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

3.1 TOXICOKINETICS

Toxicokinetic data on the chlorophenols discussed in this profile are available primarily from studies in animals exposed orally or by intraperitoneal injection. There are few human data on the toxicokinetics of chlorophenols. In addition, no toxicokinetic information was located for 2,5-DCP, 3,4-DCP, 3,6-DCP, 2,4,5-TCP, 2,3,4,5-TeCP, or 2,3,5,6-TeCP. Inferences that can be drawn from the available data are briefly summarized below.

- Absorption of the subject chlorophenols after oral, dermal, or inhalation exposure is rapid and virtually complete. Quantitative estimates of fractional absorption based on radioactivity in urine after oral administration of radiolabeled chlorophenols in animals range between 69 and 100%. Estimates of fractional dermal absorption in humans vary widely between 30 and 100%. No quantitative estimates of the fractional absorption of chlorophenols following inhalation were identified.

- Chlorophenols are widely distributed in the body, with the highest concentrations in the liver, kidney, and spleen. The extent of plasma protein binding, which is a major determinant of both the body burden and elimination kinetics, increases with increasing chlorination.

- Rapid metabolism to glucuronide and sulfate conjugates appears to be the predominant route of chlorophenol metabolism. The relative proportions of these conjugates may vary by species, dose, and exposure route. Metabolism of chlorophenols via cytochrome P-450 isozymes can also produce reactive quinone and semiquinone intermediates. Finally, there is evidence that 2,4,6-TCP is isomerized in rats to other trichlorophenols.

- Chlorophenols are rapidly excreted in the urine after oral, dermal, or intraperitoneal injection exposure. Half-lives in the range of hours to a few days have been estimated. Elimination rates tend to decrease with increasing chlorination, likely due to increased plasma protein binding with increased chlorination. No information pertaining to excretion after inhalation exposure was located.

- No physiologically based pharmacokinetic (PBPK) models of any of the subject chlorophenols were identified in the literature reviewed.

3.1.1 Absorption

Inhalation Exposure. Information pertaining to the absorption of inhaled chlorophenols is limited to indirect evidence. The identification of 2,4,6-TCP and 2,3,4,6-TeCP in the serum and urine of workers exposed while treating lumber indicates that 2,4,6-TCP and 2,3,4,6-TeCP are absorbed through inhalation and/or dermal routes (Pekari et al. 1991).

Oral Exposure. The animal data indicating rapid and complete absorption of chlorophenols are from studies reporting recovery of all or most of the orally administered chlorophenols in the urine. Spencer and Williams (1950) recovered 100% of a single oral dose of 2- or 4-CP (emulsified in water) given to rabbits. Approximately 69% of an oral dose of radiolabelled 2,4-DCP (in deionized water) was recovered in the urine of rats within 48 hours of exposure (Pascal-Lorber et al. 2012). Five days after three daily gavage treatments of rats with radiolabelled 2,4,6-TCP (vehicle not reported), 82.3% of the administered radioactivity was recovered in the urine (Korte et al. 1978). In a 15-day study in rats exposed to 25 µg/day radiolabelled 2,4,6-TCP, 92% of the administered radioactivity was recovered in the urine collected during exposure (Bahig et al. 1981).

Dermal Exposure. *In vivo* and *in vitro* data indicate that the chlorophenols are readily absorbed following dermal exposure. In an industrial accident, 20 minutes after a worker was splashed with a pure solution of 2,4-DCP on <10% of his body (arm and leg), he collapsed and shortly thereafter died (Kintz et al. 1992). Postmortem blood and urine concentrations of 2,4-DCP were 24.3 and 5.3 mg/L, respectively. Using a fluorescent tracer, and measures of urinary excretion of TeCP in lumber mill workers exposed to a wood preservative (20% TeCP, 3% pentachlorophenol, <0.4% other CPs), Fenske et al. (1987) estimated that 30–100% of the 2,3,4,6-TeCP deposited on the skin is absorbed. Absorption occurred through the hands and forearms despite the use of chemical-resistant gloves. Fenske et al. (1987) also indicated that the skin regions with greatest exposure, the hands and forearms, were in frequent contact with wood so that abrasion may have reduced the barrier properties of the stratum corneum.

Dermal absorption can be inferred from *in vivo* animal studies resulting in death and/or adverse systemic effects following dermal exposure to 2-CP (Monsanto 1975) and 2,4-DCP (Carreon et al. 1980a, 1980b; Hencke and Lockwood 1978; Monsanto 1976).

The results of diffusion experiments using hydrated human cadaver epidermis also indicate that the chlorophenols readily cross the skin at low concentrations. The permeability coefficients determined in excised human abdominal epidermis were 5.5, 6.1, 10.0, and 9.9 cm/minute x $1x10^{4}$, respectively, for 2-CP, 4-CP, 2,4-DCP, and 2,4,6-TCP (Roberts et al. 1977). Xiao et al. (2012) reported an *in vitro* permeability rate of 0.021 cm/hour for 2,4-DCP in an experiment with fresh human skin. 2-CP and 4-CP were reported to damage the skin, determined by an increase in the permeability coefficient at aqueous concentrations of 0.8 and 0.75% (w/v), respectively, while no damage was observed with 2,4-DCP and 2,4,6-TCP at concentrations up to saturation. In a study using abdominal skin exposed to air, absorption

of 2,3,4,6-TeCP over 24 hours was 33% from an aqueous medium (1.54% 2,3,4,6-TeCP) and 63% from a diesel-oil-based medium (0.96 2,3,4,6-TeCP) (Horstman et al. 1989). These values were determined by assuming that the amount of the applied dose that was not recovered from the skin's surface was the amount absorbed. The actual amounts recovered in the skin and receiving solutions were 9.5 and 3.9% for the aqueous- and oil-based medium, respectively. The authors attribute low recovery to difficulties in extracting 2,3,4,6-TeCP from the skin.

Chlorophenols are also readily permeable in rodent skin *in vitro* preparations. At solution pHs between 5.0 and 5.74, the apparent permeability constants for 2-CP, 2,4-DCP, and 2,4,6-TCP in a hairless mouse skin preparation over a concentration range of 0.05–0.5% varied from 0.14 to 0.36 cm/hour in whole skin and from 0.136 to 0.276 cm/hour in skin stripped of the stratum corneum (Huq et al. 1986). The investigators proposed that permeability is probably greater in the more highly vascularized human tissue because the extensive network of surface capillaries in humans reduces the thickness of the diffusional barrier. In another *in vitro* diffusion study of 4-CP, 87.4–90.5% of the applied dose crossed rat epidermal preparations in 72 hours, indicating extensive absorption (Hughes et al. 1993). Those phenols (both chlorophenols and other substituted phenols) with log K_{ow}, values between 1.4 and 3.5 showed the greatest amount of permeability through the dermal membrane. Although specific data were not identified, dermal absorption of chlorophenols should also be greater for the neutral acid form than for the phenolate anion as ions do not readily cross cell membranes.

An experiment with rabbits showed that 2,4,6-TCP is absorbed through the cornea to a minor degree following ocular application (Ismail et al. 1977).

3.1.2 Distribution

Distribution in Blood. The concentration of 2,4-DCP in blood was 24.3 mg/L in a worker who collapsed and died shortly after being splashed with pure 2,4-DCP on his right arm and leg (Kintz et al. 1992). Peak concentrations of 2,4,6-TCP were observed in blood 30 minutes after rats were given a single intraperitoneal injection of 25 mg/kg 2,4,6-TCP (Pekari et al. 1986).

The results of in vitro binding studies using human serum proteins indicate that both 2,4-DCP and 2,4,6-TCP strongly bind to serum proteins, including albumin and globulin (Judis 1982). The percentage of compound bound to albumin was slightly greater for 2,4,6-TCP (94.1%) than for 2,4-DCP (87.7%).

Distribution to Extravascular Tissues. Liver 2-CP concentrations were 2.2, 3.2, and 0.8 ppm, and kidney 2-CP concentrations were 2.6, 2.4, and 2.2 ppm in female rats exposed to 2-CP in the drinking water for 16 weeks at 5, 50, and 500 ppm, respectively (Exon and Koller 1982). The investigators did not provide an explanation for the low value (0.8 ppm) found in the livers of rats receiving the high dose, and did not indicate whether these values were wet or dry weight concentrations. Radioactivity was not recovered in the liver, lung, or subcutaneous fat of rats after three daily gavage doses of radiolabelled 2,4,6-TCP (Korte et al. 1978) or in unspecified tissues of rats at the end of 15 days of exposure to radiolabelled 2,4,6-TCP by gavage (Bahig et al. 1981).

The highest concentrations of 2,3,4,6-TeCP were found in the spleen, followed by the kidneys and liver, 24 hours after a single oral dose was given to rats (Hattula et al. 1981). In a 55-day study in which rats were treated by gavage with 2,3,4,6-TeCP at 10, 50, or 100 mg/kg/day, tissue levels, measured 24 hours after the last dose, increased with dose. For all doses, the concentrations of 2,3,4,6-TeCP in the brain and muscle were lower than those found in the kidney, liver, and spleen. At the 100 mg/kg/day dose, the kidney had the highest 2,3,4,6-TeCP concentrations (5.1 ppm) followed by the spleen (3.2 ppm), liver (2.2 ppm), brain (1.2 ppm), and muscle (0.46 ppm) (Hattula et al. 1981). At the 10 mg/kg/day dose, 2,3,4,6-TeCP was not detected in the brain or muscle (detection limit not stated), while low levels were found in the spleen (0.04 ppm), kidney (0.03 ppm), and liver (0.01 ppm).

Intravenously-administered 2,4-DCP rapidly distributed to the kidney, liver, fat, and brain in rats, with the highest concentrations in the kidney and liver (Somani and Khalique 1982). Similarly, in rats given a single intraperitoneal injection of 25 mg/kg 2,4,6-TCP, the kidneys exhibited the highest concentration (329±117 nmol/g tissue), followed by blood, liver, fat, muscle, and brain (Pekari et al. 1986). Concentrations of 2,4,6-TCP in the tissues peaked 30 minutes after exposure.

In rabbits, following ocular exposure, radiolabelled 2,4,6-TCP was distributed to various compartments of the eye (Ismail et al. 1977). At 30 minutes post exposure, the applied radioactivity was detected in the cornea (4%), aqueous humor (0.37%), lens (0.037%), iris (0.18%), choroid (0.04%), vitreous (0.01%), conjunctiva (2.14%), limbus (0.96%), and sclera (0.35%).

3.1.3 Metabolism

Both human and animal studies indicate that sulfation and glucuronidation are the main metabolic pathways of chlorophenols. Gulcan et al. (2008) showed that all of the subject chlorophenols were

substrates for human hydroxysteroid sulfotransferase 2A1 (hSULT2A1) when expressed in E. coli and tested *in vitro*. The highest rates of sulfation were observed with the tri- and tetrachlorophenols, with lower rates for mono- and dichlorophenols, indicating that hSULT2A1 likely contributes little to the sulfation of the mono- and dichlorophenols.

Monochlorophenols. A number of rabbit studies (Azouz et al. 1953; Bray et al. 1952a, 1952b; Spencer and Williams 1950) have shown that metabolism of the monochlorophenols occurs principally via conjugation. In the study by Spencer and Williams (1950), groups of six rabbits were treated by gavage with 171.3 mg/kg of 2-CP or 4-CP emulsified in water as a single dose. For both isomers, the 24-hour urine analysis indicated that between 78.1 and 88.3% of the administered dose was excreted as the glucuronide, and between 12.8 and 20.6% of the administered dose was excreted as the sulfate. A total of 101.7 and 101.1% of the administered 2-CP or 4-CP doses, respectively, was accounted for as urinary glucuronide and sulfate conjugates. Metabolism was further investigated in four rabbits, each treated by gavage with an average dose of 395 mg/kg/day of 4-CP. After 36 hours, 54.1% of the administered dose appeared in the urine as the glucuronide conjugate, and 10.4% of the administered dose appeared in the sulfate fraction. Only 0.1% of the administered dose was excreted as 4-chlorocatechol. The low total recovery (64.5%) in the latter experiment limits conclusions. Other rabbit studies indicated that chlorocatechols constituted only 1.5–4.5% of the administered doses of 300 mg/kg 2-CP or 500 mg/kg 4-CP (Azouz et al. 1953).

In a limited study in dogs (Coombs and Hele 1926), about half of an oral dose of 2- or 4-CP was excreted in the urine as the sulfate. No evidence for metabolism to mercapturic acid was found. In contrast to the study in dogs, Phornchirasilp et al. (1989a) proposed that 4-CP could be metabolized in mice by cytochrome P-450 enzymes to intermediates that react with glutathione to form glutathionyl adducts, based on the observation that 4-CP treatment of mice depleted liver thiol stores. The depletion of liver thiol stores was prevented by a P-450 inhibitor (SKP 525-A), suggesting that P-450 activity was required for this effect.

Dichlorophenols. A study in rats found that glucuronides and other unspecified conjugates were formed following a single intravenous dose of 2,4-DCP (10 mg/kg) (Somani and Khalique 1982). Although other unspecified conjugates were found in the fat, glucuronide conjugates were not found in the fat at any time interval.

Two minor metabolites of 2,4-DCP, both dichloromethoxy phenols, have been identified in studies using isolated perfused rat livers (Somani et al. 1984). In microsomal fractions and whole cells of yeast *S. cerevisiae* expressing human cytochrome P-450 3A4, 2,4-DCP has been shown to be metabolized to two major metabolites identified as 2-chloro-1,4-hydroxyquinone and 2-chloro-1,4-benzoquinone (Mehmood et al. 1997). Another metabolite, 1,2,4-hydroxybenzene, was also detected during biotransformation by whole cells, but was not observed in microsomal fractions. Thus, human CYP3A4 can remove either or both chlorine atoms from the aromatic ring of a 2,4-DCP molecule, forming 2-chloro-1,4-hydroxyquinone and 1,2,4-hydroxybenzene, respectively. 2-Chloro-1,4-hydroxyquinone was probably acted on by dehydrogenase from yeast microsomes, forming 2-chloro-1,4-benzoquinone (Mehmood et al. 1997).

Trichlorophenols. Among sawmill workers exposed to tri-, tetra-, and penta-chlorophenols, virtually all the absorbed chlorophenols were excreted in the urine as conjugated metabolites, predominantly sulfate conjugates (Pekari et al. 1991). In rats, 2,4,6-TCP undergoes biotic isomerization to other trichlorophenol isomers and conjugation with glucuronic acid (Bahig et al. 1981). Male rats eliminated 63% of a gavage dose of 2,4,6-TCP in the urine as four trichlorophenol isomers, and 28% as conjugates. Three of the trichlorophenol isomers were identified as 2,4,6-TCP (parent compound), 2,3,6-TCP, and 2,4,5-TCP; the fourth isomer was not identified. Glucuronic acid accounted for approximately 80% of the conjugates detected in urine (Bahig et al. 1981). A majority (70%) of intraperitoneally administered 2,4,6-TCP detected in the blood of rats was in conjugated form (not further identified) 30 minutes after dosing. The authors speculated that the chemical was conjugated with glucuronic acid (Pekari et al. 1986). The average percentage of the metabolites of 2,4,6-TCP conjugated in the blood over the course of the study was 83±11%. Metabolism of 2,4,6-TCP by the skin was not detected in a study of hairless mouse skin tested *in vitro* (Huq et al. 1986).

In vitro studies using rat liver microsomes have shown that 2,4,5-TCP can be metabolized to 3,4,6-trichlorocatechol, 2,5-dichlorohydroquinone, and a dihydroxydichlorobenzene (not further characterized) (Butte et al. 1988; Juhl et al. 1991). Metabolites were also dimerized to a dihydroxy-hexachlorobiphenyl, a dihydroxypentachlorodiphenyl ether, two hydroxypentachlorodiphenyl ethers, a hydroxyhexachlorodiphenyl ether, and a hydroxyhexachlorodioxin or hydroxyhexachlorodiphenoquinone (Butte et al. 1988). Metabolites generated following incubation of 2,4,6-TCP with rat liver S-9 fraction were 2,6-dichloro-1,4-hydroquinone and two isomers of hydroxypentachlorodiphenyl ether (Juhl et al. 1989). The 2,6-dichloro-1,4-semiquinone free radical was also identified. Although *in vivo*, the latter

metabolite may be minor, reactive oxygen species produced during formation of the semiquinone was judged to be responsible for DNA damage in *in vitro* testing (Juhl et al. 1989).

Tetrachlorophenols. As noted earlier, virtually all of the absorbed tri- and tetrachlorophenols were excreted as conjugated metabolites (predominantly sulfate conjugates) in the urine of sawmill workers (Pekari et al. 1991). In rats exposed to TeCP isomers via intraperitoneal injection, much of the dose is excreted in the urine unchanged (Ahlborg and Larsson 1978). Following treatment with 2,3,4,5- and 2,3,4,6-TeCP, a trichlorohydroquinone was identified in the urine as a minor metabolite. Following treatment with 2,3,5,6-TeCP, about 35% of the recovered dose (total recovery 98.7%) was tetrachloro-*p*-hydroquinone, while the remaining was unchanged parent compound (Ahlborg and Larsson 1978).

3.1.4 Excretion

Routes of Excretion. Excretion of chlorophenols occurs primarily via urinary elimination of conjugated forms (glucuronide and sulfate) in both humans and animals. After occupational exposure by combined dermal and inhalation routes to a chlorophenol dipping solution, maximal urinary concentrations ranged from 1–11.8, 3.4–17.3, and 0.2–0.9 µmol/L for tri-, tetra-, and pentachlorophenol, respectively (Pekari et al. 1991).

Limited data indicate that orally-administered monochlorophenols are rapidly excreted in the urine, primarily as glucuronide and sulfate conjugates, in rats, rabbits, and dogs (Bray et al. 1952a, 1952b; Coombs and Hele 1926; Spencer and Williams 1950). Most of the administered dose is excreted in the urine within 24 hours.

Male rats administered radiolabelled 2,4,6-TCP by gavage for 3 days and observed for 5 days after dosing eliminated a total of 82.3% of the total dose in the urine and 22.2% in the feces (Korte et al. 1978). In a second study using male rats, radiolabelled 2,4,6-TCP was administered by gavage for 15 days, with sacrifice 3 days after administration ended. A total of 92.5% of the administered dose was excreted in the urine, and 6.4% was excreted unchanged in the feces (Bahig et al. 1981). In rats administered 2,4,6-TCP by intraperitoneal injection, approximately 90% of the administered dose was eliminated in the urine within 4–6 hours (Pekari et al. 1986).

Ahlborg and Larsson (1978) studied the urinary excretion of TeCP isomers in rats following intraperitoneal injection of a single dose. During the 72 hours after dosing, about 60% of the

2,3,4,5-TeCP dose was recovered in the urine. In contrast, following treatment with 2,3,4,6-TeCP, 95.9% of the dose was excreted in the urine within 48 hours, and 98.7% of the administered 2,3,5,6-TeCP was excreted in the urine within 24 hours after dosing. The investigators (Ahlborg and Larsson 1978) did not provide an explanation regarding the slower excretion of 2,3,4,5-TeCP compared to the excretion of 2,3,4,6-TeCP and 2,3,5,6-TeCP.

Limited information suggests that 2,4-DCP may be excreted in bile. 2,4-DCP was measured at concentrations of 5.3, 18.7, and 1.2 mg/L, respectively, in the urine, bile, and stomach contents of a worker who collapsed (within 20 minutes) and died shortly after being splashed with pure 2,4-DCP on his right arm and leg (Kintz et al. 1992).

Rates of Elimination. Little data on rates of chlorophenol elimination in humans were available. After occupational exposure by combined dermal and inhalation routes to a chlorophenol dipping solution, elimination half-lives were 18 hours, 4.2 days, and 16 days for tri-, tetra-, and pentachlorophenol, respectively. The renal clearance rate of 2,3,4,6-TeCP was approximately 5 times faster than the clearance rate of pentachlorophenol, reflecting the increased plasma protein binding of the higher chlorinated compound (Pekari et al. 1991). The clearance rate of 2,4,6-TCP could not be calculated because of highly variable serum concentrations (Pekari et al. 1991).

Studies in rats, rabbits, and dogs (Bray et al. 1952a, 1952b; Coombs and Hele 1926; Spencer and Williams 1950) demonstrate rapid elimination of monochlorophenols after oral exposure; in these studies, most of the administered dose was excreted in the urine within 24 hours. At oral doses of 150–450 mg/kg in rabbits, excretion of the glucuronide conjugate of 4-CP followed first-order kinetics (Bray et al. 1952a). The rate of glucuronide excretion relative to remaining body burden was 0.41/hour.

A study in rats showed rapid clearance from the kidney, liver, fat, brain, and plasma of both the parent compound and metabolites after intravenous administration of 10 mg/kg/day 2,4-DCP in an aqueous solution (Somani and Khalique 1982). Half-lives for 2,4-DCP and its conjugates ranged from 4 to 30 minutes in these tissues, with the highest values in kidney, followed by the liver, fat, plasma, and brain (Somani and Khalique 1982). The elimination half-time for plasma was approximately 10 minutes. No detectable amounts were found in the brain at 60 minutes.

In male rats administered radiolabeled 2,4,6-TCP by gavage for 15 days, the excretion of radioactivity declined rapidly after dosing ended; by the third day postexposure, only 4.3% of the radioactivity in a

daily dose was detected in the urine, and 1.9% in the feces (Bahig et al. 1981). When rats were exposed to 2,4,6-TCP by intraperitoneal injection, about 90% of the administered dose had been eliminated via the urine within 4–6 hours of exposure, and only trace amounts of trichlorophenol were detected in tissues 10 hours after dosing (Pekari et al. 1986). The authors estimated the biological half-life of conjugated 2,4,6-TCP (the predominant form found in blood) as 1.4 hours in blood and from 1.4 to 1.8 hours in other tissues (Pekari et al. 1986).

Ahlborg and Larsson (1978) observed slower excretion of 2,3,4,5-TeCP compared to 2,3,4,6-TeCP and 2,3,5,6-TeCP in rats following intraperitoneal injection of a single dose; only 51% of the dose of 2,3,4,5-TeCP was excreted in the urine within 24 hours, while ≥93.7% of the doses of other isomers was excreted in that same time period.

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.

No PBPK models for chlorophenols were identified.

3.1.6 Animal-to-Human Extrapolations

Studies of health effects in humans exposed to chlorophenols are limited by coexposures to other compounds; thus, there are few data to inform a comparison between humans and animals. Extrapolating animal toxicity data to predict human risk from chlorophenol exposure appears to be reasonable based on similarities in metabolic pathways. It is possible that humans may be more sensitive than animals to the toxic effects of 2,4-dichlorophenol, based on the human deaths following dermal and/or inhalation exposures.

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

Susceptibility of Infants and Children. No direct information is available regarding the health effects of chlorophenols observed in children. However, health effects observed in adults are also expected to be of potential concern in children. The available studies of developmental effects in animals exposed to chlorophenols examined limited endpoints, but have generally shown effects only at doses inducing maternal toxicity (Chernoff et al. 1990; Exon and Koller 1982, 1983a, 1983b, 1985; Exon et al. 1984; Hood et al. 1979; Rodwell et al. 1989). The one exception is a study in which maternal exposure of rats to 500 mg/kg/day 2,4,6-TCP produced a transient reduction in the body weight of offspring (Blackburn et al. 1986).

However, one study (Hasegawa et al. 2005) clearly showed that neonatal rats exposed from PNDs 4 to 21 were more susceptible to the toxic effects of 2- and 4-CP than young (5–6 weeks old) rats exposed for 28 days. In this study, a dose of 500 mg/kg/day 4-CP was lethal to nearly all (7/8) neonatal rats, while all 24 young rats survived 4 weeks at this dose. In experiments with 2-CP, tremors were seen in neonatal rats exposed to 300 mg/kg/day, while young rats did not exhibit tremors at 500 mg/kg/day; tremors were seen in young rats at 1,000 mg/kg/day (Hasegawa et al. 2005).

Maternal exposure to chlorophenols prior to pregnancy is unlikely to lead to exposure of the fetus or a nursing neonate due to the relatively rapid metabolism and excretion of chlorophenols (Keith et al. 1980) and evidence for limited to no accumulation in animals after oral exposure (Bahig et al. 1981; Korte et al. 1978). More lipophilic chlorophenols may accumulate in the body; 2,3,4,6-TeCP was detected in adipose tissues from Finnish people not occupationally exposed to chlorophenols (Mussalo-Rauhamaa et al. 1989). Chlorophenols and/or their metabolites might cross the placenta, based on evidence for embryo- and/or fetotoxicity (decreased litter sizes or increased stillborn pups) in rats exposed to 2-CP, 2,4-DCP, or 2,4,6-TCP (Exon and Koller 1982, 1983a, 1983b, 1985; Exon et al. 1984), but these could be indirect effects on the fetus.

Metabolism of chlorophenols has not been studied in infants or children. However, sulfation and glucuronidation are the main metabolic pathways for chlorophenols in both human and animal studies. The conjugated metabolites are then eliminated in urine. In humans, activity of some hepatic UDP-glucuronosyltransferase (responsible for glucuronide conjugates) isoforms does not reach adult levels until adolescence, although others reach adult levels within a month (Badée et al. 2019). Activity of sulfotransferases (responsible for sulfate conjugates) seems to develop earlier, although again, it is isoform-specific (Coughtrie 2015). The activity of some human hepatic sulfotransferase isoforms may be greater than that of adults during infancy and early childhood (Ladumor et al. 2019). It is possible that chlorophenols might be eliminated at a slower rate in infants or children, resulting in increased susceptibility of children to their toxicity.

Potential Susceptibility of Other Subpopulations. No specific population with particular susceptibility to chlorophenol intoxication has been identified; however, toxicokinetic and target organ information suggest some possibilities. For example, Huq et al. (1986) suggested that 2,4,6-TCP absorbed through the skin could be more toxic than a similar ingested dose because the ingested compound is partially converted to glucuronide conjugates; thus, persons with dermal exposure could be more susceptible to toxicity than those with oral exposure. Because of the extensive hepatic conjugation and renal clearance of these compounds, individuals with liver or kidney dysfunction may be more sensitive than healthy persons. In particular, individuals with Gilbert's disease or Crigler-Najjar syndrome, inherited deficiencies of bilirubin UDP-glucuronyl transferase, may have increased sensitivity due to their impaired ability to conjugate chlorophenols (de Morais and Wells 1988; de Morais et al. 1992). Finally, evidence from rat studies (Exon and Koller 1985; Exon et al. 1984) suggests that the cell-mediated and humoral immune systems are sensitive to 2,4-DCP. Thus, persons with immune system deficiencies may be more susceptible to the adverse effects of 2,4-DCP exposure.

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 chlorophenols 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 chlorophenols 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 chlorophenols 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

No specific, reliable biomarkers of chlorophenol exposure have been identified. Urinary concentrations of the parent compounds and dechlorinated derivatives have been used as biomarkers of chlorophenol exposure; however, these extracts are not unique to chlorophenol exposure. For example, conjugated forms of higher chlorophenols have been observed after laboratory administration of hexachlorocyclohexanes (Engst et al. 1976; Koransky et al. 1975), indicating that urinary chlorophenol levels are not specific to chlorophenol exposure. Similarly, the presence of chlorophenols or their metabolites in urine is not necessarily diagnostic for chlorophenol exposure because these compounds are also detectable in urine after exposure to certain other compounds, such as lindane (Karapally et al. 1973), VC-13 (Shafik et al. 1973), 2,4-D, and 2,4,5-T (Hill et al. 1989). Importantly, 2,5-DCP in urine is considered to be a reliable biomarker for exposure to *p*-dichlorobenzene (Yoshida et al. 2002) rather than a marker for exposure to 2,5-DCP. Finally, metabolic dechlorination of higher chlorophenols to lower chlorophenols occurs under some conditions (Renner and Mucke 1986). Consequently, urinary chlorophenol concentrations cannot be considered specific, reliable measures of potential exposure in the absence of measured concentrations in exposure media (air, water, soil).

3.3.2 Biomarkers of Effect

Specific biomarkers of effect induced by chlorophenols have not been identified.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Only two studies of the interaction of chlorophenols with other chemical substances, or among different chlorophenols, were located. Using an *in vitro* rat liver microsomal preparation, Arrhenius et al. (1977) noted that 2,4-DCP, 2,4,6-TCP, and 2,3,4,6-TeCP in the concentration range of 0.03–3 mM shifted the metabolism of aromatic amines from C-oxygenation to N-oxygenation. The carcinogenic metabolites of aromatic amines can be formed by N-oxygenation. Therefore, Arrhenius et al. (1977) suggested that the chlorophenols could act synergistically to enhance the carcinogenicity of aromatic amines.

Liu et al. (2020b) measured the influence of lead co-exposure on the cytotoxicity of disinfection byproducts including 4-CP, 2,6-DCP, and 2,4,6-TCP in human epithelial colorectal adenocarcinoma (Caco-2) and neuroblastoma (SH-SY5Y) cells *in vitro*. In SH-SY5Y cells, coexposure to each chlorophenol with lead chloride resulted in a statistically significant reduction in the median lethal

concentrations (LC_{50}). In Caco-2 cells, synergistic results were seen with 2,6-DCP and 2,4,6-TCP, but lead chloride exposure did not affect the LC_{50} for 4-CP (Liu et al. 2020b).

Because Phase II conjugation is involved in the detoxification of chlorophenols, it is plausible that compounds capable of inhibiting sulfation or glucuronidation reactions could potentiate the toxicity of chlorophenols, while compounds that stimulate these reactions could mitigate toxicity. Similarly, compounds that induce effects on identified target organs of chlorophenols (e.g., liver, central nervous system, reproductive system, and immune system) or exert effects through a similar mechanism may interact with chlorophenols. For example, several chlorophenols have been shown to uncouple oxidative phosphorylation; thus, exposure to chlorophenols with other compounds that operate via this mechanism (e.g., dinitrophenol) may result in additive or synergistic effects.