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

3.1 TOXICOKINETICS

- Inhaled *n*-hexane is readily absorbed in the lungs, while absorption by the oral and dermal route has not been well characterized.
- Inhaled *n*-hexane distributes throughout the body; based on blood-tissue partition coefficients, preferential distribution would be in the order: body fat>>liver, brain, muscle>kidney, heart>lung, blood.
- *n*-Hexane is metabolized by cytochrome P-450 enzymes in the liver to several metabolites, including the neurotoxicant, 2,5-hexanedione.
- Approximately 10–20% of absorbed *n*-hexane is excreted unchanged in exhaled air, and 2,5-hexanedione is the major metabolite recovered in urine.

3.1.1 Absorption

n-Hexane is absorbed by passive diffusion in the lungs. Alveolar *n*-hexane reaches a steady state with the *n*-hexane in blood. As *n*-hexane is distributed and metabolized in the body, more is absorbed from the alveolar air. In studies with humans, there was no evidence of saturation up to 204 ppm (Veulemans et al. 1982). During exercise in this study, the alveolar uptake rate decreased, but total intake increased slightly because of the higher ventilation rate. The absorption of inhaled *n*-hexane has been investigated in six healthy male volunteers (Veulemans et al. 1982). Three different trials were performed on each volunteer: 4-hour exposure at 102 ppm *n*-hexane, 4-hour exposure at 204 ppm, and 4-hour exposure during exercise on a stationary bicycle ergometer at 102 ppm. Each trial was done at least 2 weeks apart. Lung clearance (from alveolar air to blood) and retention were calculated from *n*-hexane concentrations in inhaled and exhaled air. After exposure, *n*-hexane in exhaled air was measured for up to 4 hours to determine respiratory elimination. Retention of *n*-hexane (calculated from lung clearance and respiratory minute volume) was approximately 20–25% of the *n*-hexane in the inhaled air. This resulted in calculated average absorption rates of 0.84 mg/minute at 102 ppm and 1.59 mg/minute at 204 ppm. Physical exercise at 102 ppm caused a significant increase in lung clearance and at peak loads (60 watts), was more than twice the value at rest, resulting in an increase in absorption rate. Pulmonary excretion of *n*-hexane after exposure ended appeared to be biphasic, with a fast drop in the first 30 minutes and a slower drop for the remainder of the 4-hour observation period.

In a workplace study, lung uptake and excretion of *n*-hexane were studied in 10 workers (sex not specified, 18–30 years old) in a shoe factory (Mutti et al. 1984). Simultaneous samples of inhaled and alveolar air (last 100 mL of the tidal volume) were collected 6 times during an 8-hour workday. Breathing-zone air was collected with personal samplers. Median time-weighted average (TWA) *n*-hexane concentrations were 243 mg/m^3 (69 ppm). 2-Methylpentane, 3-methylpentane, cyclohexane, and *n*-heptane were also present in the air. Alveolar excretion was monitored during a 6-hour post-exposure period. Uptake was calculated from lung ventilation, the retention coefficient (1-[Calv/Cinh]), and environmental concentrations. The total amount of exhaled *n*-hexane was calculated by integration of the decay curve for the concentration of exhaled *n*-hexane. About 25% of inhaled *n*-hexane was retained in the alveoli. Absorption into the blood in relation to total respiratory uptake was about 17%, taking into account the retention coefficient and alveolar ventilation.

No studies were located that specifically addressed absorption of *n*-hexane after oral exposure in humans or animals. Absorption of *n*-hexane by the oral route in humans can be inferred from the appearance of *n*-hexane in exhaled air and 2,5-hexanedione in urine of volunteers receiving 0.24 or 0.81 mg/kg via a gastric feeding tube (Baelum et al. 1998). Absorption of toxicologically significant amounts by this route can be inferred since neurological effects occurred in rats receiving *n*-hexane by gavage (Krasavage et al. 1980; Ono et al. 1981). Significant serum levels of the *n*-hexane metabolite, 2,5-hexanedione, were also measured in rats receiving *n*-hexane by gavage (Krasavage et al. 1980).

The permeability of human skin to *n*-hexane has been determined *in vitro* in flow-through diffusion cells (Loden 1986). Pieces of full-thickness human skin were exposed to [^{3}H]*n*-hexane in human serum, and the appearance of label in the trans compartment measured for 0.5 or 12 hours. The skin was then sectioned with a microtome into 0.25 mm slices and the quantity of label in the skin was measured. The rate of resorption (uptake of substance by the receptor fluid beneath the skin [i.e., the amount that passes through the skin]) was calculated. The rate of resorption for *n*-hexane through human skin was calculated to be 0.83 (μg·cm^2/hour). The permeability of *n*-hexane through human skin was much lower (approximately 100-fold) than for other chemicals tested in this study. For example, rates of resorption (in μg·cm^2/hour) were 99 for benzene and 118 for ethylene glycol.

No information is available on whether absorption of *n*-hexane by children differs from that of adults. Since absorption by all routes appears to be by passive diffusion, it is probable that absorption in children is similar to that of adults.

3.1.2 Distribution

The distribution of *n*-hexane is a function of its high lipid and very low water solubility. *n*-Hexane is transported in blood mainly by partitioning into hydrophobic regions of blood proteins (Lam et al. 1990). Transfer to tissues occurs via a similar partitioning process. *n*-Hexane can also leave the blood through the lungs via the pulmonary circulation depending on the alveolar air *n*-hexane concentration.

Partition coefficients established in human tissues indicate a distribution pattern at equilibrium of body fat>>liver, brain, muscle>kidney, heart>lung, blood (Perbellini et al. 1985). The following partition coefficients for *n*-hexane were determined: olive oil/air, 146; blood/air, 0.80; liver/air, 5.2; kidney/air, 3; brain/air, 5; fat/air, 104; muscle/air, 5; heart/air, 2.8; and lung/air, 1. Saline/air partition was not reported separately for *n*-hexane, but was very low for the range reported for the entire group of compounds (0.1–0.4). Partition coefficients for *n*-hexane in male Fischer 344 rats have been reported (blood/air, tissue/air): blood, 2.29; liver, 5.2; muscle, 2.9; and fat, 159 (Gargas et al. 1989).

In a study where blood *n*-hexane concentrations were determined in volunteers during exposure to 102 or 204 ppm for 4 hours, blood *n*-hexane reached steady state within 50 minutes and was stable until the end of exposure. Concentrations of *n*-hexane in blood at 50 minutes were 0.183 mg/L at 102 ppm and 0.3347 mg/L at 204 ppm (Veulemans et al. 1982).

In Fischer 344 rats exposed to up to 10,000 ppm *n*-hexane for 6 hours, *n*-hexane achieved an apparent steady state in all tissues within 2 hours (Baker and Rickert 1981). Steady-state concentrations were proportional to dose only in blood and liver. In brain, sciatic nerve, kidney, lung, and testes, exposure to 1,000 ppm resulted in a disproportionately greater concentration than exposure at 500 ppm. Peak blood concentrations of *n*-hexane were 1, 2, 8, and 21 μg/mL, and peak sciatic nerve concentrations were 12, 48, 130, and 430 μg/g at 500, 1,000, 3,000, and 10,000 ppm, respectively. In a study that addressed possible accumulation of *n*-hexane in tissues, *n*-hexane was not detected in any tissue besides sciatic nerve after 2 hours post-exposure in either 1- or 5-day exposures to *n*-hexane at 1,000 ppm for 6 hours/day (Bus et al. 1981). Initial concentrations after a single exposure were: sciatic nerve, 46 μg/g; kidney, 5.8 μg/g; liver, 1.2 μg/g; brain, 3 μg/g; and blood, 0.5 μg/mL. Initial concentrations after five daily exposures were similar. No studies were located regarding distribution of *n*-hexane after oral or dermal exposure in humans or animals.

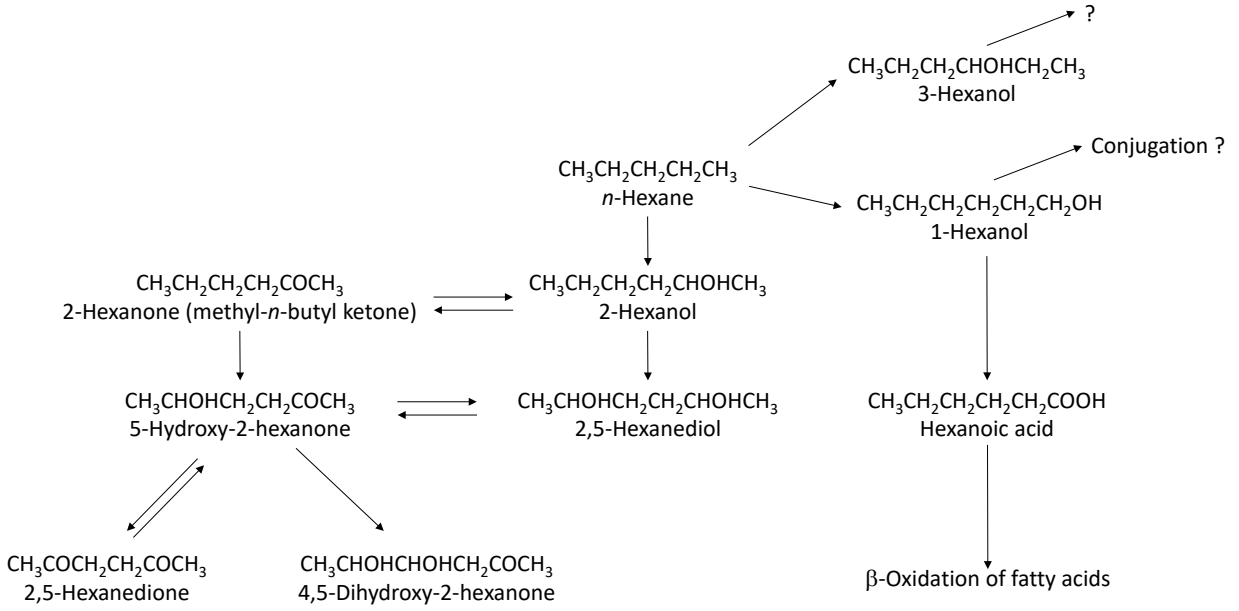
No information is available on whether distribution of *n*-hexane in children differs from that of adults. Transfer across the placenta has been demonstrated in rats for *n*-hexane and two resulting metabolites, 2-hexanone and 2,5-hexanedione (Bus et al. 1979); no preferential distribution to the fetus was observed for either *n*-hexane or the metabolites. Concentrations of *n*-hexane and its metabolites were similar between maternal tissues and the fetal tissues following a 6-hour exposure to 1,000 ppm *n*-hexane on GD 20 (Bus et al. 1979), indicating that transfer across the placenta takes place.

n-Hexane has not been measured in breast milk, although partition coefficients have been reported for human milk from a group of eight volunteers (Fisher et al. 1997). The milk/air coefficient was 4.66 and the blood/air coefficient was 2.13. A milk/blood partition coefficient of 2.10 was calculated from these data, indicating that there would be preferential distribution to this compartment. Due to its relatively rapid metabolism, storage of *n*-hexane in body fat does not appear to occur at air concentrations to which humans are exposed; thus, mobilization of stored *n*-hexane upon pregnancy or during lactation is unlikely. The toxic metabolite of *n*-hexane, 2,5-hexanedione, can probably be distributed to germ cells as demonstrated by the testicular effects observed in male rats after drinking water exposure to 2,5-hexanedione. High air concentrations of *n*-hexane can also produce these effects in rats, presumably via 2,5-hexanedione.

3.1.3 Metabolism

The metabolism of *n*-hexane takes place in the liver. The initial reaction is oxidation by cytochrome P-450 isozymes to hexanols, predominantly 2-hexanol. Further reactions convert 2-hexanol to 2-hexanone, 2,5-hexanediol, 5-hydroxy-2-hexanone, 4,5-dihydroxy-2-hexanone, and the neurotoxicant, 2,5-hexanedione. Hydroxylation at the 1- and 3- positions can be considered detoxification pathways; hydroxylation at the 2- position is a bioactivation pathway. A diagram of the proposed pathway for mammalian metabolism of *n*-hexane is presented in Figure 3-1.

Figure 3-1. Proposed Scheme for the Metabolism of *n*-Hexane



Adapted from Fedtke and Bolt (1987) and NTP (1991)

Approximately 10–20% of *n*-hexane absorbed by inhalation is excreted unchanged in exhaled air; the remainder is metabolized. Metabolism takes place via mixed-function oxidase reactions in the liver. In a study in which metabolites were measured in workers exposed to *n*-hexane (Perbellini et al. 1981), mean concentrations of *n*-hexane metabolites in urine were: 2,5-hexanedione, 5.4 mg/L (including 4,5-dihydroxy-2-hexanone because acid treatment of the urine converts 4,5-dihydroxy-2-hexanone to 2,5-hexadione); 2,5-dimethylfuran, 3.7 mg/L; gamma-valerolactone, 3.3 mg/L; and 2-hexanol, 0.19 mg/L (2,5-dimethylfuran and gamma-valerolactone are believed to be artifacts of sample preparation and analysis rather than true metabolites of *n*-hexane [Perbellini et al. 1981]). The first reaction that takes place is hydroxylation of *n*-hexane at the 2- position to form 2-hexanol. Further reactions result in 2,5-hexanedione, presumably through transient intermediates, including 2-hexanone, 2,5-hexanediol, and 5-hydroxy-2-hexanone. Correlations between concentrations of *n*-hexane in air and urinary metabolites were best for total *n*-hexane metabolites (r=0.7858), followed by 2-hexanol (r=0.6851) and 2,5-hexanedione (r=0.6725).

The time-course of the metabolism of inhaled *n*-hexane in a group of 19 volunteers has been estimated by determining serum 2,5-hexanedione during and after a 15.5-minute exposure to 60 ppm *n*-hexane (van Engelen et al. 1997). The time to reach the peak concentration varied from 16.2 to 19.8 minutes after the

start of exposure (i.e., 1–4 minutes following the cessation of exposure). The rate at which 2,5-hexanedione appeared in the blood ranged from 1.89 to 4.48 μM/hour.

Further studies in humans indicate that a large proportion of the 2,5-hexanedione detected in urine after *n*-hexane exposure is the result of an artifact resulting from treatment with acid to hydrolyze urinary conjugates (Fedtke and Bolt 1987). When urine from a male volunteer exposed to 217 ppm *n*-hexane for 4 hours was hydrolyzed enzymatically with β-glucuronidase, excretion of 4,5-dihydroxy-2-hexanone was approximately 4 times higher than that of 2,5-hexanedione. When the urine was hydrolyzed with acid, 4,5-dihydroxy-2-hexanone was not detected, but the amount of 2,5-hexanedione in the urine increased, indicating conversion of 4,5-dihydroxy-2-hexanone to 2,5-hexanedione by the acid treatment. The fraction of 2,5-hexanedione determined after complete acid hydrolysis minus the 2,5-hexanedione originally present was equal to the 4,5-dihydroxy-2-hexanone. Only "minor" amounts of 2-hexanol were reported.

2,5-Hexanedione has also been detected after acid hydrolysis of the urine of individuals unexposed to *n*-hexane (Fedtke and Bolt 1986; Perbellini et al. 1993). 2,5-Hexanedione was not detected without acid hydrolysis, indicating that it is formed as a result of conversion of 4,5-dihydroxy-2-hexanone. It is possible that small amounts of *n*-hexane are produced in the body by lipid peroxidation, as has been demonstrated for *n*-pentane (Filser et al. 1983). Urinary excretion of 2,5-hexanedione ranged from 0.3 to 1.2 mg in 24 hours for unexposed individuals; workers exposed to approximately 50 ppm *n*-hexane excreted 3–4 mg/24 hours (Perbellini et al. 1993).

When male Wistar rats were exposed to *n*-hexane at concentrations up to 3,074 ppm for 8 hours, analysis of urine showed that 2-hexanol was the major metabolite, accounting for about 60–70% of the total metabolites collected over the 48-hour collecting period (Fedtke and Bolt 1987). This is in contrast to humans, in which the major urinary metabolite is 2,5-hexanedione (Perbellini et al. 1981). The amounts of metabolites excreted were linearly dependent on the exposure concentration, up to an exposure of about 300 ppm. 2-Hexanol and 2-hexanone were detected in the first sample (obtained during the 8-hour exposure); excretion of 2,5-hexanedione was delayed and was not detected until 8–16 hours after exposure began. The amount of 2,5-hexanedione detected depended on sample treatment; total excreted amounts over 48 hours were approximately 350 μg/kg 2,5-hexanedione without acid treatment and 3,000 μg/kg with total acid hydrolysis, indicating conversion of 4,5-dihydroxy-2-hexanone with acid treatment.

The metabolism of *n*-hexane in rat lung and liver microsomes has been investigated (Toftgard et al. 1986). In liver microsomes, the formation of 1-, 2-, and 3-hexanol from *n*-hexane was best described kinetically by a two-enzyme system, while for lung microsomes, single-enzyme kinetics were indicated for each metabolite. For conversion to 1-hexanol, apparent K, values were 0.4 and 300 μM, and V_{max} values were 0.09 and 1.2 nmol/mg protein/minute, respectively. For conversion to 2-hexanol, apparent K_m values were 6 and 1,100 μM, and V_{max} values were 1 and 4.6 nmol/mg protein/minute, respectively. Insufficient information was available to estimate the high-affinity activity for 3-hexanol, the low-affinity activity had an apparent K_m of 290 pM and a V_{max} of 0.5 nmol/mg protein/minute. In the lung, K_m values were 9, 50, and 65 μM for 1-, 2-, and 3-hexanol, respectively; V_{max} values were 2.2, 1.3, and 0.2 nmol/mg protein/minute, respectively. Prior induction of cytochrome P-450 enzymes with phenobarbital markedly increased the rate of formation of 2-hexanol in liver microsomes (1.8 nmol/mg/minute control versus 15 nmol/mg/minute with phenobarbital) and that of 3-hexanol (0.4 nmol/mg/minute control versus 2.8 nmol/mg/minute), while the rate of formation of 1-hexanol fell slightly (2 nmol/mg/minute control versus 0.7 nmol/mg/minute). Antibodies to cytochrome P-450 isozymes PB-B (CYP2B1-inducible by phenobarbital) and BNF-B (CYP1A1-inducible by β-naphthoflavone) were used as inhibitors to investigate the specificity of the reactions. In control liver microsomes, anti-PB-B showed no inhibitory effects while anti-BNF-B inhibited the formation of 2- and 3-hexanol by 25 and 40%, respectively, but had no effect on the formation of 1-hexanol. In microsomes from rats induced with phenobarbital, the anti-PB-B antibody reduced the formation of hexanols back to control levels. Purified cytochrome P-450 isozymes were also tested for their ability to hydroxylate *n*-hexane. The highest activity (nmol metabolite/nmol P-450/minute) was found with P-450-PB-B (CYP2B1), followed by P-450-PB-D (CYP2B2) and P-450-BNF-B (CYP1A1). Formation of 2,5-hexanediol from 2-hexanol was catalyzed by a cytochrome P-450 isozyme different from cytochrome P-450-PB-B (as judged by antibody inhibition) that was present in liver microsomes, but not in lung microsomes. This process was unaffected by prior induction of cytochrome P-450. Furthermore, alcohol dehydrogenase activity with hexanols or 2,5-hexanediol as the substrate was found exclusively in liver cytosol. These results suggest that inhaled *n*-hexane must be transported to the liver either intact or in the form of 2-hexanol before the neurotoxic metabolite, 2,5-hexanedione, can be formed. The large increase in hydroxylation of *n*-hexane upon induction (which would favor the production of 2,5-hexanedione via 2-hexanol) is a likely explanation for the potentiating effects of MEK on *n*-hexane neurotoxicity in humans and rats (Altenkirch et al. 1977, 1982) and of methyl isobutyl ketone in chickens (Abou-Donia et al. 1985).

Mortensen and Nilsen (1998) conducted an *in vitro* species comparison of *n*-hexane metabolism in the liver. Humans had the highest K_m (132 μmol/L) and rats had the lowest K_m (25 μmol/L), with guinea

pigs and mice having similar values as rats; however, there were no statistically significant differences between the species. V_{max} values were similar in the four species, ranging from 0.19 to 0.36 µmol/g/minute.

The tissue and cytochrome P-450 isoform specificity of *n*-hexane hydroxylation to hexanols has been investigated in rat tissues and cell lines expressing specific cytochrome P-450 isoforms (Crosbie et al. 1997). The highest activity per mg protein for the production of 2-hexanol (which can be further metabolized to 2,5-hexanedione) was in liver, followed by lung (about 25% of liver activity), muscle, and brain. Activity in muscle and brain was very low compared to the liver. Membrane preparations from cells expressing human CYP2E1 had the same *n*-hexane 2-hydroxylation activity as control cells. In contrast, cells expressing human CYP2B6 had approximately 100 times the 2-hydroxylation activity of the CYP2E1 or control cells. Specific induction of the cytochrome P-450 isozyme, CYP2E1, has been reported in male Wistar rats after intraperitoneal injection of *n*-hexane (Nakajima et al. 1991). No effects on total liver microsomal protein or total cytochrome P-450 content were observed.

trans-1,2-Dichloroethylene, a specific inhibitor of CYP2E1 in rats, has also been shown to affect the metabolism of *n*-hexane (Mathews et al. 1997). Rats exhale a large number of endogenous VOCs, including *n*-hexane. When CYP2E1 was inhibited by intraperitoneal injection of 1,2-dichloroethylene, levels of exhaled *n*-hexane increased approximately 25-fold within 4 hours and returned to pre-dose levels at approximately 24 hours, closely paralleling the inhibition and resynthesis time-course for CYP2E1. No increase in lipid peroxidation was observed, indicating that the increase in exhaled *n*-hexane was the result of inhibition of metabolism.

It is probable that many cytochrome P-450 isoforms are capable of hydroxylating *n*-hexane (both *in vivo* and under laboratory conditions); it is not possible at this time to specify which forms are definitely involved in *n*-hexane metabolism *in vivo*. The results of a study in CYP2E1 knockout and wild-type mice found more extensive metabolism of *n*-hexane to 2,5-hexanedione in wild-type mice, as compared to the knockout mice (Iba et al. 2000). The study also found that 2,5-hexanedione formation increased with duration in the wild-type mice but was unchanged in the knockout mice. The formation of 2,5-hexanedione in the knockout mouse suggests that other cytochromes P-450s can also metabolize *n*-hexane; the lack of increase in 2,5-hexanedione levels in the knockout mice suggests that these other cytochrome P-450s are not likely to be inducible by *n*-hexane.

The effect of concentration on the fate of [^{14}C]*n*-hexane after inhalation exposure has been studied in Fischer 344 rats (Bus et al. 1982). The disposition of radioactivity was concentration-related with the amount of the acquired body burden excreted increasing with increasing concentrations; 12, 24, 38, and 62% of the acquired body burden was excreted at exposure concentrations of 500, 1,000, 3,000, and 10,000 ppm, respectively. In contrast, 38 and 18% of the body burden of radioactivity was recovered as exhaled CO_2 and 35 and 18% was recovered in the urine at *n*-hexane concentrations of 500 and 10,000 ppm (exhaled air and urine were collected for 72 hours after exposure). Radioactivity remaining in the tissues and carcass 72 hours after exposure was 6.1 and 5.4% of the body burden at 500 and 10,000 ppm, respectively. The decreased total $^{14}CO_2$ and urinary ^{14}C excretion after exposure to 10,000 ppm, as compared to lower concentrations, was likely due to an inhibition of *n*-hexane metabolism rather than saturation of *n*-hexane excretion by the lungs or the kidneys.

In a study in which pregnant rats received a single 6-hour exposure to 1,000 ppm *n*-hexane on GD 12 or 20 (Bus et al. 1979), *n*-hexane was rapidly and extensively metabolized to methyl-*n*-butyl ketone (2-hexanone) and 2,5-hexanedione. 2-Hexanone and 2,5-hexanedione (the only metabolites measured) were detected in the maternal liver, kidney, brain, and blood. Fetal concentrations of *n*-hexane and its metabolites (entire fetus) were similar to those in maternal blood at all times after exposure. Results were similar on both GDs 12 and 20. *n*-Hexane and 2-hexanone were rapidly eliminated from maternal tissues and the fetus, with minimal or nondetectable concentrations reached 8 hours after exposure. In contrast, tissue concentrations of 2,5-hexanedione increased between 0 and 4 hours after exposure and thereafter exhibited a significantly slower elimination rate compared to *n*-hexane and 2-hexanone. 2,5-Hexanedione was not detected in the blood or tissues 24 hours after exposure. The half-life of 2,5-hexanedione in maternal blood was significantly greater than *n*-hexane and 2-hexanone (3.9 hours versus 1.24 and 0.99 hours, respectively).

Concentration time curves for *n*-hexane in a closed exposure system indicated that metabolism in rats was proportional to air concentration up to about 300 ppm (Filser et al. 1987). Metabolism was nonlinear above 300 ppm and appeared to be saturated at concentrations ≥3,000 ppm.

Little information is available on the metabolism of *n*-hexane after oral exposure, although it appears to be qualitatively similar to that after inhalation exposure. Peak serum concentrations of the *n*-hexane metabolite, 2,5-hexanedione, of 24, 44, and 53 μg/mL were observed in rats after a single gavage exposure to 570, 1,140, and 4,000 mg/kg *n*-hexane, respectively (Krasavage et al. 1980). Serum

2,5-hexanedione concentrations rose slowly to a peak at 12–16 hours and returned to baseline by 24 hours.

No information is available as to whether metabolism of *n*-hexane in children differs from that of adults. No studies were located comparing metabolism in young and adult animals. The toxicity of *n*-hexane results from biotransformation yielding the active metabolite, 2,5-hexanedione. The initial step is an oxidation to 2-hexanol catalyzed by a cytochrome P-450 enzyme. Some cytochrome P-450 enzymes are developmentally regulated (Leeder and Kearns 1997). As the above discussion indicates, it is not completely clear which cytochrome P-450 enzymes are involved in *n*-hexane metabolism.

3.1.4 Excretion

Elimination half-lives of *n*-hexane and its metabolites in the body and several tissues have been evaluated in humans and animals. In a study of workers exposed to *n*-hexane, the post-exposure alveolar excretion of *n*-hexane was about 10% of the total uptake and was in two phases: a fast phase with a half-life of 11 minutes and a slow phase with a half-life of 99 minutes (Mutti et al. 1984). Veulemans et al. (1982) estimated the elimination half-life of *n*-hexane in blood following a 4-hour inhalation exposure of humans to 102 ppm *n*-hexane. After exposure, there was a rapid fall to about 50% of the level at the end of exposure in the first 10 minutes, followed by a slower exponential time course with a half-life of 1.5–2 hours. A physiologically based pharmacokinetic (PBPK) model estimated a half-life of *n*-hexane in fat tissue of approximately 64 hours in humans (Perbellini et al. 1986). A half-life for the urinary excretion of 2,5-hexanedione was estimated by Perbellini et al. (1986) using data from workers. A study in rats exposed to 500 ppm [^{14}C] *n*-hexane for 6 hours estimated a urinary half-time for excretion of radioactivity of 12.7 hours (Bus et al. 1982).

Excretion of *n*-hexane after oral exposure in humans can be inferred based on elevated levels of 2,5-hexanedione in urine of volunteers receiving 0.24 or 0.81 mg/kg via a gastric feeding tube (Baelum et al. 1998). No studies were located regarding excretion of *n*-hexane or *n*-hexane metabolites following oral exposure to *n*-hexane in animals. No studies were located regarding excretion of *n*-hexane or *n*-hexane metabolites following dermal exposure to *n*-hexane.

No information is available as to whether excretion of *n*-hexane and its metabolites in children differs from that of adults. No studies were located comparing excretion in young and adult animals.

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

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and 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 (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). 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 (Clewell 1995).

Summary of PBPK Models. The Perbellini et al. (1986, 1990b) model simulates the absorption, distribution, biotransformation, and excretion of *n*-hexane during inhalation exposure. The excretion kinetics of the neurotoxic metabolite of *n*-hexane, 2,5-hexanedione, are also simulated.

A model describing transfer of *n*-hexane via lactation from a mother to a nursing infant is also available (Fisher et al. 1997). Human milk/blood partition coefficients for 19 VOCs, including *n*-hexane, were experimentally determined using samples from volunteers. These parameters were used to estimate the amount of *n*-hexane an infant would ingest from milk if the mother was occupationally exposed to *n*-hexane at the Threshold Limit Value (TLV) throughout a workday.

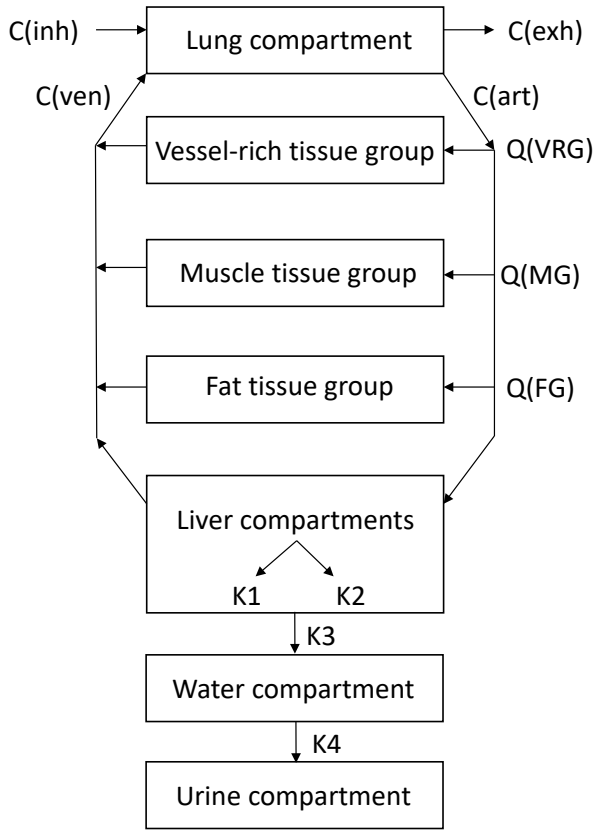
n-Hexane PBPK Model Comparison. The Perbellini et al. (1986, 1990b) model is the only validated model for this chemical identified in the literature. The Fisher et al. (1997) model was intended for risk assessment to predict which of 19 VOCs may be present in milk at a high enough level after workplace exposure to raise health concerns for a nursing infant.

The Perbellini et al. (1986, 1990b) Model

Risk Assessment. The Perbellini et al. (1986, 1990b) model successfully described alveolar air and venous blood concentration of *n*-hexane following inhalation exposure in humans. Simulations indicated that exposure to 50 ppm for an 8-hour workday, 5-day workweek would result in a gradual accumulation of *n*-hexane in body fat, which is not completely cleared during the weekend.

Description of the Model. The Perbellini et al. (1986, 1990b) model has eight compartments (see Figure 3-2) representing lung, liver, fat, muscle, a lumped compartment representing richly perfused tissues, urine, and a "water." The water compartment was included to simulate the transfer of the liver metabolite, 2,5-hexanedione, to urine.

Figure 3-2. Perbellini et al. (1986, 1990b) Physiologically Based Pharmacokinetic Model



C(ven) = concentration of *n*-hexane in venous blood; C(inh) = concentration of *n*-hexane in inhaled air; C(exh) = concentration of *n*-hexane in exhaled air; C(art) = *n*-hexane in arterial blood; FG = fat tissue group; MG = muscle tissue group; VRG = vessel-rich tissue group

Source: Perbellini et al. 1986

Absorption from the lung is assumed to be flow-limited and governed by the air concentration, a blood/air partition coefficient, and blood flow to the lung. Exchanges between blood and tissues are assumed to be flow-limited and governed by the concentration gradient between blood and tissue, the tissue/blood partition coefficient, and tissue blood flow. Metabolism of *n*-hexane was attributed to the liver and was simulated as two first-order pathways (minute^{-1}), one of which results in production of 2,5-hexanedione; the other pathway represents all other metabolism elimination processes. The metabolite, 2,5-hexanedione, is transferred to the water compartment and, from the water compartment, to urine, with both transfers assumed to be first order (minute^{-1}).

Physiological parameters for volumes and blood flow of the compartments are listed in Table 3-1. Physiologic constants (compartment volume, blood flows, etc.) were taken from published values. Values for the solubility of *n*-hexane in blood and tissues (partition coefficients) are taken from human tissue (Perbellini et al. 1985). Rate constants (Table 3-1, Figure 3-2) were estimated from animal and human data and are all assumed to be first order.

Table 3-1. Parameters Used in the Perbellini et al. (1986, 1990b) Physiologically Based Pharmacokinetic Model for *n*-Hexane

Parameters	Human
	Compartment volumes (L)
Liver	1.7
Lung	1.0
Fat	11.5
Vessel-rich compartment	7.1
Muscle compartment	36.3
	Flows (L/minute)
Alveolar ventilation	6
Cardiac output	6.3
	Percentage of cardiac output
Liver	30
Fat	4.4
Vessel-rich compartment	50
Muscle compartment	16

Table 3-1. Parameters Used in the Perbellini et al. (1986, 1990b) Physiologically Based Pharmacokinetic Model for *n*-Hexane

Parameters	Human
	Partition coefficients
Blood/air	0.8
Liver/blood	6.5
Fat/blood	130
Vessel-rich compartment	5
Muscle/blood	6.2
	Metabolic constants ($minute^{-1}$)
K_1 (catabolism of *n*-hexane to metabolites)	0.3
K_2 (synthesis of 2,5-hexanedione to *n*-hexane)	0.012
K_3 (synthesis of 2,5-hexanedione to body water)	0.009
K_4 (transfer of 2,5-hexanedione from body water to urine)	0.0009

Validation of the Model. The Perbellini et al. (1986, 1990b) model was validated using a data set for venous blood *n*-hexane values in volunteers exposed for 4 hours (Veulemans et al. 1982). The range in the study was 334–368 µg/L during exposure to 204 ppm; the model predicted values that were within 1 standard deviation of the observed means. After 4 hours of exposure to 102 ppm, the predicted value for venous blood *n*-hexane concentration was about 10% below the actual observed means. Blood *n*-hexane concentrations and air *n*-hexane concentration have shown to be strongly correlated in workers exposed to *n*-hexane (Perbellini et al. 1986). The model predicted a blood *n*-hexane level in workers exposed to 102 ppm (182 µg/L) that was similar to values predicted from the observed air-blood correlation (176 µg/L). The urinary excretion rate of 2,5-hexanedione predicted by the model was also compared to a data set from 13 workers followed for 24 hours from the beginning of a workday. The model successfully predicted the rate of 2,5-hexanedione urinary excretion.

Target Tissues. Target tissues were not specifically addressed in Perbellini et al. (1986, 1990b). The target tissue for *n*-hexane is peripheral nerve (via the neurotoxic metabolite 2,5-hexanedione).

Species Extrapolation. Species extrapolation was not addressed in Perbellini et al. (1986, 1990b). Results from *in vitro* studies in rat liver homogenates were used to estimate kinetic parameters for the catabolism of *n*-hexane and synthesis of 2,5-hexanedione.

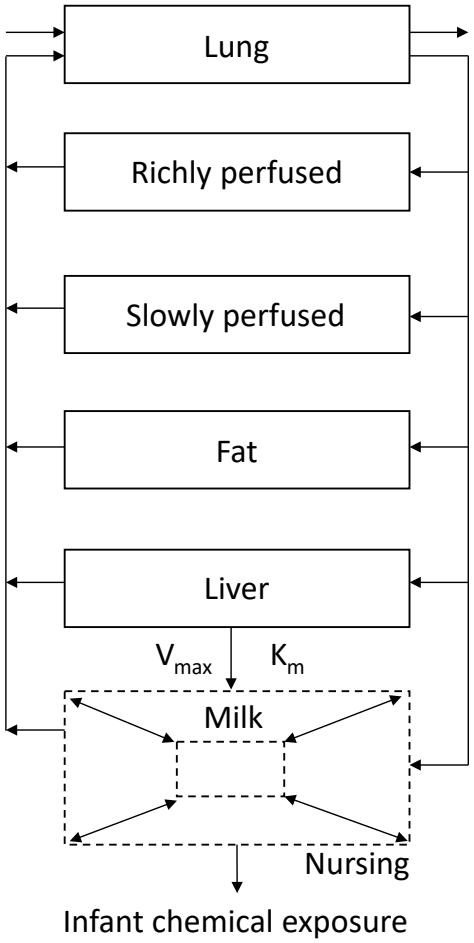
Interroute Extrapolation. Interroute extrapolation was not addressed in Perbellini et al. (1986, 1990b).

The Fisher et al. 1997 Model

Risk Assessment. The purpose of this study was risk assessment. The transfer of 19 chemicals to milk was simulated to predict those that may result in exposures to infants higher than the EPA Drinking Water Health Advisory values for chronic ingestion of contaminated water by a 10-kg child. The model predicted an ingestion rate of 0.052 mg/day for *n*-hexane, which was below this value.

Description of the Model. The Fisher model simulates the transfer of *n*-hexane from a mother to a nursing infant during and after occupational exposure via inhalation (Fisher et al. 1997). The model contains seven compartments: alveolar space, lung blood, fat, slowly perfused tissues, rapidly perfused tissues, liver, and milk (see Figure 3-3). Absorption from the lung is assumed to be flow-limited and governed by the air concentration, a blood/air partition coefficient, and blood flow to the lung. Exchanges between blood and tissues are assumed to be flow-limited and governed by the concentration gradient between blood and tissue, the tissue/blood partition coefficient, and tissue blood flow. Metabolism of *n*-hexane was attributed to the liver and was simulated as a capacity-limited process (K_M, V_{max}). Fate of the metabolites were not simulated in the model. Transfer of *n*-hexane to breast milk was simulated as a first-order process ($hour^{-1}$). Standard literature values were used for most parameters while blood/air and milk/air partition coefficients were determined experimentally from milk samples from nine volunteers (Table 3-2). The milk/blood partition coefficient was derived from the blood/air and milk/air coefficients. Maximum rates of hepatic metabolism (V_{max}) and the K_m value for *n*-hexane were taken from a study in rats. The milk compartment included changes in volume in response to nursing; milk letdown from nursing is assumed to be a first-order process and milk production a zero-order process. Minimum and maximum volumes for the milk compartment were 0.010 and 0.125 L, respectively. The amount of *n*-hexane ingested by the infant was predicted using simulations run assuming an *n*-hexane air level of 50 ppm (based on the TLV) for an 8-hour working period containing two 0.5-hour and one 1-hour break periods without exposure and eight 12-minute nursing periods over 24 hours.

Figure 3-3. Fisher et al. (1997) Physiologically Based Pharmacokinetic Model



Milk compartment volume changes due to nursing.

Source: Fisher et al. 1997

Table 3-2. Parameters Used in the Fisher et al. (1997) Physiologically Based Pharmacokinetic Model for *n*-Hexane

Parameters	Human
	% BW (BW=60 kg)
Liver	1.5
Richly perfused	10
Slowly perfused	54
Fat	25
Milk	10–125 mL
	Flows (L/minute)
Alveolar ventilation	$24xBW^{0.74}$
Cardiac output	$15xBW^{0.74}$

Table 3-2. Parameters Used in the Fisher et al. (1997) Physiologically Based Pharmacokinetic Model for *n*-Hexane

Parameters	Human
	Percentage of cardiac output
Liver	29
Fat	10
Richly perfused	35
Slowly perfused	19
Milk	7
	Partition coefficients
Blood/air	2.13
Liver/blood	2.45
Fat/blood	74.74
Richly perfused blood	2.45
Slowly perfused blood	1.36
Milk/air	4.66
Milk/blood	2.10
	Metabolic constants
V_{max}	6.0 mg/kg/hour
K_m	0.3 mg/L
	Milk compartment
Nurse[a]	20/hour
Prod[b]	0.06 L/hour

[a]Nurse is a first-order term to describe the rate of ingestion of breast milk by a nursing infant.
[b]Prod is a zero-order term to describe the rate of breast milk production at 1.3–3 months of lactation.

BW = body weight

Validation of the Model. Fisher et al. (1997) did not validate that model against observations of *n*-hexane in blood, tissues, or breast milk.

Target Tissues. Target tissues (peripheral nervous system) were not specifically addressed in Fisher et al. (1997).

Species Extrapolation. Species extrapolation was not reported in Fisher et al. (1997).

Interroute Extrapolation. Interroute extrapolation was not reported in Fisher et al. (1997).

3.1.6 Animal-to-Human Extrapolations

The rat is the major model system for human *n*-hexane neurotoxicity. Inhalation of *n*-hexane in this species produces clinical and histopathological effects similar to those seen in workers exposed to *n*-hexane. However, the toxicokinetics in rats are somewhat different than humans; for example, less 2,5-hexanedione and more 2-hexanol is produced in rats as a proportion of total urinary metabolites compared to humans (Fedtke and Bolt 1987; Frontali et al. 1981). Mice do not develop clinical signs of neurotoxicity after exposure to *n*-hexane, although histopathological changes (paranodal axonal swellings) have been observed (NTP 1991). A single study in rabbits exposed to high levels of *n*-hexane (3,000 ppm) showed no evidence of neurotoxicity in this species (Lungarella et al. 1984). Since 2,5-hexanedione is the likely causative agent of the *n*-hexane-induced peripheral neuropathy, the species differences in metabolism could result in species differences in toxicity, with humans being more sensitive than animals.

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 *n*-hexane are discussed in Section 5.7, Populations with Potentially High Exposures.

Age. Cases of *n*-hexane toxicity in humans have occurred as the result of workplace exposure and solvent misuse (Spencer et al. 1980). Some of these cases of peripheral neuropathy have occurred in teenagers (particularly with solvent misuse); however, none of the clinical reports indicate differences in physical

signs or functional tests between this group and adults (Altenkirch et al. 1977; Yamamura 1969). While no reports of *n*-hexane toxicity in young children were located, it is probable that similar toxicity would occur if exposure was comparable to that in affected adults. Specific information is not available on whether children are more susceptible than adults to the effects of *n*-hexane.

Animal studies provide limited further information. Only two studies were located where the responses to *n*-hexane were compared between young animals and adults. In a study in Fischer 344 rats directly comparing the effects of exposure to 1,000 ppm *n*-hexane 24 hours/day, 6 days/week for 11 weeks in weanlings (21 days old) and young adults (80 days old), peripheral neuropathy occurred in both groups, although onset was more rapid in the young adult group (Howd et al. 1983). For example, mild, slight ataxia was observed in 2/10 young adults and in 0/10 weanlings after 7 weeks of exposure; mild, slight ataxia was observed in 2/10 weanlings after 8 weeks of exposure. Age-related differences in severity were also observed. After 11 weeks of exposure, severe hindlimb ataxia, inability to stand, and flaccid hindlimbs were observed in all surviving young adults; in contrast, all of the weanling animals displayed mild, slight ataxia. No deaths were observed over the 11-week exposure period and 3-week recovery period in weanling rats. In young adults, however, 5 of 10 rats died as the result of severe neuropathy. The study authors suggested that the relative resistance of the weanling rats may have been due to shorter, smaller-diameter axons, or to a greater rate of growth and repair in their peripheral nerves compared to those of adults. In contrast, an oral LD_{50} study in Sprague Dawley rats showed that 14-day-old rats were more susceptible to the lethal effects of a large dose of *n*-hexane than young adults (Kimura et al. 1971). LD_{50} values for *n*-hexane were 15,840 mg/kg for 14-day-old rats and 32,340 mg/kg for the young adults. Clinical signs and time to death were not reported. Comparison of the findings in the Howd et al. (1983) neurotoxicity study and the Kimura et al. (1971) LD_{50} study is limited by differences in endpoint examined, exposure routes, and rat strains.

n-Hexane has not caused teratogenic effects in rodent models, although some developmental effects (decreased fetal weight, decreased live fetuses per litter) have been reported in rats and mice exposed during pregnancy to ≥1,000 ppm (API 1979; Bus et al. 1979; Marks et al. 1980; NIEHS 1987, 1988c). Observation of the offspring after birth to maturity was not performed. No information is available on whether parental exposure to *n*-hexane can cause transgenerational effects in children. This appears unlikely since *n*-hexane has tested negative for genotoxicity in a number of *in vivo* and *in vitro* tests. One area of potential concern is that very high air concentrations of *n*-hexane (±1,000 ppm) administered for 21–24 hours/day resulted in signs of testicular damage in rats (De Martino et al. 1987; Nylen et al. 1989). These signs are also found in rats after large oral doses (Krasavage et al. 1980) and the administration of

the *n*-hexane metabolite, 2,5-hexanedione, in drinking water (Chapin et al. 1982; Gillies et al. 1981). Severe neurotoxicity was evident in all these cases. It is not known whether or not this is a species-specific effect since examination of sperm in a worker population with exposure to *n*-hexane and elevated 2,5-hexanedione urinary levels has not been reported.

No information is available as to whether *n*-hexane or its metabolites cross the placenta in humans. Transfer across the placenta has been demonstrated in rats for *n*-hexane and two resulting metabolites, 2-hexanone and 2,5-hexanedione (Bus et al. 1979); no preferential distribution to the fetus was observed for either *n*-hexane or the metabolites. Due to its relatively rapid metabolism, storage of *n*-hexane in body fat does not appear to occur at air concentrations to which humans are exposed; thus, mobilization of maternally stored *n*-hexane upon pregnancy or during lactation is unlikely. Data to support this assumption were not located as no studies evaluating levels of *n*-hexane in amniotic fluid, meconium, cord blood, or neonatal blood were identified. There are no studies dealing with exposure or body burden measurements in children. Given the absence of such studies targeted at children, it is unknown whether children are different in their weight-adjusted intake responses to *n*-hexane.

Hexanes (C_6H_{14}) have been detected in samples of human breast milk (Pellizzari et al. 1982); however, *n*-hexane was not quantified, nor was any attempt made to assess the subjects' exposure. A human milk/blood partition coefficient of 2.10 (Fisher et al. 1997) indicates that there would be preferential distribution to this compartment if significant absorption occurred; however, no pharmacokinetic experiments have been done to confirm that *n*-hexane or its metabolites are actually transferred to mammalian breast milk following confirmed exposure to *n*-hexane. No quantitative information is available on *n*-hexane metabolites in breast milk.

No information is available on the toxicokinetics of *n*-hexane in children or in young animals compared to adult animals. No information is available as to whether metabolism of *n*-hexane in children differs from that of adults; it is noted that some cytochrome P-450 enzymes are developmentally regulated (Leeder and Kearns 1997). No studies were located comparing metabolism in young and adult animals. The toxicity of *n*-hexane results from biotransformations that yields the active metabolite, 2,5-hexanedione (see Section 3.1.3 for additional information on the metabolism of *n*-hexane).

No information is available on whether biomarkers for exposure or effect of *n*-hexane validated in adults (exhaled *n*-hexane, 2,5-hexanedione in urine) also are valid for children. Interactions of *n*-hexane with other chemicals have not been reported in children but have occurred in adults (Altenkirch et al. 1977).

Since interactions in adults are dependent on toxicokinetic parameters, predicting interactions in children requires greater understanding of the metabolism of *n*-hexane in children.

Children, like adults, have background exposures to *n*-hexane resulting from emissions from the combustion of motor fuels or heating oil or other uses of petroleum products. Some products used in the home, such as rubber cement, contain *n*-hexane and could pose exposure risks to children from inhalation. In addition to inhalation exposures through the normal use of such products in poorly ventilated interior areas, children may engage in "glue sniffing" substance-misuse behaviors that could pose serious inhalation exposure risks. Dermal exposures are also possible from hexane-containing household products. For very small children, accidental ingestion of hexane-containing materials is also a potential exposure risk. Other potential exposures are possible from hazardous waste sites. No studies in children have examined potential secondary exposure to *n*-hexane or take-home exposures from materials transferred from the parents' workplace on clothes, skin, hair, tools, or other. Such exposure risks are not expected to be a concern with *n*-hexane because it is highly volatile.

Pre-existing Conditions, Diseases, and Exposure to Other Substances. No population has been identified that is unusually susceptible to toxic effects resulting from *n*-hexane exposure. It is possible that individuals with diminished peripheral nerve function may be more susceptible to *n*-hexane neurotoxicity than the general population. This group would include diabetics, persons with alcohol use disorder, and the aged.

Genetic Polymorphisms. A case-control study examined the association between metabolic gene polymorphisms and risk of peripheral neuropathy in workers exposed to *n*-hexane (Zhang et al. 2006). The cases included 22 offset printing factory workers in China diagnosed with peripheral neuropathy, while the controls included 163 workers in the same shop but with no signs or symptoms. An association was observed between CYP2E1 Dra polymorphism and peripheral neuropathy, with 18% of cases having the CYP2E1 Dra homozygous mutation compared to 3.7% in controls. No other associations were identified.

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 2006).

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 *n*-hexane from this report are discussed in Section 5.6, General Population Exposure.

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 2006). 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 *n*-hexane are discussed in Section 3.3.1.

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 2006). 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 *n*-hexane 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

n-Hexane can be measured in exhaled breath and blood during and following exposure (Mutti et al. 1984; Raymer and Pellizzari 1996; Veulemans et al. 1982; White et al. 1979). At exposure concentrations of 100–200 ppm, *n*-hexane can be detected in exhaled air for about 12–24 hours. While this is the most direct method to identify and quantify exposure to *n*-hexane, these measurements require specialized equipment and are used mainly in research studies.

Exposure to *n*-hexane results in the production of metabolites by microsomal oxidative enzymes in the liver. In humans, the major metabolite in urine is the neurotoxic metabolite, 2,5-hexanedione. The amount of this metabolite in urine has shown a good correlation with concentrations of *n*-hexane in the workplace air (Mayan et al. 2001, 2002; Mutti et al. 1984). Urinary metabolite concentrations were lowest at the beginning of the shift, highest at the end of the shift, and still elevated the next morning. There was a strong correlation (r=0.967) between TWA *n*-hexane air concentration and end-of-shift 2,5-hexanedione in the urine; end-of-shift samples gave the best estimate of overall exposure. In this study, it was found that about 3 mg 2,5-hexanedione/g creatinine would correspond to about 50 ppm of *n*-hexane in the air.

Since *n*-hexane and its metabolites are cleared from the body within a few days, a test for 2,5-hexanedione in the urine is only a biomarker for recent exposure. Another neurotoxic solvent, 2-hexanone (methyl *n*-butyl ketone), also has 2,5-hexanedione as a metabolite; therefore, exposure to this chemical would have to be ruled out before exposure to *n*-hexane could be confirmed. 2-Hexanone is also a metabolite of *n*-hexane but is present in much smaller quantities in urine after exposure than 2,5-hexanedione (Fedtke and Bolt 1987).

2,5-Hexanedione levels in urine measure the excretion of 2,5-hexanedione (about 10% of the total) and levels of 4,5-dihydroxy-2-hexanone that are converted to 2,5-hexanedione upon acid treatment (acidification of urine samples is routinely performed in order to hydrolyze conjugates that can interfere with analysis). 2,5-Hexanedione has also been detected after acid treatment of urine from individuals not occupationally exposed to *n*-hexane (Fedtke and Bolt 1986; Perbellini et al. 1993); because the urine was acid-treated, the reported value is related to the total of 2,5-hexanedione plus 4,5-dihydroxy-2-hexanone. A reference value for 2,5-hexanedione in acid-treated urine in a non-occupationally exposed Italian population (n=123, 60 males, 63 females) has been determined (Bavazzano et al. 1998). This value, defined as the upper unilateral 95% tolerance interval at 95% confidence, was 0.795 mg 2,5-hexanedione/L in males and 0.627 mg/L for females. It is possible that small amounts of *n*-hexane are produced in the body by lipid peroxidation, as has been demonstrated for *n*-pentane (Filser et al. 1983). Urinary excretion of 2,5-hexanedione was 3–4 mg in 24 hours in workers exposed to approximately 50 ppm *n*-hexane, as compared to 0.3–1.2 mg in 24 hours in unexposed individuals (Perbellini et al. 1993).

Pyrrolidation of proteins appears to be a necessary step in *n*-hexane neurotoxicity, and the targets relevant to toxicity are thought to be neuronal axon proteins (Graham et al. 1995). However, *n*-hexane metabolites

can pyrrolidate a variety of proteins at lysine residues, which upon oxidation can become crosslinked. Pyrrolidated proteins in rat hair have been measured after intraperitoneal administration of 2,5-hexanedione (Johnson et al. 1995). Serial analysis of nose hairs taken during 2,5-hexanedione administration showed a progression with time of the region staining positively for pyrroles. Studies in rats found significant increases in serum, urine, and hair pyrrole adducts in rats exposed to *n*-hexane (Li et al. 2018, 2020b). The levels of serum and urine pyrrole adducts were dose- and exposure duration-related (Yin et al. 2014). This method may eventually be useful as a biomarker for past exposure to *n*-hexane in humans. An *in vitro* species comparison found that pyrrole formation in human serum was approximately 2 times higher than in rats (Yin et al. 2014). A more sensitive and rapid biomarker for 2,5-hexanedione exposure is the crosslinking of erythrocyte spectrin, where the altered migration of crosslinked spectrin is easily observable in polyacrylamide gels (Anthony et al. 1983). Further research is needed to determine whether exposure to *n*-hexane also results in adduct formation and/or crosslinking of spectrin via metabolism to 2,5-hexanedione.

3.3.2 Biomarkers of Effect

There are currently no subtle or sensitive biomarkers of effect associated specifically with exposure to *n*-hexane, although this is an active area of research. Electroneuromyographic testing may prove useful in the detection of nerve conduction abnormalities in their early stages before they are accompanied by clinical manifestations. In a study of 15 women who had been exposed to *n*-hexane in a shoe factory, all nerve conduction velocities (motor and sensory) were significantly slowed in exposed workers compared to controls (Mutti et al. 1982b); however, the effects of the *n*-hexane may have been exacerbated by co-exposure to MEK. None of these women had clinical signs of peripheral neuropathy. In a study of workers with relatively high urinary 2,5-hexanedione levels (indicating exposure), clinical exams were negative for neuropathy (Pastore et al. 1994). Sensory and motor nerve conduction velocities and distal latencies were normal in all nerves tested; however, significant decreases were found in sensory nerve action potential amplitude when compared with an unmatched control group. Neither the level of 2,5-hexanedione in urine nor the age of the workers correlated with the changes in amplitude; however, there was a significant correlation between years worked and decreased amplitude. In contrast, no correlation was found with the length of exposure in another study of asymptomatic workers where 14 of 40 showed abnormalities on electrophysiological testing. Levels of 2,5-hexanedione in the urine correlated with a numerical index for abnormalities (Governa et al. 1987).

Pyrrolidation and crosslinking of proteins can be considered biomarkers of either exposure or effect and are discussed in the previous section. As noted in Section 3.3.1, increases in serum, urine, and hair pyrrole adducts were observed in rats administered *n*-hexane (Li et al. 2018, 2020b); the level of pyrrole adducts, particularly in hair, correlated with a gait abnormality score. The results suggest that hair pyrrole levels may be a biomarker for *n*-hexane-induced peripheral neuropathy. A 24-week study in rats found correlations between gait score and levels of serum and urine pyrrole adducts and an inverse correlation between time to paralysis and serum and urine pyrrole levels (Yin et al. 2014).

3.4 INTERACTIONS WITH OTHER CHEMICALS

Because many other chemicals can affect the enzymes responsible for *n*-hexane metabolism, the possibility of interactions is a significant concern. The initial step in *n*-hexane metabolism is oxidation to a hexanol by a cytochrome P-450 isozyme; other chemicals can induce these enzymes, possibly increasing the rate of metabolism to the neurotoxic 2,5-hexanedione or competing with *n*-hexane and its metabolites at enzyme active sites, reducing the rate of metabolism. Interactive effects can be concentration- and/or duration-dependent.

Co-exposure to acetone, MEK, toluene, xylenes, and phenobarbital have been shown to influence the neurotoxicity of *n*-hexane.

Acetone. Evidence for an effect of co-exposure to acetone on *n*-hexane metabolism in humans has been described in a study of workers at a shoe manufacturing facility (Cardona et al. 1996). A statistically significant correlation was found between air levels of acetone and the ratios of free and total 2,5-hexanedione to air levels of *n*-hexane. Multiple regression analysis indicated that at a given level of *n*-hexane exposure, co-exposure to acetone increases the level of free 2,5-hexanedione in urine while reducing the level of 4,5-dihydroxy-2-hexanone.

Oral administration of acetone has been reported to potentiate the neurotoxicity caused by exposure to the *n*-hexane metabolite, 2,5-hexanedione, in rats (Ladefoged et al. 1989, 1994). It is possible that acetone may potentiate *n*-hexane neurotoxicity by decreasing body clearance of 2,5-hexanedione (Ladefoged and Perbellini 1986). Acetone also influences the action of many chemicals by its induction of the cytochrome P-450 isozyme, CYP2E1 (Patten et al. 1986). *n*-Hexane is metabolized by cytochrome P-450 isozymes, so induction by acetone may result in an increased production of the neurotoxic metabolite, 2,5-hexanedione.

MEK. The addition of MEK to paint thinner appears to have been the cause of an outbreak of peripheral neuropathy in Berlin in the 1970s (Altenkirch et al. 1977). The *n*-hexane proportion was reduced from 31 to 16%, but the study authors hypothesized that the addition of MEK had caused a synergistic effect to occur, resulting in *n*-hexane neurotoxicity. The potentiation of *n*-hexane neurotoxicity by co-exposure to MEK may be duration-dependent, as suggested by an experiment in volunteers (van Engelen et al. 1997). Simultaneous exposure to 60 ppm *n*-hexane and either 200 or 300 ppm MEK for 15.5 minutes had no effect on exhaled *n*-hexane concentrations, and actually lowered 2,5-hexanedione serum concentrations about 3-fold. Time to peak 2,5-hexanedione concentrations was approximately doubled (18–30 minutes). These results are consistent, with MEK inhibiting the metabolism of *n*-hexane during a single acute-duration exposure.

In experiments with male Wistar rats, co-exposure to *n*-hexane and MEK for 9 weeks resulted in an earlier onset of signs of neurotoxicity than with *n*-hexane alone (Altenkirch et al. 1982). Similarly, co-exposure to *n*-hexane and MEK over 20 weeks significantly enhanced clinical and electrophysiological signs of neurotoxicity in Wistar rats compared to *n*-hexane alone (Ichihara et al. 1998). This was accompanied by an approximate doubling in urinary 2,5-hexanedione concentrations. Co-exposure to *n*-hexane and MEK also resulted in a greater decrease in motor conduction velocity and mixed nerve conduction velocity in Wistar rats exposed for 20–22 weeks, as compared to rats only exposed to *n*-hexane (Takeuchi et al. 1983). Oral exposure to MEK prior to inhalation exposure to *n*-hexane significantly increased blood levels and sciatic nerve levels of the neurotoxic metabolite, 2,5-hexanedione, and 2,5-dimethylfuran (a metabolite of 2,5-hexanedione) in Fischer 344 rats (Robertson et al. 1989). Levels of 2,5-hexanedione in blood were approximately 10-fold higher than control immediately after *n*-hexane exposure in rats and fell rapidly to approximately 2-fold after 6 hours. In sciatic nerve, increases in 2,5-hexanedione were approximately 6-fold at 2 hours and 3-fold at 4 hours. Although some studies have identified higher levels of *n*-hexane metabolites following co-exposure to MEK (Zhao et al. 1998), other studies have found that serum 2,5-hexanedione levels are decreased with increasing co-exposure to MEK (Shibata et al. 2002; van Engelen et al. 1997; Yu et al. 2002).

The mechanism of action of MEK on the neurotoxicity of *n*-hexane remains unclear.

Toluene. Co-exposure of *n*-hexane and toluene resulted in a reduction in auditory sensitivity in rats compared to controls (Nylen et al. 1994). Exposure to *n*-hexane alone caused a marked decrease in peripheral nerve conduction velocities, while co-exposure with toluene prevented these effects. In a

similar study where both peripheral and central nervous system effects were measured in rats co-exposed to *n*-hexane and toluene (Pryor and Rebert 1992), toluene exposure prevented the peripheral neurotoxicity (decreased grip strength and nerve conduction velocities) caused by exposure to *n*-hexane alone. There was no reciprocal action of *n*-hexane on the motor syndrome (shortened and widened gait and widened landing foot splay) and hearing loss caused by toluene. Brainstem auditory response amplitudes were decreased by *n*-hexane, while co-exposure to toluene did not block this effect.

Co-exposure to approximately equal concentrations of toluene prevented *n*-hexane-induced testicular atrophy in rats (Nylen et al. 1989). The protective effects of toluene on peripheral neuropathy and testicular atrophy caused by *n*-hexane may result from competition for metabolism, resulting in a slowing of *n*-hexane conversion to 2,5-hexanedione. A study evaluating the toxicokinetics of toluene and *n*-hexane exposure found increased blood toluene concentrations and reduced 2,5-hexanedione urinary concentrations (Ali and Tardif 2006).

The interaction of *n*-hexane with toluene and trichloroethylene has also been examined in volunteers (Baelum et al. 1998). Exposure in these experiments was via a gastric feeding tube at controlled rates equivalent to what the study authors stated would be delivered to the liver by inhalation exposure at Danish occupational exposure limits (50 ppm *n*-hexane, 50 ppm toluene, and 30 ppm trichloroethylene). Co-exposure to toluene and trichloroethylene slightly increased the area under the curve (AUC) representing concentration versus time for end exhaled *n*-hexane air concentration, but urinary excretion of 2,5-hexanedione was unchanged. The only statistically significant interaction observed with *n*-hexane was an 18% decrease in the urinary excretion of hippuric acid, a toluene metabolite.

Xylene. Co-exposure to *n*-hexane and xylene resulted in a loss of auditory sensitivity in male Sprague-Dawley rats (Nylen and Hagman 1994) as measured by the auditory brainstem response. Exposure to *n*-hexane or xylene alone caused a slight loss of auditory sensitivity when measured 2 days after the end of exposure. Simultaneous exposure to *n*-hexane and xylene caused a greater and persistent loss of auditory sensitivity that was greater than the sum of effects of exposure to *n*-hexane and xylene separately. These effects were still observed 4 and 10 months after exposure ended. In contrast, combined exposure to *n*-hexane and xylene partially reversed the decreased nerve conduction velocities and action potential amplitudes observed in the group treated with *n*-hexane alone. These effects were persistent from 2 days to 10 months after cessation of exposure. And like toluene, co-exposure to xylene also prevented *n*-hexane-induced testicular atrophy in rats (Nylen et al. 1989). Rats pretreated with xylene and then exposed to *n*-hexane by inhalation exhibited a markedly increased peak serum

concentration of 2,5-hexanedione (Toftgard et al. 1983). Peak serum concentrations were approximately 4 μg/mL in control rats and 11 μg/mL in xylene-induced rats. Peaks were reached in 1–2 hours. The half-life for elimination from serum was approximately 1 hour for both pretreated and untreated rats. The high serum 2,5-hexanedione concentrations were correlated with an induction of liver microsomal cytochrome P-450 content (0.56 nmol/mg protein in control rats and 1.03 nmol/mg in xylene-induced rats).