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

3.1 TOXICOKINETICS

- 2,4-D is readily absorbed from the gastrointestinal tract and is expected to be readily absorbed from the respiratory tract; however, dermal absorption is relatively low.
- 2,4-D distributes widely in tissues following oral exposure, but does not accumulate in tissues.
- 2,4-D is subject to limited metabolism.
- 2,4-D is relatively rapidly eliminated in the urine.
- The toxicokinetics of 2,4-D is species- and sex-dependent largely due to differences in renal clearance of 2,4-D. The differential capacity for excreting 2,4-D plays an important role in the susceptibility to 2,4-D-induced effects between species.

3.1.1 Absorption

No studies were located regarding absorption of 2,4-D following inhalation exposure.

Evidence of gastrointestinal absorption of 2,4-D in humans comes from analysis of 2,4-D in tissues and fluids from cases of intentional or accidental ingestion of commercial products containing 2,4-D that resulted in death and from studies with volunteers. Quantitative data are available from the latter studies.

Results from studies in volunteers have shown that oral absorption of 2,4-D in humans is rapid and virtually complete. For example, oral administration of a single dose of 5 mg/kg 2,4-D in a gelatin capsule to six male volunteers resulted in a significant amount of the compound in plasma 1 hour after dosing and in a maximum of approximately 30 µg/mL 7–24 hours after dosing (Kohli et al. 1974). Assuming first rates of absorption and clearance, the investigators estimated a plasma half-life of 33 hours. A similar study in which five male volunteers were administered 5 mg/kg analytical-grade 2,4-D reported that plasma levels achieved a maximum of 10–30 µg/g approximately 6 hours after dosing (Sauerhoff et al. 1977). Elimination from plasma appeared to follow a one-compartment model for two subjects and a one- or two-compartment model for the third subject. Two subjects were not modeled. The volumes of distribution for the former were 238 and 294 mL/kg, and 83 and 218 mL/kg for the third subject if a two-compartment model was assumed; these data suggested relatively limited distribution to

tissues. The pooled half-life value for clearance of 2,4-D from plasma was 11.6 hours. Based on recovery data, it was estimated that absorption ranged from 87.6 to 106.3% of the administered dose.

Oral absorption in animals is fast and complete, particularly at relatively low doses (≤50 mg/kg), as assessed by early detection of 2,4-D in tissues and almost complete recovery of the dose in urine (i.e., Khanna and Fang 1966).

Dermal absorption of 2,4-D in humans is low compared to oral absorption. Male volunteers who received a topical application of 4 μg/cm^2 of 2,4-D in acetone on the ventral forearm excreted only 5.8% of the applied dose in the urine over a 5-day monitoring period (Feldmann and Maibach 1974). The application site was not protected and the subjects were asked not to wash the site for 24 hours. These results are consistent with those from a similar study in male volunteers who reported that 4.5% of an applied dose of 10 mg 2,4-D in acetone/water over a 9 cm^2 area on the dorsum of the hand was absorbed over a 144-hour period (Harris and Solomon 1992). Using data from Feldmann and Maibach (1974) in an exponential saturation model with lag time, Thongsinthusak et al. (1999) estimated dermal absorption of 2,4-D in humans to be 21.2–21.7% of the applied dose. In a review of the literature, however, it was noted that because the results of Harris and Solomon (1992) indicated that excretion of 2,4-D was essentially complete by 144 hours, using models much beyond 120 hours will overpredict absorption (Ross et al. 2005), so the results of Thongsinthusak et al. (1999) are not reliable.

Based on recovery of 2,4-D in the urine, a comparative study showed that rabbits absorbed a higher percentage (36% of the dose) of the applied dose than monkeys and that absorption rate can vary with the application site (Moody et al. 1990). Monkeys absorbed almost twice the amount of 2,4-D when the compound was applied on the forehead (29% of the dose) than when applied on the forearm (15% of the dose). Another study in monkeys reported an absorption rate of 8.6% of the dose when 2,4-D in acetone was applied on the abdomen of the animals (Wester et al. 1996). Application of 2,4-D in soil onto a 12-cm^2 area of abdominal skin lightly clipped resulted in absorption of 9.8% of the dose when the soil load was 1 mg/cm^2 and 15.9% when the soil load was 40 mg/cm^2. Because the dose of 2,4-D applied was the same with both soil loads, the results showed that, under the conditions of the study, dermal absorption from soil was not significantly affected by soil load (Wester et al. 1996). However, a study with human skin *in vitro* in which the concentration of 2,4-D in soil was 5 ppm (5 mg 2,4-D/kg soil) reported that dermal absorption of 2,4-D was dependent on both soil load and the type of soil (Duff and Kissel 1996).

In a comparative study in which rats and guinea pigs were applied ^{14}C-2,4-D onto the skin, rats and guinea pigs absorbed a total of 49 and 40%, respectively, of the applied dose over a 14-day monitoring period (Moody et al. 1994). The value estimated for the rat in this *in vivo* study was consistent with a 40% absorption estimated in a dermatomed skin preparation *in vitro*, but not so for the guinea pig in which only 14% of a 2,4-D dose was absorbed through a skin preparation *in vitro*. For comparison purposes, 19% of a dose of 2,4-D in acetone was absorbed through human skin *in vitro* and 14% through pig skin *in vitro* (Moody et al. 1994). Approximately 2% of 2,4-D in soil was absorbed through human skin *in vitro* (Wester et al. 1996). However, when using acetone as a vehicle, 19% of an applied dose of 2,4-D was absorbed (Moody et al. 1994).

In mice, approximately 7% of a dose of 1 mg/kg of ^{14}C-2,4-D in acetone penetrated the body (disappeared from the covered site of application) in 1 hour and about 21% in 24 hours (Grissom et al. 1985).

A series of studies by Brand and coworkers (Brand et al. 2002, 2003, 2004, 2007a, 2007b) examined factors that can influence the dermal absorption of 2,4-D in animal models. Using hairless mice skin *in vitro*, the investigators reported that six out of nine commercially available sunscreens significantly increased the total penetration of 2,4-D through the skin over a 24-hour period (Brand et al. 2002). Total penetration of 2,4-D ranged from 39.1% for no sunscreen used to 81.0% for the sunscreen that facilitated penetration the most. Subsequent studies showed that ultraviolet (UV) absorbers in sunscreens significantly enhanced the transdermal absorption of 2,4-D (Brand et al. 2003; Pont et al. 2004). The investigators also showed that dietary exposure of rats to ethanol for 6–8 weeks resulted in increased penetration of 2,4-D through the rat skin in an *in vitro* diffusion system, most likely due to altering the properties of the dermal barrier, possibly by inducing changes in lipid peroxidation and increasing transepidermal water loss (Brand et al. 2004, 2007a). Results from an additional study showed that the combination of sunscreen use and ethanol ingestion enhanced penetration of 2,4-D in rats' skin in an additive manner (Brand et al. 2007b).

Analysis of plasma from rats following an intravenous injection of 5 mg/kg 2,4-D showed a significantly smaller volume of distribution in females (50.2 mL) than in males (80.6 mL), consistent with significantly higher plasma concentration of 2,4-D (Griffin et al. 1997a). In addition, clearance (mL/minute) was about 10-fold lower in females than in males, whereas elimination half-lives from plasma were significantly higher in females.

3.1.2 Distribution

No information was located regarding distribution of 2,4-D following inhalation exposure of humans or animals.

Distribution data for 2,4-D following oral ingestion by humans are available in case reports that resulted in death; the results showed wide distribution in tissues. For example, reports by Dudley and Thapar (1972), Nielsen et al. (1965), Osterloh et al. (1983), and Keller et al. (1994) showed measurable amounts of 2,4-D in all organs that were examined, including the brain, liver, kidney, spleen, muscle, body fat, pancreas, heart, and lungs.

Studies in animals have shown that 2,4-D is widely distributed in tissues after oral dosing. In a study in rats, some 2,4-D-derived radioactivity was detected in all 12 tissues examined as early as 1 hour after gavage dosing (Khanna and Fang 1966). Rats were given approximately 3 or 240 mg/kg 2,4-D. With the low dose, peak concentration in tissues was achieved 6–8 hours after dosing. Elimination was fast (half-life 0.58 hours), with no detectable radioactivity in tissues 24 hours after dosing. Aside from the stomach, the kidneys had the highest amount of radioactivity and the brain had the least; no radioactivity could be detected in the brain within the first 4 hours after dosing. In high-dose rats, peak concentrations in tissues occurred 8 hours after dosing and could still be detected in tissues 41 hours after dosing. Elimination half-lives ranged from 3 to 3.5 hours; the brain had the lowest amount of label at all times and the kidneys had the highest. Examination of the intracellular distribution of 2,4-D in the nuclear, mitochondrial, microsomal, and soluble fractions of the kidneys, liver, spleen, brain, heart, and lungs showed that all fractions contained significant radioactivity. Regardless of the dose, most radiolabel was found in the soluble and nuclear fractions, while the microsomal and mitochondrial fractions only contained 1.4–6.7% of the total radioactivity. Because the radioactivity in the soluble fraction from all tissues could easily be extracted with ether, Khanna and Fang (1966) suggested that the 2,4-D molecule in the soluble fraction was not protein- or peptide-bound.

A comparative study in rats, mice, and hamsters of both sexes showed that ^{14}C-2,4-D-derived radioactivity was widely distributed in tissues following a single oral dose (5 or 200 mg/kg) of 2,4-D, but differences between sexes were apparent in rats and hamsters (Griffin et al. 1997a). In general, over a 72-hour monitoring period, liver and kidneys appeared to have the most radioactivity at early time points (2–8 hours); skin and fat showed relatively high amounts of radioactivity throughout the monitoring period in animals given the high dose of 2,4-D. Tissues levels of radioactivity were consistently higher in

female rats than in male rats, although the differences were not always statistically different. In hamsters, tissue levels of radioactivity were more often than not higher in males than in females. No clear differences in disposition of radioactivity were established between male and female mice.

A study in rats showed that postnatal dietary maternal exposure to 2,4-D can result in transfer of 2,4-D to the offspring via the milk (Stürtz et al. 2006). Over a dose range of 15–70 mg/kg, the concentrations of 2,4-D in dams' serum, milk, and 16-day-old pups' serum were dose-dependent, but were significantly lower in pups' serum than in maternal media. The study also showed that maternal exposure to 2,4-D altered the contents of lipids (30% decreased at 25 mg 2,4-D/kg/day) and of some proteins in the milk. More recently, Saghir et al. (2013a) also demonstrated excretion of 2,4-D in rat's milk following perinatal exposure to 2,4-D via the diet. On lactation day 4, the concentration of 2,4-D in milk was 1.7–6.3 times lower than the concentration in the dams' plasma. The ratio was reduced to 1.5–2.5 times lower on lactation day 14 due to an approximate doubling of the dams' intake of 2,4-D in the 10-day interval. The concentration of 2,4-D in pups' plasma also increased from PND 4 to 10. Over the range of dietary concentrations tested (10–1,600 ppm 2,4-D), the ratios of pups' plasma 2,4-D/maternal plasma 2,4-D increased greatly on PND 14 relative to PND 4.

No information was located regarding distribution of 2,4-D following dermal exposure of humans or animals. However, since dermal absorption occurs, it is reasonable to assume that 2,4-D will distribute in a manner similar to that reported in oral animal studies.

In adult male rats, subcutaneous administration of a dose of 250 mg/kg 2,4-D followed by intravenous dosing of radiolabeled 2,4-D resulted in most of the radiolabel in the plasma, kidneys, and liver about 2 hours after dosing (Elo and Ylitalo 1979). Somewhat lower amounts were reported in the lungs and heart, and significantly lower amounts were found in the brain, muscle, testes, and cerebral spinal fluid. In a study that only evaluated brain distribution, subcutaneous administration of 300 mg/kg 2,4-D (half the LD_{50}) followed by intravenous radioactive 2,4-D resulted in radioactivity widely distributed in various brain areas (cerebral cortex, striatum, medulla oblongata, cerebellum, and midbrain brain, including hippocampus, hypothalamus, and thalamus) without any one area showing preferential accumulation of radioactivity (Tyynelä et al. 1990). In adult rabbits, administration of a single intraperitoneal low dose of ^{14}C-2,4-D resulted in wide distribution of radioactivity throughout the brain 2 hours after dosing, and ranged from 2.8% of plasma in the hypothalamus to 4.58% in the brainstem (Kim et al. 1988).

Intravenously injected 2,4-D to pregnant mice tended to accumulate in the visceral yolk sac and after passing to the fetus, was eliminated from all tissues within 24 hours (Lindquist and Ullberg 1971). Another study in pregnant mice given an intraperitoneal injection of ^{14}C-2,4-D on GD 17 showed that 3 hours after dosing, radioactivity was distributed in various brain regions and ranged from a low of 2.8% of that of plasma in the caudate nucleus to 4.6% in the brainstem (Kim et al. 1988). Fetal brain as a whole contained 5.8% of the amount in plasma, suggesting that the brain barrier forms early in fetal life. Intravenous injection of ^{14}C-2,4-D to pregnant rabbits on GDs 28–30 resulted in rapid transfer of radioactivity to fetal plasma and brain (Sandberg et al. 1996). Peak concentrations of radiolabel were achieved in fetal plasma approximately 30 minutes after injection and remained relatively constant for the remainder of the 2-hour sampling period. Except for radiolabel in plasma, maternal kidneys and uterus showed the highest tissue AUCs. In maternal brain, lateral and ventricular choroid plexus had the highest concentration of radioactivity (about 10 times higher than any other brain region). Fetal brain had the lowest concentration of label of any maternal or fetal organ sampled. However, the concentration in fetal brain tissue was 7% of that in fetal plasma compared to 2% of that in maternal plasma, suggesting possible increased vulnerability of the fetus. In general, maternal and fetal tissue AUCs increased proportionally as the dose of 2,4-D increased from 1 to 10 mg/kg; however, in fetal tissues, it also increased 10-fold when the maternal dose increased from 10 to 40 mg/kg. The investigators suggested that because only unbound compound was available for placental transfer, the greater increase in fetal AUCs suggested saturation of maternal 2,4-D plasma protein binding (Sandberg et al. 1996).

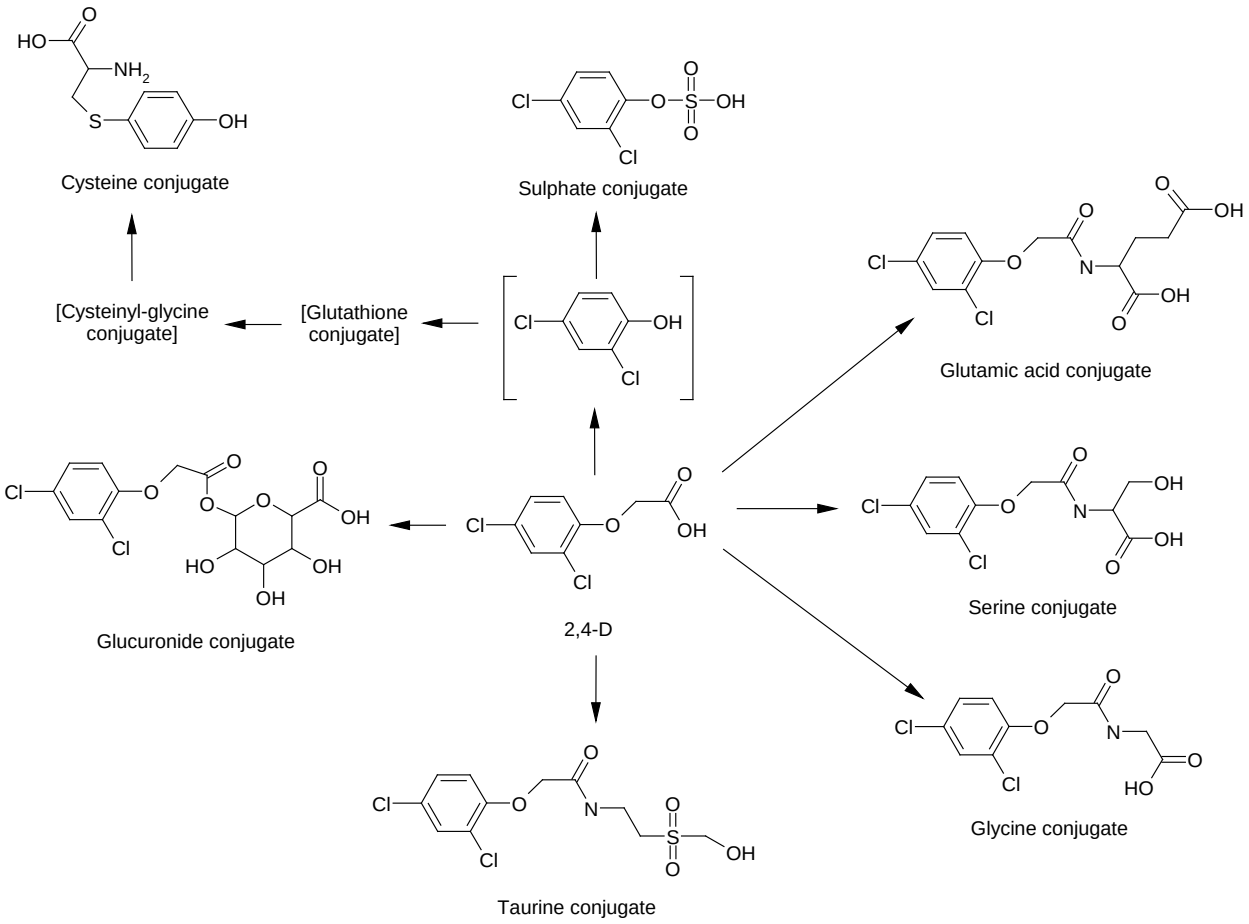
Transfer of 2,4-D to the offspring was also observed in rats following intraperitoneal injections to nursing dams every other day up to PND 16 (Stürtz et al. 2000). Transfer to 2,4-D was evident already in 4-day-old pups. In general, 2,4-D residues in pups' stomach contents, blood, kidney, and brain were dose- and exposure-time-dependent. The stomach content (milk) and the kidneys always contained the highest concentrations of 2,4-D. Levels of 2,4-D in kidneys in 8-day-old offspring from high-dose dams (100 mg/kg) increased 6-fold compared to 4-day-old pups. Pups' brain always had the lowest concentration of 2,4-D, which varied 10-fold between low-dose (50 mg/kg) 4-day-old pups and high-dose 16-day-old pups. The latter gained significantly less weight than controls, which the investigators attributed to diminished milk intake and/or a direct toxic effect of 2,4-D. Unlike Stürtz et al. (2006), these investigators discounted the quality of milk as a reason for less weight gain.

3.1.3 Metabolism

Studies in humans and animals have shown that 2,4-D undergoes limited metabolism in the body based on identification and quantification of products in the urine. For example, in a group of six male volunteers, only unchanged 2,4-D was detected in urine samples over a 1-week period after receiving a single oral dose of 5 mg/kg 2,4-D in a gelatin capsule (Kohli et al. 1974). In a similar study, analysis of urine samples from five volunteers following ingestion of 5 mg/kg 2,4-D showed mostly unchanged parent compound (mean 82.3% of the administered dose) with smaller amounts (mean 12% of the dose) excreted as a 2,4-D conjugate over a 6-day period (Sauerhoff et al. 1977).

Studies in animals have shown that, depending on the species, 2,4-D does not undergo metabolism, or if it does, as in dogs, it undergoes phase II metabolism to form conjugates that are excreted mainly in the urine; the biliary system plays only a minor role (Griffin et al. 1997b). Griffin et al. (1997a) studied the metabolism of 2,4-D in rats, mice, and hamsters and reported qualitative and quantitative differences in metabolite profiles between species, but not between sexes. Following administration of an oral dose of 5 or 200 mg/kg ^{14}C-2,4-D, the parent compound was the major urinary metabolite in the three species. A glycine conjugate was identified in the urine from mice and hamsters, a taurine conjugate was present in the urine from mice and male hamsters, and a glucuronide was detected only in urine from hamsters. Male mice metabolized 2,4-D to the glycine conjugate to a greater extent than female mice. A more recent comparative study in rats and dogs administered a single oral dose of 5 or 50 mg/kg ^{14}C-2,4-D reported that 2,4-D was excreted unmetabolized in the urine as parent compound (van Ravenzwaay et al. 2003). In dogs, however, 2,4-D formed taurine, serine, glycine, glutamic acid, cysteine, sulfate, and glucuronide conjugates, which were excreted in the urine; dog plasma only contained unchanged 2,4-D. In general, although conjugation is minimal, it favors elimination in the urine. Figure 3-1 shows a proposed metabolic pathway for 2,4-D in dogs.

Figure 3-1. Proposed Metabolic Pathway of 2,4-D in Dogs



2,4-D = 2,4-dichlorophenoxyacetic acid

Source: Van Ravenzwaay et al. 2003

3.1.4 Excretion

No data were located regarding elimination of 2,4-D in humans or in animals following inhalation exposure. However, 2,4-D has been measured in the urine of workers who experienced multi-route exposure, including inhalation (see Section 3.3.1 Biomarkers of Exposure).

In six healthy male volunteers administered a gelatin capsule with 5 mg/kg 2,4-D, unchanged compound was detected in the urine as early as 2 hours after ingestion; >75% of the parent compound was excreted in the urine in 96 hours (Kohli et al. 1974). A similar study with volunteers reported that most of a single

oral dose of 5 mg/kg 2,4-D was excreted unchanged in the urine within 3 days of dosing (Sauerhoff et al. 1977). Over a 6-day period after dosing, recovery of the administered dose was almost complete. The half-life elimination from urine ranged from 10.2 to 28.5 hours. The estimated fraction of the dose eliminated in the urine as free 2,4-D over the 6-day period ranged from 47.8 to 96.5%.

Studies in animals show that 2,4-D is eliminated mainly in the urine as unchanged compound or as conjugate, as it occurs in dogs.

In urine from rats collected every 10 hours after gavage administration of a single dose of 2.6 mg/kg 2,4-D as the sodium salt by gavage in water, peak concentration of 2,4-D occurred in the 20-hour spot sample (Knopp and Schiller 1992). Gradual decline occurred over the next 10 hours, and by 40 hours after dosing, approximately 90% of the administered dose had been accounted for in the urine. In an earlier study in rats administered doses of approximately 3–30 mg/kg ^{14}C-2,4-D by gavage, excretion of 2,4-D was virtually complete within 48 hours of dosing and 93–96% of the dose was excreted in the first 24 hours (Khanna and Fang 1966). Almost all of the radioactivity corresponded to parent compound and was excreted in the urine; no radioactivity could be detected in expired air. Administration of higher doses (~60–300 mg/kg) resulted in a linear decrease in recovery of radiolabel in urine and feces, and increased amounts were recovered in the second 24 hours after dosing. Excretion of the higher dose was still incomplete 144 hours after dosing.

In a comparative study in rats, mice, and hamsters administered a single dose of 5 or 200 mg/kg ^{14}C-2,4-D, urine was the main route of elimination of radiolabel in the three species (Griffin et al. 1997a). In rats, <4% of the administered radioactivity appeared in the feces during the 72-hour monitoring period. No 2,4-D metabolites were detected in the urine or feces from rats. Mice excreted 10–24% of administered radioactivity in the feces, and of this, 13.3% was the taurine conjugate. Hamsters excreted 6–16% of the administered radioactivity in the feces and all of it was unchanged 2,4-D. In the three species, expired air contained <1% of the administered radioactivity. In a similar study in rats and dogs administered 5 or 50 mg/kg ^{14}C-2,4-D, irrespective of the dose, rats excreted almost all of the administered radioactivity in the urine, and excretion was virtually complete 24 hours after dosing (van Ravenzwaay et al. 2003). Dogs metabolized 2,4-D (Figure 3-1). Low-dose dogs excreted approximately 38% of the dose in the urine and 10–13% in the feces over the 120-hour monitoring period. High-dose dogs excreted about equal amounts of the dose (20–25%) in the urine and feces. Excretion was not complete in dogs after the 120-hour sampling time. No significant differences regarding rates or routes of excretion between male and female animals were observed.

An oral exposure study provides suggestive evidence of sex-related differences in the elimination of 2,4-D from plasma (van Ravenzwaay et al. 2003). In rats administered 5 mg/kg, no differences in plasma elimination half-lives were observed between males and females. However, at 50 mg/kg, the elimination half-life was almost twice as long in the female rats (3.35 hours) than in the males (1.50 hours). In contrast, the plasma elimination half-times were similar between male and female dogs administered 5 or 50 mg/kg (van Ravenzwaay et al. 2003).

In volunteers applied a dermal dose of 4 μg/cm^2 2,4-D in acetone, most of the absorbed dose was eliminated in the urine within 72 hours of dosing (Feldmann and Maibach 1974). In a similar study, subjects applied a dose of 10 mg of 2,4-D in acetone over a 9 cm^2 area excreted most of the absorbed dose in 96 hours; an average of 84.8% of the applied dose was recovered in the urine in 96 hours. The approximate mean urinary excretion half-life was 39.5 hours (Harris and Solomon 1992).

Application of an aqueous solution of 2.6 mg/kg 2,4-D sodium salt to the shaved back of rats resulted in significantly lower urinary concentration of 2,4-D than when the dose was administered orally (Knopp and Schiller 1992). Peak urinary concentration of 2,4-D occurred at about 40 hours after dosing and declined gradually thereafter. As a percentage of the applied dose, 2,4-D in the urine increased steadily over a 116-hour period after dosing, reaching a cumulative maximum of about 10.5% of the applied dose. In rabbits, 36% of a dose of 4 μg/cm^2 of 2,4-D in acetone applied to the shaved back was recovered in the urine over a 14-day period (Moody et al. 1990). In the same study with monkeys and rabbits, 15 and 29% of the dose applied to the forearm and forehead, respectively, was recovered in the urine over the same time period. Urinary excretion half-lives ranged from 1.47 days for the monkeys' forehead application to 2.14 days for the rabbits' back application.

In rats, fecal excretion of ^{14}C-2,4-D represented only a minor elimination route following dermal application of the chemical, with only 2% of the applied dose accounted for in the feces over a 14-day sampling period (Moody et al. 1994). In the same time period, guinea pigs excreted 9% of a dermal dose of 2,4-D in the feces (Moody et al. 1994). Mice applied a dose of 1 mg/kg ^{14}C-2,4-D in acetone on the shaved back excreted small amounts of radiolabel in the feces and as CO_2, although the authors did not provide the specific amounts (Grissom et al. 1985). In 24 hours, 93% of 2,4-D that had penetrated the application site (almost 21% of the applied dose) was accounted for in the excreta.

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

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

PBPK models for 2,4-D in rabbits, rats, and humans have been reported (Durkin et al. 2004; Kim et al. 1994, 1995, 1996, 2001). The Kim et al. (1994, 1995, 1996, 2001) models were developed with the primary objective of simulating regional brain distribution of 2,4-D. These models included compartments for various brain regions, while all other tissues were aggregated into a single compartment. The rat and human models developed by Durkin et al. (2004) have compartments for liver and kidney, but no separate compartment for brain. The model has been applied to interspecies and route-to-route dosimetry calculations for deriving Hazard Quotients (ratio of a measure of exposure to a chemical to an established benchmark such as a reference dose [RfD] or reference concentration [RfC]) in forestry workers who spray 2,4-D based on dose equivalence for plasma peak and average 2,4-D concentrations. The Durkin et al. (2004) and Kim et al. (2001) models differ in several other important ways. In the Durkin et al. (2004) model, exchanges of 2,4-D between plasma and tissues are flow-limited with partitioning of the non-ionized species (e.g., protonated acid) between interstitial and intracellular fluid in tissues. Uptake of the anionic base is attributed to differences in extracellular and intracellular pH, which result in intracellular pH-trapping of the anionic base. In the Kim et al. (2001) models, exchanges between plasma and tissues are diffusion limited and no distinction is made between the protonated and anionic species. Another important difference concerns the simulation of urinary excretion of 2,4-D. In the Durkin et al. (2004) model, renal clearance of 2,4-D is dependent on plasma 2,4-D concentration, with renal clearance decreasing as plasma 2,4-D concentration increases. This approach accommodated results of studies in animals that found dose-dependent inhibition of urinary excretion of 2,4-D. In the Kim et al. (2001) model, urinary excretion is simulated as a capacity-limited transfer of 2,4-D to urine. Both models include a “deep” compartment, which exchanges 2,4-D with plasma very slowly. In the Durkin et al. (2004) model, the deep compartment is assigned to red blood cells; in the Kim et al. (2001) models, the deep compartment is assigned a subcompartment of the lumped

body compartment representing all tissues other than brain. The Durkin et al. (2004) model also includes parameters for simulating binding of 2,4-D to plasma protein. Although very different in structure, both models yielded similar predictions of plasma elimination kinetics when optimized to the same intravenous dosing studies in rats (Durkin et al. 2004).

Rabbit (Kim et al. 2001)

Kim et al. (1994, 1995, 1996, 2001) developed a PBPK model for predicting uptake and distribution of 2,4-D in rabbit and rat brain. The model includes compartments for plasma, brain, and a single lumped body compartment representing all tissues other than brain. The brain compartment includes subcompartments representing the hypothalamus, caudate nucleus, hippocampus, forebrain, brainstem, cerebellum, brain plasma, and cerebrospinal fluid (CSF). The six brain compartments have distinct mass transfer clearance coefficients for plasma-brain and brain-CSF. The body compartment includes a deep subcompartment and a compartment representing the rest of the body (excluding brain). Exchanges of 2,4-D between plasma and brain are simulated as four process: (1) flow-limited exchange between central plasma and brain plasma, governed by blood flow and the brain/plasma partition coefficient; (2) diffusion-limited exchange between plasma and brain tissue governed by a mass transfer clearance coefficient; (3) diffusion-limited exchange between brain tissue and CSF; and (4) capacity-limited transfer from CSF to plasma, representing transport through the choroid plexus, governed by a V_{max} and K_m. Exchange of 2,4-D between plasma and the rest of the body is flow-limited. Excretion of 2,4,-D is represented as capacity-limited transfer from the body compartment (V_{max}, K_m).

Partition coefficients for the rabbit model were estimated from tissue/plasma concentration ratios measured in rabbits following a single intraperitoneal dose of 40 or 100 mg/kg ^{14}C-2,4-D (Kim et al. 1995). These same values were used in the rat model. Transfer coefficients for the rabbit model were optimized with data from the same study (Kim et al. 1995). Transfer coefficients for the rat model were optimized with data on plasma and brain concentrations in rats following intravenous injection of 10, 50, or 150 mg/kg 2,4-D or subcutaneous implantation of osmotic mini-pumps that delivered 2,4-D doses of 1 or 10 mg/kg day (Patterson et al. 2000). The rabbit model was evaluated by comparing observed and predicted time courses for plasma, CSF, and brain region 2,4-D concentrations. Data for an individual rabbit is displayed in Kim et al. (1995), and these plots show time profiles that are similar to observations. The rat model predicted plasma and brain regions concentration of 2,4-D that were within ±2 standard deviations of the mean observations (Kim et al. 2001).

A maternal-fetal model was developed based on the rabbit model (Kim et al. 1996). The model includes placental and amniotic fluid compartments and fetal tissue compartments representing fetal CSF, fetal brain tissue, and fetal brain plasma. Exchanges between maternal plasma and placenta are flow-limited. Exchanges between fetal plasma and brain include the same four flow-limited, diffusion-limited, and capacity-limited processes as in the maternal model. 2,4-D in amniotic fluid undergoes diffusion-limited exchange with 2,4-D in the fetal body compartment and with the placenta. Transfer coefficients were optimized based on data from a study in which anesthetized pregnant rabbits received intravenous doses of 1, 10, or 40 mg/kg ^{14}C-2,4-D on GD 30. The study provided time-course data on 2,4-D in maternal and fetal plasma, amniotic fluid, and fetal brain. The optimized model predicted the dose-dependent time course for 2,4-D fetal and maternal plasma, amniotic fluid, fetal brain, and maternal brain regions.

Human and Rat (Durkin et al. 2004)

Durkin et al. (2004) developed a PBPK model of 2,4-D for predicting internal exposures resulting from ingestion exposures in rats and dermal exposures in humans. The model includes compartments for plasma, red blood cells, skin, kidney, liver, gastrointestinal tract, and a lumped compartment representing other tissues. The blood compartment includes a red cell compartment, which exchanges 2,4-D slowly with plasma (first order). The plasma compartment includes saturable binding to two classes of binding sites. The free unbound fraction exchanges with tissues. Exchanges of 2,4-D between plasma and tissues are flow-limited with partitioning of the non-ionized species (e.g., protonated acid) between interstitial and intracellular fluid in tissues. Dissociation of the acid into its anionic base is calculated based on the Henderson-Hasselbalch equation, pKa for 2,4-D (2.87), pH of interstitial fluid (7.0), and intracellular fluid (7.4). The lower intracellular pH favors intracellular trapping of the anion. The liver compartment includes a term for first-order transfer of 2,4-D into the gastrointestinal tract representing biliary secretion. Excretion of 2,4-D is simulated as four processes: (1) delivery of 2,4-D into tubule fluid from glomerular filtration; (2) saturable transport of the anionic base from plasma into kidney (V_{max}, K_m); (3) secretion of the anionic based from kidney into urine (first order); and (4) excretion of 2,4-D in tubule fluid into urine (first order). Studies conducted in animals have found that urinary excretion of 2,4-D is inhibited by increasing concentrations of plasma 2,4-D (Orberg 1980). Although the mechanism for this apparent self-inhibition in not understood, the inhibition affects both glomerular filtration and renal secretion of 2,4-D, suggesting that it may represent a vascular effect resulting in depression of glomerular filtration and/or renal blood flow (Durkin et al. 2004). The pharmacodynamics of inhibition of urinary excretion are represented in the model as an adjustment to parameters that govern glomerular filtration, transport from plasma into kidney, and secretion of 2,4-D into urine. The adjustment factor is a variable

that changes in value as a function of plasma 2,4-D concentration. Dependence of the adjustment factor on plasma 2,4-D concentration results in renal clearance of 2,4-D decreasing with increasing plasma 2,4-D concentration. The adjustment factor was empirically derived from animal studies (Orberg 1980). Absorption pathways in the model are from the gastrointestinal tract and skin surface. The gastrointestinal tract model includes compartments representing stomach lumen, gastrointestinal tract lumen (representing the tract distal to stomach), and gastrointestinal tract tissue. Absorption from the stomach and transfer to feces are first-order processes. 2,4-D deposited on skin is subject to first-order transfer to the environment (fugitive loss) or first-order absorption into skin tissue from where it can undergo flow-limited exchange with plasma.

The model was parameterized to simulate rats, and subsequently extrapolated to humans. The rat model was based primarily on intravenous and oral studies (Durkin et al. 2004). Rats were administered a single intravenous dose (5 or 90 mg/kg) or oral dose (10, 25, 50, or 150 mg/kg). A study conducted in goats was used to estimate the effects of 2,4-D dose on 2,4-D renal clearance and glomerular filtration (Orberg 1980). Protein binding parameters were based on data from studies conducted in rats (Ylitalo et al. 1990), goats (Orberg 1980), and bovine serum albumin (Kolberg et al. 1973). Partition coefficients were estimated from physical-chemical properties of 2,4-D and tissue composition (Poulin and Krishnan 1995) and adjusted based on measured values for brain/plasma (Kim et al. 1995). The rat model was initially optimized based on data from the rat intravenous study and then applied to the rat oral study to estimate values for gastrointestinal tract absorption parameters. By parameterizing the model to achieve decreasing renal clearance in association with increasing plasma 2,4-D concentrations, the model predicted the observed nonlinear dose-dependence of urinary excretion and plasma concentration as well as time-dependent changes in kinetics of 2,4-D removal from plasma and excretion in urine following dosing (Durkin et al. 2004).

The human model was optimized to data from studies conducted in humans (Feldmann and Maibach, 1974; Sauerhoff et al. 1977). In the Feldmann and Maibach (1974) study, urinary ^{14}C was measured following a single intravenous (tracer) dose of ^{14}C-2,4-D or dermal dose to the forearm (4 µg/cm^2). In the Sauerhoff et al. (1977) study, plasma levels and urinary excretion of 2,4-D were measured following a single oral dose of 2,4-D (5 mg/kg). Data from the human studies were used to optimize values for parameters controlling the absorption rate from the gastrointestinal tract, absorption rate from skin, V_{max} uptake to kidney, and k_e for urinary excretion. All other parameters were allometrically scaled from the rat model.

The human model was evaluated by comparing observed and predicted urinary excretion of 2,4-D in forestry workers who sprayed 2,4-D from backpack sprayers (Lavy et al. 1987). The study provided data on application rates and urinary excretion of 2,4-D over a 5-day period. Skin deposition rates were estimated from data contained in the Pesticide Handlers Exposure Database (Durkin et al. 2004). Predictions from the optimized model encompassed observed cumulative urinary excretion of 2,4-D.

The model was applied to an interspecies and route-to-route dosimetry extrapolation. The model was used to predict plasma 2,4-D concentrations corresponding to a rat NOAEL and LOAEL estimated from a 90-day feeding study (Durkin et al. 2004). Average and peak plasma concentrations in rats corresponding to the NOAEL were predicted to be 3.6 and 7.2 µM, respectively. Average (2-week) plasma 2,4-D concentrations in forestry workers were predicted to range from 1.4 to 7.3 µM and peak concentration were predicted to range from 2.5 to 13 µM

3.1.6 Animal-to-Human Extrapolations

As mentioned previously, it has been proposed that the dog might not be a relevant species for evaluation of human health risk because of the relatively low capacity to excrete 2,4-D (Timchalk 2004). The implication is that, at equivalent doses of 2,4-D, more 2,4-D will remain in plasma and potentially reach tissues in dogs than in other species, particularly at lower doses since clearance may become saturated in most species at higher doses. This was illustrated by van Ravenzwaay et al. (2003) who reported that equivalent doses of 5 and 50 mg 2,4-D/kg given to rats and dogs resulted in plasma 2,4-D AUCs 125- and 15-fold greater, respectively, in dogs than in rats.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic

makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to 2,4-D are discussed in Section 5.7, Populations with Potentially High Exposures.

Epidemiological studies of farming communities where 2,4-D has been used, which have included monitoring of children, have not provided convincing evidence of associations between 2,4-D and adverse health outcomes in children. For example, no significant association was found between 2,4-D and birth weight in the AHS (Sathyanarayana et al. 2010), birth defects in the Ontario Farm Family Health Study (Weselak et al. 2008), or birth defects and congenital anomalies in a study of pesticide applicators in the San Joaquin Valley of California (Yang et al. 2014). Studies of state-licensed, private pesticide applicators in Minnesota found a significant increase in birth defects among children conceived during the herbicide application season (Garry et al. 1996, 2002). However, chemical-specific analyses were not conducted.

Further evaluation of children born to participants in the Ontario Farm Family Health Study showed a significant increased risk of hay fever or allergies associated with maternal exposure to 2,4-D during pregnancy among male offspring, but not among female offspring (Weselak et al. 2007). No significant association was found between exposure to 2,4-D and asthma or persistent cough or bronchitis.

Studies of children from parent participants in the AHS did not find significant associations between 2,4-D exposure and NHL, Hodgkin's disease, or leukemia (Flower et al. 2004). In a study of exposure to 2,4-D in house dust in California, childhood leukemia was not associated with 2,4-D (Metayer et al. 2013).

Animal studies have shown that 2,4-D can be transferred to the offspring through the placenta and via the mother's milk and that it distributes widely in fetal or neonatal tissues (Lindquist and Ullberg 1971; Marty et al. 2013; Saghir et al. 2013a; Sandberg et al. 1996; Stürtz et al. 2000, 2006). Therefore, it seems reasonable to assume that the same could happen in humans.

As summarized in Section 2.17, Developmental Effects, studies in rodents have shown that, for the most part, adverse developmental effects (i.e., mainly reduced body weight in the offspring) occur at maternal dose levels that induced maternal toxicity, mainly reduced maternal weight during pregnancy. Reduced

offspring weight was reported in a study in rats administered a relatively low postpartum dose of 2.5 mg 2,4-D/kg/day (Stürtz et al. 2010). This was attributed to 2,4-D affecting the suckling-induced hormone release milk transfer to the litter. However, no such effect has been reported in other studies that exposed dams to considerably higher doses (approximately 29 mg 2,4-D/kg/day) for periods that included gestation and postpartum (Marty et al. 2013).

2,4-D has not been found to cause teratogenicity in animal studies (Charles et al. 2001; Marty et al. 2013; Schwetz et al. 1971).

Children may be exposed to 2,4-D during and after its use in residential and recreational areas, such as on lawns or park grasses. Children may also be exposed when swimming in bodies of water that have been treated with 2,4-D (EPA 2005a). Children who live with farmworkers may also be exposed to 2,4-D from the clothing, boots, or containers brought into the home by household residents after a workday and spray drift proximal to fields, forests, and orchards (Arcury et al. 2007).

In a biomonitoring study of exposure to 2,4-D in farm families with licensed applicators in Minnesota and South Carolina, 24-hour urine 2,4-D concentrations were collected 1 day before through 3 days after application (Alexander et al. 2007). For children 4–17 years old (n=53), the median urine 2,4-D concentrations pre-application and 1 day after application were 1.5 and 2.9 µg/L, respectively. At baseline, 2,4-D was detectable in the urine of 62% of the children. The mean urine 2,4-D concentration in children the day before application, the day of application, 1 day after application, 2 days after application, and 3 days after application were 1.4, 2.1, 3.6, 3.5, and 3.4 µg/L, respectively. Younger children, 4–11 years old, had higher median post-application urine 2,4-D concentrations than older children, 12–17 years old (6.5 compared to 1.9 µg/L). Exposure to children was determined to be primarily attributable to the level of contact with the application process, including their presence during mixing or application of 2,4-D. Another study was performed to measure the level of pesticide urinary metabolites in 60 farmworker children 1–6 years old in North Carolina from July through August 2004 (Arcury et al. 2007). 2,4-D was detected in 41.7% of the 60 urine samples collected, with a median concentration of 0.23 µg/g creatinine.

Nishioka et al. (2001) performed a study to determine exposure to 2,4-D to young children (ages 5–14 years) in air and on surfaces (floors, tabletops, and windowsills) inside single-story Midwestern residences both before and after lawn application. 2,4-D was detected in indoor air and on all surfaces after application. It was determined that the main transport routes of 2,4-D into the home were from the

homeowner applicator and by an active dog. No 2,4-D was detected in indoor air samples before application. The maximum indoor air concentrations during and after application were 17.7 and 10.8 ng/m^3, respectively. Post application, floor dust was concluded to be the major source of 2,4-D in the air, on tables, and on windowsills by resuspension. Postapplication floor dust concentrations ranged from approximately 1 to 200 µg/m2, compared to 0.2–1.0 µg/m2 for dust levels prior to application. The concentrations of 2,4-D measured in occupied homes postapplication on carpets, bare floors, tabletops, and windowsills were <0.1–228, <0.01–23, 0.3–27, and 0.5–22 µg/m^2, respectively. It was estimated that dietary ingestion was the main source of exposure for young children before lawn application of 2,4-D, but during postapplication periods, dietary ingestion (53%), nondietary ingestion (41%), and dermal penetration (4%) were the main pathways. Postapplication exposure levels from nondietary ingestion by contact with floors and contact with tabletops were estimated to be 1–10 and 0.2–30 µg/day, respectively, which are estimated to be about 10 times higher than levels before application. Dust samples collected from the homes of 513 subjects residing in Detroit, Michigan, the state of Iowa, Los Angeles, California, and Seattle, Washington had an arithmetic mean and geometric mean concentration of 2,422 and 419 ng/g, respectively (Colt et al. 2004). Seventy-eight percent of all of the samples tested were positive for 2,4-D. Samples collected in Iowa had the greatest geometric mean concentration of 2,4-D (1,512 ng/g), followed by Detroit (606 ng/g), Seattle (374 ng/g), and Los Angeles (87 ng/g).

NHANES uses biomonitoring to provide estimates of exposure to the civilian U.S. population. Chemicals and their metabolites are measured in subsets of participants aged 6–59 years old, meant to be a representative sample of the population. Urinary levels of 2,4-D in children 6–11 and 12–19 years old were measured in NHANES samples assessing exposure from years 1999–2010 (CDC 2015). For survey years 1999–2000 and 2001–2002, no geometric mean urinary concentration of the 2,4-D could be calculated because the proportion of results below the detection limit was too high to provide a valid result. These results are summarized in Tables 5-7 and 5-8 (CDC 2015). The results suggest that urinary levels of 2,4-D in children have remained relatively unchanged over the temporal period, but slightly higher levels have been observed in children as compared to adults.

In the Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study, the exposures of 135 preschool children and their adult caregivers to 2,4-D at their homes in North Carolina and Ohio were examined in 2000 and 2001 (Morgan et al. 2008). Monitoring was performed over a 48-hour period, and personal (hand wipes and food) and environmental (air, soil, and dust) samples were collected. 2,4-D was detected in all types of environmental samples, with the highest frequency in carpet dust samples at 83% (median concentration of 47.5 ng/g) and 98% (median

concentration of 156 ng/g) in North Carolina and Ohio, respectively. Detection frequencies in North Carolina and Ohio were 38 and 49% (maximum concentrations of 3.7 and 2.0 ng/m^3) for indoor air, 19 and 34% (maximum concentrations of 1.7 and 3.2 ng/m^3) for outdoor air, and 17 and 45% (maximum concentrations of 30.5 and 13.3 ng/g) for soil, respectively. Maximum concentrations of 2,4-D in personal exposure samples for children in North Carolina and Ohio were 0.04 and 0.1 ng/cm^2 for hand wipes and 4.4 and 20.2 ng/g for solid food, respectively. 2,4-D was detected in >85% of the total samples collected. The median 2,4-D urinary concentrations in children were 0.5 and 1.2 ng/mL in North Carolina and Ohio, respectively. Morgan et al. (2014) estimated the potential intakes of 2,4-D from different routes using data from 129 preschool children from North Carolina in the CTEPP study. The daily intake dose was calculated as 4.981 ng/kg/day, with the largest intake arising from dietary exposure (4.84 ng/kg/day).

In a study of urine collected from 197 children in Arkansas, 20% had detectable levels of 2,4-D, and the 95th percentile and maximum concentrations were reported as 3 and 9 µg/L, respectively (Hill et al. 1989).

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to 2,4-D are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for 2,4-D from this report are discussed in Section 5.6, General Population Exposure.

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

tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by 2,4-D 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

As mentioned in Section 3.1.3, Metabolism, 2,4-D undergoes limited metabolism in humans and can thus be measured as unchanged parent compound in body fluids and tissues from humans. Information regarding levels in human tissues is available from cases of acute intentional or accidental oral intoxication with commercial products that contained 2,4-D that resulted in deaths (i.e., Dudley and Thapar 1972; Keller et al. 1994; Nielsen et al. 1965; Osterloh et al. 1983). Tissue levels of 2,4-D determined in these and other case reports are typically not representative of occupational or environmental exposure to 2,4-D.

2,4-D can be readily measured in urine, and with the benefit of non-invasive collection procedure, urine is a widely used and accepted media to ascertain exposure to 2,4-D. Because 2,4-D is rapidly eliminated from the body (Kohli et al. 1974; Sauerhoff et al. 1977), urinary levels of 2,4-D reflect recent exposure, within days.

There are many reports that provide information regarding urinary levels of 2,4-D in workers, especially farmers and herbicide applicators, and in members of the general population. Providing detailed information from the extensive number of studies available is beyond the scope of this document, but pertinent data have been extracted from recent reviews (Burns and Swaen 2012; von Stackelberg 2013). Additional information on this topic is presented in Chapter 5.

Burns and Swaen (2012) noted that large studies designed to be representative of the United States (CDC 2009; population surveyed years 1999–2002) and Canadian (Health Canada 2010) populations (surveyed years 2007–2009) did not detect 2,4-D at the 50th percentile (<1 µg/L) (in 50% of the samples, the concentration of 2,4-D was below 1 µg/L of urine). In general, urinary levels of 2,4-D in groups of individuals considered bystanders varied from less than the limit of detection (0.2 µg/L) to 3 µg/L. Bystanders were individuals who did not mix, load, or apply 2,4-D, but who occasionally could have experienced greater exposure than the general population. These included spouses and children of applicators, and applicators of other herbicides. Levels of 2,4-D in the urine from individuals who experienced direct exposures, such as those who applied 2,4-D on crops, forests, and turf, as well as those involved in the manufacture of 2,4-D, varied greatly. Geometric means between 5 and 45 µg/L were reported for crop and forestry applicators; maximum levels varied from 410 to 2,500 µg/L 2,4-D among these groups. A highest maximum of 12,963 µg/L was reported in a study of German manufacturers in the 1980s (Knopp 1994). The wide ranges reported are not surprising considering the number of factors that can determine the extent of exposure, including type of application method, glove use, repairing equipment, size of the area treated, and personal hygiene practices. A study reported that these factors explained 16% of the between-worker variance and 23% of the within-worker variance of urinary 2,4-D concentrations (Bhatti et al. 2010), suggesting that other determinants remained unexplained. It is worth noting that urinary pH is an important determinant of 2,4-D urinary levels.

Knowing the urinary levels of 2,4-D is important to determine whether someone has been exposed to excessive amounts of 2,4-D. This information is particularly useful if it can be used to estimate an absorbed dose of 2,4-D that can be compared to exposure guidance values. For example, Mage et al. (2004) collected data on urinary creatinine concentration and excretion rate from 978 volunteer participants in NHANES, 1988–1994, computed for their age, gender, height, and weight and determined that none of the subjects were exposed to 2,4-D at a rate above the reference dose (RfD) of 5 µg/kg/day established by EPA (EPA 2005a). A number of assumptions were made in this exercise, including assuming that the subjects had a relatively constant intake of 2,4-D and a constant dietary intake of red meat, and that the tubular secretion transport mechanism was not saturated. Under these conditions, the body would excrete approximately constant amounts of 2,4-D and creatinine per day. A similar approach was used by Alexander et al. (2007) to estimate systemic doses in farm families using urine samples collected from the application day through the third day after application. Subjects were participants in the Farm Family Exposure Study, a study of licensed applicators in Minnesota and South Carolina. The geometric means systemic doses (µg/kg/day) were as follows: 2.46 for applicators, 0.8 for spouses, 0.22 for children (all ages included), 0.32 for children 4–11 years of age, and 0.12 for children ≥12 years

of age. Exposure to family members was determined primarily by the potential for direct contact with the application process or chemical, although for many spouses and most children, it is more likely to be due to indirect exposure (contamination of surfaces, drift from application areas, in household dust) than direct exposure. Some factors found to be predictive of exposure were use of gloves, size of application, and having to repair equipment. The estimated systemic dose for applicators is consistent with a value of 2.7 µg/kg/day estimated for applicators in a study of participant in the AHS (Thomas et al. 2010b). Scher et al. (2008) developed a simple pharmacokinetic model from 2,4-D urinary excretion data from the Farm Family Exposure Study to evaluate the feasibility of reconstructing absorbed dose of 2,4-D. The model was a one-compartment model with single first-order absorption and elimination rate constants that adequately described the pharmacokinetic disposition of 2,4-D in humans as reported in studies with volunteers (Feldmann and Maibach 1974; Harris and Solomon 1992; Kohli et al. 1974; Sauerhoff et al. 1977). The final analysis was conducted on data from 14 farmers, and the results showed that the model accurately simulated measured urinary output and adequately described the data at early and late time points.

More recent studies have examined the use of biomonitoring equivalents to assess whether exposure to 2,4-D exceeds levels of concern (Aylward and Hays 2015; Aylward et al. 2010, 2013; Hays et al. 2012). Studies included both general population adults and children as well as farmers and farm family members. Biomonitoring equivalents are defined as a concentration of a chemical or its metabolite in a human biological medium (usually blood or urine) that is consistent with existent exposure guidance values (i.e., RfDs). The results of these studies showed that current exposures to 2,4-D are well below exposure guidance values for 2,4-D.

3.3.2 Biomarkers of Effect

Adverse effects, including death, have been observed in humans who intentionally or accidentally ingested herbicide formulations containing 2,4-D. Adverse effects were also reported following cases of accidental dermal exposure to 2,4-D. Some reported effects included tachypnea, tachycardia, vomiting, leukocytosis, liver and kidney congestion in fatal cases, metabolic acidosis, and neurological effects characterized by sensory and motor abnormalities. None of these conditions is specific for 2,4-D; any of these effects or combination of them can be caused by exposure to other chemicals or can be due to conditions unrelated to chemical exposures.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Limited information was located regarding interactions of 2,4-D with other chemicals. 2,4-D was found to increase the expression of some CYP1 cytochromes in rat liver, kidney, and mammary gland (Badawi et al. 2000) and of some microsomal enzymes in the liver of mice (Chaturvedi et al. 1991) and rats (Hietanen et al. 1983), and decrease some phase II enzymes in rat liver (Hietanen et al. 1983). This suggests that, in general, the toxicity of chemicals that are metabolized by the affected enzymes will increase or decrease depending on whether metabolism produces a reactive intermediate or a detoxification product. In general, in mice, 2,4-D combined with toxaphene seemed to have additive effects regarding microsomal enzyme induction and liver toxicity; the same, but to a lesser extent, occurred with the combination 2,4-D and parathion (Chaturvedi et al. 1991; Kuntz et al. 1990). Given that exposure to 2,4-D could coexist with exposure to other pesticides, more information on potential interactions would be useful.