CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Information on the toxicokinetics of acetone are available from studies of humans and animals.

- Following exposure from exogenous sources, acetone is rapidly and passively absorbed from the lungs and gastrointestinal tract. Acetone can also be absorbed through the skin.
- Acetone is highly water soluble and is widely distributed to tissues and organs throughout the body, especially to tissues with high water content. It is not likely to accumulate with repeated exposure. Acetone can also undergo transplacental transfer to the fetus. In addition, acetone is distributed to mother's milk, and represents a source of excretion from the mother and exposure for infants.
- Metabolism of acetone is independent of route of exposure and similar in humans and animals. It involves three separate gluconeogenic pathways, with ultimate incorporation of carbon atoms into glucose and other products of intermediary metabolism, with generation of carbon dioxide. Metabolism takes place primarily in the liver. Physiological status, such as diabetes and fasting, and genetic predisposition for obesity can alter the pattern of metabolism.
- The main route of excretion is via the lungs regardless of the route of exposure with very little excreted in the urine. Acetone is excreted both unchanged and, following metabolism, mainly as carbon dioxide. Elimination is generally complete in 48–72 hours after the last exposure, depending on the exposure concentration, duration, and factors such as biological sex and level of physical activity.

Although the focus of this profile is on the effects of exposure to acetone from exogenous sources, a full understanding of the toxicokinetics requires consideration of the metabolic fate of endogenous acetone. Acetone is one of three ketone bodies that occurs naturally throughout the body (Le Baron 1982; Vance 1984). Under normal conditions, the production of ketone bodies occurs almost entirely within the liver and to a smaller extent in the lung and kidney (Gavino et al. 1987; Le Baron 1982; Vance 1984). The process is continuous, and the three products are excreted into the blood and transported to all tissues and organs of the body where they can be used as a source of energy. Two of these ketone bodies, acetoacetate and β-hydroxybutyrate, are organic acids that can cause metabolic acidosis when produced in large amounts. Acetone, in contrast, is nonionic and is derived endogenously from the spontaneous and enzymatic breakdown of acetoacetate (Dabek et al. 2020; Kimura et al. 1986; Koorevaar and Van Stekelenburg 1976; Lopez-Soriano and Argiles 1985; Lopez-Soriano et al. 1985; Reichard et al. 1979; Van Stekelenburg and Koorevaar 1972). Endogenous acetone is eliminated from the body either by excretion into urine and exhaled air or by enzymatic metabolism (Charbonneau et al. 1986c; Haggard et al. 1944; Owen et al. 1982; Reichard et al. 1986; Wigaeus et al. 1981). Under normal circumstances,

metabolism is the predominant route of elimination for endogenous acetone and handles 70–80% of the total body burden.

Levels of endogenous acetone can fluctuate greatly due to normal diurnal variations (Wildenhoff 1972). In addition, circulating levels of endogenous acetone can fluctuate greatly depending on a person's age (Paterson et al. 1967; Peden 1964), nutritional status and fasting (Jones 1987; Kundu et al. 1993; Levy et al. 1973; Lewis et al. 1977; Neiman et al. 1987; Reichard et al. 1979; Rooth and Carlstrom 1970; Williamson and Whitelaw 1978), and degree of physical activity (Koeslag et al. 1980). These physiological states all place high energy demands upon the body, which result in increased fatty acid utilization and higher-than-normal blood levels of acetone. Infants and young children typically have higher acetone blood levels than adults due to their higher energy expenditure (Peden 1964). Pregnancy and lactation can also lead to higher-than-average blood levels of acetone (Bruss 1989; Paterson et al. 1967). In addition to these normal physiological conditions, a number of clinical states can result in acetonemia and/or acetonuria in humans. In each of these conditions, the ketosis can be traced to the increased mobilization and utilization of free fatty acids by the liver. The conditions include diabetes (Kobayashi et al. 1983; Levey et al. 1964; Reichard et al. 1986; Rooth 1967; Rooth and Ostenson 1966), trauma (Smith et al. 1975), and alcohol use disorder (Phillips et al. 1989; Tsukamoto et al. 1991).

3.1.1 Absorption

Studies of inhalation exposures in humans indicate that, due to its high blood-air partition coefficient (167–330), acetone is rapidly and passively taken up by the respiratory tract and absorbed into the bloodstream (Fiserova-Bergerova and Diaz 1986; Haggard et al. 1944; Paterson and Mackay 1989; Sato and Nakajima 1979). Experiments in humans exposed to 23–4,607 ppm for up to 4 hours have measured pulmonary uptakes ranging from ≈30 to 80% (DiVincenzo et al. 1973; Landahl and Herrmann 1950; Nomiyama and Nomiyama 1974a; Pezzagno et al. 1986; Wigaeus et al. 1981). The reason for the wide range in reported values involves the unique aqueous wash-in/wash-out effect when acetone is inhaled, which can lead to spurious results (Schrikker et al. 1985, 1989). During this phenomenon, acetone, which is highly water soluble, will dissolve in epithelial cells during inspiration (wash-in) and evaporate during expiration (wash-out). This could account for the lower than expected pulmonary absorption based on the high blood/air partition coefficient (Johanson 1991; Wigaeus et al. 1981). Exhaled breath levels of acetone in humans rose during exposure and reached steady-state within ≈2 hours during exposure to concentrations between 125 and 250 ppm (Brown et al. 1987; Nomiyama and Nomiyama 1974a).

Uptake and absorption of acetone in humans following inhalation exposures depends on factors such as concentration, duration, and level of physical activity. Uptake was directly proportional to exposure concentration and duration (DiVincenzo et al. 1973; Wigaeus et al. 1981). Uptake also increased as the level of physical activity increased, due to increased pulmonary ventilation (DiVincenzo et al. 1973; Haggard et al. 1944; Jakubowski and Wieczorek 1988; Wigaeus et al. 1981). Lungs (including the mouth and trachea) retained a greater percentage of inspired acetone (55%) than the nasal cavity (18%) in humans, indicating that the nasal cavity absorbs acetone less readily than the rest of the respiratory system (Landahl and Herrmann 1950). Blood levels of acetone rose rapidly during exposure for up to 4 hours with no indication that steady-state was reached (Brown et al. 1987; Dick et al. 1989; DiVincenzo et al. 1973), suggesting that during exposure, the rate of absorption exceeded the rate of distribution and elimination. In humans exposed to 100 or 500 ppm acetone for 2 or 4 hours, 75–80% of the amount of acetone inspired was absorbed by blood after 15 minutes of exposure, and 20–25% remained in the dead space volume (DiVincenzo et al. 1973). Higher inspired amounts resulted in higher blood levels (DiVincenzo et al. 1973; Haggard et al. 1944; Matsushita et al. 1969a; Pezzagno et al. 1986). A correlation between blood level at the end of exposure and exposure concentration was found in humans exposed to 23–208 ppm for 2–4 hours (Pezzagno et al. 1986). No significant difference in uptake or retention was found between men and women (Brown et al. 1987). External temperature appears to alter absorption of inhaled acetone. An experimental study in five human subjects showed that for a constant air concentration of acetone (495 ppm), blood levels of acetone increased as the external temperature increased from 21 to 30°C (Marchand et al. 2021). After a 4-hour exposure at 21°C, the mean venous blood acetone concentration was 22.14 mg/L, compared to 27.65 ppm at 30°C, an increase of approximately 25%. The study authors suggested that the increased venous blood concentrations at higher temperatures was due to physiological changes associated with thermoregulation.

Studies on absorption of acetone following oral exposure in humans are limited but likewise indicate high levels of absorption, as with inhalation exposure. In a series of experiments conducted in male volunteers given acetone orally at 40–80 mg/kg, an estimated 65–93% of the administered dose was eliminated via metabolism, with the remainder excreted in the urine and expired air in about 2 hours, indicating rapid and extensive gastrointestinal absorption (Haggard et al. 1944). In a human who ingested 137 mg/kg acetone on an empty stomach, the blood level of acetone rose sharply to a peak 10 minutes after dosing (Widmark 1919). In other experiments, the subject ingested the same dose 10 or 12 minutes after eating porridge. The blood acetone level rose slowly over 48–59 minutes to levels of about one-half to two-thirds of that achieved after taking acetone on an empty stomach. Thus, the presence of food in the gastrointestinal tract may lead to a slower rate of absorption.

Measurement of acetone in blood and urine of patients who accidentally or intentionally ingested acetone indicated that acetone was absorbed, but the percentage absorbed cannot be determined from the data. In one case, a man ingested liquid cement that provided an approximate dose of acetone of 231 mg/kg (Sakata et al. 1989). His plasma acetone level was about 110 μg/mL and his urinary level was 123 μg/mL 5 hours after ingestion, but he had been subjected to gastric lavage. In another case, a woman who had ingested nail polish remover had a blood acetone level of 0.25 g/100 mL (2.5 mg/mL) upon admission to the hospital (Ramu et al. 1978). The authors estimated that her body burden was 150 g acetone at the time of admission. The serum acetone level of a 30-month-old child was 445 mg/100 mL (4.45 mg/mL) 1 hour after ingestion of a 6-ounce bottle of nail polish remover (65% acetone) (Gamis and Wasserman 1988).

Dermal absorption of acetone has also been demonstrated in humans. Application of cotton soaked in acetone to a 12.5 cm^2 uncovered area of skin of volunteers for 2 hours/day for 4 days resulted in blood levels of acetone of 5–12 μg/mL, alveolar air levels of 5–12 ppm, and urinary concentrations of 8–14 μg/mL on each day (Fukabori et al. 1979). Higher blood, alveolar air, and urinary levels were obtained when the daily exposure increased to 4 hour/day: 26–44 μg/mL in blood, 25–34 ppm in alveolar air, and 29–41 μg/mL in urine. The absorption was fairly rapid, with peak blood levels appearing at the end of each daily application. Although precautions were taken to limit inhalation of acetone vapors, the study authors noted that it was not possible to completely prevent inhalation, and the acetone concentration in the breathing zone of one subject was found to be 0.4–0.6 ppm. From the alveolar air and urine concentrations, it was estimated that a 2-hour dermal exposure over 12.5 cm^2 of skin was equivalent to a 2-hour inhalation exposure to 50–150 ppm, and a 4-hour dermal exposure was equivalent to a 2-hour inhalation exposure to 250–500 ppm acetone.

Similar to humans, animals also absorb acetone rapidly during inhalation exposure. Measurement of blood acetone levels in 13-week-old male and female rats after 4–6 hours of exposure to various concentrations shows that blood levels correlate well with exposure concentrations (Charbonneau et al. 1986a, 1991; NTP 1988) and are highest immediately after exposure (NTP 1988). In male rats exposed to 1.50 ppm for 0.5–4 hours, measurement of blood acetone concentrations during exposure revealed that blood levels increased steadily for 2 hours and then remained constant for the next 2 hours of exposure (Geller et al. 1979b). Blood acetone levels also correlated well with exposure concentration in dogs exposed for 2 hours (DiVincenzo et al. 1973). Blood levels were 4, 12, and 25 mg/L after exposures to 100, 500, and 1,000 ppm, respectively. Comparison of uptakes in dogs and humans revealed that humans

absorbed a greater absolute quantity under comparable exposure conditions, but when expressed in terms of kg body weight, male and female dogs absorbed 5 times more than humans. In anesthetized dogs allowed to inhale concentrated vapors of acetone spontaneously from a respirator at various ventilation rates, uptake by the respiratory tract was 52% at flow rates of 5–18 L/minute and 42% at ventilation rates of 21–44 L/minute (Egle 1973). Retention in the lower respiratory tract was 48% at 4–18 L/minute and 37.5% at 21–50 L/minute. Retention by the upper respiratory tract was 57% at 4–18 L/minute. The effect of exposure concentration on total uptake was studied at a range of ventilation rates equated with exposure concentrations. Percent uptakes were 52.1% at a mean concentration of 212 ppm, 52.9% at 283 ppm, and 58.7% at 654 ppm. These results indicate the respiratory uptake of acetone by dogs is similar to human uptake values reported by Landahl and Herrmann (1950). The retention in the upper respiratory tract was higher than in the lower respiratory tract of dogs (Egle 1973). Exposure concentration had little effect on retention. The absorption of acetone by the nasal walls of anesthetized dogs, in which the nasal passage was isolated, increased when the airflow rate was increased (Aharonson et al. 1974). This suggests that increased airflow decreases the proportion of acetone that reaches the lungs.

In rats exposed continuously to 2,210 ppm for 9 days, peak acetone blood levels of approximately 1,020–1,050 mg/L were reached in 3–4 days and remained at this level for the duration of exposure (Haggard et al. 1944). In rats exposed to 4,294 ppm for 12 days, acetone blood levels plateaued at 2,420–2,500 mg/L in 4 days. Blood levels in rats exposed to these concentrations for 8 hours/day were about half of those reached during continuous exposure. The amount of acetone absorbed in the first 8 hours exceeded the amount eliminated in the next 16 hours of exposure to fresh air, leading to a small accumulation. However, the accumulation during intermittent exposure did not reach the levels achieved during continuous exposure. In other experiments of rats exposed to 2,105–126,291 ppm, the time to peak blood level decreased as the exposure concentration increased (Haggard et al. 1944).

As was found in humans (Landahl and Herrmann 1950) and dogs (Egle 1973), disposition of acetone in the upper respiratory tract of rats, mice, guinea pigs, and hamsters indicates that relatively little acetone is absorbed from the upper respiratory tract (Morris 1991; Morris and Cavanagh 1986, 1987; Morris et al. 1986, 1991). The deposition efficiency was greater in Sprague-Dawley rats than in Fischer-344 rats. Deposition was similar in B6C3Fl mice and Fischer-344 rats, and greater than in Hartley guinea pigs and Syrian golden hamster. No difference was found between male and female Sprague-Dawley rats (Morris et al. 1991). The differences among strains and species could not be attributed to differences in metabolism because acetone is not significantly metabolized in the upper respiratory tract of these species

(Morris 1991). Rather, the difference was attributed to differences in upper respiratory tract perfusion rates (Morris 1991; Morris and Cavanagh 1987).

Experiments in rats indicated that acetone is rapidly and almost completely absorbed from the gastrointestinal tract after oral exposure. No studies were located regarding absorption of acetone in other animal species after oral exposure to acetone. A rat given ^{14}C-acetone at a dose of 1.16 mg/kg expired 47.4% of the dose as ^{14}C-carbon dioxide over the 13.5-hour collection period (Price and Rittenberg 1950). Another rat given about 7.11 mg/kg ^{14}C-acetone by gavage once a day for 7 days expired 67–76% of the administered radioactivity as ^{14}C-carbon dioxide and 7% as ^{14}C-acetone over a 24-hour period after the last dose. From these data, absorption of least 74–83% of the administered dose can be inferred. A rat dosed with 6.19 mg/kg ^{14}C-acetone expired 4.24% of the radiolabel as unchanged ^{14}C-acetone over 5.5 hours, indicating rapid absorption. In rats given a single gavage dose of 1,177 mg/kg acetone, the maximum blood level of 850 µg/mL was reached in 1 hour and declined gradually to about 10 µg/mL over 30 hours (Plaa et al. 1982). In another experiment, peak blood levels and the time to peak blood levels were compared after various gavage doses to rats. After a dose of 78.44 mg/kg, the maximum blood level of acetone of about 200 µg/mL was reached in 3 hours and declined to 10 µg/mL at 12 hours, where it remained for the next 12 hours. After a dose of 196.1 mg/kg, the peak blood level was 400 µg/mL at 6 hours and declined biphasically to 50 µg/mL at 12 hours and to 30 µg/mL at 18 hours where it remained for the next 6 hours. After a dose of 784.4 mg/kg, the peak level was 900 µg/mL at 1 hour and declined to 300 µg/mL at 12 hours, 110 µg/mL at 18 hours, and 50 µg/mL at 24 hours. After a dose of 1,961 mg/kg, the peak level was 1,900 µg/mL at 3 hours and declined slowly to 400 µg/mL at 24 hours. In other studies where rats were given similar or higher doses of acetone, plasma acetone levels rose proportionately with dose in rats given acetone as single doses by gavage (Charbonneau et al. 1986a; Lewis et al. 1984) or in the drinking water for 7 days (Skutches et al. 1990).

In a study comparing blood levels of acetone in fasting male rats to those in male rats that received oral doses of acetone, peak blood levels of acetone of about 35 and 110 µg /mL were reached within about 3 hours after dosing of rats with 78 and 196 mg/kg acetone, respectively (Miller and Yang 1984). The levels returned to background levels within the next 16 hours. At an acetone dose of 20 mg/kg, the blood level increased to about 5 µg/mL over 19 hours, when the rats were sacrificed. In rats fasted for 48 hours, blood acetone levels increased continuously to about 13 µg/mL. While the maximal blood concentrations of the treated rats differed considerably from that of the fasting group, the areas under the curve for the 78 and 196 mg/kg groups were comparable to the fasting groups.

Conflicting data were located regarding the effect of vehicle on the gastrointestinal absorption of acetone. In one study, maximum blood levels were higher and achieved earlier in male rats given acetone by gavage in water than in rats given acetone by gavage in corn oil (Charbonneau et al. 1986a). The slower absorption of acetone in corn oil may have resulted from a delayed gastric emptying due to the presence of corn oil (fat) in the stomach. In a later study, however, very little difference in blood and liver levels of acetone were found in male rats given the same dose of acetone in water or in corn oil (Charbonneau et al. 1991).

There are little data regarding the absorption of acetone in animals after dermal exposure. One study reported a permeability coefficient (K_p) for acetone of 0.00249 cm-hour^{-1} when administered to the skin of newly deceased piglets (Schenk et al. 2018). The findings of cataract formation in guinea pigs exposed dermally (Rengstorff and Khafagy 1985; Rengstorff et al. 1972) (see Section 2.12) indicated that acetone was absorbed from the skin of the guinea pigs.

3.1.2 Distribution

There are limited data regarding distribution of acetone or its metabolites in humans. Biomonitoring conducted in workers at a plastics factory found similar regression slopes between air concentrations or acetone and its levels in serum, whole blood, and urine, indicating that acetone is evenly distributed throughout the body (Mizunuma et al. 1993). Acetone was detected in cerebrospinal fluid in a 55-year-old man who ingested 1 L of acetone (Gregoire et al. 2018). In addition, acetone is well absorbed into the blood from the respiratory and gastrointestinal tract of humans (see Section 3.1.1) and is highly water soluble. Therefore, widespread distribution, especially to tissues with high water content, is expected.

Biomonitoring studies in humans indicate that maternal-fetal transfer and maternal-infant transfer of acetone occur. Acetone was identified in maternal and cord blood collected at the time of delivery, indicating transplacental transfer (Dowty et al. 1976). Of eight samples of breast milk from lactating women from four urban areas, all were found to contain acetone (Pellizzari et al. 1982). Whether the source of acetone was endogenous or exogenous could not be determined. Nevertheless, the data indicate that acetone is distributed to mother's milk, and represents a source of excretion from the mother and exposure for infants.

The distribution of acetone has been studied in mice exposed to acetone by inhalation (Wigaeus et al. 1982). Mice were exposed to 500 ppm ^{14}C-acetone for 1, 3, 6, 12, and 24 hours or for 6 hours/day for 1,

3, or 5 consecutive days, after which they were immediately killed. Radioactive unmetabolized acetone and total radioactivity were found in blood, pancreas, spleen, thymus, heart, testes, vas deferens, lungs, kidneys, brain, liver, muscle, brown adipose tissue, subcutaneous adipose tissue, and intraperitoneal adipose tissue. A common feature was an increase in tissue concentration of acetone and total radioactivity during the first 6 hours after exposure, with no further accumulation observed after 6 hours except in the liver and brown adipose tissue. The continued accumulation of radioactivity in these tissues could be the result of high metabolic turnover. Only about 10% of the radioactivity in the liver at 24 hours was unmetabolized acetone. When the mice were exposed intermittently on 3 or 5 consecutive days, most tissues showed no or only a small additional increase in radioactivity after more than 1 day of exposure; however, the concentration in adipose tissue increased significantly with increasing exposure duration up to 5 days. Elimination of acetone was fastest in blood, kidneys, lungs, brain, and muscles with half-lives of about 2–3 hours during the first 6 hours after exposure. The slowest elimination was in subcutaneous adipose tissue with a half-time of >5 hours. Elimination of acetone was complete in all tissues by 24 hours after exposure, but total radioactivity, indicative of metabolites, was still present in all tissues except blood and muscle. These data indicate that acetone is not selectively distributed to any tissues but is more evenly distributed in body water. In addition, acetone is not likely to accumulate with repeated exposure.

In another study of inhalation exposure, rats (n=4) were exposed to 1,000 ppm of acetone for 8 hours/day for 3 consecutive days (Scholl and Iba 1997). Plasma concentrations of acetone were 122, 107, and 125 μg/mL at 30 minutes after exposure on days 1, 2, and 3, respectively. Plasma elimination followed first-order kinetics in rats that were terminated after exposure to 1,000 ppm for 3 hours/day for 3 days. The half-life for elimination was 4.5 hours, and the area under the curve (AUC) was 950 μg-hour/mL. Inhalation exposure of the rats to 1,000 ppm of acetone for 3 hours/day for 10 days resulted in concentrations of 35.3 μg/g of acetone in plasma, 13.2 μg/g in liver, 11.4 μg/g in the lung, and 21.8 μg/g in the kidney (Scholl and Iba 1997).

Acetone has also been detected in the plasma of rats following oral exposures. In rats receiving acetone in drinking water (7.5% v/v) for 11 consecutive days, plasma concentrations of acetone on day 1 were in the range of 315–800 μg/mL. The plasma concentration appeared to plateau at about 1,200 μg/mL by day 4 (Scholl and Iba 1997). Acetone was additionally found in the liver of rats after oral exposure (Charbonneau et al. 1986a, 1991). No other studies were located regarding the distribution of acetone or its metabolites in animals. However, acetone is well absorbed from the gastrointestinal tract (see

Section 3.1.1) and is highly water soluble. Therefore, widespread distribution, especially to tissues with high water content, is expected.

No studies were located regarding distribution of acetone or its metabolites in animals after dermal exposure. However, the findings of cataract formation in guinea pigs exposed dermally (Rengstorff and Khafagy 1985; Rengstorff et al. 1972) (see Section 2.12) indicated that acetone was absorbed from the skin of the guinea pigs and distributed to the eyes.

Similar to humans, there is evidence of transplacental transfer of acetone in animals. Acetone and its metabolites were found in fetuses from rats injected intravenously with 100 mg/kg acetone on GD 19 (Peinado et al. 1986).

One study investigated the pharmacokinetics of intravenously injected radiolabeled acetone in baboons (Gerasimov et al. 2005). Acetone uptake in the brain was rapid in both the cerebellum and white matter, reaching peaks of approximately 0.02% of the injected dose within 1–2 minutes after injection. Acetone had a half-time of clearance from peak uptake of 31 and 38 minutes in the cerebellum and white matter, respectively. The brain/blood uptake ratio for acetone reached a peak of 1.0 and plateaued around 4–5 minutes after injection.

3.1.3 Metabolism

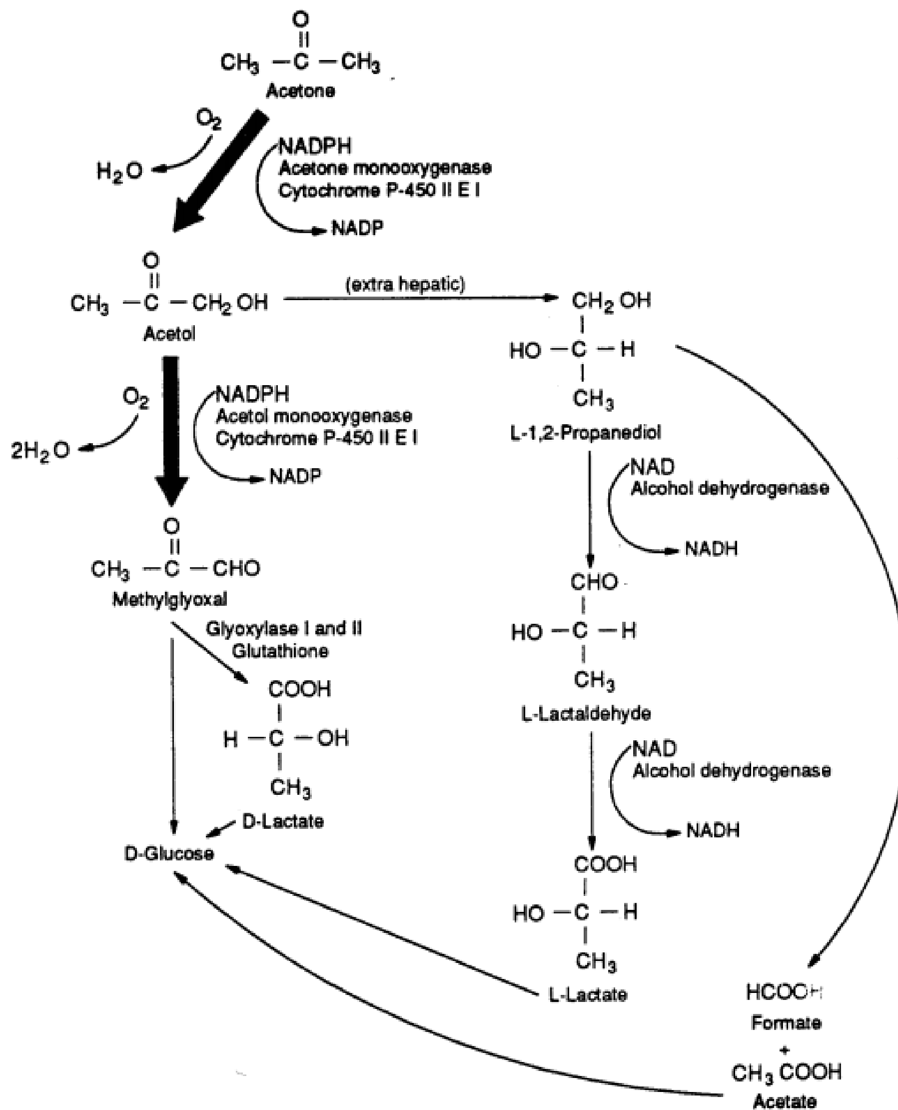
The metabolic fate of acetone is independent of route of administration and involves three separate gluconeogenic pathways, with ultimate incorporation of carbon atoms into glucose and other products and substrates of intermediary metabolism with generation of carbon dioxide. The metabolic pathways appear to be similar in humans and animals. The primary (major) pathway involves hepatic metabolism of acetone to acetol and hepatic metabolism of acetol to methylglyoxal, while two secondary (minor) pathways are partially extrahepatic, involving the extrahepatic reduction of acetol to L-1,2-propanediol. Some of exogenous acetone is unmetabolized and is excreted primarily in the expired air with little acetone excreted in urine (see Section 3.1.4).

The only studies located regarding the metabolism of acetone in humans were conducted in non-obese fasted, obese fasted, and male and female diabetic patients (Reichard et al. 1979, 1986). The involvement of gluconeogenesis was demonstrated in non-obese patients fasted for 3 days (n=6), obese patients fasted for 3 days (n=6), and obese patients fasted for 21 days (n=3) before intravenous injection of

2-[^{14}C]-acetone (Reichard et al. 1979). The percentages of ^{14}C-glucose in plasma derived from ^{14}C-acetone were 4.2, 3.1, and 11.0% in the three respective groups, suggesting the involvement of gluconeogenesis. Cumulative ^{14}C-carbon dioxide excretion by the lungs during the 6-hour collection period accounted for 17.4, 21.5, and 4.9% in the three respective groups. Radioactivity was also incorporated into plasma lipids and plasma proteins. Unmetabolized acetone in the expired air accounted for 14.7, 5.3, and 25.2%; urinary excretion of acetone accounted for 1.4, 0.6, and 1.3%, respectively; and *in vivo* metabolism accounted for 83.9, 94.1, and 73.5%, respectively, of the radioactivity. Intravenous infusion of 2-[^{14}C]-acetone into patients with diabetic ketoacidosis resulted in a mean plasma acetone turnover rate of 6.45 μmol/kg/minute (Reichard et al. 1986). Analysis of glucose in urine revealed a labeling pattern in five of the six patients consistent with the involvement of pyruvate in the gluconeogenic pathway. A different pathway may have operated in the other patient. Acetol and 1,2-propanediol were also detected in the plasma, and the concentrations of these metabolites were directly related to the plasma level of acetone. The results demonstrated high plasma acetone levels in decompensated diabetic patients. The suggested pathway of acetone metabolism in these patients was acetone to acetol to 1,2-propanediol to pyruvate and ultimately to glucose, but other pathways may exist between subclasses of diabetic patients.

The metabolism of acetone has been studied extensively in animals, primarily in rats, and three separate pathways of gluconeogenesis have been elucidated (Figure 3-1). These pathways are consistent with the metabolic fate of acetone in humans, discussed above. The elucidation of these pathways has been performed in experiments in which rats, mice, or rabbits were exposed by inhalation, by gavage, via drinking water, or by intravenous, subcutaneous, or intraperitoneal injection of nonradiolabeled acetone or to acetone labeled with ^{14}C in the methyl groups, number 2 carbon atom, or all three carbon atoms (Casazza et al. 1984; Hallier et al. 1981; Hetenyi and Ferrarotto 1985; Johansson et al. 1986; Koop and Casazza 1985; Kosugi et al. 1986a, 1986b; Mourkides et al. 1959; Price and Rittenberg 1950; Puccini et al. 1990; Rudney 1954; Sakami 1950; Sakami and LaFaye 1951; Skutches et al. 1990). In these experiments, identification of metabolites in liver, plasma, or urine, the labeling patterns of ^{14}C incorporation into metabolites from ^{14}C-acetone in plasma or in liver, or the results of enzyme reactions using microsomes from acetone treated animals have led to the pathways illustrated in Figure 3-1.

Figure 3-1. Proposed Metabolic Pathway for Acetone in Humans



Sources: Casazza et al. 1984; Dietz et al. 1991; Kosugi et al. 1986a

In the first step, acetone is oxidized (hydroxylation of a methyl group) to acetol by acetone monooxygenase (also called acetone hydroxylase), an activity that is associated with CYP2E1 and requires oxygen and NADPH (Casazza et al. 1984; Johansson et al. 1986; Koop and Casazza 1985; Puccini et al. 1990). CYP2E1 can be induced by fasting, experimental diabetes, or exposure to ethanol or acetone (Johansson et al. 1988; Patten et al. 1986; Puccini et al. 1990). When the rate of acetone oxidation was evaluated in microsomes with acetone added to the incubation system, microsomes from rats (Johansson et al. 1986) and mice (Puccini et al. 1990) pretreated with acetone had a 7–8 times greater acetone oxidation rate than microsomes from control rats or mice. Thus, acetone induces its own metabolism.

The formation of acetol is common to all three pathways. Subsequent conversion of acetol to methylglyoxal in microsomes is catalyzed by acetol monooxygenase (also called acetol hydroxylase), an activity that is also associated with CYP2E1 and requires oxygen and NADPH (Casazza et al. 1984; Johansson et al. 1986; Koop and Casazza 1985). Methylglyoxal can then be converted to D-glucose by an unidentified pathway, and/or possibly by catalysis by glyoxalase I and II and glutathione to D-lactate, which is converted to D-glucose (Casazza et al. 1984). The conversion of methylglyoxal to D-lactate by the actions of glyoxalase I and II is well established (Racker 1951), but may represent a minor pathway in the metabolism of acetone (Casazza et al. 1984; Kosugi et al. 1986a, 1986b; Thornalley 1990). The unidentified pathway by which methylglyoxal is converted to D-glucose may involve conversion of methylglyoxal to pyruvate by 2-oxoaldehyde dehydrogenase, an activity identified using aqueous extracts of sheep liver acetone powders (Monder 1967).

Investigations that included CYP2E1-null mice have confirmed the importance of CYP2E1 in acetone catabolism *in vivo* (Bondoc et al. 1999; Chen et al. 1994). In the study of Bondoc et al. (1999), acetone levels were measured in non-fasted and 48-hour-fasted wild type and CYP2E1-null mice. Fasting is known to result in the elevation of acetone levels in the blood. Blood acetone levels in non-fasted wild type and CYP2E1-null mice were not significantly different from one another. However, fasted CYP2E1-null mice exhibited 24-fold increased blood acetone levels compared to their non-fasted controls. The wild-type fasted mice, on the other hand, exhibited only a 2- to 4-fold increase in blood acetone levels compared to their non-fasted controls. Chen et al. (1994) assessed the role of CYP2E1 in acetone catabolism by measuring acetone levels at different time points in rats that had been treated with diallyl sulfide (DAS, a CYP2E1 inhibitor) at a variety of dose levels. The study authors noted DAS dose-dependent increases in the time to peak blood acetone level and in the time to return to pre-dose levels, suggesting an important role of CYP2E1 in acetone catabolism.

In the second and third pathways, acetol is converted to L-1,2-propanediol by an extrahepatic mechanism that has not been characterized (Casazza et al. 1984; Kosugi et al. 1986a, 1986b; Rudney 1954; Skutches et al. 1990). The two pathways then diverge from the point of production of 1,2-propanediol. In the second pathway, 1,2-propanediol formed extra-hepatically returns to the liver where it is converted to L-lactaldehyde via nicotinamide adenine dinucleotide (NADH)-dependent alcohol dehydrogenase (Casazza et al. 1984; Kosugi et al. 1986a, 1986b), and L-lactaldehyde, in turn, is converted to L-lactate (Casazza et al. 1984; Ruddick 1972; Rudney 1954) via NADH-dependent aldehyde dehydrogenase (Casazza et al. 1984). L-lactate can then be converted to D-glucose (Casazza et al. 1984). In the third

pathway, the L-1,2-propanediol formed extra-hepatically returns to the liver where it is degraded by an uncharacterized mechanism to acetate and formate (Casazza et al. 1984; Ruddick 1972).

Several studies have traced the labeling patterns of ^{14}C from 2-[^{14}C]-acetone or 1,3-[^{14}C]-acetone to gluconeogenic precursors and formate to incorporation of ^{14}C into glycogen, glycogenic amino acids, fatty acids, heme, cholesterol, choline, and urea (Mourkides et al. 1959; Price and Rittenberg 1950; Sakami 1950). The pattern of labeling suggested the involvement of the "acetate and formate" pathway. The ultimate fate of glucose is entry into glycolysis or into the tricarboxylic acid cycle, via pyruvate and acetyl coenzyme A (CoA) with the liberation of carbon dioxide, and subsequent electron transport and oxidative phosphorylation with the production of ATP (Lehninger 1970). Fatty acids, amino acids, and glycogen may also enter stages of intermediary metabolism.

The relative importance of the three pathways in the metabolism of acetone may depend upon the amount of acetone administered. When a trace amount of 2-[^{14}C]-acetone was administered intravenously to rats, the pattern of incorporation of ^{14}C into glucose was consistent with the production of glucose via the methylglyoxal/lactate pathway (Kosugi et al. 1986a). When a higher dose of 2-[^{14}C]-acetone (325 mg/kg) was injected, the pattern of incorporation was more consistent with the 1,2-propanediol pathway. These results suggest that at low doses of acetone or endogenous acetone, the methylglyoxal and lactate pathways predominate, but at higher doses, these pathways become saturated and metabolism is shunted to the formate-acetate branch of the 1,2-propanediol pathway.

In addition to the pathways illustrated in Figure 3-1, 2,3-butanediol (Casazza et al. 1984) and isopropyl alcohol (Lewis et al. 1984) were detected in the blood of rats after oral dosing with acetone and were deemed to be unrelated to the ingestion of these chemicals. The positions of 2,3-butanediol and isopropyl alcohol in the metabolic scheme are not clear.

That acetone is extensively metabolized has been demonstrated by the finding of high percentages of ^{14}C-carbon dioxide in the expired air of animals exposed to ^{14}C-acetone (Mourkides et al. 1959; Price and Rittenberg 1950; Sakami 1950; Sakami and LaFaye 1951; Wigaeus et al. 1982) (see Section 3.1.4).

Although the liver is the primary site of acetone metabolism, radioactive unmetabolized acetone and total radioactivity were found in blood, pancreas, spleen, thymus, heart, testes, vas deferens, lungs, kidneys, brain, liver, muscle, brown adipose tissue, subcutaneous adipose tissue, and intraperitoneal adipose tissue of mice after inhalation exposure to ^{14}C-acetone (Wigaeus et al. 1982) (see Section 3.1.2). The fraction of

total radioactivity that was not still acetone represented metabolites. Elimination of acetone was complete in all tissues by 24 hours after exposure, but total radioactivity, indicative of metabolites, was still present in all tissues except blood and muscle. Whether these tissues (other than the liver) were capable of metabolizing acetone or whether the metabolites themselves were distributed to the tissues was not clear. However, microsomes from the lungs of hamsters exposed to acetone in drinking water for 7 days had a 500% increased activity of aniline hydroxylase, an enzyme associated with CYP2E1 (Ueng et al. 1991). Furthermore, the level of CYP2E1 increased 6-fold in microsomes from the nasal mucosa of rabbits exposed to acetone in drinking water for 1 week (Ding and Coon 1990). In hamsters given drinking water containing acetone for 7 days (Ueng et al. 1991) or 10 days (Menicagli et al. 1990), microsomes prepared from kidneys had increased levels of CYP and cytochrome b. These results suggest that acetone metabolism, which involves CYP2E1, may occur in the lungs and kidneys of hamsters and the nasal mucosa of rabbits. Incubation of acetone with homogenates of nasal mucosa from mice indicated that acetone was metabolized via a NADPH-dependent pathway *in vitro*, but no evidence of *in vivo* metabolism of acetone by the upper respiratory tract was found in mice, rats, guinea pigs, or hamsters (Morris 1991). Injection of pregnant rats with acetone on GD 19 resulted in high levels of 1,2-propanediol and acetol in the fetuses (Peinado et al. 1986). Whether these findings reflect transfer of the metabolites from the dams or metabolism of transferred acetone by the fetuses was not resolved.

Very few differences have been found among species in the metabolism of acetone. The pathways illustrated in Figure 3-1 appear to operate in rats, mice, and rabbits. In microsomes from rabbits exposed to acetone via drinking water, it was found that the oxidation of acetol could be catalyzed by cytochromes P-4503b (IIC3), 2 (IIB6), and 4(IA2), as well as by cytochrome P-4503a (P-450IIE1) (Koop and Casazza 1985). Only CYP2E1 could catalyze the oxidation of acetone to acetol. No studies were located regarding the ability of other isoenzymes of CYP to catalyze these reactions in other species.

Physiological or genetic status may alter the metabolism of acetone. When nondiabetic and diabetic 6-week-old male rats were treated by gavage with 99.5% pure acetone (containing <0.01% isopropyl alcohol) at doses of 1,000, 2,000, or 4,000 mg/kg, isopropyl alcohol deemed unrelated to the ingestion of the chemical was detected in the blood (Lewis et al. 1984). The levels of isopropyl alcohol and acetone increased with increasing dose in the diabetic rats, although with plateaus for both acetone and isopropyl alcohol at 1,000 and 2,000 mg/kg doses, but leveled off in the nondiabetic rats, indicating either saturation of the metabolic pathway from acetone to isopropyl alcohol or a reversibility of the conversion at high doses. It was suggested that in the diabetic rats, acetone and NADH, both needed for isopropyl alcohol production from acetone, presumably via alcohol dehydrogenase, may be diverted to gluconeogenic

pathways to meet the diabetic rat's need for glucose, resulting in the short plateau. The subsequent rise of both compounds at the high dose of acetone in the diabetic rats could be accounted for by greater generation of NADH from fatty acid oxidation in the diabetic rat, which reduces acetone to isopropyl alcohol, accounting for the rising level of isopropyl alcohol. Liver homogenates from mice heterozygous for the obesity gene treated with acetone were more effective in converting acetone to lactate than liver homogenates from non-obese homozygous mice treated with acetone (Coleman 1980). The more effective conversion by heterozygous mice may account for their prolonged survival on the starvation regimen, compared with non-obese mice. In pregnant and virgin rats (either fed or fasted) injected intravenously with acetone, plasma acetol levels were not significantly different between fasted and nonfasted rats, but pregnant rats had significantly lower levels than virgin rats (Peinado et al. 1986). Liver levels of acetol were also significantly lower in pregnant rats than in nonpregnant rats. Methylglyoxal levels were very high in the livers and plasma of nonfasted rats (pregnant or nonpregnant), but fasting resulted in much lower levels. No major differences were found in the expiration of carbon dioxide between fasted and diabetic rats injected intraperitoneally with acetone (Mourkides et al. 1959) or in the labeling pattern of ^{14}C derived from ^{14}C-acetone into glucose among nonfasted diabetic, fasted diabetic, nondiabetic nonfasted, and fasted nondiabetic rats injected intravenously with ^{14}C-acetone (Kosugi et al. 1986a, 1986b).

3.1.4 Excretion

The main route of excretion of acetone is via the lungs, regardless of the route of exposure. Acetone is excreted both unchanged and, following metabolism, mainly as carbon dioxide. Studies have been conducted in humans exposed by inhalation, but these studies have followed the elimination only of unchanged acetone from blood and the excretion of unchanged acetone in the expired air and urine.

A case report of a 55-year-old man who ingested 1 L of acetone estimated a terminal half-life of 17 hours (Gregoire et al. 2018). In humans exposed to acetone up to 1,250 ppm for up to 7.5 hours/day in a complex protocol for 16 weeks, the concentration of acetone in venous blood was directly related to the vapor concentration and duration of exposure, and inversely related to the time elapsed following exposure (Stewart et al. 1975). The rate of elimination of acetone from blood was constant regardless of blood acetone concentration (DiVincenzo et al. 1973). Half-times for blood elimination of 3–3.9 hours have been estimated in humans exposed to 100–500 ppm for 2–4 hours (Brown et al. 1987; DiVincenzo et al. 1973; Wigaeus et al. 1981). A study of workers exposed to 0.34 ppm during an 8-hour shift found an estimated half-life of 5.8 hours for acetone in blood (Wang et al. 1994). Elimination half-times of 3.9

and 6.2 hours have been estimated for arterial and venous blood, respectively, in volunteers (n=8) exposed to acetone concentrations ranging from 712 to 1,309 ppm (Wigaeus et al. 1981). No differences in elimination half-times were found between men and women (Brown et al. 1987). The elimination from blood was found to be complete in 24 hours after a 6-hour exposure in subjects exposed to 250 ppm, in 32 hours in subjects exposed to 500 ppm, and in 48 hours in subjects exposed to 1,000 ppm (Matsushita et al. 1969a). When 22 year-old men were exposed to 250 ppm for 6 hours/day for 6 days, the blood levels of acetone rose each day and returned to baseline levels in the morning after each daily exposure (Matsushita et al. 1969b). At an exposure concentration of 500 ppm, however, the blood levels declined each day, but not to baseline levels. At the end of the 6-day exposure, blood acetone levels declined to baseline within 2 days for the 250 ppm group and within 3 days for the 500 ppm group. Another study measured urinary acetone on the morning after occupational exposure to acetone concentrations above 300 ppm and found elevated concentrations relative to baseline (Satoh et al. 1995). However, by the end of the second day of exposure, there was no evidence of significant acetone accumulation; urinary acetone concentrations were similar to those at the end of the first day. From the half-time and the data on time to return to baseline levels, it appears that at higher concentrations, acetone may accumulate slightly in the blood during daily intermittent exposure, such as would be experienced by workers.

The rate and pattern of respiratory excretion of acetone is influenced by exposure concentration, duration, the level of physical activity during exposure, and biological sex. In humans exposed to acetone up to 1,250 ppm for up to 7.5 hours/day in a complex protocol for up to 6 weeks, the rate of respiratory excretion was a function of the duration, and the concentration of acetone in breath after exposure was directly related to the time-average concentration during exposure, with constant duration (Stewart et al. 1975). The length of time after exposure in which acetone could be detected in the expired air was related to the magnitude of exposure, with acetone still readily detectable 16 hours after exposure to 1,000 or 1,250 ppm for 7.5 hours. Excretion of acetone by the lungs was complete within 20 hours postexposure in humans exposed to 237 ppm for 4 hours (Dick et al. 1989). During exposure for 2 hours, the acetone concentration in expired air rose to 20 ppm in humans exposed to 100 ppm and to 90–100 ppm in those exposed to 500 ppm (DiVincenzo et al. 1973). After exposure to 100 ppm, the expired air concentration of acetone declined biphasically over the next 7 hours to 5 ppm. However, after exposure to 500 ppm, the expired air concentration dropped sharply to 2 ppm and declined to 1 ppm over the next 7 hours. DiVincenzo et al. (1973) observed prolonging the exposure duration to 4 hours resulted in a <2-fold increase in acetone levels in postexposure expired air, which may reflect a greater loss of acetone through metabolism and urinary excretion. Exercise during the exposure period increased the elimination almost 2-fold. In humans exposed to acetone at rest, during exercise at a constant workload, or during exercise

with step-wise increments in workload, expiration of acetone via the lungs amounted to 63, 74, and 138 mg, respectively, at approximately 0.25–4 hours postexposure and to 48, 77, and 197 mg, respectively, over the next 4–20 hours (Wigaeus et al. 1981). Excretion of acetone from the lungs and kidneys (combined) amounted to 16, 20, and 27% of the amount absorbed in the three respective groups of subjects. Urinary excretion amounted to only 1% of the total uptake. Women expired acetone more slowly than men after a 4-hour exposure to 127–131 ppm, but the percentages excreted by the lungs were not statistically significantly different between men and women (17.6% for men, 15.0% for women) (Nomiyama and Nomiyama 1974b).

Very little unchanged acetone is excreted in the urine (DiVincenzo et al. 1973; Kawai et al. 1992; Vangala et al. 1991; Wigaeus et al. 1981). Urinary excretion is biphasic (Pezzagno et al. 1986). Peak urinary excretion occurred between 1 and 3.5 hours after exposure (Matsushita et al. 1969b; Wigaeus et al. 1981). In male volunteers exposed to 497 or 990 ppm acetone for 4 hours, cumulative acetone excretion in urine at 18 hours after cessation of exposure was 89.5 mg, suggesting slow excretion of acetone in the urine (Vangala et al. 1991). The amount of acetone excreted in the urine is influenced by the exposure concentration, duration of exposure, and level of physical activity during exposure. The acetone concentration in the urine ranged from 0 to 17.5 mg/L at the end of the 8-hour workshift in 45 workers exposed to 0–70 ppm acetone (baseline urinary concentration in 343 nonexposed subjects averaged 1.5 mg/L) (Kawai et al. 1992). Acetone levels in the preshift urine samples were significantly higher than baseline levels when acetone exposure on the previous day was >15 ppm. There was no significant difference between baseline urine levels and preshift urine levels when the previous day's exposure was <15 ppm. In humans exposed for 6 hours, peak urinary levels were found within the first hour after exposure and were 5.2 mg/dL in subjects exposed to 1,000 ppm, 2.9 mg/dL in subjects exposed to 500 ppm, and 1.8 mg/dL in subjects exposed to 250 ppm (Matsushita et al. 1969b). The returned to baseline levels occurred within 48 hours for the 1,000 ppm group, within 32 hours for the 500 ppm group, and within 24 hours in the 250 ppm group. When human subjects were exposed for 6 hours/day for 6 days, urinary levels of acetone rose each day and declined to baseline levels by the following morning each day when the exposure concentration was 250 ppm (Matsushita et al. 1969a). At an exposure level of 500 ppm, however, urinary levels declined each day, but not to baseline levels. At the end of the 6-day exposure period, urinary acetone levels returned to baseline within 2 days for the 250 ppm group and within 3 days for the 500 ppm group. Therefore, excretion was more complete after exposure to lower concentrations, and at higher concentrations, acetone may accumulate somewhat during daily intermittent exposure, such as would be experienced occupationally. Total 24-hour urine content of acetone was 1.25 mg in subjects exposed to 100 ppm for 2 hours and 3.51 mg in subjects exposed to 500 ppm for

2 hours (DiVincenzo et al. 1973). Prolonging the duration to 4 hours in the 100-ppm group resulted in a total of 1.99 mg acetone in the urine. A slight increase in the urinary content of acetone (1.39 mg) was found when humans exposed to 100 ppm for 2 hours exercised during the exposure. The nature of physical activity during exposure also influenced the urinary excretion. At 3–3.5 hours after exposure, 8.5, 8.5, and 13.4 mg were excreted by the kidneys in subjects exposed at rest, during exercise at a constant workload, and during exercise with stepwise increments in workload, respectively (Wigaeus et al. 1981).

One human controlled exposure study on excretion was identified, in which volunteers ingested 60–80 mg/kg acetone (Haggard et al. 1944). The elimination of acetone in expired air and urine was determined; acetone concentration in expired air was measured 1 hour after administration and 30 minutes thereafter and in urine, acetone concentrations were determined 2 hours later. Over an observation period of 4 hours, the authors estimated that 64–93% of the administered dose was metabolized and 7–36% was eliminated via urine and expired air.

The only other information regarding excretion of acetone in humans after oral exposure is from case reports of accidental or intentional ingestion of materials containing acetone plus other components that may have influenced the elimination of acetone. In a man who ingested liquid cement containing 18% acetone (231 mg/kg), 28% 2-butanone, and 29% cyclohexanone and 720 mL sake (alcoholic beverage), the plasma level of acetone was ≈1,120 μg/mL 5 hours after ingestion and declined to 65 μg/mL at 18 hours, 60 μg/mL at 24 hours, and <5 μg/mL at 48 hours (Sakata et al. 1989). A first-order plasma elimination rate constant of 0.038/hour and a half-time of 18.2 hours were calculated. The urinary level of acetone decreased gradually from about 123 μg/mL at 5 hours after ingestion to about 61 μg/mL at 19 hours. In a case of an individual known to have alcohol use disorder who had ingested nail polish remover and whose blood acetone level was 0.25 g/dL (2.5 mg/mL) upon admission to the hospital, the blood level of acetone declined in a log-linear manner to about 0.06 g/dL (0.6 mg/mL) about 86 hours after admission, with a half-life of 31 hours (Ramu et al. 1978). The calculated clearance of acetone from the lungs was 29 mL/minute or 0.39 mL/minute/kg. A half-time of 25 hours for lung clearance was calculated, which is in agreement with the observed plasma elimination half-time of 31 hours. The serum acetone level of a 30-month-old male child was 445 mg/100 mL (4.45 mg/mL) 1 hour after ingestion of a 6-ounce bottle of nail polish remover (65% acetone) and declined to 2.65 mg/mL at 117 hours, to 0.42 mg/mL at 48 hours, and to 0.04 mg/mL at 72 hours (Gamis and Wasserman 1988). The half-time of acetone in this patient was 19 hours in the severe early stage and 13 hours in later stages of intoxication, which suggested to the authors greater metabolism and/or excretion in children, compared with adults.

Information regarding excretion of acetone after dermal exposure of humans is limited, but the main route of excretion is via the lungs, with little excreted in the urine. Application of an unspecified quantity of acetone to a 12.5 cm^2 area of skin of volunteers for 2 hours/day for 4 days resulted alveolar air levels of 5–12 ppm and urinary concentrations of 8–14 μg/mL on each day (Fukabori et al. 1979). These levels returned to baseline levels by the next day after each exposure. Higher alveolar air and urinary levels were obtained when the daily exposure increased to 4 hours/day: 25–34 ppm in alveolar air, and 29–41 μg/mL in urine, but these levels also returned to baseline each day.

Physiological status may influence the disposition of endogenous and exogenous acetone in humans. In groups of nonobese patients fasted for 3 days, obese patients fasted for 3 days, and obese patients fasted for 21 days and injected intravenously with ^{14}C-acetone, 8–29% of the urinary acetone was ^{14}C-labeled (Reichard et al. 1979). The concentrations of urinary acetone were 1.2, 0.4, and 2.6 μmol/mL in 3-day-fasted nonobese, 3-day-fasted obese, and 21-day-fasted obese patients, respectively. The rates of urinary acetone excretion were 1.2, 0.4, and 1.7 μmol/minute, respectively, suggesting marked renal reabsorption or back-diffusion. The percentages of measured acetone production that could be accounted for by excretion via the lungs were 14.7, 5.3, and 25.2%, respectively. The percentages that could be accounted for by urinary excretion were 1.4, 0.6, and 1.3%, respectively. Cumulative excretion of ^{14}C-carbon dioxide during the 6-hour turnover study periods accounted for 17.4, 21.5, and 4.9%, respectively. Thus, nonobese subjects fasted for 3 days excreted more acetone at higher rates than did obese subjects fasted for 3 days. However, excretion by the obese patients fasted for 21 days exceeded that by both 3-day-fasted groups. These differences are probably related to the effect that the degree of starvation ketosis has on the metabolism and overall disposition of acetone.

As in humans, acetone is excreted mainly by the lungs of animals. Studies in animals have followed the elimination of acetone from blood and tissues, excretion of acetone and carbon dioxide in expired air, and the urinary excretion of formic acid.

Blood levels of acetone were highest immediately after a 4-hour exposure of rats to acetone (Charbonneau et al. 1986b). In rats exposed to 10,000 ppm, the blood level dropped from 2,114 to 5 μg/mL in 25 hours. In rats exposed to 15,000 ppm, the blood level dropped from 3,263 to 50 μg/mL after 25 hours. Elimination from blood was biphasic in rats exposed to 10,000 and 15,000 ppm, perhaps indicating saturation. Elimination from blood was triphasic in rats exposed to 1,000, 2,500, or 5,000 ppm and was complete within 17–25 hours. In dogs exposed to 100, 500, or 1,000 ppm acetone for 2 hours,

blood levels declined in a log-linear manner with a half-time of 3 hours, similar to that observed in humans (DiVincenzo et al. 1973). Blood levels declined from 25 mg/L immediately after exposure to 10 mg/L at 5 hours postexposure for the 1,000 ppm group, from 12 to 3 mg/L for the 500 ppm group, and from 4 to 1.5 mg/L for the 100 ppm group. Elimination of radioactivity and ^{14}C-acetone was fastest from blood, kidney, lung, brain, and muscle tissues of mice exposed to 500 ppm ^{14}C-acetone for 6 hours, with half-times of 2–3 hours during 6 hours postexposure (Wigaeus et al. 1982). Elimination of acetone was complete in 24 hours in all tissues, but radioactivity (indicative of metabolites) was still present in all tissues except blood and muscle. When rats were exposed for 5 days, acetone tended to accumulate in adipose tissue.

Excretion of acetone in air followed pseudo-first-order kinetics in rats exposed to <20 ppm acetone for 1–7 days, while at higher concentrations, saturation kinetics were observed (Hallier et al. 1981). In male rats exposed to 500 ppm ^{14}C-acetone for 6 hours, 42 µmol of radioactive acetone and 37 µmol ^{14}C-carbon dioxide were excreted in the expired air during a 12-hour postexposure period, with 95 and 85%, respectively, recovered in the first 6 hours postexposure (Wigaeus et al. 1982). Radioactive acetone accounted for 52% and radioactive carbon dioxide accounted for 48% of the expired radioactivity. The concentration of acetone in the expired breath of dogs exposed to 100, 500, or 1,000 ppm acetone for 2 hours declined in a log-linear manner (DiVincenzo et al. 1973). The breath levels were directly related to the magnitude of exposure. Breath levels declined from 1.6 ppm at 30 minutes after exposure to 0.3 ppm at 300 minutes in the 100-ppm group, from 6.8 to 1.5 ppm in the 500 ppm group, and from 15 to 4 ppm in the 1,000 ppm group.

Urinary excretion of formic acid was followed for 7 days in rats exposed to 62,000 ppm acetone for 2 days. The rate of formic acid excretion was 344 µg/hour compared with 144 µg /hour in controls (Hallier et al. 1981).

Information regarding the excretion of acetone after oral exposure in animals is available only for rats. As is the case after inhalation exposure, acetone, mainly as carbon dioxide, is excreted primarily by the lungs. In a rat given 1.16 mg/kg ^{14}C-acetone by gavage in water, expiration of ^{14}C-carbon dioxide totaled 47.4% of the administered radioactivity over the 13.5-hour collection period (Price and Rittenberg 1950). In another experiment, a rat was given 7.11 mg/kg radioactive acetone. A small amount of radioactive acetone (10%) was found in the expired air. Radioactive carbon dioxide and acetate were also detected. In a rat made diabetic by alloxan and given 6.15 mg/kg ^{14}C-acetone, a total of 7.29% of the administered radioactivity was expired as acetone and 51.78% as carbon dioxide. Radioactive acetate was detected in

the urine. These data indicate that very little acetone (<10%) was excreted by the lungs after small doses of acetone. A major fraction was oxidized to carbon dioxide and some of the derived carbon was used for acetylation. The diabetic rat was also able to oxidize acetone, but only to ≈70% of that in the non-diabetic rat.

The dose of acetone influences the elimination of acetone from blood (Plaa et al. 1982). At a dose of 78.44 mg/kg, the maximum blood level of 200 µg/mL at 3 hours declined to 10 µg/mL at 12 hours, where it remained for the next 12 hours (data inadequate to calculate total body clearance). At a dose of 196.1 mg/kg, the maximum blood level of 400 µg/mL at 6 hours declined biphasically to 50 µg/mL at 12 hours and to 30 µg/mL at 18 hours where it remained at 24 hours (total body clearance of 64 mL/hour). At a dose of 784.4 mg/kg, the maximum blood level of 900 µg/mL at 1 hour declined to 300 µg/mL at 12 hours, to 110 µg/mL at 18 hours, and to 50 µg/mL at 24 hours (total body clearance of 86 mL/hour). At a dose of 1,961 mg/kg, the maximum blood level of 1,900 µg/mL at 3 hours declined slowly to 400 µg/mL at 24 hours (total body clearance of 75 mL/hour). Thus, total body clearance was independent of dose, but the half-time for elimination increased from 2.4 hours for 196.1 mg/kg, to 4.9 hours for 784.4 mg/kg, and 7.2 hours for 1,961 mg/kg.

The vehicle (corn oil or water) in which acetone is administered has little influence on the elimination of acetone from blood (Charbonneau et al. 1986a). After gavage treatment of rats with 78, 196, 392, 784, or 1,177 mg/kg acetone in corn oil or water, elimination was biphasic for the two higher doses and triphasic for the lower doses. Acetone elimination from blood declined to <5 to <10 µg/mL by 18–26 hours for all doses, but minor differences were found between water and corn oil as vehicle. The blood concentration curves from rats given acetone in water more closely resembled those from rats exposed by inhalation.

No studies were located regarding excretion of acetone by animals after dermal exposure.

Contrary to evidence from human studies, no major differences were observed among fed non-diabetic rats, fasted non-diabetic rats, and fed diabetic rats in the excretion of ^{14}C-carbon dioxide from the lungs after intraperitoneal injections of ^{14}C-acetone (Mourkides et al. 1959). However, the dose level influenced the pattern of metabolism and, hence, the excretion of carbon dioxide. Rats that received 9.3–22.7 mg/kg radioactive acetone rapidly metabolized acetone, as evidenced by exhalation of 24–43% of the administered radioactivity as ^{14}C-carbon dioxide within the first 3 hours after dosing. Rats that received 258–460 mg/kg radioactive acetone exhaled only 2.1–5.7% of the radiolabel as carbon dioxide in the first 3 hours, 2.8–7.8% in the next 3–6 hours, and 16–29% in the next 6–24 hours. Rats injected

subcutaneously with ^{14}C-acetone also excreted the derived radioactive carbon mainly as carbon dioxide. In rats fasted for 24 hours and given 170 mg/kg radioactive acetone, 27% of the radiolabel was excreted as carbon dioxide in 4 hours (Sakami and LaFaye 1951). Rats fasted for 48 hours before the subcutaneous dose of 174 mg/kg radioactive acetone excreted 53% of the radiolabel as carbon dioxide over a 14-hour collection period (Sakami 1950).

Fasted pregnant rats had an enhanced capacity for acetone elimination compared with fasted or fed virgin rats or fed pregnant rats, after intravenous dosing with 100 mg/kg (Peinado et al. 1986). While the elimination of acetone from plasma was biphasic in all groups, the fasted pregnant rats eliminated acetone at a faster rate than the other groups.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

PBPK models have been developed to simulate the behavior of acetone in rats and humans exposed by various routes (Clewell et al. 2001; Gentry et al. 2002; Huizer et al. 2012; Kumagai and Matsunaga 1995; Mörk and Johanson 2006). Clewell and coworkers (Clewell et al. 2001; Gentry et al. 2002) developed a PBPK model intended to simulate the behavior of isopropanol and its major metabolite, acetone, in rats and humans for intravenous, intraperitoneal, oral, inhalation, and dermal exposure. The model was specifically intended to be used for human health risk assessment for isopropanol. The model is capable of simulating exposures to acetone as well (Gentry et al. 2003) and was expanded to simulate exposure to isopropanol during pregnancy (Gentry et al. 2002). Validation of acetone metabolism was performed by use of intravenous, oral, and inhalation exposure data from rats and by use of inhalation and oral exposure data from humans. Huizer et al. (2012) used a similar model to investigate uncertainty and variability in biological parameters after simulated occupational exposure to isopropanol. The PBPK model developed

by Mörk and Johanson (2006) accounts for variation in workload by separating working and resting muscle groups.

The PBPK model of Kumagai and Matsunaga (1995) was designed to account for uptake of acetone in the mucous layer of the respiratory tract. By adjusting the value for the volume of the mucous layer and the rate of respiration, the study authors found that the simulated acetone concentrations in arterial blood, end exhaled air, urine, and fatty tissues were well matched to experimental data.

Mörk and Johanson (2006) designed a PBPK model for acetone to account for differences in the behavior of acetone in blood and exhaled air at different levels of physical exercise. The model involves deeper parts of the mucous membrane in absorption and desorption of acetone than the ones used in previous modeling exercises and includes separate compartments for working and resting muscles. In a follow-up study, the authors used Bayesian population analysis to derive improved estimates of population variability and uncertainty in the PBPK model parameters (Mörk et al. 2009). Using the PBPK model, Mörk and Johanson (2010) derived chemical-specific adjustment factors (CSAFs) for acetone by Monte Carlo simulations. According to the simulations, CSAFs for occupational exposure were 1.6, 1.8, and 1.9 for 90th, 95th, and 97.5^{th} percentiles, respectively. The corresponding CSAFs for the general population were 2.1, 2.9, and 3.8. CSAFs for children from 3 months of age to 10 years of age were 4.2–4.8, 4.7–5.0, and 5.0–5.9 for the 90th, 95th, and 97.5^{th} percentiles, respectively.

3.1.6 Animal-to-Human Extrapolations

Acetone appears to have similar target organs in animal and humans, such as the hematological system and the CNS. Toxicokinetic studies have been conducted in both humans and animals, especially in humans exposed by inhalation. There appears to be very few differences between animal species, and the dog appears to be a good model for extrapolating absorption results to humans (DiVincenzo et al. 1973). Metabolic pathways have been elucidated primarily in rats, but mice and rabbits have also been studied. Metabolism involves three different pathways of gluconeogenesis (Casazza et al. 1984; Kosugi et al. 1986a, 1986b). The first step in the metabolism of acetone is dependent on CYP2E1 (Casazza et al. 1984), which acetone induces, and this induction has been demonstrated in rats (Johansson et al. 1988), mice (Bánhegyi et al. 1988), hamsters (Ueng et al. 1991), and rabbits (Ding and Coon 1990). It appears that the metabolic pathways operate in all tested species. The distribution of acetone has been studied only in mice exposed by inhalation (Wigaeus et al. 1982). Acetone was widely distributed to organs and

tissues throughout the body. This is expected to be true for all species by virtue of its high water solubility, facilitating distribution through the water compartments of the body.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to acetone are discussed in Section 5.7, Populations with Potentially High Exposures.

Several lines of evidence from studies in animals indicate that sex differences exist in the susceptibility to effects caused by acetone. Male rats were more susceptible than female rats to acetone's hematological, hepatic, and renal effects, and effects on reproductive organs (American Biogenics Corp. 1986; NTP 1991). In a study of humans exposed to acetone in air during light exercise for 2 hours, women had higher levels of acetone in blood, saliva, and exhaled air than men (Ernstgard et al. 2003). While results in animals cannot always be extrapolated to humans, it is possible that men may be more susceptible than women to the hematological, hepatic, renal, and reproductive effects of acetone. Furthermore, acetone may exacerbate preexisting hematological, liver, kidney, or reproductive disorders in humans.

In a lethality study among male and female newborn rats, male and female 14-day-old rats, and male adult rats, susceptibility to the lethal effects of acetone generally decreased with increasing maturity (Kimura et al. 1971).

Pregnant rats exposed to acetone by inhalation during gestation had reduced body weights (NTP 1991), while nonpregnant rats exposed to a higher concentration for a longer duration did not show any effects on body weight (Goldberg et al. 1964). Pregnant rats also had lower plasma and liver levels of acetol, the first intermediate in the overall metabolism of acetone, than virgin rats (Peinado et al. 1986), suggesting differences in the rate of acetone metabolism. It is possible that the condition of pregnancy made these rats more susceptible to body weight changes, and this susceptibility might apply to humans. Exposure to acetone and increased endogenous ketone levels through gestational diabetes are known to increase hepatic CYP2E1 in pregnant mothers; however, gestational diabetes does not appear to increase placental CPY2E1 in humans (Pasanen 1988).

The role of acetone in fasting and diabetes is complicated and not well understood (Reichard et al. 1979, 1986). Acetone is produced endogenously and, as demonstrated in humans, more acetone is produced endogenously by fasting, which can result in ketosis (Reichard et al. 1979). In one human study, subjects on various ketogenic diets with lower body mass index produced more acetone (Prabhakar et al. 2015). In diabetics, ketoacidosis has been identified as the main pathological response to malabsorption of glucose (Dabek et al. 2020). Ketogenic diets, which induce a mild ketonemia, have been shown to promote endogenous ketone body production (Dabek et al. 2020). Acetone exposure of rats resulted in a reduced insulin-stimulated glucose oxidation rate, and the reduction was greater in fasted rats than in fed rats, indicating that the insulin resistance indigenous to fasting may be attributed in part to metabolic influences of acetone (Skutches et al. 1990).

Diabetics may also be more susceptible to the effects of acetone. Acetone-induced insulin resistance (Skutches et al. 1990) might also result in greater hyperglycemia in diabetics. Patients with diabetic ketoacidosis have higher plasma levels of endogenous acetone (Reichard et al. 1986), and exposure to exogenous acetone may increase the levels further. Similar results were found in rats. Diabetic rats had higher plasma acetone levels than nondiabetic rats after treatment with the same doses of acetone, due to the higher endogenous level of acetone in the diabetic rats and differences in the metabolism of acetone to isopropyl alcohol (Lewis et al. 1984). Diabetic rats were also less able to oxidize acetone than nondiabetic rats (Price and Rittenberg 1950). While research suggests that the metabolic pathway for acetone is similar in rats and humans, studies of acetone exposure in diabetic and obese animals have been conducted at higher doses than expected background human environmental exposures. That said, a human study showed decreasing breath acetone levels along with decreasing insulin levels in type 1 diabetic patients during a hypoglycemic clamp, a technique involving infusion of insulin and glucose to

produce a stepwise reduction in blood glucose (Turner et al. 2009). Results of this study indicate that variation in metabolic status and/or changes in glycaemia in type 1 diabetics may affect acetone levels.

Groups of obese and lean mice maintained on high-fat diets were given acetone in drinking water (2%) for 2 weeks to induce CYP2E1 (Dey and Cedebaum 2007). This study used homozygous obese C57BL/6J ob/ob mice in the obese groups, which are leptin-deficient mice that are bred to exhibit obesity (Drel et al. 2006). Controls consisted of obese and lean mice maintained on the same diet as the experimental mice but not given acetone. Acetone induced more extensive fatty changes, and mild necrosis in the livers of the obese mice compared with the livers of both control lean and control obese mice. The acetone-treated obese mice also had higher caspase-3 activity; numerous apoptotic hepatocytes; increased protein carbonyls, malondialdehyde, 4-hydroxynonenal-, and 3-nitrotyrosine-protein adducts; and elevated levels of inducible nitric oxide synthase. These results suggest that obesity contributes to liver toxicity and that the damage is enhanced by exposure to acetone through its induction of CYP2E1.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to acetone are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for acetone from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of

tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by acetone are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Acetone concentrations in expired air, blood, and urine have been monitored in a number of studies of humans exposed to acetone in the workplace as well as in controlled laboratory situations, and studies show that acetone levels in the body are an accurate indicator of acetone exposure (Leung and Paustenbach 1988). A study of 659 factory workers exposed to acetone occupationally reported a strong positive correlation between acetone levels in workplace air and acetone levels in workers' urine after their shift (Ghittori et al. 1987). However, acetone is cleared from breath, urine, and blood within 1–3 days, so these methods are useful for monitoring only for recent exposure to acetone. In addition, these methods can be used to detect or confirm relatively high exposure to acetone, such as what might occur in the workplace or from accidental ingestion; but they cannot be used to detect lower environmental exposures in the general population at levels that are expected to be lower than those observed in occupational settings. The detection of acetone odor in the breath can alert a physician that a nondiabetic patient has been exposed to acetone (Harris and Jackson 1952; Strong 1944). It should be noted that exposure to other chemicals that are metabolized to acetone, such as isopropyl alcohol, could also lead to elevated blood, expired air, or urinary levels of acetone.

Levels of endogenous acetone can fluctuate greatly due to normal diurnal variations (Wildenhoff 1972). In addition, physical exercise (Koeslag et al. 1980), nutritional status and fasting (Jones 1987; Kundu et al. 1993; Levy et al. 1973; Lewis et al. 1977; Neiman et al. 1987; Reichard et al. 1979; Rooth and Carlstrom 1970; Williamson and Whitelaw 1978), trauma (Smith et al. 1975), and pregnancy and lactation (Bruss 1989; Paterson et al. 1967) place high energy demands upon the body, resulting in

increased fatty acid utilization and higher than average blood levels of acetone. Diabetes (Kobayashi et al. 1983; Levey et al. 1964; Reichard et al. 1986; Rooth 1967; Rooth and Ostenson 1966) and alcohol use (Phillips et al. 1989; Tsukamoto et al. 1991) may result in higher levels of endogenous acetone. Infants and young children typically have higher acetone in their blood than adults due to their higher energy expenditure (Peden 1964). These factors and physiological states can complicate measuring acetone levels in blood, breath, and urine for biomonitoring purposes.

In a group of 115 workers, alveolar air samples obtained during the workshift were collected at the same time as breathing zone acetone concentrations (Brugnone et al. 1980). The mean ratio of alveolar air acetone and breathing zone acetone was 0.288. Correlations were high between alveolar air concentrations and breathing zone concentrations. Because the alveolar air samples and breathing zone concentrations were collected at the same time, and because the equilibration of alveolar air with environmental air requires some time, the alveolar samples might not necessarily reflect the environmental concentration. Similar results were obtained in a group of 20 workers in a shoe factory in which the mean environmental air concentrations ranged from 10 to 12 ppm at four sampling times (Brugnone et al. 1978). The mean alveolar concentrations ranged from 2.75 to 3.75 ppm at three sampling times during the workshift. The correlation was good between workroom air concentration and alveolar air concentration, indicating that alveolar air concentrations of acetone are useful for monitoring concurrent occupational exposure to acetone. In a group of 110 male workers exposed to acetone for an average of 14.9 years, alveolar air samples were collected before work and at the end of work on 2 consecutive days (Fujino et al. 1992). The breathing zone concentrations of acetone were measured for each individual with personal monitors and ranged from 0 to about 1,200 ppm, with most concentrations between 100 and 500 ppm. The average concentration of acetone in alveolar air before exposure on the first day was 2.95 ppm. Alveolar air concentrations at the end of the workday (range of about 20–300 ppm, average not reported) correlated strongly with exposure concentrations (r=0.65). It was estimated that the alveolar air concentrations corresponding to 750 ppm (the current American Conference of Governmental Industrial Hygienists [ACGIH] TLV for short-term exposure to acetone) and to the Japan Association of Industrial Health acceptable concentration of 200 ppm were 177 and 56.2 ppm, respectively.

Expired air concentrations of acetone have also been studied in volunteers exposed to acetone in controlled laboratory situations. In 11 men and 11 women exposed to 237 ppm acetone for 2 or 4 hours, alveolar breath samples collected immediately after exposure contained mean levels of acetone of 21.5 ppm in those exposed for 2 hours and 25.8 ppm in those exposed for 4 hours (Dick et al. 1989). The

alveolar air concentrations of acetone dropped to 12.8 ppm by 90 minutes after the 4-hour exposure and to baseline levels of 0.6 ppm by 20 hours postexposure. In humans exposed to acetone at up to 1,250 ppm for up to 7.5 hours/day in a complex protocol for up to 6 weeks, the rate of respiratory excretion was a function of the duration, and the concentration of acetone in breath after exposure was directly related to the time-average concentration during exposure, with constant duration (Stewart et al. 1975). The length of time after exposure in which acetone could be detected in breath was related to the magnitude of exposure; acetone was still readily detectable 16 hours after exposure to 1,000 or 1,250 ppm for 7.5 hours. Breath analysis can be used as a rapid method to estimate the magnitude of recent acetone exposure, but should only be used to assess recent exposures because the elimination of acetone in expired air is generally complete within 1 day.

As discussed in Section 3.1.4, the level and nature of physical activity, the exposure concentration, the duration of exposure, and biological sex can influence the rate and amount of acetone elimination in the breath (DiVincenzo et al. 1973; Nomiyama and Nomiyama 1974a, 1974b; Pezzagno et al. 1986; Wigaeus et al. 1981). In general, more acetone is expired faster following exposure to high concentrations than to low concentrations (DiVincenzo et al. 1973). Doubling the duration of exposure almost doubles the total amount of acetone expired. Exercise during exposure eliminates nearly twice the amount in expired air compared with exposure to the same concentration at rest, due to increased uptake from increased pulmonary ventilation. Furthermore, exercising at stepwise increments in workload during exposure results in greater respiratory elimination than exercising at a constant workload (Wigaeus et al. 1981). Women appeared to expire acetone more slowly than men, but the total expired by women was not statistically significantly different than the total expired by men (Nomiyama and Nomiyama 1974a, 1974b).

Acetone is mainly excreted in the expired air after oral exposure as well as after inhalation exposure (see Section 3.1.4). Because urinary clearance of acetone is minimal, the calculated clearance of acetone from the lungs was 29 mL/minute or 0.39 mL/minute/kg for a patient who ingested nail polish remover using an average minute ventilation of 9.65 L/minute based on the patient's age, weight, and sex (Ramu et al. 1978). With a volume of distribution of 0.82 L/kg, the calculated half-life was 25 hours.

Monitoring of expired air for acetone exposure should take into consideration baseline levels of acetone, because acetone is produced endogenously in the body, especially during fasting and in diabetics. In addition, the ingestion of ethanol can influence the breath levels of acetone. Endogenous levels of acetone in normal humans averaged 0.56 ppm (Phillips and Greenberg 1987). Endogenous levels of

acetone in alveolar air in a group of volunteers in an experimental study averaged 0.108 ppm (Wigaeus et al. 1981). Breath sampling of volunteers under normal conditions found a mean alveolar gradient (difference between concentrations in exhaled air and inhaled ambient air) of 27.91 for acetone, indicating that the rate of *in vivo* synthesis is greater than the rate of clearance (Phillips et al. 1999). In healthy men who had fasted for 12 hours, the breath acetone levels ranged from 0.96 to 1.7 ppm (Jones 1987). Fasting for 36 hours resulted in average acetone breath levels of 14–66 ppm. However, in fasting men who ingested 0.25 g/kg of ethanol, the breath acetone levels decreased by 40% after a 12-hour fast and by 18% after a 36-hour fast (Jones 1988).

Acetone is metabolized to carbon dioxide (see Section 3.1.3), which is eliminated in expired air (see Section 3.1.4). However, because carbon dioxide is the main constituent of normal expired air, expired carbon dioxide has not been monitored to determine acetone exposure.

Although unchanged acetone is excreted mainly by the lungs, urinary levels are sufficiently high for monitoring purposes. In a group of 104 workers employed at factories in which breathing zone levels of acetone ranged from <242 to <1,452 ppm, urine was collected before the workshift and 4 hours after the shift started (Pezzagno et al. 1986). A close correlation was found between the TWA workroom concentration and the urinary concentration of acetone. The equation obtained was: urinary concentration (µmol/L) = 0.033 x TWA environmental concentration (µmol/m^3) - 0.005 (r=0.94, n=104). In another study of 28 workers, personal breathing zone monitoring revealed wide variation depending on the type of job and ranged from <1 to 30 ppm (Kawai et al. 1990a). Results of stationary monitoring revealed workroom concentrations ranging from 1.4 to 16.2 ppm. Urine was collected at the end of the workshift, and acetone was detected in the urine of all the workers. The concentration of acetone in urine was linearly correlated with the breathing zone concentration as follows: acetone in urine (mg/L) = 0.10 + 0.40 x breathing zone concentration (ppm) (r=0.90, p<0.01). Therefore, urinary levels of acetone are useful for monitoring occupational exposure. In another study, postshift urinary levels of acetone in 45 workers exposed to 0–70 ppm acetone ranged from 0 to 17.5 mg/L (Kawai et al. 1992). The baseline urinary level of acetone in nonexposed subjects was 1.5 mg/L. Acetone levels in preshift urine samples were significantly higher than baseline levels when acetone exposure on the previous day was >15 ppm, but there was no significant difference between baseline urine levels and preshift urine levels when acetone exposure on the previous day was <15 ppm. In a group of 110 male workers exposed to acetone for an average of 14.9 years, urine samples were collected before work and at the end of work for 2 consecutive days (Fujino et al. 1992). The breathing zone concentrations of acetone were measured for each individual with personal monitors and ranged from 0 to about 1,200 ppm, with most concentrations

between 100 and 500 ppm. The average urinary concentration before exposure on the first day was 2.44 mg/L. Urinary levels at the end of the workshift (range of about 5–150 ppm, average not reported) correlated with exposure concentration (r=0.71). It was estimated that the urinary concentrations corresponding to 750 ppm (the current ACGIH TLV for short-term exposure to acetone) and to the Japan Association of Industrial Health acceptable concentration of 200 ppm were 76.6 and 21.6 mg/L, respectively.

Acetone has also been detected in the urine of 15 men and women exposed to acetone under controlled laboratory conditions. In volunteers exposed to 23–208 ppm for 2–4 hours, the urinary concentrations of acetone immediately after exposure ranged from 18.8 to 155.2 µmol/L and displayed statistically significant linear relationships with the exposure concentrations (Pezzagno et al. 1986). The regression equation for subjects exposed for 2 hours at rest was: acetone in urine (µmol/L) = 0.0125 x environmental concentration (µmol/m^3) + 5.87 (r=0.98, n=5). For subjects exposed for 4 hours at rest the equation was: acetone in urine (µmol/L) = environmental concentration (µmol/m^3) + 6.97 (r=0.96, n=5). For the subjects exposed for 2 hours with exercise, the equation was: acetone in urine (pmol/L) = environmental concentration (µmol/m^3) - 4.52 (r=0.99, n=5). At 4 hours after the cessation of exposure, the urine concentration increased to 120% of that measured immediately after exposure, then fell to 65% at 7 hours, 45% at 9 hours, 35% at 12 hours, and 15% at 20 hours post-exposure. Urinary acetone was completely cleared within 20 hours from subjects exposed to 242 or 542 ppm for 2 hours, regardless of whether or not they had exercised during exposure (Wigaeus et al. 1981). In a group of subjects exposed to acetone vapors for about 6 hours, urinary levels of acetone peaked within the first hour after exposure to 1.8 mg/dL at 250 ppm, 2.9 mg/dL at 500 ppm, and 5.3 mg/dL at 1,000 ppm and declined rapidly post-exposure to control levels within 24, 32, and 48 hours, respectively (Matsushita et al. 1969b). In subjects exposed to 250 ppm for 6 hours/day for 6 days either at rest or during exercise, the urinary levels returned to pre-exposure levels by the next morning each day and within 48 hours after the last exposure day, regardless of whether or not they had exercised (Matsushita et al. 1969a). However, in subjects exposed to 500 ppm 6 hours/day for 6 days, the level of acetone in the urine fell each day, but not to pre-exposure baseline levels. After the last day of exposure, urinary levels returned to baseline levels within 3 days. Baseline urinary levels of acetone in these subjects were about 0.1 mg/dL. Therefore, the rate of urinary clearance is dependent on the magnitude of exposure.

Acetone can also be detected in urine after oral exposure. In a male patient who was admitted to the hospital in a comatose condition after ingesting sake (alcoholic beverage) and liquid cement containing 18% acetone (231 mg/kg), urinary clearance of acetone was followed, but after he had been subjected to

gastric lavage (Sakata et al. 1989). Urine levels of acetone decreased gradually from 123 µg/mL at 5 hours after ingestion to about 61 µg/mL at 19 hours. Acetone then disappeared more rapidly from the urine.

Formic acid was detected in the urine of rats collected for 7 days after exposure to 62,000 ppm acetone in air, and was excreted at a rate of 344 µg formic acid/hour, compared with controls that excreted formic acid at a rate of 144 µg/hour (Hallier et al. 1981). The authors concluded that the low rate of formic acid excretion by rats suggests that 24 hours is an insufficient period of time for following formic acid excretion in order to biomonitor acetone exposure in humans.

Blood levels of acetone can also be useful for exposure monitoring, but blood sampling is less desirable because it is more invasive. In a group of 110 male workers exposed to acetone for an average of 14.9 years, blood samples were collected before work on the first day and at the end of work on the second day (Fujino et al. 1992). The breathing zone concentrations of acetone were measured for each individual with personal monitors and ranged from 0 to about 1,200 ppm, with most concentrations between 100 and 500 ppm. The average blood concentration before exposure on the first day was 3.80 mg/L. Blood levels at the end of the workshift (range of about 2 to 225 mg/L, average not reported) correlated strongly with exposure concentration (r=0.65). It was estimated that the blood concentrations corresponding to the current ACGIH TLV for short-term exposure to acetone of 750 ppm and to the Japan Association of Industrial Health acceptable concentration of 200 ppm were 118 and 41.4 mg/L, respectively. Subjects exposed to 100 or 500 ppm for 2 or 4 hours had a blood acetone clearance half-life of 3 hours (DiVincenzo et al. 1973). The rate of blood elimination was constant regardless of blood acetone concentration. In volunteers exposed to 237 ppm acetone, blood levels of acetone averaged 2.0 µg/mL preexposure, 9.0 µg/mL after 2 hours of exposure, 15.3 µg/mL after 4 hours of exposure, 11.9 µg/mL at 90 minutes postexposure, and 1.5 µg/mL at 20 hours postexposure (Dick et al. 1989). Therefore, elimination of acetone from blood was complete 20 hours after exposure. Results were similar for subjects exposed to acetone vapors for 6 hours (Matsushita et al. 1969b). Maximum blood levels of acetone achieved and blood clearance of acetone were exposure concentration related, but not in direct proportion. At an exposure level of 250 ppm, the maximum blood level was 2 mg/dL and returned to baseline levels within 24 hours. At an exposure level of 500 ppm, the maximum blood level was 4.7 mg/dL and returned to baseline levels within 32 hours. At an exposure level of 1,000 ppm, the maximum blood level of 6.0 mg/dL returned to baseline levels within 48 hours. In subjects exposed 6 hours/day for 6 days, maximum blood levels on each day were similar to those seen in the subject exposed only 1 day (Matsushita et al. 1969a). Blood levels returned to baseline levels on the morning

after exposure on each day when the exposure concentration was 250 ppm. With an exposure concentration of 500 ppm, however, blood levels declined each day, but not to baseline levels. As with urinary clearance, blood clearance of acetone at the end of the 6-day exposure period returned to baseline within 2 days at 250 ppm and within 3 days at 500 ppm. Baseline blood levels of acetone in these subjects were about 0.1 mg/dL. In subjects exposed to 242 or 542 ppm for 2 hours, the arterial blood concentration 1 hour post-exposure plotted as a function of total uptake gave a linear relationship, indicating that an arterialized capillary sample during or after exposure may be useful for exposure monitoring (Wigaeus et al. 1981). In humans exposed to acetone up to 1,250 ppm for up to 7.5 hours/day in a complex protocol for up to 6 weeks, the concentration of acetone in venous blood was directly related to the vapor concentration and duration of exposure and inversely related to the time elapsed following exposure (Stewart et al. 1975). Using a physiologically-based pharmacokinetic model, Leung and Paustenbach (1988) calculated a biological exposure index of 35 mg acetone/L blood for occupational exposure. The authors reported a baseline acetone blood level of 2 mg/L. This value is in agreement with baseline levels determined in other studies: 0.016 mM (0.93 mg/L) (Gavino et al. 1986), 0.03 mmol/L (1.74 mg/L) (Trotter et al. 1971), and 2,100 ppb (2.1 mg/L) (Ashley et al. 1992).

Similar rates of blood acetone clearance occur after oral exposure. In a patient admitted to the hospital in a comatose condition after ingesting liquid cement containing 18% acetone (231 mg/kg), the plasma level of acetone was 110 µg/mL at 5 hours after ingestion and declined to 65 µg/mL at 18 hours, to 60 µg/mL at 20 hours, and to <5 µg/mL at 48 hours (Sakata et al. 1989). The gastric contents of a patient were analyzed using infrared spectrophotometry and found to contain 1 mL acetone/100 mL (Fastlich 1976). This analytical method was developed to detect volatile solvents in gastric contents due to accidental ingestion of these solvents.

Acetone has been identified in breast milk of lactating women (Pellizzari et al. 1982).

3.3.2 Biomarkers of Effect

The most consistently observed effect of acetone exposure in animals is the induction of microsomal enzymes, particularly of CYP2E1 (see Sections 2.21, 3.1.3, and 3.4). The enzyme induction has been associated with increased liver weights and hepatocellular hypertrophy due to the increased protein content (NTP 1991). Acetone itself is only moderately toxic to the liver of animals, as most studies have found no clinical or histological evidence of liver damage. However, increased levels of serum alanine aminotransferase beyond the expected range, which constitutes clinical evidence of liver damage, have

been found in rats in one study (American Biogenics Corp. 1986). CYP2E1 is associated with the metabolism of acetone itself, but acetone is not metabolized to toxic intermediates (see Section 2.3). However, the induction of this enzyme by acetone is the mechanism by which acetone potentiates the hepatotoxicity, nephrotoxicity, genotoxicity, and perhaps the reproductive and hematological toxicity of other chemicals (see Section 2.6). CYP2E1 can be induced by a variety of other factors, such as exposure to ethanol, fasting, and experimental diabetes (Johansson et al. 1986; Puccini et al. 1990); therefore, the induction is not specific to acetone. Moreover, the detection of enzyme induction might require invasive methods, such as liver biopsy.

Exposure of animals to acetone has resulted in degeneration of apical microvilli in renal tubules (Brown and Hewitt 1984) and enhancement of nephropathy commonly seen in aging rats (NTP 1991), but these effects have not been associated with increased levels of blood urea nitrogen.

As is typical of many organic solvents, acetone is irritating to respiratory mucosa, the skin, and eyes. Acetone exposure can also result in such nonspecific narcotic effects such as headache, dizziness, lightheadedness, confusion, unconsciousness (DiVincenzo et al. 1973; Matsushita et al. 1969a, 1969b; Nelson et al. 1943; Raleigh and McGee 1972; Ross 1973), some neurobehavioral and hematological effects (Dick et al. 1989; Matsushita et al. 1969a; Stewart et al. 1975), and perhaps menstrual disorders (Stewart et al. 1975). In addition, patients who had hip casts applied with acetone as the setting fluid became nauseous, vomited blood, and had a strong odor of acetone in the breath. These symptoms were associated with the subsequent development of unconsciousness (Harris and Jackson 1952; Strong 1944). The detection of a strong acetone odor on the breath and nausea could alert physicians to the development of more serious sequelae, such as gastrointestinal hemorrhage and narcosis.

Because acetone is a ketone, acetone exposure can lead to ketosis and other diabetes-like symptoms in humans (Gitelson et al. 1966) and to reduced insulin-stimulated glucose oxidation in animals (Skutches et al. 1990). Again, the detection of a strong odor of acetone on the breath, or high levels of acetone in blood or urine can alert physicians to these effects.

In male rats, acetone exposure resulted in anemia as detected by hematological parameters (American Biogenics Corp. 1986; NTP 1991), and in increased testes weight, decreased sperm motility, caudal and epididymal weight, and increased incidences of abnormal sperm (NTP 1991). Hematological tests and tests for sperm motility and abnormalities could be used to screen humans for possible hematological and fertility effects.

Dermal exposure of humans to acetone irritated the skin, which when examined by light and electron microscopy, showed signs of degenerative changes in the epidermis (Lupulescu and Birmingham 1976; Lupulescu et al. 1972, 1973). Decreased protein synthesis was also found (Lupulescu and Birmingham 1975). Overt signs of skin irritation could alert physicians to possible degenerative changes. Allergic reactions to acetone can be detected by patch testing (Tosti et al. 1988).

As most of the effects from acetone exposure are not specific to acetone, there is no reliable biomarker of effect that can be used to detect or screen for possible effects from exposure to acetone at levels reasonably likely to occur outside the workplace or from accidental ingestion.

3.4 INTERACTIONS WITH OTHER CHEMICALS

While acetone by itself is only moderately toxic, it potentiates the toxicity of a variety of chemicals, including halogenated alkanes and alkenes, benzene, dichlorobenzene, ethanol, 2,5-hexanedione, nitrosamines, acetonitrile, and acetaminophen. The most extensively studied interactions are those with carbon tetrachloride and chloroform. In most of the interactions discussed below, acetone exerts its potentiating effect by inducing microsomal mixed function oxidases, in particular CYP2E1 and CYP2E1-dependent enzyme activities, that bioactivate the other chemicals to reactive metabolites.

Halogenated Alkanes and Alkenes. No studies were located regarding the effects of coexposure of humans to acetone and carbon tetrachloride. However, acetone, a metabolite of isopropyl alcohol, was implicated in a study of workers in an isopropyl alcohol packaging plant who became ill after accidental exposure to carbon tetrachloride (Folland et al. 1976). Fourteen workers became ill (nausea, vomiting, headache, and weakness or abdominal pain, dizziness, diarrhea, and blurred vision). Workers in closer proximity to isopropyl alcohol were especially affected. Renal failure and hepatitis developed in four of the workers with closer proximity to isopropyl alcohol. Expired air samples subsequently taken from workers during isopropyl alcohol bottling revealed strikingly elevated levels of acetone (means of 19 ppm in workers on the bottling line and 7.5 ppm in more remote workers). Their blood acetone levels were 3–30 times higher than the normal range. Thus, it appeared that isopropyl alcohol, by way of acetone, predisposed the workers to the hepatotoxicity and renal toxicity induced by carbon tetrachloride.

The potentiation of carbon tetrachloride-induced hepatotoxicity and renal toxicity by acetone has been well documented in rats. Pretreatment of rats by gavage with acetone enhanced the hepatotoxicity of

carbon tetrachloride, as evidenced by the statistically significantly increased relative liver weights, increased severity of histopathological lesions (necrosis, hepatocellular swelling, lipid droplets), activities of serum alanine aminotransferase and ornithine carbamoyltransferase, the serum concentration of bilirubin, and/or the liver concentration of triglycerides compared with the liver toxicity induced by carbon tetrachloride alone in several studies (Charbonneau et al. 1985, 1986a, 1986c, 1988, 1991; Plaa and Traiger 1972; Plaa et al. 1973, 1982; Traiger and Plaa 1973, 1974). Acetone treatment alone had no effect on these parameters. The potentiation increased in a dose-related manner at single doses of acetone >0.25 mL/kg (>196 mg/kg); doses of <0.10 mL/kg (<78 mg/kg) are ineffective (Charbonneau et al. 1986a; Plaa et al. 1982; Traiger and Plaa 1973). In rats given the minimal effective dose of acetone (196 mg/kg) twice daily for 3 days (total dose 1,177 mg/kg), carbon tetrachloride-induced liver toxicity was further enhanced over that of a single dose of 196 mg/kg acetone (Plaa et al. 1982). However, repetitive dosing of acetone (6x196 mg/kg = 1,177 mg/kg; 12 hours between doses) potentiated the liver toxicity of carbon tetrachloride to a lesser extent than a single dose of 1,177 mg/kg. Administration of the noneffective dose (78 mg/kg) twice a day for 3 days (total dose 468 mg/kg) did not affect the liver toxicity of carbon tetrachloride, even though the cumulative dose of 468 mg/kg, if given as a single dose, would have been high enough to cause significant potentiation. When a dose of acetone of 1.5 mL/kg (1,177 mg/kg) was given once, divided into 6 doses of 0.25 mL/kg (196 mg/kg) over 3 days (cumulative dose 1,177 mg/kg), or into 12 doses of 0.125 mL/kg (98 mg/kg) over 3 days (cumulative dose 1,177 mg/kg), or infused intravenously over 3 days, before challenge with carbon tetrachloride, the most severe potentiation occurred with the single dose, followed by 6 divided doses, and then by 12 divided doses. The intravenous infusion did not enhance the toxicity of carbon tetrachloride. The maximum blood levels calculated from pharmacokinetic parameters for the different acetone treatment regimens showed a direct relationship with the degree of potentiation. The results indicate that threshold blood, and hence liver, concentrations must be exceeded before potentiation occurs. Acetone pretreatment also prolonged the time required for complete recovery induced by carbon tetrachloride exposure (Charbonneau et al. 1985). With carbon tetrachloride alone, the severity of liver toxicity increased temporally in rats sacrificed 24 and 48 hours after dosing, but liver toxicity was no longer observed at 96 hours. Following pretreatment with acetone, the liver toxicity induced by carbon tetrachloride was enhanced and increased in severity at all sacrifice times, even at 96 hours. Gavage pretreatment of rats with 1,452 mg/kg/day acetone in corn oil twice weekly for <12 weeks, followed by carbon tetrachloride challenge, resulted in decreased body weight gain and 35% mortality, compared with no effect on body weight gain and 5% mortality in rats given corn oil and challenged with carbon tetrachloride (Charbonneau et al. 1986c). Rats treated with acetone plus carbon tetrachloride also had statistically significantly decreased relative liver weights and statistically significant increased kidney weights at all

four sacrifice times, compared with corn oil plus carbon tetrachloride rats. Bilirubin concentrations and collagen content were also enhanced. Histological examination revealed fully developed cirrhosis in the acetone plus carbon tetrachloride rats, compared to less severe cirrhosis with corn oil plus carbon tetrachloride. Renal toxicity was also enhanced, as evidenced by statistically significantly elevated blood urea nitrogen levels in the acetone pretreated rats, compared with corn oil pretreated rats. It should be noted that acetone displays a greater degree of potentiation when it is administered in corn oil than in water (Charbonneau et al. 1986a, 1991), and it appears that corn oil alone can be toxic to the liver (Charbonneau et al. 1991).

Inhalation exposure of rats to acetone vapors also displays a threshold effect (Charbonneau et al. 1986a). In rats exposed to 1,000, 2,500, 5,000, 10,000, or 15,000 ppm acetone for four hours, and challenged 18 hours later with carbon tetrachloride, the liver toxicity of carbon tetrachloride was enhanced in a concentration-related manner at ≥2,500 ppm acetone. The noneffective concentration was 1,000 ppm. No cumulative effect of repetitive inhalation exposure to acetone on the carbon tetrachloride-induced liver toxicity was found, but maximum blood levels of acetone correlated with the degree of potentiation.

When rats were challenged with a mixture of trichloroethylene and carbon tetrachloride, the minimal effective dose of acetone required to enhance the liver toxicity of carbon tetrachloride decreased at least five-fold, indicating that mixtures of haloalkanes can cause severe liver injury, and prior exposure to acetone can markedly affect the response produced by the mixtures (Charbonneau et al. 1988).

The mechanism of acetone potentiation of carbon tetrachloride-induced liver toxicity involves the induction of mixed function oxidase microsomal enzymes. In microsomes prepared from rats treated by gavage with acetone at 2.5 mL/kg (1,961 mg/kg) and incubated with ^{14}C-carbon tetrachloride, covalent binding of radioactivity to microsomal protein increased 3–4 times that of control microsomes (Sipes et al. 1973). The time course followed the increased activity of N-nitrosodimethylamine N-demethylase, indicating enzyme induction. Furthermore, aminothiazole, an inhibitor of CYP and mixed function oxidase induction, reduced the potentiation by acetone of carbon tetrachloride-induced liver toxicity (Traiger and Plaa 1973). Studies have indicated that the effects of acetone on the toxicity of carbon tetrachloride are caused by induction of CYP forms belonging to at least two gene subfamilies, CYP2B and CYP2E (Johansson et al. 1988; Kobusch et al. 1989). Complementary DNA and protein sequencing analyses have shown that these CYP gene subfamilies are similar in rats and humans (Song et al. 1986). Acetone treatment caused a nine-fold increase in CYP2E1 accompanied by a similar increase in the rate of NADPH-dependent metabolism of carbon tetrachloride (Johansson et al. 1988). Acetone treatment

also increased the amount of messenger ribonucleic acid (mRNA) and apoprotein of CYP2B1 10- to 30-fold, suggesting regulation of CYP2B1 at the transcriptional level. mRNA coding for CYP2E1 was increased by a combination of fasting and acetone treatment, but not by treatment with acetone alone. The results suggested an enhanced rate of CYP2E1 gene transcription, CYP2E1 mRNA stabilization, or other posttranscriptional mechanisms. The exact mechanism by which acetone increases the CYP subfamilies is a subject of recent and on-going investigations. The findings that pretreatment of rats or rabbits with acetone results in increases in CYP2E1 and associated enzyme activities, but has no effect on the level of CYP2E1 mRNA, suggests that regulation of acetone-induced CYP2E1 occurs at the posttranscriptional level (Hong et al. 1987; Johansson et al. 1988; Porter et al. 1989; Ronis and Ingelman-Sundberg 1989; Ronis et al. 1991; Song et al. 1986, 1989). In microsomal and ribosomal preparations from rats administered acetone intraperitoneally, the polyribosomal distribution of CYP2E1 mRNA shifted, compared with controls, suggesting that the induction of CYP2E1 by acetone involved enhanced translation efficiency through increased loading of ribosomes of CYP2E1 mRNA (Kim et al. 1990). However, in another study, incorporation of 3H-leucine into CYP2E1 in microsomes from rats treated with acetone was lower than that in control microsomes, but the rate of translation of the CYP2E1 mRNA was about the same in both sets of microsomes, indicating that CYP2E1 is not induced by an increase in the rate of translation of its mRNA (Song et al. 1989). Furthermore, incorporation of $NaH^{14}CO^{3}$ was three-fold less in acetone induced CYP2E1 protein than in controls. The rate of disappearance of radiolabel from CYP2E1 in controls was biphasic, with half-lives of 7 and 37 hours for the fast and slow phase, respectively. However, in acetone-treated rats, the fast phase was absent, with a monophasic half-life of 37 hours. These results demonstrated that the induction of CYP2E1 by acetone is due primarily to protein stabilization. In microsomes and lysosomes from rats treated with acetone, CYP2E1 and CYP2B1 increased, but the increase was greater in microsomes (Ronis and Ingelman-Sundberg 1989; Ronis et al. 1991). Quantification of the proteins in lysosomes indicated that CYP2E1 and CYP2B1 are degraded via an autophagosomal/autolysosomal pathway. The study authors speculated that CYP2E1 is catalytically inactivated in microsomes prior to degradation in lysosomes and that acetone may interfere with the inactivation. Thus, the induction CYP2B1 appears to occur at the transcriptional level, while the induction of CYP2E1 by acetone appears to occur through stabilization of the apoprotein.

One study located suggests that induction of CYP may not be the only mechanism responsible for the interaction between acetone and carbon tetrachloride (Raymond and Plaa 1996). In an investigation of rats, examination of the liver membranes showed that acetone altered the effects of carbon tetrachloride on plasma membrane enzymes and membrane fluidity. Thus, the mechanism may involve the effects of co-exposure to acetone on membrane integrity.

Pretreatment of mice with carbon tetrachloride has been found to enhance the toxicity of acetone. In mice intraperitoneally pretreated with olive oil, the oral LD_{50} of acetone was 5,250 mg/kg, but in mice pretreated with a 20% solution of carbon tetrachloride in olive oil, the LD_{50} of acetone was 4,260 mg/kg (Tanii et al. 1986). The dose of carbon tetrachloride alone did not result in any death. The authors suggested that carbon tetrachloride inactivated the microsomal monooxygenase system, thereby inhibiting the inactivation of acetone.

Acetone also potentiates the hepato- and nephrotoxicity of chloroform. Pretreating rats with 15 mmol/kg (871 mg/kg) acetone in corn oil by gavage 18 hours prior to a challenge dose of 0.5 mL/kg chloroform in corn oil statistically significantly increased the relative kidney weight, inhibited lactate stimulated accumulation of p-aminohippurate and the accumulation of tetraethylammonium ion in kidney slices, and resulted in vacuolar degeneration in the tubular epithelium, but not necrosis, compared with corn oil controls (Hewitt et al. 1980). No effects on these parameters were observed with acetone alone or in rats pretreated with corn oil and challenged with chloroform. Acetone pretreatment also statistically significantly increased the plasma activities of alanine aminotransferase (32-fold) and ornithine carbamoyltransferase (134-fold), compared with corn oil pretreated controls challenged with chloroform, and caused balloon cells with pyknotic nuclei in the centrilobular region of the liver. Acetone alone and chloroform alone did not cause liver lesions. In rats treated by gavage with acetone in corn oil at 58, 290, 436, 581, 726, or 871 mg/kg and challenged with 0.5 mL/kg chloroform in corn oil, acetone showed a dose-dependent decrease in p-aminohippurate uptake and an increase in plasma creatinine levels, with maximum effects seen at doses between 290 and 581 mg/kg acetone (Brown and Hewitt 1984). Renal necrosis, hyaline bodies, and/or tubular casts were seen in 3/6 rats at 58 mg/kg acetone and in 4/6–5/6 rats at higher doses. Acetone pretreatment also statistically significantly increased plasma activities of alanine aminotransferase at ≥290 mg/kg. Balloon cells and necrosis were observed in 2/6 rats pretreated with 58 mg/kg and in most of the rats pretreated with ≥290 mg/kg. The effects of acetone pretreatment and chloroform challenge were greater than the effects of corn oil pretreatment and chloroform challenge. Pretreating rats by gavage with 0.5 mL/kg acetone (871 mg/kg) in corn oil prior to a challenge dose of chloroform (0.5 mL/kg) in corn oil statistically significantly increased the plasma activities of ALT and ornithine carbamoyltransferase above that seen in rats pretreated with corn oil and challenged with chloroform, and the potentiation was maximal at 18 hours (Hewitt et al. 1987). Microsomes from the acetone treated rats showed increased activities of ethoxycoumarin O-deethylase and NADPH-dependent cytochrome c reductase and statistically significantly increased rates of covalent binding of radioactivity from ^{14}C-chloroform in the reaction medium, compared with control microsomes. Results were similar

with microsomes prepared from rats treated by gavage with 2.5 mL/kg (1,961 mg/kg) acetone in corn oil. Acetone enhanced the covalent binding of radioactivity of ^{14}C-chloroform two-fold compared with control microsomes and increased the activity of N-nitrosodimethylamine N-demethylase (Sipes et al. 1973), an activity associated with CYP2E1. Thus, acetone increased the biotransformation of chloroform.

The involvement of CYP2E1 was confirmed with microsomes from rats given a gavage dose of acetone (871 mg/kg) in corn oil (Brady et al. 1989). Acetone statistically significantly increased the CYP content, the activity of N-nitrosodimethylamine N-demethylase, and the content of CYP2E1, but not CYP2B1, compared with control microsomes. Furthermore, no effect was seen on the activity of benzphetamine demethylase, an activity associated with CYP2B1. The acetone-induced microsomes also showed a three-fold enhancement of CYP2E1-dependent chloroform metabolism, but the activity required the presence of cytochrome b5. No increased CYP2B1-dependent metabolism was seen. The involvement of CYP2E1 was further demonstrated by inhibition of the reaction with a monoclonal antibody to CYP2E1 and by alternate substrates for CYP2E1 such as pyrazole, benzene, nitrosodimethylamine, and diallyl sulfate.

Acetone also potentiates the toxicity of other halogenated alkanes. In rats injected intraperitoneally with acetone in saline at doses of 581, 1,162, 1,742, or 2,323 mg/kg 48 hours prior to a gavage dose of dichloromethane (0.4 mL/kg), statistically significant increased blood levels of carboxyhemoglobin were observed at 21,742 mg/kg acetone, compared with controls challenged with dichloromethane (Pankow and Hoffmann 1989). The results indicated that acetone increased the metabolism of dichloromethane to carbon monoxide. Results obtained with fasting rats or rats pretreated with isoniazid, which also induces CYP2E1, produced similar potentiation of dichloromethane-induced carboxyhemoglobinemia, thus implicating induction of CYP2E1 as the mechanism whereby acetone increased the metabolism of dichloromethane to carbon monoxide.

While neither bromodichloromethane or dibromochloromethane were hepatotoxic (assessed by relative liver weight and plasma activities of alanine aminotransferase and ornithine carbamoyltransferase) in rats at the sublethal doses used, acetone pretreatment at 871 mg/kg by gavage in water resulted in liver toxicity at lower challenge doses of these compounds (Hewitt et al. 1983). Neither bromodichloromethane nor dibromochloromethane alone displayed appreciable toxicity to the kidney (assessed by relative kidney weight, accumulation of p-aminohippurate and tetraethylammonium ion in kidney slices, and blood urea nitrogen). However, pretreatment with acetone resulted in statistically significant increased kidney weight, inhibition of p-aminohippurate uptake, and increased levels of blood

urea nitrogen after challenge with bromodichloromethane. With a challenge dose of dibromochloromethane, only blood urea nitrogen was significantly increased by acetone pretreatment. Acetone pretreatment of rats by gavage at doses of 196 and 392 mg/kg, prior to challenge with 1,1,2-trichloroethane, potentiated 1,1,2-trichloroethane-induced increased activity of plasma alanine aminotransferase (MacDonald et al. 1982a). However, higher pretreatment doses of acetone did not potentiate the toxicity and may have decreased the severity. Pretreatment of rats with acetone (392 mg/kg) followed by a challenge dose of ^{14}C-1,1,2-trichloroethane did not increase covalent binding of radioactivity to microsomal proteins but resulted in a greater decline in the content of reduced glutathione. When ^{14}C-trichloroethane was added *in vitro*, covalent binding of the radiolabel statistically significantly increased in microsomes from rats treated with acetone, compared with control microsomes (MacDonald et al. 1982b). The *in vitro* covalent binding was inhibited 80% by the addition of reduced glutathione. It was suggested that acetone alters the bioactivation and the detoxification of 1,1,2-trichloroethane, but the exact mechanism is unclear.

Acetone also potentiated the hepatotoxicity of chlorinated alkenes. Inhalation exposure of adult male rats to 10,000 ppm acetone vapor for 2 hours prior to or during concomitant inhalation exposure to 2,000 ppm 1,1-dichloroethene resulted in statistically significant increased activity of serum alpha-ketoglutarate transaminase, compared with that induced by 1,1-dichloroethene alone (Jaeger et al. 1975). A biphasic pattern of potentiation of the liver toxicity induced by 1,1-dichloroethene was observed in rats pretreated orally with acetone at several dose levels (Hewitt and Plaa 1983). At doses of 290 and 581 mg/kg acetone prior to challenge with 1,1-dichloroethene, statistically significant increased activities of plasma alanine aminotransferase and ornithine carbamoyltransferase were observed, compared with water pretreated rats challenged with 1,1-dichloroethene. At higher pretreatment doses of acetone (≥871 mg/kg), the effect on these parameters diminished and acetone appeared to have a protective effect. Treatment of rats with 1,1-dichloroethene did not result in any evidence of nephrotoxicity, but acetone pretreatment statistically significantly reduced the accumulation of tetraethylammonium ion in kidney slices. The biphasic pattern of potentiation/protection may be related to alterations in the rate and/or pattern of 1,1-dichloroethene bioactivation, such as, bioactivation to reactive intermediates or decreased detoxification by decreasing hepatic glutathione levels at the potentiating doses of acetone.

Benzene. Although potentiation of benzene toxicity by acetone has not been specifically tested, microsomes from rats treated with acetone (3,922 mg/kg) for 1 or 2 days produced an 8-fold increase in the rate of NADPH-dependent oxidation of benzene and induced CYP, in particular CYP2E1 (Johansson et al. 1988; Johansson and Ingelman-Sundberg 1988). Addition of inhibitors of CYP2E1 inhibited the

oxidation of benzene in microsomes from acetone treated rats, providing further evidence that this form of CYP is involved. In addition, antibodies to rabbit CYP2E2 and rat CYP2E1 inhibited the oxidation of benzene by 80–100% in microsomes prepared from rabbits and rats treated with acetone. In hepatocytes from rabbits given acetone (863 mg/kg/day) in drinking water for 7 days, immunoblot analysis identified three distinct cytochromes: CYP2E1, CYP2A1, and CYP2A2 (Schnier et al. 1989). In bone marrow cells from the treated rabbits, CYP2E1 and CYP1A1 were identified. Quantitative analysis revealed that acetone treatment resulted in a 7.3-fold induction of CYP2E1 in liver and a 12.9-fold induction of CYP2E1 in bone marrow cells. Acetone slightly decreased the concentration of CYP reductase in bone marrow, and increased the ratio of CYP2E1 to reductase by 16.4 times and the ratio of CYP1A1 to reductase by 2 times. Hepatic microsomes from acetone-treated rabbits were 4.8 times more active than control microsomes in benzene hydroxylation, an activity of CYP2E1. Acetone-induced marrow microsomes were 9.4 times more active in benzene hydroxylation. In a study of mice, acetone pretreatment via drinking water was associated with significant increases in the percent and mass of hydroxylated benzene metabolites after exposure to benzene at 600 ppm (Kenyon et al. 1998). Thus, the stimulation of benzene metabolism by acetone occurs by a mechanism similar to that of the stimulation of carbon tetrachloride metabolism by acetone. The results suggest that acetone may potentiate the toxicity of benzene, because bioactivation is required for the expression of hematotoxicity of benzene (Sammett et al. 1979; Snyder et al. 1975). It should be noted that commercial acetone may contain low levels of benzene (Pubchem 2021).

Dichlorobenzene. Inhalation exposure of rats to acetone vapors at 4,785, 10,670, or 14,790 ppm for 4 hours increased the CYP contents and the activity of glutathione-S-transferase, with the greatest increases occurring at the 4,785 ppm level (Brondeau et al. 1989). When the rats were challenged 18 hours later by inhalation exposure to 1,2-dichlorobenzene, the level of CYP and the activity of glutathione-S-transferase were no different from that seen with acetone alone. However, acetone preexposure potentiated the liver toxicity of 1,2-dichlorobenzene at the lowest exposure, reduced it at 10,670 ppm, and suppressed it at 14,790 ppm. In mice exposed to 6,747, 8,910, or 14,345 ppm acetone for 4 hours, followed by a challenge by 1,2-dichlorobenzene, acetone preexposure caused an interactive glucose-6-phosphatase response in the mediolobular area of the liver. It was suggested that, at low concentrations, acetone induces the microsomal enzymes that convert 1,2-dichlorobenzene to toxic intermediates. However, because the glutathione-S-transferase activity did not increase in rats preexposed to acetone and challenged with 1,2-dichlorobenzene, the diminished liver toxicity induced by 1,2-dichlorobenzene after preexposure to the higher concentrations cannot be explained by detoxification via enhanced glutathione conjugation. Instead, two microsomal enzymes may be involved, in which, at

low concentrations of acetone, one (activating) enzyme is induced, but at higher concentrations concomitant induction of the second enzyme system could result in protection.

Ethanol. Acetone potentiated the CNS toxicity of ethanol in mice (Cunningham et al. 1989). Mice were pretreated with an intraperitoneal injection of acetone in corn oil at 581, 1,162, or 2,323 mg/kg, and 30 minutes later injected with 4,000 mg/kg ethanol. At 1,162 and 2,323 mg/kg, acetone statistically significantly prolonged the duration of the loss of righting reflex induced by ethanol. In mice given 2,323 mg/kg acetone prior to 2,000 mg/kg ethanol, the blood level of ethanol was statistically significantly higher at all time intervals measured, and acetone pretreatment significantly decreased the mean elimination rate of ethanol.

In vitro, acetone inhibited the activity of liver alcohol dehydrogenase, a reaction responsible for 90% of ethanol elimination. It was suggested that acetone produced a prolongation of the CNS toxicity of ethanol by reducing its elimination.

Other Ketones. The neurological and reproductive effects of coexposure to acetone and 2,5-hexanedione has been studied in animals. In rats exposed to 0.5% 2,5-hexanedione, 0.5% acetone (650 mg/kg/day), or to a combination of 0.5% 2,5-hexanedione and 0.5% acetone in drinking water for 6 weeks, peripheral motor nerve conduction velocity was measured weekly from the third week of dosing (Ladefoged et al. 1989). Acetone alone reduced the nerve conduction velocity compared with controls only at 6 weeks, while 2,5-hexanedione alone significantly reduced it from the third week on. The combination treatment resulted in a statistically significant greater reduction than was seen with 2,5-hexanedione alone on the fourth and sixth week. Acetone alone had no effect on balance time in the rotorod test, but balance time was statistically significantly reduced from the second week with the combination treatment, and the reduction was greater than that with 2,5-hexanedione alone from the fourth week on. In a similar dosing regimen for 7 weeks, coexposure to 2,5-hexanedione and acetone statistically significantly inhibited acquisition, but not performance of spatial learning (assessed in the radial arm maze) above that seen with 2,5-hexanedione alone (Lam et al. 1991). Brain weights of rats exposed to 2,5-hexanedione alone or to the combination were significantly reduced, with greater reduction in the coexposed group. Both treatments reduced synaptosomal 5-hydroxytryptamine uptake rate, but the combination treatment did not reduce the uptake below that seen with 2,5-hexanedione alone. In a companion report of these treatment groups, there was no significant difference on the number and size of neurons in the cerebral cortex between rats treated with 2,5-hexanedione alone or rats coexposed to 2,5-hexanedione and acetone (Strange et al. 1991).

In follow-up experiments, Ladefoged et al. (1994) included histological examination of the sciatic and tibial nerves in rats immediately after a 6-week exposure period and in rats 10 weeks after cessation exposure (recovery period). As in previous experiments, acetone potentiated 2,5-hexanedione-induced effects in open field ambulation and rearing balance in the rotarod tests, and grip strength. The ambulation effects were reversible during the recovery period by all treatments, but the effects on rearing and balance were reversible in the 2,5-hexanedione group only. That is, the potentiation by acetone persisted. Histological examination revealed that after exposure, giant axon swelling was induced by 2,5-hexanedione and the combination of 2,5-hexanedione and acetone, and a change in the distribution of fiber area size occurred in rats exposed to 2,5-hexanedione alone. The lesions observed in the co-exposure group were statistically similar to the effects of 2,5-hexanedione alone, but appeared aggravated by co-exposure, as seen by conventional pathological evaluation. After the 10-week recovery period, the nerve tissues appeared normal. The investigators concluded that neurotoxicity of the combined exposure was not reversible and that the mechanism of acetone potentiation is probably an effect on the toxicokinetics of 2,5-hexanedione.

Acetone alone had no effect on indices of fertility in male rats but potentiated the reproductive toxicity of 2,5-hexanedione when coadministered, compared with that seen with 2,5-hexanedione alone (Larsen et al. 1991). The rats were exposed to drinking water containing 0.13, 0.25, or 0.5% 2,5-hexanedione or in combination with 0.5% acetone for 6 weeks. Fertility was assessed by mating the exposed males with nonexposed females. 2,5-Hexanedione alone or the combination had no effects on the number of matings. 2,5-Hexanedione alone at 0.5% statistically significantly decreased the number of pregnancies, the number of fetuses, and the testicular weight. The combination treatments further reduced all indices, and at 0.5% 2,5-hexanedione plus 0.5% acetone, complete infertility occurred. Morphological assessment of the testes revealed mild to moderate vacuolization, chromatin margination, epithelial disruption, multinucleated giant cells, and/or atrophy in rats exposed to 2,5-hexanedione alone after 6 weeks of treatment, and the combination increased the severity of these lesions. When assessed 10 weeks after the end of treatment, the lesions were still present.

The mechanism by which acetone potentiates or adds to the toxicity of 2,5-hexanedione in rats is not known, but coexposure of rabbits to 2,5-hexanedione and acetone altered the pharmacokinetic parameters of 2,5-hexanedione (Ladefoged and Perbellini 1986). The combined treatment decreased the body clearance of 2,5-hexanedione, compared to the clearance of 2,5-hexanedione alone.

In a neurobehavioral study in volunteers, 11 men and 11 women exposed to 237 ppm acetone, 12 men and 13 women exposed to 200 ppm 2-butanone (methyl ethyl ketone), and 8 men and 13 women exposed simultaneously to acetone (125 ppm) and 2-butanone (100 ppm) for 4 hours were subjected to psychomotor tests (choice reaction time, visual vigilance, dual task, memory scanning), sensorimotor tests (postural sway), and psychological tests (profile of mood states) (Dick et al. 1989). Acetone exposure alone produced small but statistically significant changes in performances from controls in two measures of auditory tone discrimination (increased response time and increased false alarm) and hostility in men only. Neither 2-butanone alone nor the combination of acetone and 2-butanone produced any statistically significant changes. Furthermore, no interactions between acetone and 2-butanone on the uptake or elimination of acetone or 2-butanone were found in the same human subjects (Brown et al. 1987). From this limited information, it appears that acetone and 2-butanone do not interact to produce neurological effects.

Styrene. Information regarding interactions between acetone and styrene in the expression of toxic effects in animals is limited. In rats exposed to 2.2 mmol/kg styrene by intraperitoneal injection, a co-injection of 2.2 mmol/kg acetone was ineffective at attenuating symptoms of toxicity (Ikeda and Hirayama 1978). Several studies in humans have reported that coexposure to acetone and styrene produce different changes in the content or activity of biotransformation enzymes in the liver and lungs, compared with the changes seen with styrene alone (Elovaara et al. 1990, 1991; Vainio and Zitting 1978). A study of 19 male workers exposed to styrene and acetone in air at work for 4 hour intervals reported an inverse correlation between acetone concentration in air and styrene metabolite levels in subjects' urine post-shift, suggesting that acetone may slow metabolism of styrene (Marhuenda et al. 1997). However, in 23–34 year-old men exposed for 2 hours to 293 mg/m^3 styrene alone or to a mixture of 301 mg/m^3 styrene and 1,240 mg/m^3 (517 ppm) acetone, there was no indication that acetone alters the uptake, distribution, metabolism, or elimination of styrene (Wigaeus et al. 1984).

Nitrosamines. Acetone potentiated the hepatotoxicity of N-nitrosodimethylamine in rats pretreated by gavage with 2.5 mL/kg (1,961 mg/kg) acetone in water 24 hours prior to a challenge intraperitoneal dose of 75 mg/kg N-nitrosodimethylamine (Lorr et al. 1984). The acetone pretreatment doubled the plasma activity of alanine aminotransferase ($p<0.005$) and increased the extent and severity of liver necrosis and hemorrhage, compared with that seen with N-nitrosodimethylamine alone. Microsomes prepared from rats treated with N-nitrosodimethylamine had diminished N-nitrosodimethylamine-N-demethylase activity, compared with microsomes from untreated mice. The results indicate that N-nitrosodimethylamine N-demethylase, an activity associated with CYP2E1, is responsible for the

activation of N-nitrosodimethylamine to a toxic intermediate, and that the induction of this enzyme by acetone potentiates the hepatotoxicity. Similar results were seen in Ko et al. (1987), where rat liver microsomes pre-treated with acetone inhibited nitrosodimethylamine demethylase activity up to 70%, and rat P450 pre-treated with acetone was inhibited up to 92%. Microsomes from mice given 2,614 mg/kg acetone increased the covalent binding of radioactivity from [^{14}C]-N-nitrosodimethylamine to microsomal DNA, RNA, and protein (Sipes et al. 1978). Microsomes from rats pretreated with acetone had a four-fold increased activity of N-nitrosodimethylamine demethylase and a six-fold increase in DNA methylation compared with control microsomes (Hong and Yang 1985). Several studies have shown that acetone given to rats or mice enhances the microsomal activity of N-nitrosodimethylamine N-demethylase in a dose-related manner (Miller and Yang 1984; Patten et al. 1986; Sipes et al. 1973, 1978; Tu et al. 1983; Yoo et al. 1990), and this activity is associated with CYP (Miller and Yang 1984; Tu et al. 1983; Yoo et al. 1990), in particular CYP2E1 (Patten et al. 1986; Yoo et al. 1990). Acetone pretreatment of rats also enhanced the denitrosation of N-nitroso-dimethylamine in microsomes, and antibodies against CYP2E1 inhibited this activity (Yoo et al. 1990). Similar results were obtained with N-nitrosodiethylamine deethylation and denitrosation. The rates of both types of reactions depended upon the concentration of the nitrosamine in the reaction mixture, leading to the conclusion that CYP2E1 has a role in the metabolism of low concentrations of these nitrosamines, and that this form of the enzyme is important in the carcinogen activation.

In Ames assays, addition of acetone to the S-9 mix inhibited the mutagenicities of N-nitrosodimethylamine, N-nitrosodiethylamine, and 6 oxidative derivatives of these two chemicals in *Salmonella typhimurium* TA100 at a concentration of <5.2 mg/0.1 mL (52,000 mg/L) nitrosamines (Mori et al. 1985). Acetone also inhibited the metabolism of N-nitrosodimethylamine, N-nitrosomethyl (2-hydroxypropyl) amine, and N-nitrosomethyl (2-oxopropyl) amine *in vitro*. In contrast, another study found that the S-9 mix prepared from mice treated with acetone strongly enhanced the mutagenicity of N-nitrosodimethylamine in the Ames assay in *S. typhimurium* TA92, which was more sensitive to N-nitrosodimethylamine than TA100 (Glatt et al. 1981). This assay used concentrations of the nitrosamine at <20 mM (1,491 mg/L). However, acetone did not enhance the mutagenicity in the host-mediated assay. The authors explained that *in vitro*, the activity of the dilute metabolizing system is limiting for the activity of N-nitrosodimethylamine, such that induction increases mutagenicity, whereas *in vivo*, N-nitrosodimethylamine is completely metabolized in both induced and noninduced animals. The reason for the different effects of acetone on the mutagenicity of nitrosamines in the studies by Mori et al. (1985) and Glatt et al. (1981) could be related to differences in the assay system (e.g., acetone added to medium versus acetone-induced S-9), to the difference in concentration of the nitrosamines, or to the different

strains of *S. typhimurium*. Microsomes from rats pretreated with acetone increased the activity of N-nitrosodimethylamine demethylase and increased the mutagenicity of N-nitrosodimethylamine in Chinese hamster lung V79 cell cultures at low substrate concentrations (0.1 and 4 mM or 5.8 and 232 mg/L) compared with untreated microsomes (Yoo and Yang 1985). However, a slight decrease in mutagenic activity was found at a N-nitrosodimethylamine concentration of 200 mM (11,616 mg/L). Acetone induced microsomes also enhanced the mutagenic activity of N-nitrosomethylethylamine, N-nitrosodiethylamine, and N-nitrosomethylbutylamine, but not N-nitrosomethylbenzylamine or N-nitrosomethylaniline. The findings that lower concentrations enhanced the mutagenicity of N-nitrosodimethylamine (Glatt et al. 1981; Yoo and Yang 1985) are consistent with the conclusions of Yoo et al. (1990) that CYP2E1 is important in the activation of the carcinogen at low concentrations.

Acetonitrile. Acetone also potentiates the toxicity of acetonitrile. When rats were given a 1:1 mixture of acetone plus acetonitrile by gavage, the acute LD_{50} was 3–4 times lower than the predicted LD_{50} for additive toxicity (Freeman and Hayes 1985). The LD_{50} values of these chemicals alone were 5,800 mg/kg for acetone and 4,050 mg/kg for acetonitrile, while the LD_{50} for the mixture was 1,160 mg/kg, compared with the predicted value of 4,770 mg/kg. However, deaths occurred later with the mixture than with either acetone or acetonitrile alone. Blood cyanide (a toxic metabolite of acetonitrile) levels were higher, but peaked at a later time, in the rats given the mixture than in those given acetonitrile alone. Administration of a second dose of acetone 30 hours after administration of the mixture protected the rats from lethality to a degree similar to that seen with a dose of sodium thiosulfate (an antidote used for cyanide poisoning). It was suggested that, initially, acetone competitively inhibits the metabolism of acetonitrile to cyanide but later induces an isoenzyme of CYP that catalyzes the metabolism of acetonitrile to cyanide, hence explaining the greater toxicity of the mixture seen at a later time. To test this hypothesis, the metabolism of acetonitrile by microsomes from rats treated with acetone at the same dose that potentiated the toxicity was compared with that by noninduced microsomes (Freeman and Hayes 1988). The metabolism of acetonitrile required oxygen and NADPH and was inhibited by known inhibitors of CYP. Microsomes from acetone pretreated rats increased the Vmax, while acetone added to the reaction mixture *in vitro* competitively inhibited the conversion of acetonitrile to cyanide. The *in vitro* metabolism of acetonitrile was competitively inhibited by ethanol (which also induces CYP2E1), by dimethyl sulfoxide (which inhibits CYP2E1-dependent metabolism of ethanol), and by aniline (a substrate for CYP2E1). Thus, the mechanism for the potentiation of the toxicity of acetonitrile by acetone also appears to involve CYP2E1.

A case report describes a woman who was asymptomatic for 24 hours after ingesting an overdose of acetonitrile and acetone, but subsequently developed cardiovascular collapse and profound acidosis, and eventually died (Boggild et al. 1990). It was suggested that acetone delayed the onset of symptoms by initially inhibiting the metabolism of acetonitrile to cyanide, which is consistent with the mechanism proposed by Freeman and Hayes (1988).

Acetaminophen. Acetone has been reported to increase the hepatotoxicity of acetaminophen *in vitro* (Moldeus and Gergely 1980) and *in vivo* (Jeffery et al. 1991). The addition of acetone to phenobarbital-induced rat liver hepatocytes caused a three-fold increase in acetaminophen-glutathione conjugation due to enhanced CYP-dependent activation of acetaminophen to a toxic metabolite (Moldeus and Gergely 1980). The addition of acetone to the reaction system also caused loss of hepatocyte viability, which was not seen when acetone or acetaminophen were excluded from the system. According to the suggested mechanism, acetone enhanced a CYP-dependent activation of acetaminophen to a metabolite that conjugates with glutathione, thereby depleting hepatic glutathione stores, leading to accumulation of the reactive metabolite. In contrast, pretreatment of rats with 813 or 1,975 mg/kg acetone 18 hours and 1 hour prior to administration of acetaminophen resulted in an increased blood half-life of acetaminophen, a decreased rate constant for acetaminophen mercapturate formation, decreased acetaminophen sulfate formation, and decreased renal elimination of acetaminophen (Price and Jollow 1983). Acetone also decreased the incidence and severity of liver necrosis induced by acetaminophen. The authors suggested that acetone decreased the formation of an acetaminophen reactive metabolite. However, in mice pretreated orally with acetone at 1,900 mg/kg/day for 10 days and then given 600 mg/kg acetaminophen intraperitoneally 6 hours before sacrifice, a greater portion of the liver lobules with necrosis and hemorrhage was observed than when acetaminophen was administered alone (Jeffery et al. 1991). Acetone pretreatment followed by saline injection resulted in no hepatic lesions. When dimethylsulfoxide (DMSO), an inhibitor of CYP2E1, was incubated with microsomes prepared from the acetone-pretreated, acetaminophen-treated mice, a 91% inhibition of acetaminophen-glutathione conjugation was found compared to when DMSO was excluded from the incubation mixture. Presumably, the inhibition of glutathione conjugation by DMSO was due to inhibition of CYP2E1 to form the active metabolite of acetaminophen. Activation of acetaminophen to a reactive metabolite, N-acetyl-P-benzoquinone imine, which can bind to tissue macromolecules leading to necrosis at high doses of acetaminophen, is known to be dependent on CYP2E1 (Morgan et al. 1983; Raucy et al. 1989). NAPQI can also be detoxified via conjugation with glutathione. The addition of acetone to the reaction system enhances the formation of the glutathione conjugate in rat liver microsomes (Liu et al. 1991). These results support a mechanism whereby acetone enhances the CYP2E1-dependent conversion of acetaminophen to NAPQI, which in

turn conjugates with glutathione to deactivate it. Thus, acetone could decrease the toxicity of acetaminophen. However, if the dose of acetaminophen is high (leading to more N-acetyl-p-benzoquinone imine than can be handled by glutathione detoxification), glutathione is depleted and NAPQI accumulates. Thus, the induction of CYP2E1 by acetone to produce enough NAPQI from acetaminophen to deplete glutathione would result in an enhancement of acetaminophen-induced toxicity.

Pyridine. Iba et al. (1993) prepared microsomes from lungs and livers of rats exposed to 20 ppm pyridine by inhalation for 5–6 hours/day for 10 days, to acetone (7.5%, v/v) in drinking water for 10 days or by inhalation to 50% aqueous acetone for 5–6 hours/day for 10 days, or to acetone in combination with pyridine administered separately as above. Controls received water for inhalation and oral exposures. In the liver microsomes, there was induction of ethoxyresorufin O-deethylase (EROD) activity for oral acetone by 2.5-fold, for pyridine by inhalation by 2.8-fold, and for the combination of acetone and pyridine by 7.6-fold, indicating greater-than-additive interaction. The levels of CYP1A1 were induced by acetone, pyridine, and the combination by 8.3-, 6.6-, and 32.7-fold, respectively. These results indicated even greater synergistic interaction. Similar greater-than-additive interaction results were also found for methoxyresorufin O-demethylase (MEROD) and CYP1A2 in the liver microsomes. Microsomal EROD was induced by all treatments in the lung, and a synergistic interaction was even greater in the lung, with an increase that was 4-fold for acetone, 21-fold for pyridine, and 115.5-fold for the combination. CYP1A1 was also induced synergistically by acetone and pyridine in the lung microsomes.

Miscellaneous Chemicals. 9,10-Dimethyl-1,2-benzanthracene (DMBA) in acetone was more effective as a carcinogen than DMBA in mineral oil when applied to the tongues of hamsters (Marefat and Shklar 1977). In a study of 10 male volunteers, ingestion of 500 mg chlorzoxazone prior to inhalation of 250 ppm acetone for 2 hours resulted in slight but significant increases in steady state blood level and area under the blood concentration-time curve for acetone (Ernstgard 1999). A dose of 581 mg/kg acetone prior to administration of N-(3,5-dichlorophenyl)succinimide (NDPS), a fungicide, enhanced the NDPS-induced increase in blood urea nitrogen and kidney weight, but had no effect on NDPS-induced changes in urine volume or content, organic ion uptake by kidney slices, or renal pathology (Lo et al. 1987). Lower doses of acetone were ineffective. Because NDPS requires bioactivation by CYP-dependent microsomal enzymes in the liver before renal toxicity occurs, it appears that acetone potentiated the renal toxicity of NDPS by inducing a CYP capable of the bioactivation. Pretreatment of rats with acetone prior to administration of thiobenzamide enhanced the degree of liver necrosis and serum activity of alanine aminotransferase, while coadministration of acetone and thiobenzamide reduced the extent of liver damage (Chieli et al. 1990). In addition, liver microsomes from acetone treated rats statistically

significantly increased the rate of thiobenzamide-S-oxidation, which was dependent on a CYP enzyme. Thiobenzamide competitively inhibited acetone monooxygenase activity, which is highly specific for CYP2E1. The results indicated that pretreatment of rats with acetone induces CYP2E1, leading to enhanced bioactivation of thiobenzamide to a reactive metabolite and enhanced thiobenzamide-induced liver damage. However, when acetone and thiobenzamide were administered together, competition for the enzyme may have led to less bioactivation of thiobenzamide, thereby affording the protective effect of acetone. Acetone appears to afford protection against other toxic effects of other chemicals. Pretreatment of rats with acetone produced complete protection against clonic tonic convulsions induced by isonicotinic acid and electroshock-induced convulsion (Kohli et al. 1967). Because the protective action of acetone was nonspecific, a biochemical mechanism did not seem likely.

Acetone also increased the toxicity of oxygen (Tindberg and Ingelman-Sundberg 1989) and chromate (Cr[VI]) (Mikalsen et al. 1991). Pretreatment of rats with acetone prior to oxygen exposure potentiated the NADPH-dependent microsomal lipid peroxidation in the liver and lung and decreased the survival of the rats (Tindberg and Ingelman-Sundberg 1989). Oxygen also induced CYP2E1, indicating a role for CYP2E1 in oxygen-mediated tissue toxicity.

Coexposure of rats to acetone and sodium chromate (Cr[VI]) resulted in some macroscopic alterations in the liver (not otherwise described), whereas no liver toxicity was noted with chromate or acetone alone (Mikalsen et al. 1991). Cytochrome CYP2E1 exhibited high chromate reductase activity, and biochemical studies indicated that acetone caused the induction of microsomal Cr(V1) metabolism. While the interactions discussed above involve the potentiation of the toxicity of other chemicals by acetone, acetone has been found to antagonize the toxicity of semicarbizide (Jenney and Pfeiffer 1958). In mice injected intraperitoneally with 168 mg/kg semicarbizide, 93% had convulsions and 91% died. Pretreatment with 4,000 mg/kg acetone orally reduced the percentage of the semicarbizide-induced convulsions and mortality to 0%. A dose of 1,800 mg/kg acetone reduced the percentage of mice convulsing to 31%, delayed the onset of convulsions by 286%, reduced the percentage that exhibited unmodified seizure from 98 to 40%, reduced the mortality to 12.5%, and delayed the time to death by 125%. The authors attributed the protective effect of acetone to the presence of the keto group.

A study of co-exposure to acetone and di(ethylhexyl)phthalate in drinking water of rats for 4 or 9 weeks found no significant interactions between the two chemicals with regard to reproductive toxicity (Dalgaard et al. 1999). However, the general toxicity of di(ethylhexyl)phthalate, as assessed by clinical

signs, decreases in body weight, and increases in mortality, was slightly increased by co-exposure to acetone.