Peterson (1981a) exposed male Sprague-Dawley rats to 19,000 ppm

for 3 hours/day, 5 days/week, for 8 weeks and noted that differences in kidney weights at 4 weeks were not apparent at 8 weeks. The authors noted no kidney lesions in the acetone-treated group.

Developmental toxicity studies in mice and rats (Mast et al., 1988) produced reduced body weights in fetuses in rats, but there was no statistically significant increase in incidence of malformation or number of live births. In mice there was a slight but significant increase in the number of resorptions at the highest level of exposure tested but no change in the number of live births. As such the significance of the effects is uncertain.

There are no studies that address the issue of acetone and cancer. The one epidemiological study that is available has several limitations. There are no lifetime animal studies.

6.2. DOSE RESPONSE

Quantitative estimates of human risk as a result of exposure to acetone are based on laboratory animal exposures because no human data are available. The reference dose of ingested acetone (RfD) is 0.3 mg/kg/day. The RfD is based on a subchronic oral study in the rat (NTP, 1991; Dietz et al., 1991). 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 database, however, is low because no chronic, developmental, or reproductive oral studies are available.

The human chronic concentration of inhaled acetone (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 database 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 database deficiency. Testicular lesions were observed in male rats following oral exposure, an increase in abnormal sperm was seen in male workers, and a premature menstrual period occurred in women exposed experimentally by inhalation. The significance of these endpoints of reproductive toxicity in men and women is unknown at this time.

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