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

3.1 TOXICOKINETICS

Human studies of 2-butanone provide primarily qualitative information on absorption following inhalation exposure and limited quantitative data on urinary excretion kinetics following inhalation. 2-Butanone toxicokinetics have been studied in rats following oral and inhalation exposure. An overview of these data is summarized below.

- 2-Butanone is rapidly absorbed following inhalation and dermal exposure in humans. Experiments in rats indicate that 2-butanone is rapidly absorbed and eliminated after oral administration.
- Distribution has not been extensively studied following *in vivo* exposure; however, *in vitro* determinations of the 2-butanone tissue:air solubility ratios for human kidney, liver, muscle, lung, heart, fat, blood, and brain show similar solubility in all tissues. 2-Butanone did not accumulate in perirenal fat following repeat inhalation exposure in rats.
- Urinary metabolites of 2-butanone in humans include 3-hydroxy-2-butanone and 2,3-butanediol. In guinea pigs, 2-butanone was metabolized by both oxidative and reductive pathways. Oxidation produces 3-hydroxy-2-butanone, which is then reduced to 2,3-butanediol, and 2-butanone reduction produces 2-butanol. The metabolites of 2-butanone in guinea pigs were excreted in the urine as O-glucuronides or O-sulfates. 2-Butanone exposure induces CYP in the liver.
- 2-Butanone is removed rapidly from the blood and is excreted unchanged in expired air and urine. Metabolites of 2-butanone (3-hydroxy-2-butanone and 2,3-butanediol) with or without conjugation are also excreted in urine.

3.1.1 Absorption

2-Butanone is well absorbed during inhalation exposure in humans. Pulmonary uptake ranged from 41 to 56% of the inspired quantity (Liira et al. 1988a, 1988b, 1990a). Exercise increased the pulmonary uptake due to the greater ventilatory rate (Liira et al. 1988b). Several investigators have reported that exposure concentrations of 2-butanone are significantly correlated with blood concentrations in humans (Brown et al. 1986; Brugnone et al. 1983; Ghittori et al. 1987; Liira et al. 1988a, 1988b; Lowry 1987; Miyasaka et al. 1982; Perbellini et al. 1984; Tolos et al. 1987). Exposure of humans to 200 ppm 2-butanone for 4 hours resulted in blood concentrations of 3.5–7.2 μg/mL (Liira et al. 1988a, 1988b; Lowry 1987). In two subjects exposed to 25, 200, and 400 ppm on separate days for 4 hours/day (Liira et al. 1990b), blood levels increased continuously with increasing 2-butanone exposure. The increase in blood concentration

was steeper during exposure for 200 and 400 ppm compared to 25 ppm. Slower elimination from the blood after cessation of exposure was also seen at 400 ppm. These concentration-dependent changes in blood kinetics suggest that metabolic saturation may occur at higher exposure concentrations. Using physiologically based pharmacokinetic (PBPK) model simulations for 8-hour exposures, the investigators estimated that metabolic saturation may be approached at concentrations near 100 ppm at rest and 50 ppm during exercise (Liira et al. 1990b). Occupational concentrations are significantly correlated with blood and urine concentrations of unmetabolized 2-butanone (Brugnone et al. 1983; Ghittori et al. 1987; Miyasaka et al. 1982). Blood levels of 2-butanone are also significantly correlated with breath levels (Brown et al. 1986).

Information on the absorption of 2-butanone by animals after inhalation exposure is limited. Pulmonary and nasal uptake in dogs exposed to 500 ppm 2-butanone for 30 minutes was 25 and 36% of the total inhaled vapor concentration (Dahl et al. 1991). Rats that were exposed to 600 ppm 2-butanone for 6 hours on 1 day or for 6–10 hours/day for 8 days had blood concentrations of 1,041 μmol/L after a single exposure and 1,138 μmol/L after repeated exposure (Liira et al. 1991).

The high blood:air solubility ratio of 2-butanone also favors absorption (Saida et al. 1976; Perbellini et al. 1984). Blood:air partition coefficients determined for humans, rats and dogs ranged from 138 to 208 (Beliveau and Krishnan 2000; Dahl et al. 1991; Fisher et al. 1997; Mahle et al. 2007; Thrall et al. 2002). The human blood:air partition coefficient was 2–4% higher in male and female pediatric subjects compared with adults (Mahle et al. 2007). A similar age-related pattern was observed in rats with a 4–6% higher blood:air coefficient observed in PND 10 males compared with adult and aged male rats.

A woman who had metabolic acidosis after having accidentally ingested 2-butanone stored in a rum bottle had a blood concentration of 95 mg/100 mL (13.2 mM) (Kopelman and Kalfayan 1983). A man who intentionally ingested 100 mL of liquid cement containing a mixture of acetone (18%), 2-butanone (28% or about 37 mg/kg), and cyclohexanone (39%) had a plasma level of 2-butanone of about 110 μg/mL at 5 hours after ingestion (Sakata et al. 1989). These reports provide qualitative evidence that 2-butanone is absorbed following oral exposure in humans, but do not provide information regarding the extent of absorption. In the first case, the quantity ingested was unknown, while in the second case, the man was treated by gastric lavage at 2 hours after ingestion.

Experiments in rats indicate that 2-butanone is rapidly absorbed and eliminated after oral administration. Gavage administration of 1,690 mg/kg 2-butanone in rats resulted in a plasma concentration of

94 mg/100 mL at 4 hours (Dietz and Traiger 1979). Within 18 hours, the plasma concentration decreased to 6.2 mg/100 mL (Dietz and Traiger 1979). A second, similar experiment in rats showed that, after oral administration of 1,690 mg/kg 2-butanone, the plasma concentration was 95 mg/100 mL; the concentration decreased to 7 mg/100 mL by 18 hours (Dietz et al. 1981). The peak exhaled breath concentration of 2-butanone was measured within 1 hour of gavage dosing with 50 mg/kg (Thrall et al. 2002). Concentrations in expired breath decreased slowly over the next 3 hours.

2-Butanone was rapidly absorbed following dermal exposure to the forearm skin of volunteers and was detected in expired breath within 2–3 minutes of exposure (Munies and Wurster 1965; Wurster and Munies 1965). Dermal penetration was enhanced by hydration and was lower when applied to dry skin. In subjects exposed to 200 ppm airborne 2-butanone for 4 hours, dermal absorption contributed approximately 1.2–9.6% (mean of 3.10–3.5%) of absorbed dose (Brooke et al. 1998). A dermal permeability constant (Kp) of 53 g/m^2/hour was reported for 2-butanone across excised human skin (Ursin et al. 1995). Schenk et al. (2018) reported an *in vitro* steady-state flux of 0.00143 g/cm^2/hour (14.3 g/m^2/hour) and permeability coefficient of 0.00175 cm/hour for 2-butanone across pig skin.

3.1.2 Distribution

No studies were located regarding the distribution of 2-butanone following inhalation, oral, or dermal exposure in humans. *In vitro* determinations of the 2-butanone tissue:air solubility ratio for human kidney, liver, muscle, lung, heart, fat, and brain show that the solubility is similar in all tissues, and that the ratio is nearly equal to 200 (Perbellini et al. 1984). Blood:tissue solubility ratios are all near unity; therefore, 2-butanone is not expected to concentrate in any one tissue (Perbellini et al. 1984). In rats, tissue:air partition coefficients were similar for liver, kidney, fat, muscle, and brain (Mahle et al. 2007; Thrall et al. 2002). Tissue:air partition coefficients for muscle and brain were higher in PND 10 male rats compared with adult and aged male rats; however, older rats exhibited higher tissue:air partition coefficients for liver, kidney and fat (Mahle et al. 2007). 2-Butanone has been detected in human breast milk (Giroux et al. (1992).

Information regarding distribution of 2-butanone in animals after inhalation exposure is limited. Rats that were exposed to 600 ppm 2-butanone for 6 hours on 1 day or for 6–10 hours/day for 8 days had perirenal fat concentrations of 0.71 μmol/g after a single exposure and 0.70 μmol/g after repeated exposure. The similarity in fat concentrations after single and repeated intermittent exposure indicates that 2-butanone does not accumulate (Liira et al. 1991). Cosnier et al. (2018a) repeatedly exposed rats to 2-butanone by

inhalation at 20, 200, or 1,400 ppm. Similar blood levels of 2-butanone were observed following a single 6-hour exposure or repeated exposures for up to 1 month. Brain concentrations were only slightly increased by repeated exposures for 1 month.

3.1.3 Metabolism

Few studies exist regarding the metabolism of 2-butanone in humans. Two metabolites of 2-butanone have been identified in human urine after inhalation exposure. They are 3-hydroxy-2-butanone (Brugnone et al. 1983; Perbellini et al. 1984) and 2,3-butanediol (Liira et al. 1988a, 1988b, 1990a). The urinary concentrations of these metabolites, however, represent only about 0.1–2% of the absorbed 2-butanone. 2-Butanol was found in the blood of male volunteers exposed to 200 ppm 2-butanone for 4 hours (Liira et al. 1990a).

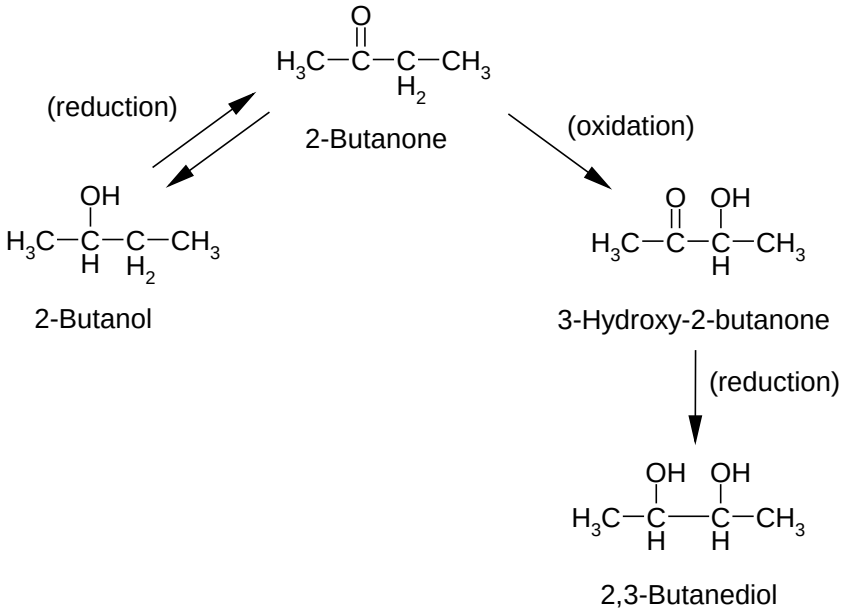
3-Hydroxy-2-butanone, 2,3-butanediol, and 2-butanol have also been found in the blood in guinea pigs (DiVincenzo et al. 1976) and rats (Dietz et al. 1981) exposed to 2-butanone. About 30% of the 2-butanone administered orally in rats was converted to 2,3-butanediol; 4% was converted to 2-butanol, and 4% was converted to 3-hydroxy-2-butanone (Dietz et al. 1981).

In guinea pigs, 2-butanone was metabolized by both oxidative and reductive pathways (Figure 3-1). Oxidation produces 3-hydroxy-2-butanone, which is then reduced to 2,3-butanediol (DiVincenzo et al. 1976). Reduction of 2-butanone produces 2-butanol. The metabolites of 2-butanone in guinea pigs were excreted in the urine as O-glucuronides or O-sulfates (DiVincenzo et al. 1976). Cosnier et al. (2018a) detected 2-butanone, 2-butanol, and 3-hydroxy-2-butanone in rat urine, but only at 2-butanone inhalation levels resulting in metabolic saturation. Thrall et al. (2002) demonstrated that 2-butanone metabolism in rats is not completely eliminated by inhibition of the oxidative pathway using pyrazole.

Several studies have shown that 2-butanone has the ability to induce microsomal liver enzymes. Acute oral treatment of rats with 2-butanone at doses of 1,080–1,500 mg/kg/day for 1–7 days resulted in increased levels of CYP protein, increased activities of CYP-dependent monooxygenases (Brady et al. 1989; Raunio et al. 1990; Robertson et al. 1989; Traiger et al. 1989), and proliferation of the smooth endoplasmic reticulum (Traiger et al. 1989). 2-Butanone also induced specific CYP isozymes in rat liver (CYP2B1 and CYP2B2) following daily intraperitoneal injections of 5 mmol/kg for 4 days (Imaoka and Funae 1991). Induction of microsomal enzymes did not occur in rats exposed to 2-butanone by inhalation. After exposure of rats to 800 ppm 2-butanone for 5 weeks (Toftgard et al. 1981) or 600 ppm

n-butanone for 8 days (Liira et al. 1991), no changes were observed in the content of hepatic CYP or in the CYP isozyme profile. However, Cosnier et al. (2018a) exposed rats to 2-butanone by inhalation and reported the exposure-related induction of both CYP1A2 and CYP2E1 enzymes, although total hepatic P450 enzyme concentration was not altered. Furthermore, exposure to 2-butanone at 1,400 ppm resulted in decreased hepatic glutathione concentration and glutathione S-transferase activity.

Figure 3-1. Proposed Metabolic Pathways for 2-Butanone



Source: DiVincenzo et al. 1976

3.1.4 Excretion

Urinary excretion of unchanged 2-butanone and its metabolites, 3-hydroxy-2-butanone and 2,3-butanediol, accounts for only 5% or less of the 2-butanone absorbed by inhalation in humans (Kawai et al. 2003; Liira et al. 1988a, 1990a; Perbellini et al. 1984) and rats (Cosnier et al. 2018a). Unchanged 2-butanone is excreted primarily through the lungs; the quantity eliminated by this route is an estimated 20–40% (Browning 1965; Riihimaki 1986); however, only about 3% of absorbed 2-butanone was excreted unchanged in the expired air of humans exposed to 200 ppm for 4 hours (Liira et al. 1988a, 1990a). 2-Butanone is rapidly cleared from the blood with a reported plasma half-life in humans of 49–96 minutes (Brown et al. 1986; Liira et al. 1988a; Lowry 1987) and an apparent clearance rate of 0.60 L/minute (Liira et al. 1990a). Therefore, 2-butanone would not be expected to accumulate with chronic exposure (Lowry 1987). Tomicic et al. (2011) measured urinary 2-butanone concentrations

before during and after a 6-hour exposure to 100 ppm 2-butanone. The urinary 2-butanone concentration was highest immediately following exposure and returned to pre-exposure levels by 6 hours after the cessation of exposure (urinary half-life was not determined). 2-Butanone concentrations were highest in women without hormonal contraceptives compared to women with hormonal contraceptives and men, suggesting an influence of sex hormones on 2-butanone metabolism.

Information regarding the excretion of 2-butanone after oral exposure in humans is limited. A man who intentionally ingested 100 mL of liquid cement containing a mixture of acetone (18%), 2-butanone (28% or about 37 mg/kg), and cyclohexanone (39%) had a plasma level of 2-butanone of about 110 µg/mL at 5 hours after exposure (Sakata et al. 1989). The plasma level declined to about 95 µg/mL at 12 hours and to <20 µg/mL at 18 hours, where it remained until about 25 hours and slowly declined to <5 µg/mL at 48 hours. Urine levels of 2-butanone decreased gradually from 123 µg/mL at 5 hours to 61 µg/mL at 19 hours. Disappearance from the urine then became more rapid with about 10 µg/mL excreted at 48 hours. While this study provided information on the elimination of 2-butanone from plasma and urine of a human orally exposed, coexposure to the other components of the cement could have influenced the elimination.

No studies were located regarding the rate or extent of excretion of 2-butanone in animals following inhalation or oral exposure.

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.

Several PBPK models of 2-butanone have been reported. These include human models simulating 2-butanone kinetics following inhalation exposure (Liira 1990b; Jongeneelen et al. 2013; Tomicic and Vernez 2014) and rat models using data from multiple exposure routes (Dietz et al. 1981; Thrall et al.

2002). Risk assessment applications of these models are limited by the small number of data sets available for testing and model calibration.

Liira et al. (1990b)

Liira et al. (1990b) developed a PBPK model using blood concentration data for two male subjects exposed to 25, 100, or 200 ppm 2-butanone for 4 hours. Blood samples were collected during exposure and for 8 hours after exposure. The pulmonary ventilation rate of the subjects was measured at rest and during exercise. 2-Butanone metabolism was assumed to occur in the liver only and followed Michaelis-Menten kinetics. The K_m (2 μM) and V_{max} (30 μmol/minute) were calculated from the best fit of the simulated blood concentrations. 2-Butanone was detected in blood (0.2–0.3 μM) prior to exposure suggesting some endogenous formation of this compound. This was treated as a continuous inhalation exposure in the PBPK model (1.25 ppm).

The elimination of 2-butanone from blood is slower at higher exposure concentrations, which is suggestive of metabolic saturation. The PBPK model was used to simulate blood concentrations for an 8-hour continuous exposure at rest and during exercise. Metabolic saturation was estimated to occur at 2-butanone concentrations of 100 ppm at rest and 50 ppm during exercise.

Tomicic and Vernez (2014); Jongeneelen et al. (2013)

Tomicic and Vernez (2014) and Jongeneelen et al. (2013) both utilized a generic PBPK model to evaluate human urinary biomarker data for 2-butanone obtained from volunteers exposed to 100 ppm for 6 hours (Tomicic et al. 2011). Tominic and Vernez (2014) used an inhalation model that describes absorption from air into a central compartment (representing the total body water) and distribution between the central compartment and a peripheral or storage compartment (representing fatty tissues). Absorption into the central compartment was calculated as a product of the mass concentration in air, the alveolar ventilation rate scaled to body weight, and the fraction absorbed by the lung (pulmonary retention of 0.56 from Liira et al. 1988a). Metabolism is described by Michaelis-Menten kinetics (K_m 45 mg/L and V_{max} 22 mg/(hour*$kg^{0.75}$) from Thrall et al. 2002) and elimination is represented by metabolism and excretion in expired air or urine.

The sensitivity analysis, obtained by increasing each toxicokinetic parameter of the PBPK model by 10% indicated that the urinary 2-butanone concentration was especially sensitive to metabolism parameters

(K_m and V_{max}), cardiac output, and liver blood flow. A comparison of experimental data and model simulations showed adequate goodness of fit during the 6 hours exposure with poorer fit during the urinary elimination phase. Predictive simulations done for a work week (8 hours/day, 5 days at the Threshold Limit Value [TLV] concentration of 200 ppm) showed an overestimation of urinary 2-butanone concentration for women without hormonal contraceptives compared to women with hormonal contraceptives and men.

Jongeneelen et al. (2013) used a generic model with 11 body compartments (lung, heart, brain, skin, adipose tissues, muscles, bone, bone marrow, stomach and intestines, liver, and kidney). This model used Michaelis-Menten kinetic constants for liver metabolism of 2-butanone (K_m 4 μmol/L, V_{max} 1,800 μmol/kg tissue/hour) and liver metabolism of 2,3-butanediol (K_m 50 μmol/L, V_{max} 300 μmol/kg tissue/hour). Model simulations for women were similar to the experimental data for women volunteers from the Tomicic et al. (2011) study. The model predicted higher urinary 2-butanone concentrations in men compared with experimental data.

Dietz et al. (1981)

A flow-limited PBPK model was used to describe blood concentrations of 2-butanol, 2-butanone, 3-hydroxy-2-butanone, and 2,3-butanediol in Sprague-Dawley rats after gavage administration of 2-butanol (1,776 mg/kg) or 2-butanone (1,776 mg/kg) or intravenous injection of 3-hydroxy-2-butanone (400 mg/kg) and 2,3-butanediol (800 mg/kg) were administered by intravenous injection. The model assumed distribution to liver and body water (including blood) and metabolism in liver only. Michaelis-Menten kinetics were used to describe metabolism, and rate constants for each metabolite were estimated by curve fitting of the experimental blood concentration data. The model was adjusted to account for the lower than expected concentration of 3-hydroxy-2-butanone in blood suggested to result from partitioning, binding, or decreased transport from the liver. The competitive inhibition of 2-butanone oxidation by 2-butanol was also accounted for. Model adjustments were shown to improve the fit of the simulation compared with the experimental data used to derive the model.

Thrall et al. (2002)

The PBPK model developed by Thrall et al. (2002) consisted of four tissue compartments (fat, liver, rapidly perfused tissues, and slowly perfused tissues) and a description of the exchange of 2-butanone between lung blood and alveolar air. Pulmonary uptake of 2-butanone was evaluated in Fisher 344 rats

exposed to concentrations ranging from 100 to 2,000 ppm. Exhaled breath concentration were considered a surrogate measure of blood concentration. Michaelis-Menten metabolic rate constants were obtained by model simulation of the gas uptake data (best fit values of K_m 0.63 mg/L, V_{max} 5.44 mg/hour/kg). The PBPK model was calibrated using experimental data for expired breath concentrations in rats exposed by intravenous injection (25 mg/kg), intraperitoneal injection (50 mg/kg), and gavage (50 mg/kg). Rate constants calculated for oral and intraperitoneal absorption were 1.9 and 0.91 $hours^{-1}$, respectively.

3.1.6 Animal-to-Human Extrapolations

The toxicokinetics of 2-butanone in humans are similar to those that have been observed in rats and guinea pigs. Metabolites of both oxidation and reduction reactions are found in all species.

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 2-butanone are discussed in Section 5.7, Populations with Potentially High Exposures.

The human blood:air partition coefficient was 2–4% higher in male and female pediatric subjects compared with adults and a similar age-related pattern was observed in rats, with a 4–6% higher blood:air coefficient observed in PND 10 males compared with adult and aged male rats (Mahle et al. 2007). These data suggest that pulmonary uptake following inhalation may be slightly higher in children compared to adults. Tomicic et al. (2011) suggested that individuals with a genetic polymorphism in the gene for

CYP2E1 (mutant allele CYP2E1*6) may exhibit enhanced oxidative metabolism of 2-butanone; however, the findings were limited by the small number of study participants (n=25). Experimental animal studies suggest that inhalation exposure to 2-butanone during pregnancy may lead to developmental effects; however, these effects were only seen at very high concentrations (>2,000 ppm).

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 2-butanone 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 2-butanone 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 2-butanone 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

Inhalation exposure to 2-butanone correlates well with blood, breath, and urinary concentrations of unchanged 2-butanone (Brown et al. 1986; Brugnone et al. 1983; Ghittori et al. 1987; Kawai et al. 2003; Miyasaka et al. 1982; Sia et al. 1991). Personal dosimetry was used to measure exposure to 2-butanone among 27 furniture makers (Kawai et al. 2003), 50 magnetic videotape factory workers (Sia et al. 1991), 62 printing plant workers (Miyasaka et al. 1982), 72 printing plant workers (Yoshikawa et al. 1995), and 659 workers in plastic boat, chemical, plastic button, paint, and shoe factories (Ghittori et al. 1987). The correlation between exposure levels and urinary concentration of unchanged 2-butanone was strong in each study (r values ranging from 0.774 to 0.889). Miyasaka et al. (1982) concluded, however, that estimating exposure from urinary levels was reliable on a group basis, but not an individual basis. In a study of eight aircraft maintenance workers, Lemasters et al. (1999) suggested that breath measurements were more sensitive than urine and blood measurements following low-level exposure to 2-butanone (<20 ppm).

A significant correlation between workroom and urinary 2-butanone concentrations was observed in shoe factory workers ($r=0.6877$, $p<0.001$) (Brugnone et al. 1983). In the same study, a more significant correlation was observed between workroom concentrations and a 2-butanone urinary metabolite, 3-hydroxy-2-butanone ($r=0.8179$, $p<0.001$). Another 2-butanone metabolite, 2,3-butanediol, has also been identified in the urine of humans (Liira et al. 1988a, 1988b); however, no studies have examined the correlation between exposure to 2-butanone and urinary levels of this metabolite. A third metabolite, 2-butanol, was identified in guinea pig blood; however, no attempt was made to correlate 2-butanol blood levels with exposure to 2-butanone (DiVincenzo et al. 1976). Metabolism of alcohols, hydrocarbons, and other ketones may also yield 2-butanone, 3-hydroxy-2-butanone, and 2,3-butanediol (Dietz and Traiger 1979; Tsukamoto et al. 1985); therefore, these compounds may confound assessment of exposure to 2-butanone. The urinary concentration of 2-butanone measured immediately after a 6-hour exposure to 100 ppm 2-butanone was similar in women using hormonal contraceptives and men of similar age, but was higher in women not using hormonal contraceptives (Tomicic et al. 2011). This finding suggests that the presence of sex hormones may increase 2-butanone metabolism by CYP2E1; however, interpretation of study findings is limited by the small number of study participants (n=25). Creatinine adjustment of

this exposure biomarker was not necessary due to the passive process of elimination by the kidney (i.e., dependent on urine flow rate only).

Blood and breath levels of 2-butanone were significantly correlated ($r=0.78$, $p<0.001$) in volunteers exposed to 200 ppm 2-butanone for 4 hours (Brown et al. 1986). Measurements of tissue, blood, and excreta levels may not be an accurate indication of past exposure to 2-butanone. Accumulation in target tissues does not occur because tissue/blood solubility ratios are all near unity; therefore, 2-butanone will not concentrate in specific tissues (Perbellini et al. 1984). The serum half-life of 2-butanone in humans is very short; estimates range from 49 to 96 minutes (Liira et al. 1988a; Lowry 1987). Furthermore, 2-butanone was not detectable in blood or breath measurements reported the morning after a 4-hour exposure to 200 ppm (Brown et al. 1987).

2-Butanone is endogenously produced during the catabolism of isoleucine and is considered a normal constituent of urine (Tsao and Pfeiffer 1957). A study of National Health and Nutrition Examination Survey (NHANES) participants found an association between daily consumption of >20 mL alcohol and blood 2-butanone levels (Churchill et al. 2001).

3.3.2 Biomarkers of Effect

2-Butanone induces hepatic microsomal enzymes in rats after oral exposure (Brady et al. 1989; Raunio et al. 1990; Robertson et al. 1989; Traiger et al. 1989), but this enzyme induction has not been associated with more severe liver effects. No other subtle biochemical effects of 2-butanone have been identified that would be useful as biomarkers to characterize effects of 2-butanone.

3.4 INTERACTIONS WITH OTHER CHEMICALS

The neurological and hepatic effects of 2-butanone alone are minimal (Altenkirch et al. 1978; Saida et al. 1976). This compound, however, is frequently mixed with other chemicals such as n-hexane or methyl-n-butyl ketone for various commercial and industrial applications, which can then lead to serious toxic effects. Exposure to mixed solvents is most likely to occur in occupational settings or at a hazardous waste site. Clinical reports, animal studies, and some *in vitro* tests have shown that 2-butanone potentiates or enhances the neurotoxicity of ethanol, n-hexane, and methyl-n-butyl ketone, ethyl-n-butyl ketone, and toluene; the hepatotoxicity of carbon tetrachloride, chloroform, n-hexane, and

dimethylformamide; and the renal toxicity of methanol, and chloroform. These studies emphasize the potential public health hazard of mixed solvent exposure including 2-butanone.

Interactions Potentially Influencing Neurotoxicity. Based on several case studies and clinical case reports, there is some evidence to suggest an interaction between 2-butanone and n-hexane and 2-butanone and methyl-n-butyl ketone, which potentiates neurotoxic effects. Altenkirch et al. (1977) investigated a large outbreak of toxic polyneuropathies in a group of West Berlin "glue sniffers." Until the fall of 1975, the major constituents of the glue were n-hexane, toluene, ethyl acetate, and benzene. The development of neuropathies (muscular atrophy, paresthesia, paresis, quadriplegia) coincided with the addition of 2-butanone to the mixture. Similar outcomes were described in a clinical case report of three men exhibiting “glue sniffing neuropathy” following the addition of 2-butanone to the glue formulation (King et al. 1985), and also in workers from a coated fabrics plant who exhibited peripheral nephropathy when a methyl-n-butyl ketone solvent was introduced that contained high concentrations of 2-butanone (Allen et al. 1975; Billmaier et al. 1974). In another case, a 39-year-old woman who had worked for several years gluing shoes using a glue containing 20% 2-butanone and 8% n-hexane, developed polyneuropathy after a few weeks of work in a poorly ventilated shop (Vallat et al. 1981).

Laboratory animal studies demonstrated 2-butanone potentiation of n-hexane, methyl-n-butyl ketone, and ethyl-n-butyl ketone neurotoxicity (Altenkirch et al. 1978, 1982; O'Donoghue et al. 1984; Saida et al. 1976; Schmidt et al. 1984). A study exposing rats to either 10,000 ppm n-hexane or a combination of 1,000 ppm 2-butanone and 9,000 ppm n-hexane reported that the co-exposed animals developed paresis more rapidly and in greater numbers than rats exposed to n-hexane only (Altenkirch et al. 1978). In the same study, rats exposed to 6,000 ppm 2-butanone alone showed no signs of neurotoxicity up to 7 weeks, when all of the rats in this group died suddenly of bronchopneumonia. These results were confirmed in a second study; mixtures of 500 ppm n-hexane and 2-butanone (4:1 or 3:2) or 700 ppm (5:2) caused clinical signs of neuropathy 1–5 weeks earlier than 500 ppm n-hexane alone (Altenkirch et al. 1982). Histological examination revealed morphological changes in the rats similar to those found in youths suffering from glue sniffing neuropathy, including paranodal axon swelling, accumulation of neurofilaments in the cytoplasm, and demyelination. Takeuchi et al. (1983) observed a significant decrease in motor nerve and mixed nerve conduction velocity in rats exposed to 300 ppm n-hexane:2-butanone (1:2) compared with rats exposed to 200 ppm 2-butanone alone or 100 ppm n-hexane alone (measured after 20 and 24 weeks of exposure). Finally, male Wistar rats exposed to n-hexane or a combination of n-hexane and 2-butanone developed ultrastructural changes in the intrapulmonary nerves characteristic of hexacarbon neurotoxicity (Schmidt et al. 1984).

A marked potentiation of peripheral neurotoxicity was reported when rats were exposed to methyl-n-butyl ketone:2-butanone (225:1,125 ppm) (Saida et al. 1976). Rats exposed to methyl-n-butyl ketone alone developed paralysis by 66 days. The combination caused paralysis in 25 days, while 2-butanone alone had no effect up to 5 months. Histological examination of neurons revealed morphological changes similar to those reported by Altenkirch et al. (1982), which included paranodal axon swelling, accumulation of neurofilaments, and demyelination. Subcutaneous injection of methyl-n-butyl ketone alone increased distal motor latency and decreased motor fiber conduction velocity in male Donryu strain rats; these effects were enhanced with concomitant exposure to 2-butanone (Misumi and Nagano 1985). Oral administration of ethyl-n-butyl ketone in rats for several weeks caused paranodal axon swelling and neurofilamentous hyperplasia characteristic of n-hexane and methyl-n-butyl ketone neurotoxicity (O'Donoghue et al. 1984). Oral administration of 2-butanone potentiated the development of clinical and histological signs of ethyl-n-butyl ketone neurotoxicity.

In vitro studies support the hypothesis that 2-butanone potentiates both n-hexane and methyl-n-butyl ketone neurotoxicity. Veronesi et al. (1984) observed that, in tissues cultured from fetal mouse spinal cord, dorsal root ganglia, and muscle, the combination of 2-butanone and n-hexane produced giant axonal swellings more rapidly than cultures treated with n-hexane alone. Furthermore, cultures exposed to nontoxic concentrations of n-hexane also developed giant axonal swellings when 2-butanone was administered concomitantly.

The precise mechanisms behind 2-butanone potentiation of n-hexane and methyl-n-butyl ketone neurotoxicity remain unclear; however, several studies suggest that 2-butanone alters the metabolism and elimination kinetics of these compounds. Biotransformation of n-hexane, methyl-n-butyl ketone, and ethyl-n-butylketone can produce the neurotoxic metabolite, 2,5-hexanedione (2,5-HD) (Couri et al. 1978; DiVincenzo et al. 1976; Robertson et al. 1989). The concentrations of the n-hexane metabolites, 2,5-HD and 2,5-dimethylfuran, were significantly higher in the blood and sciatic nerves of rats pretreated by gavage with 2-butanone before inhalation exposure to n-hexane, compared to concentrations in rats exposed to n-hexane alone (Robertson et al. 1989). Shibata et al. (1990a, 1990b) observed changes in urine n-hexane metabolite profiles in rats co-exposed to 2-butanone for 8 hours, indicating an overall decrease in both the production and clearance of 2,5-HD. Similar urine metabolite changes were seen in a controlled, acute 2-butanone/n-hexane co-exposure inhalation study in four human subjects (Shibata et al. 2002). In other acute single-dose studies in laboratory animals, concomitant oral administration of 2-butanone and 2,5-HD in rats reduced blood 2,5-HD clearance (Ralston et al. 1985). In Wistar rats co-

exposed interperitoneally to 2,5-HD and 2-butanone, reductions in of 2,5-HD clearance was observed in all tissues examined including serum, urine, and sciatic nerve tissue (Aoki et al. 1996; Yasui et al. 1995; Zhao et al. 1998a, 1998b). Concomitant inhalation exposure to ethyl-n-butyl ketone and 2-butanone (700:700 ppm) for 4 consecutive days caused a 2.6-fold increase in the serum concentration of 2,5-heptanedione, which can be further metabolized to 2,5-HD (O'Donoghue et al. 1984). A metabolic study *in vitro* was done to evaluate the effect of 2-butanone on n-hexane metabolism in rat liver S9 fractions using a head-space vial equilibration technique (Mortensen et al. 1998). Liver S9 fractions were isolated from rats orally exposed (pretreated) *in vivo* to a vehicle control or to 2-butanone. S9 fractions where then exposed in closed test tubes to either *n*-hexane vapors alone or to an n-hexane:2-butanone mixture. Consistent with metabolism studies done *in vivo*, the total amount of n-hexane metabolized from the head space was higher in 2-butanone pretreated liver S9 fractions than in untreated fractions, and the levels of the n-hexane metabolite, 2,5-HD, was approximately 3.5 times higher. 2,5-HD levels increased further with increasing concentrations of 2-butanone vapors added *in vitro* (Mortensen et al. 1998).

The impact of reduced 2,5-HD clearance on neurotoxicity becomes more evident in longer studies. A 20-week subchronic inhalation study in rats exposed to n-hexane and 2-butanone reported a biphasic response, and an initial decrease in 2,5-HD concentrations in urine, similar to the observations from acute studies, was followed by an overall increase in 2,5-HD over time (Ichihara et al. 1998). The rise in 2,5-HD coincided with decreased motor nerve velocity and increased distal latency of the tail nerve, measures of n-hexane neurotoxicity (Ichihara et al. 1998). Collectively, these studies indicate that the potentiating effects of 2-butantone on n-hexane, methyl-n-butyl ketone, and ethyl-n-butyl ketone neurotoxicity may be mediated by increased persistence of the 2,5-HD metabolite.

2-Butanone has also been found to potentiate the neurotoxicity of ethanol (Cunningham et al. 1989). Mice pretreated intraperitoneally with 2-butanone followed by intraperitoneal injection of ethanol 30 minutes later showed prolonged loss of righting reflex induced by ethanol. 2-Butanone decreased the rate of ethanol elimination in mice *in vivo* and inhibited the *in vitro* activity of alcohol dehydrogenase, the primary mechanism for ethanol elimination. These results suggest that 2-butanone may potentiate the neurotoxicity of ethanol by inhibiting its metabolism by alcohol dehydrogenase.

Cosnier et al. (2014) reported significant increases in blood toluene levels at both 1 and 5 days after exposure by inhalation to binary mixtures of toluene and 2-butanone, compared to those exposed to toluene alone. Cosnier et al. (2018b) found that toluene inhibited 2-butanone metabolism and that

2-butanone inhibited toluene metabolism in rats exposed by inhalation to both substances. However, the studies of Cosnier and coworkers did not include evaluation of neurological endpoints.

Interactions Potentially Influencing Liver Toxicity. 2-Butanone alone is not highly hepatotoxic but has a well-documented role in potentiating haloalkane-induced hepatotoxicity (Brown and Hewitt 1984; Dietz and Traiger 1979; Hewitt et al. 1983, 1986, 1987; Tanii et al. 1986). Intraperitoneal injection of chloroform (0.5 mL/kg) alone caused a 9-fold increase in rat ALT activity (Brown and Hewitt 1984). In contrast, chloroform injection caused a 195-fold increase in rat ALT activity if administered 18 hours after oral administration of 2-butanone. Similarly, intraperitoneal injection of chloroform increased rat plasma ornithine carbamyl transferase activity 215-fold if given 18 hours after oral administration of 2-butanone (Hewitt et al. 1983). The severity of hepatotoxicity appears to be dependent on dose and the length of time between 2-butanone pretreatment and subsequence chloroform exposure (Hewitt et al. 1987). 2-Butanone also potentiates carbon tetrachloride-induced hepatotoxicity in the rat (Dietz and Traiger 1979; Raymond and Plaa 1995a; Traiger et al. 1989). Significant increases in rat plasma ALT activity and hepatic triglyceride content, both suggestive of liver damage, were observed following the administration of 2-butanone either for 16 hours (Traiger et al. 1989) or for a duration of 3 days (Raymond and Plaa 1995a) before intraperitoneal injection of carbon tetrachloride. An *in vitro* study by Kim et al. (2014) suggests a possible interaction between 2-butanone and dimethylformamide (DMF) in HepG2 cells. Additional studies will be needed to determine what effect this interaction, if any, will have on DMF-mediated liver toxicity *in vivo*.

The potentiation of liver toxicity by ketones like 2-butanone is thought to be due to CYP induction. The maximal potentiation of carbon tetrachloride-induced hepatic injury by pretreatment with 2-butanone coincided with increased microsomal enzyme activity within the same time frame following exposure to 2-butanone alone (Traiger et al. 1989). This strongly suggests that 2-butanone potentiates the hepatotoxicity of carbon tetrachloride by enhancing its metabolism to toxic intermediates. Liver microsomes extracted from rats pretreated with 2-butanone, however, did not have increased CYP content compared with controls (Raymond and Plaa 1995b). Additionally, the mechanism of 2-butanone potentiation of chloroform-induced hepatotoxicity apparently does not involve biotransformation of chloroform to a reactive intermediate, an alteration of the CYP system, or depletion of liver glutathione (Hewitt et al. 1987). To explore other possibilities, Raymond and Plaa (1996) tested whether 2-butantone altered the adverse impacts of carbon tetrachloride treatment on liver membrane integrity. Purified liver membranes from controls or rats pretreated with 2-butanone were monitored for membrane fluidity and measured for membrane enzymes including 5’-nucleotidase, leucine aminopeptidase, and alkaline

phosphatase (Raymond and Plaa 1996). 2-Butanone had no significant impact on the membrane integrity status influenced by carbon tetrachloride; therefore, increased membrane sensitivity is not likely a mechanism contributing to 2-butanone potentiation of carbon tetrachloride hepatotoxicity.

Another hypothesis is that the observed 2-butanone potentiation of chloroform and carbon tetrachloride hepatotoxicity may be related to biotransformation of the 2-butanone to its metabolite, 2,3-butanediol. Carbon tetrachloride increased rat ALT 164-fold when injected 16 hours after oral administration of 2,3-butanediol. Replacement of 2,3-butanediol with 2-butanone increased the transaminase 66-fold. Hepatic triglyceride content was potentiated to a similar degree by both 2-butanone and 2,3-butanediol (Traiger et al. 1989).

Interactions Potentially Influencing Kidney Toxicity. Few studies have evaluated the impact of 2-butanone interactions on kidney toxicity. In a case-report, a 42-year-old male who ingested a cleaning solution that contained methanol and 2-butanone became tachycardic, with a hyperosmolar coma without anion gap metabolic acidosis. The study authors suggested that the osmolar gap, in the absence of metabolic acidosis, could be due to 2-butanone inhibition of methanol metabolism (Price et al. 1994).

Kidney toxicity, assessed by a decreased accumulation of *p*-aminohippuric acid in renal cortical slices in rats exposed to chloroform, was potentiated in rats that were pretreated with 2-butanone for 3 days prior to chloroform exposure (Raymond and Plaa 1995a). Unlike in the liver, total CYP content and aniline hydroxylase levels were increased in kidney microsomes extracted from rats pretreated with 2-butanone, compared to controls. These data suggest a role for CYP induction in the potentiation of chloroform kidney toxicity by 2-butanone (Raymond and Plaa 1995b).

Interactions Potentially Influencing Other Toxicity. Pretreatment of ddY mice with carbon tetrachloride 24 hours before oral administration of 2-butanone reduced the 2-butanone LD_{50} about 20% (Tanii et al. 1986). The mechanism of this effect was not investigated.

Exposure of pregnant rats continuously to n-hexane alone (1,000–1,500 ppm) or n-hexane and 2-butanone (1,200 ppm n-hexane, 300 ppm 2-butanone) throughout gestation and/or during the postnatal period resulted in reduced birth weight of pups, and weight gain reduction persisted during the postnatal exposure period (Stoltenburg-Didinger et al. 1990). The effect was more pronounced with the mixture of solvents. In addition, hindlimb weakness in one dam during the gestational exposure period progressing

to quadriplegia in all dams during the postpartum exposure period was observed with the solvent mixture, while only hindlimb weakness was observed in the dams exposed to n-hexane alone.

Coexposure of *S. cerevisiae* to 2-butanone, ethyl acetate, and propionitrile enhanced the induction of chromosome loss caused by 2-butanone (Zimmermann et al. 1989). Coexposure of *S. cerevisiae* to 2-butanone and nocodazole enhanced the induction of aneuploidy caused by 2-butanone alone (Mayer and Goin 1987).