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

3.1 TOXICOKINETICS

- Absorption
  - Respiratory tract: 2-Hexanone is well absorbed from the respiratory tract. A small study in humans estimated that approximately 75–92% of the inhaled dose was absorbed. Absorption of inhaled 2-hexanone has also been demonstrated in rats.
  - Gastrointestinal tract: 2-Hexanone is well absorbed from the gastrointestinal tract. A small study in humans estimated that approximately 66% of the oral dose was absorbed. A study in rats showed that almost 100% of an oral dose of 2-hexanone was absorbed.
  - Dermal: 2-Hexanone is absorbed following dermal exposure; however, quantitative estimates of the absorption fraction are not available.
- Distribution. In humans, 2-hexanone was detected in serum, but no additional information regarding distribution was available. Studies in laboratory animals show that 2-hexanone is distributed to the brain and liver.
- Metabolism. 2-Hexanone undergoes metabolism through reduction and oxidation reactions. The metabolite, 2,5-hexanedione, is toxicologically active.
- Excretion. Expired breath and urine appear to be the main routes of excretion for 2-hexanone and its metabolites in both animals and humans.

3.1.1 Absorption

The available data indicate that 2-hexanone is well absorbed after administration via the inhalation route. 2-Hexanone was detected in expired breath of humans who inhaled 2-hexanone at 10 or 50 ppm for 7.5 hours or 100 ppm for 4 hours (DiVincenzo et al. 1978). Concentrations of 2-hexanone in expired air were lower than that of the external exposure concentrations. Analysis of serum showed that 2-hexanone was present in serum in subjects exposed to 100 ppm, but not to 10 or 50 ppm. The study authors stated that results indicate that 75–92% of the inhaled 2-hexanone vapor was absorbed by the lungs and respiratory tract; however, the basis of this quantitative assessment was not reported. Similarly, beagles that inhaled 2-hexanone at 50 or 100 ppm for 6 hours absorbed 65–68% of the inhaled vapor (DiVincenzo et al. 1978). Whole-body exposure of rats to 75, 150, or 300 ppm 2-hexanone for 4 hours resulted in exposure-related amounts of the parent compound and the metabolites, 2-hexanol and 2,5-hexanedione, in

plasma immediately after the last exposure (Duguay and Plaa 1995). At 75 and 150 ppm, the concentration of 2,5-hexanedione in plasma was approximately 5 times that of 2-hexanone; at 300 ppm, it was about 2.5 times. It should be mentioned that in rats from the mid- and high-exposure groups, the concentration of 2,5-hexanedione in plasma was significantly higher following inhalation exposure than following oral exposure (see below).

2-Hexanone also appears to be well absorbed after oral administration. Humans who ingested a single capsule containing ^{14}C-2-hexanone at 0.1 mg/kg excreted about 40% of the ^{14}C in breath and 26% in urine during the next 8 days (DiVincenzo et al. 1978). This indicates that the absorbed amount averaged at least 66% of the administered dose. Administration of 1-^{14}C-2-hexanone at 20 or 200 mg/kg by gavage to rats resulted in excretion of about 1.2% of the administered radioactivity in the feces, about 44% in the breath, 38% in urine, and 16% remaining in the carcass (DiVincenzo et al. 1977). The results were similar at either dosage level. These findings suggest that about 98% of the administered dose was absorbed and that absorption was not saturable at the range of doses administered. Similar results were reported in rats administered three gavage doses of 50, 100, or 200 mg/kg 2-hexanone (Duguay and Plaa 1995). Plasma samples analyzed 1 hour after administration of the last dose showed dose-related amounts of 2-hexanone.

2-Hexanone is also absorbed after dermal application. The excretion of ^{14}C in the breath and urine of two volunteers was measured after a 60-minute occlusive application of ^{14}C-2-hexanone to shaved forearms (DiVincenzo et al. 1978). Calculated skin absorption rates were 4.8 and 8.0 µg/minute/cm^2; however, the fraction of 2-hexanone that was absorbed was not calculated. ^{14}C-Hexanone was also applied to the clipped thorax of beagle dogs, and absorption was observed to be slow at first but increased dramatically after 20 minutes. At 60 minutes, 77 mg of 2-hexanone had penetrated the skin (DiVincenzo et al. 1978). The fraction of applied 2-hexanone that was absorbed was not calculated.

3.1.2 Distribution

Little information on the distribution of 2-hexanone in humans following inhalation exposure is available. In humans exposed to 2-hexanone via inhalation to 10 or 50 ppm for 7.5 hours or to 100 ppm for 4 hours, 2-hexanone was detected in serum in subjects exposed to 100 ppm (DiVincenzo et al. 1978). No information regarding distribution to other tissues was reported.

Studies in laboratory animals provide some information regarding distribution of 2-hexanone; however, this has not been extensively studied. 2-Hexanone and its metabolites, 2-hexanol and 2,5-hexanedione, were detected in the lungs of rats 1 hour after the last of three daily 4-hour inhalation exposures to 75, 150, or 300 ppm 2-hexanone (Duguay and Plaa 1995). Some degree of accumulation seemed to have occurred since the lungs of the mid- and high-exposure groups had 4 and 20 times more 2-hexanone, respectively, than the low-exposure group. The three compounds were also measured in the liver, but in contrast with the lung findings, the concentrations of 2-hexanone in the liver were exposure concentration-related. The lungs and liver were the only tissues examined in the Duguay and Plaa (1995) study. An additional metabolite, 5-hydroxy-2-hexanone, was detected in blood from cats following intermittent chronic exposure to 2-hexanone (O'Donoghue and Krasavage 1979). This metabolite was short-lived since it could not be detected on Mondays following 2 days exposure-free.

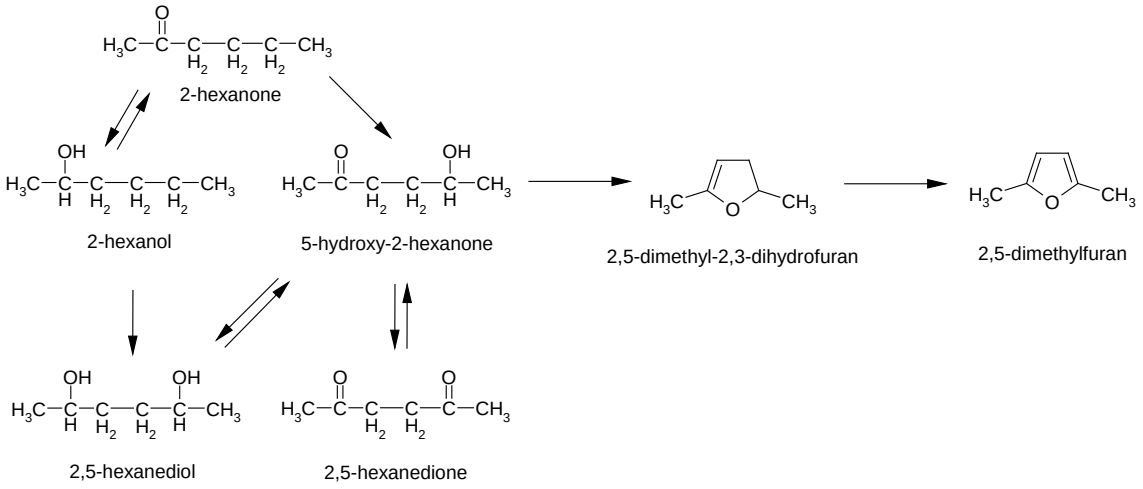
In rats administered a single oral dose of ^{14}C-2-hexanone at 200 mg/kg by gavage, tissue distribution was reported to be widespread with highest counts in the liver and blood. No quantitative data were given on tissue distribution (DiVincenzo et al. 1977). An analysis of subcellular distribution of the ^{14}C label in liver, brain, and kidney tissue indicated highest counts were associated with the crude lipid fraction and protein, with some recovery in DNA, and little or none in RNA. Gavage administration of 50, 100, or 200 mg/kg 2-hexanone to rats for 3 days resulted in measurable amounts of the parent compound and its metabolites, 2-hexanol and 2,5-hexanedione, in the liver 1 hour after the last dose (Duguay and Plaa 1995). However, in contrast to the liver findings, no 2,5-hexanedione was detected in the lungs, which led the investigators to suggest that lung metabolism of 2-hexanone might contribute to plasma metabolite levels.

2-Hexanone was shown to distribute to the brain of mice within 15–90 minutes following intraperitoneal administration of a single dose of approximately 500 mg/kg of the compound (Granvil et al. 1994). Both of its metabolites, 2-hexanol and 2,5-hexanedione, were also found in the brain. Brain concentrations of 2-hexanone seemed to be lower than those measured in blood. 2-Hexanol was detected in the brain considerably earlier than 2,5-hexanedione. The study also showed that the concentrations of 2-hexanol in the brain at the various time intervals measured were approximately twice those found in blood, which according to the investigators, might explain the lower concentrations of 2-hexanone found in brain compared to those found in blood.

3.1.3 Metabolism

The proposed phase I metabolic pathway (oxidation, reduction, and hydrolysis reactions) for 2-hexanone, based on 2-hexanone metabolites identified in blood during intraperitoneal studies in guinea pigs (DiVincenzo et al. 1976) and oral studies in rats (DiVincenzo et al. 1977), is presented in Figure 3-1. DiVincenzo et al. (1978) hypothesized that the metabolic pathway for 2-hexanone is similar in humans and experimental animals based on increases in 2,5-hexanedione in serum following inhalation exposure and radiolabeled carbon dioxide in expired air following oral exposure. The metabolism of aliphatic ketones has generally been found to proceed via reduction to the corresponding secondary alcohol, which accounts for the formation of 2-hexanol. An alternate pathway is oxidation of the 5-methylene group to the corresponding alcohol, 5-hydroxy-2-hexanone, which may be followed by further oxidation to the diketone 2,5-hexanedione. Another possibility in the metabolism of 2-hexanone is the cyclization of 5-hydroxy2-hexanone to the corresponding dihydrofuran and oxidation to 2,5-dimethylfuran (DiVincenzo et al. 1977). However, the formation of these furan moieties may be the result of thermal dehydration and cyclization during gas chromatography (DiVincenzo et al. 1977). In addition, the gamma-valerolactone found in the urine (not shown in figure) is hypothesized to result from α-oxidation of 5-hydroxy-2-hexanone to 2-keto-5-hydroxyhexanoic acid, decarboxylation and oxidation to 4-hydroxypentanoic acid, and lactonization to gamma-valerolactone (DiVincenzo et al. 1977). The specific cytochrome P-450 isozymes involved in the phase I metabolism of 2-hexanone have not been identified. The appearance of glucuronide and sulfate conjugates of 2-hexanone metabolites (Phase II metabolism) in the urine indicate that there is further metabolism; however, no additional information was identified.

Figure 3-1. Proposed Phase I Metabolic Pathway for 2-Hexanone



Source: DiVincenzo et al. 1976, 1977

3.1.4 Excretion

In humans exposed to 2-hexanone via inhalation to 10 or 50 ppm for 7.5 hours or to 100 ppm for 4 hours, unchanged 2-hexanone (but not 2,5-hexanedione) was found in expired air during exposure, and neither 2-hexanone nor any of its metabolites was found in urine during or after exposure (DiVincenzo et al. 1978). 2-Hexanone was not detected in the expired air 3 hours after exposure to 50 or 100 ppm. The study authors stated that results suggest slow clearance and possible accumulation of 2-hexanone in humans exposed by this route.

In beagle dogs exposed to 2-hexanone via inhalation at 50 or 100 ppm for 6 hours, 32 and 35%, respectively, of the inhaled vapor was excreted in the expired breath (DiVincenzo et al. 1978). By 3–5 hours after exposure, 2-hexanone was no longer detected in expired air. Excretion via other routes was not addressed.

In two humans who received a single oral dose of 1-^{14}C-2-hexanone, breath excretion of $^{14}CO_2$ reached a peak within 4 hours, then decreased slowly over the next 3–5 days. Average overall recovery of the ^{14}C-label in 8 days was 40% in breath and 26% in urine. Feces were not analyzed (DiVincenzo et al. 1978).

In rats administered a single oral dose of 1-^{14}C-2-hexanone, DiVincenzo et al. (1977) observed similar results. Radioactivity in breath accounted for about 45% of the administered dose (5% was in unchanged 2-hexanone; 40% was in $^{14}CO_2$); 35% was found in the urine; 1.5% was recovered in the feces; and about 15% remained in the carcass after 6 days. In male rats that received daily gavage doses of 2-hexanone at 400 mg/kg/day for 40 weeks, very low concentrations of free 2-hexanone were detected in the urine from the 3rd week. A maximum concentration of approximately 20 µg was reached in the 17th week (Eben et al. 1979). Similarly, free 2,5-hexanediol was found in the urine after 3 weeks and peaked in the 17th week. Free and conjugated 2,5-hexanedione was present in the urine from the 1st week of the study. The conjugated form peaked in the 7th week, whereas excretion levels of the free form were fairly consistent throughout the study. A strong correlation was observed in this study between the onset of neuropathy and the urinary concentration of 2,5-hexanedione when 2-hexanone, 2,5-hexanedione, or 2,5-hexanediol was administered orally to rats at 400 mg/kg/day.

^{14}C from 1-^{14}C-2-hexanone applied to the forearms of two volunteers was found in the breath and urine (DiVincenzo et al. 1978). In one subject, excretion was similar by both routes; in the other subject, the levels were much higher (about 3:1) in the breath. Levels of radioactivity in feces were not measured.

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 not been developed for 2-hexanone.

3.1.6 Animal-to-Human Extrapolations

2-Hexanone, via its metabolite, 2,5-hexanedione, affects mainly the nervous system (Abdel-Rahman et al. 1978; DiVincenzo et al. 1976, 1978; Eben et al. 1979). Most animal species tested have shown similar clinical signs and morphological alterations in the peripheral nervous system, as have humans exposed to 2-hexanone itself or to *n*-hexane, a chemical that is also biotransformed into 2,5-hexanedione. Comparative studies have shown the relative species sensitivity to 2-hexanone as chicken > cat > dog > primate > rat (Abdo et al. 1982; Mendell et al. 1974). While many studies have been conducted in hens/chickens and are useful for hazard identification, they are not useful for risk assessment. As mentioned earlier, because their digestive and respiratory systems are different from mammals, it is not known whether the dose-response in this species is applicable to humans.

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

There are no studies of humans that could help determine whether children are more susceptible than adults to the effects of exposure to 2-hexanone. Likewise, there are no studies in animals that examined the comparative sensitivity of young and older animals to 2-hexanone.

To the extent that the metabolism of 2-hexanone involves cytochrome P-450 enzymes, some of which are known to be developmentally regulated, infants may be at higher or lower risk of 2-hexanone toxicity depending on whether oxidative (activation) or reductive (detoxification) reactions prevail in the initial steps of 2-hexanone metabolism.

No specific population has been identified that is unusually susceptible to toxic effects resulting from exposure to 2-hexanone.

Children are expected to be exposed to 2-hexanone by the same routes that affect adults. Ingestion of foods contaminated with small amounts of 2-hexanone is the most likely route of exposure for children. No data were located regarding 2-hexanone in breast milk; therefore, an adequate determination of the importance of this route of child exposure has not been made.

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-hexanone 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-hexanone 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-hexanone 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

2-Hexanone and its various metabolic products (2-hexanol, 2,5-hexanedione, 5-hydroxy-2-hexanone, 2,5-dimethylfuran) can be measured in expired air, biological tissue, fluid, and excreta (Fedtke and Bolt 1986; Nomeir and Abou-Donia 1985; O'Donoghue and Krasavage 1979; White et al. 1979). The currently available information, however, does not indicate whether the levels of these substances can be used to calculate or estimate corresponding levels of exposure to 2-hexanone. Because exposure to other

substances, for example *n*-hexane, also produce 2,5-hexanedione as a metabolite, identification of 2,5-hexanedione in the urine does not necessarily indicate that exposure to 2-hexanone occurred.

It is worth noting that 2,5-hexanedione has been identified in the urine of subjects in Italy who had not been occupationally exposed to 2-hexanone or *n*-hexane (Bavazzano et al. 1998). The investigators proposed that 2,5-hexanedione had both an endogenous and an exogenous origin. The former is related to pollution due to exposure to solvents and the latter is based on the hypothesis that 2,5-hexanedione might be an intermediate catabolite of some biochemical physiological processes. However, the study did not provide any support for an endogenous origin.

3.3.2 Biomarkers of Effect

There are no biomarkers specific for exposure to 2-hexanone. The main effect of exposure to 2-hexanone is neuropathy. Signs of neuropathy can be monitored by non-invasive procedures such as measurement of nerve conduction velocities, amplitude of evoked muscle action potentials, and amplitude of evoked sensory action potentials. However, these signs are not exclusive to exposure to 2-hexanone. They can occur due to exposure to other chemicals or can be caused by conditions not even associated with chemical exposures.

3.4 INTERACTIONS WITH OTHER CHEMICALS

There are limited data on the effect of other chemicals on the toxicity of 2-hexanone. A study in which rats were exposed via inhalation to a combination of 2-hexanone and methyl ethyl ketone resulted in the potentiation of severe neurotoxic effects including paralysis and histopathological changes. These effects were either not observed or they occurred at much lower frequencies when either of the two compounds was administered separately (Saida et al. 1976). Similarly, dermal or inhalation exposure in hens to 2-hexanone in combination with dermal application of the pesticide, *O*-ethyl-*O*-4-nitrophenyl phenylphosphonothioate (EPN), has resulted in earlier onset and far more severe clinical and histological manifestations of neurotoxic effects than with either chemical exposure alone (Abou-Donia et al. 1985a, 1985b). The authors speculated that this potentiation effect may have been due to induction of hepatic microsomal cytochrome P-450 by EPN, leading to increased metabolism of 2-hexanone to its neurotoxic metabolite, 2,5-hexanedione. An alternate explanation is that local trauma to the nervous tissue produced by 2-hexanone and EPN might increase vascular permeability and thus increase the entry of these compounds and their metabolites from circulation.

Given that 2-hexanone and *n*-hexane have similar active metabolites, interaction studies with *n*-hexane provide information on potential for interactions for 2-hexanone. As discussed in the toxicological profile for *n*-hexane (ATSDR 1999), co-exposure of *n*-hexane with methyl ethyl ketone or acetone increased the neurotoxicity of *n*-hexane. In contrast, co-exposure of *n*-hexane with xylene or toluene prevented or reversed the decreased nerve conduction velocity that was associated with exposure to *n*-hexane only. This protective effect may have been due to metabolic competition resulting in a decrease in the metabolism of *n*-hexane to 2,5-hexanedione (ATSDR 1999). Although no studies were identified, it is likely that co-exposure to 2-hexanone and *n*-hexane would result in additive or greater-than-additive toxicity. Additionally, co-exposure to other compounds that have similar mechanisms of neurotoxicity or result in alterations that favor the production of 2,5-hexanedione (e.g., methyl isobutyl ketone) may influence the toxicity of 2-hexanone.