

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

3.1 TOXICOKINETICS

Toxicokinetic data for glyphosate are summarized below.

- Glyphosate is readily absorbed from the gastrointestinal tract; very little glyphosate is absorbed through the skin; it is assumed that glyphosate is readily absorbed from the respiratory tract.
- Absorbed glyphosate is readily distributed via the blood, but does not accumulate in any particular organ or tissue.
- Glyphosate does not undergo significant metabolism in mammals; <1% is metabolized to aminomethylphosphonic acid (AMPA).
- Approximately two-thirds of an oral dose of glyphosate is excreted in the feces as unabsorbed parent compound. Most absorbed glyphosate is rapidly excreted in the urine as parent compound.

3.1.1 Absorption

3.1.1.1 Inhalation Exposure

Limited information is available regarding the toxicokinetics of inhaled glyphosate. Observations of increased urinary glyphosate levels among 48 farmer-applicators following application of glyphosate-containing products is evidence that inhaled glyphosate can be absorbed (Acquavella et al. 2004).

However, dermal absorption was likely involved in some cases because mean urinary glyphosate was higher among those farmers (14/48) who did not use rubber gloves. Detectable levels of urinary glyphosate were also measured in children of the farmers who were present during mixing, loading, or application of the herbicide; exposures among the children may have involved inhalation and/or dermal routes. No information was located regarding the toxicokinetics of inhaled glyphosate in laboratory animals.

3.1.1.2 Oral Exposure

Information regarding the toxicokinetics of ingested glyphosate in humans is limited. The detection of glyphosate in serum and/or urine samples from individuals who had intentionally or unintentionally

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ingested glyphosate-containing products is confirmation of absorption from the gastrointestinal tract (e.g., Hiraiwa et al. 1990; Hori et al. 2003; Sribanditmongkol et al. 2012; Zouaoui et al. 2013).

Numerous reports of systemic effects following intentional or unintentional ingestion of glyphosate-containing products serve as additional evidence that ingested glyphosate is absorbed (e.g., Chang and Chang 2009; Chen et al. 2009; Hsiao et al. 2008; Kim et al. 2014; Lee et al. 2000; Menkes et al. 1991; Moon and Chun 2010; Roberts et al. 2010; Sato et al. 2011; Sawada et al. 1988; Sørensen and Gregersen 1999; Stella and Ryan 2004; Talbot et al. 1991; Tominack et al. 1991).

Several groups of investigators have evaluated the absorption of glyphosate following oral exposure in laboratory animals, particularly rats. In one study (NTP 1992), male F344/N rats were administered a single gavage dose of ^{14}C -glyphosate (purity 99%) in distilled water at 5.6 or 56 mg/kg. Other rats were administered a single dose of glyphosate at 5.6 mg/kg via intravenous injection, intraperitoneal injection, or oral (gavage) to compare 24-hour urinary and fecal elimination by these administration routes. Results from comparative studies of oral, intravenous, and intraperitoneal administration of glyphosate indicated that urinary radioactivity represented the amount of glyphosate absorbed and fecal radioactivity represented the amount of unabsorbed glyphosate following oral exposure. Although quantitative data were not included in the study report, the study authors estimated that 30% of the 5.6 mg/kg dose of ^{14}C -glyphosate was absorbed and that a slightly higher percentage (34%) of the 56 mg/kg dose was absorbed. In another study, male Sprague-Dawley rats received a single gavage dose of ^{12}C - and ^{14}C -glyphosate at 10 mg/kg (Brewster et al. 1991). Based on urinary radioactivity, it was estimated that 35–40% of the oral dose had been absorbed from the gastrointestinal tract. Anadón et al. (2009) reported an absorption half-life of 2.29 hours following administration of an oral dose of 400 mg glyphosate/kg to rats; an estimated peak plasma glyphosate of 4.62 $\mu\text{g/mL}$ was reached at 5.16 hours postdosing. Results from a number of unpublished industry studies cited in EPA (1993), FAO and WHO (2016), IPCS (1994), and/or Williams et al. (2000), but not available to ATSDR, demonstrate that single or repeated oral dosing of glyphosate to rats at doses in the range of 10–1,000 mg/kg/day result in urinary excretion of 7–36% of the administered dose during ≤ 7 days of posttreatment, which presumably represents the proportion of absorbed glyphosate.

3.1.1.3 Dermal Exposure

Limited human data are available regarding the toxicokinetics of glyphosate following dermal exposure. Increased urinary glyphosate levels among 48 farmer-applicators following application of glyphosate-containing products is evidence that glyphosate can be absorbed (Acquavella et al. 2004). Dermal

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absorption was likely involved in some cases because mean urinary glyphosate was higher among those farmers (14/48) who did not use rubber gloves.

In vitro studies using human skin samples indicate that dermal penetration of glyphosate is very low. Wester et al. (1996) applied 300 µL of a 1% aqueous dilution of analytical-grade ¹⁴C-labeled glyphosate to human cadaver skin (0.8 cm² of available skin area). The study authors reported a permeability constant of 4.59x10⁻⁴ cm/hour, with a lag time of 10.48 hours, which resulted in a calculated flux of 4.12 µg glyphosate/hour. Wester et al. (1991) used a ¹⁴C-labeled Roundup® formulation to evaluate dermal absorption of glyphosate through human skin (*in vitro*) and abdominal skin of Rhesus monkeys (*in vivo*). Undiluted application to human skin samples at doses ranging from 15.4 to 154 µg/cm² resulted in 0–0.4% dermal absorption over 8 hours postapplication; dermal absorption of glyphosate from aqueous dilutions of test substance (1:20 or 1:32 test substance:water, v/v) during 16 hours postapplication was ≤2.2%. Twelve-hour *in vivo* application of the test substance diluted 1:29 with water at concentrations of 25 or 270 µg/cm² resulted in 7-day recovery of 0.8 and 2.2% of the applied dose, respectively, in the urine and 3.6 and 0.7%, respectively, in the feces. These results indicate that approximately 3–4% of the applied dose had been absorbed. An *in vitro* study using rat skin membranes, applied glyphosate formulations, concentration and field diluted, for 8 hours at concentrations of 6.249 and 0.08 mg/cm² for MON 35012 and 6.343 and 0.08 mg/cm² for MON 0139 (van Burgsteden 2002). Over a period of 48 hours, penetration of concentrates was higher, with 10% penetration of MON 35012 concentrate through rat skin membrane and 1.3% penetration of MON 0139 concentrate.

3.1.2 Distribution

3.1.2.1 Inhalation Exposure

No human or animal data were located regarding distribution of glyphosate following absorption via the inhalation exposure route.

3.1.2.2 Oral Exposure

Limited human data were located regarding distribution of glyphosate following absorption via the oral exposure route. Menkes et al. (1991) reported measurable glyphosate in kidney, liver, blood, and brain in postmortem examination of an individual who had ingested 200–250 mL of Roundup®.

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Following oral administration, absorbed glyphosate is readily distributed and rapidly eliminated without significant accumulation in any particular tissue. In male F344/N rats administered a single gavage dose of ^{14}C -glyphosate (purity 99%) in distilled water at 5.6 or 56 mg/kg, peak blood radioactivity occurred at 1 and 2 hours postdosing, respectively, mean peak blood concentration was 30-fold higher in the high-dose group (NTP 1992). Among rats gavaged at 5.6 mg radiolabeled glyphosate/kg and evaluated for tissue distribution, total tissue radioactivity amounted to approximately 12, 11.7, 5.5, 0.9, and 0.1% of the administered dose at 3, 6, 12, 24, and 96 hours postdosing, respectively. The highest radioactivity level was found in the small intestine, reaching a peak level of approximately 10% of the administered dose at 6 hours postdosing; radioactivity in the large intestine peaked at approximately 1.2% at 3 hours postdosing. Liver, kidney, skin, and blood each accounted for <1% of the administered dose at each time point. By 24 hours postdosing, <1% of the administered dose remained in all tissues combined. Brewster et al. (1991) administered ^{12}C - and ^{14}C -glyphosate by a single gavage dose at 10 mg/kg to male Sprague-Dawley rats and found approximately 34% of the administered dose in the small intestine (not associated with intestinal content) at 2 hours postdosing, decreasing to 0.05% of the administered dose by 96 hours postdosing. Radioactivity levels in most other tissues (blood, colon, kidney, liver, stomach, abdominal fat, testicular fat) peaked at 2–6 hours postdosing; each of these tissues accounted for $\leq 1.3\%$ of the administered dose at peak and $\leq 0.06\%$ by 96 hours postdosing. Radioactivity in bone peaked at 6 hours postdosing (4.7% of the administered dose) and remained at 1.7% at 96 hours postdosing. The tissue to blood ratio for bone increased with time suggesting a slower elimination from bone compared to blood. Anadón et al. (2009) reported an absorption half-life of 2.29 hours following administration of an oral dose of 400 mg glyphosate/kg to rats; an estimated peak plasma glyphosate level of 4.62 $\mu\text{g/mL}$ was reached at 5.16 hours postdosing.

3.1.2.3 Dermal Exposure

No human data were located regarding distribution following dermal exposure to glyphosate.

Limited animal data are available. The observation of radioactivity in urine and feces collected from rhesus monkeys following dermal application of a ^{14}C -labeled Roundup® formulation is demonstration of systemic distribution following dermal absorption (Wester et al. 1991). However, at sacrifice 7 days posttreatment, no radioactivity was detected in spleen, ovaries, kidney, brain, abdominal fat, bone marrow, upper spinal column, or central nervous system fluid.

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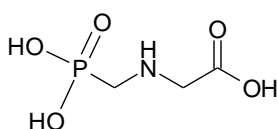
3.1.2.4 Other Routes of Exposure

Limited data are available regarding the distribution of parenterally administered glyphosate. Male and female Sprague-Dawley rats were administered ^{14}C -glyphosate via intraperitoneal injection at 1,150 mg/kg (EPA 1992h). Radioactivity measured in bone marrow samples taken 30 minutes postinjection amounted to approximately 0.0044 and 0.0075% of the administered activity for the males and females, respectively. Anadón et al. (2009) administered glyphosate (95% purity) to male Wistar rats via intravenous injection at 100 mg/kg. Plasma levels of glyphosate and its metabolite, AMPA, were measured using high-performance liquid chromatography (HPLC). Reported fast plasma distribution (half-life of 0.345 hours) and high volume of distribution at steady state (2.99 L/kg) were interpreted to indicate that glyphosate was extensively distributed to extravascular tissues.

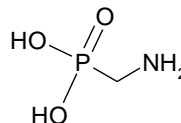
3.1.3 Metabolism

Glyphosate does not undergo significant metabolism in mammals. Available data are limited to the oral exposure route and indicate that ingested glyphosate is eliminated mostly as parent compound; only a small amount may be metabolized to AMPA. Figure 3-1 depicts the chemical structures of glyphosate and AMPA. In one human case of intentional ingestion of an herbicide in a suicide attempt, glyphosate and its metabolite, AMPA, were detected in serum and urine (Hori et al. 2003). At 16 hours postingestion, serum levels of glyphosate and AMPA were 4.4 and 0.03 $\mu\text{g/mL}$, respectively (147:1, glyphosate:AMPA). Total urinary excretion of glyphosate and its metabolite during 4 days postingestion was 3.7 g and 25 mg, respectively (148:1, glyphosate:AMPA).

Figure 3-1. Chemical Structures of Glyphosate and Aminomethylphosphonic Acid (AMPA)



Glyphosate



Aminomethylphosphonic acid (AMPA)

Results from available animal studies also indicate that very little ingested glyphosate is metabolized. Anadón et al. (2009) administered glyphosate (95% purity) to male Wistar rats by gavage at 400 mg glyphosate/kg. Plasma glyphosate peaked at 5.16 hours postdosing and measured 4.62 $\mu\text{g/mL}$; plasma AMPA peaked at 2.42 hours postdosing and measured 0.416 $\mu\text{g/mL}$. Based on the ratios between the

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area under the curve (AUC) for AMPA and the AUC for glyphosate, it was estimated that the metabolite represented 6.49% of the parent compound plasma concentration. In an unpublished study summarized by EPA (1993) and Williams et al. (2000), following oral administration of radiolabeled glyphosate (>99% purity) to Sprague-Dawley rats at 10 mg/kg, the glyphosate metabolite (AMPA) was detected in the urine (0.2–0.3% of the administered dose) and feces (0.2–0.4% of the administered dose). The formation of AMPA was thought to have occurred in the gastrointestinal tract (possibly by microflora) because AMPA was not detected in other rats administered glyphosate via intravenous injection. Following a single gavage dose of administered radiolabeled glyphosate (>99% purity) to Sprague-Dawley rats, expired air accounted for <0.27% of the administered radioactivity at 24 hours postdosing, indicating that glyphosate metabolism had occurred to a slight extent (EPA 1993).

In addition to its potential role in glyphosate metabolism, gut microflora can also be impacted by exposure to glyphosate and glyphosate-based herbicides. Though the shikimate pathway is absent in mammals, the shikimate pathway in animal gut microbes synthesizes amino acids (Aitbali et al. 2018; Nielsen et al. 2018). Rodents orally exposed to doses of glyphosate-based herbicides ranging from 5 to 500 mg/kg/d showed a significant decrease in bacteria count and changes in community composition (Aitbali et al. 2018; Dechartres et al. 2019). A study using fecal samples from Roundup®-exposed Sprague-Dawley rats also found changes in bacterial community composition (Lozano et al. 2018). Decreases in bacteria count were also observed in rodents orally exposed to 5 mg/kg/d of glyphosate technical (Aitbali et al. 2018). However, fecal samples from Sprague-Dawley rats orally exposed to doses of glyphosate or Glyfonova® found that while minimal changes in bacteria composition were observed, exposure to the glyphosate formulation appeared to have a more pronounced effect than exposure to glyphosate technical (Nielsen et al. 2018). While the implications of alterations in the gut microbiome are unclear, some animal studies suggest neurologic and behavioral changes (Aitbali et al. 2018; Dechartres et al. 2019; Lozano et al. 2018) and greater susceptibility to infection may be associated with loss of microbiome diversity, especially in cases of malnutrition (Nielsen et al. 2018; Shehata et al. 2013).

Ford et al. (2017) administered glyphosate to male C57BL/6 mice by intraperitoneal injection at 200 mg/kg/day for 7 days. Glyphosate treatment at this high dose level resulted in measurable levels of AMPA (approximately 4% of the dose of glyphosate) and an approximately 2-fold increase in hepatic glyoxylate (a reactive substance produced endogenously). Because glyoxylate is formed endogenously, the increase in glyoxylate level in the liver may be a result of glyphosate acting on mechanisms responsible for endogenous production of glyoxylate.

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3.1.4 Excretion**3.1.4.1 Inhalation Exposure**

Limited information is available regarding elimination and excretion of glyphosate in humans following inhalation exposure. In one study, urinary glyphosate levels were evaluated in 48 farmer-applicators prior to application of glyphosate-containing products, immediately following application, and for 3 days thereafter (Acquavella et al. 2004). Urinary glyphosate was detectable in 15% (7/47) of the farmers prior to application, in 60% (29/48) of the farmers immediately following application, and in only 27% (13/48) of the farmers on postapplication day 3. No information was located regarding elimination or excretion following inhalation exposure of laboratory animals to glyphosate.

3.1.4.2 Oral Exposure

Roberts et al. (2010) estimated a half-life of 3–4 hours for elimination of glyphosate from the blood of patients who had intentionally ingested large amounts of glyphosate-containing herbicide products. In other cases of poisoning victims, plasma glyphosate levels dropped rapidly (within 2–3 days) following the onset of observation (e.g., Talbot et al. 1991). Glyphosate has been detected in feces and urine of individuals who intentionally or accidentally ingested relatively large amounts of glyphosate.

Results from animal studies identify the feces and urine as major routes of elimination following oral exposure to glyphosate. For example, among male and female Sprague-Dawley rats administered ^{14}C -glyphosate (99% purity) via a single gavage dose at 10 mg/kg, during 7 days posttreatment, radioactivity recovered in the feces averaged 62.4 and 69.4% of the administered dose (males and females, respectively); another 28.6 and 22.5% of the administered dose (males and females, respectively) was recovered in the urine (IPCS 1994). Thus, feces and urine accounted for approximately 88–91% of the administered dose. HPLC analysis revealed that parent compound accounted for 98.5–99.3% of the radioactivity in feces and urine. There were no significant differences in fecal and urinary excretion among rats dosed with unlabeled glyphosate for 14 days followed by a single oral dose of radiolabeled glyphosate. Following a single gavage dosing of ^{14}C -glyphosate (>96% purity) to male and female Sprague-Dawley rats at 30 mg/kg, the feces accounted for 57–59% of the administered radioactivity and the urine accounted for 27–29% during the first 36 hours posttreatment; indicating that fecal and urinary excretion occur relatively rapidly following oral exposure to glyphosate (IPCS 1994). In male F344/N rats administered a single gavage dose of ^{14}C -glyphosate (purity 99%) in distilled water at 5.6 or 56 mg/kg, 72-hour collection of feces and urine resulted in the recovery of 91–92% of the administered

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radioactivity; 74 and 19%, respectively, at the low dose and 58 and 34%, respectively, at the high dose (NTP 1992). In one study (NTP 1992), male F344/N rats were administered a single dose of glyphosate at 5.6 mg/kg via intravenous injection, intraperitoneal injection, or oral (gavage) to compare 24-hour urinary and fecal elimination by these administration routes. Results from comparative studies of oral, intravenous, and intraperitoneal administration of glyphosate indicated that urinary radioactivity represented the amount of glyphosate absorbed and fecal radioactivity represented the amount of unabsorbed glyphosate following oral exposure. Although quantitative data were not included in the study report, the study authors estimated that 30–34% of the oral doses of ^{14}C -glyphosate was absorbed and excreted in the urine. Therefore, approximately 66–70% was unabsorbed and eliminated in the feces.

Very little ingested glyphosate is eliminated via routes other than feces and urine. Among Sprague-Dawley rats administered radiolabeled glyphosate (>99% purity) by a single gavage dose, <0.27% of the administered radioactivity was recovered in expired air at 24 hours postdosing (EPA 1993).

3.1.4.3 Dermal Exposure

No information was located regarding elimination or excretion following known dermal exposure to glyphosate in humans. However, in a study that evaluated urinary glyphosate levels in 48 farmer-applicators involved in application of glyphosate-containing products, mean urinary glyphosate was higher among those farmers (14/48) who did not use rubber gloves, indicating that some glyphosate had been absorbed through the skin (Acquavella et al. 2004). Limited information is available for laboratory animals. Wester et al. (1991) applied a ^{14}C -labeled Roundup® formulation to the abdominal skin of Rhesus monkeys (*in vivo*) to evaluate dermal absorption of glyphosate. Twelve-hour application of the test substance at concentrations of 25 or 270 $\mu\text{g}/\text{cm}^2$ resulted in 7-day recovery of 0.8 and 2.2% of the applied dose, respectively, in the urine and 3.6 and 0.7%, respectively, in the feces.

3.1.4.4 Other Routes of Exposure

Male and female Sprague-Dawley rats were administered ^{14}C -glyphosate via intraperitoneal injection at 1,150 mg/kg (EPA 1993). Assuming first-order kinetics, the half-life of elimination from the bone marrow was estimated at 7.6 and 4.2 hours for males and females, respectively. A half-life for elimination of radioactivity from plasma was approximately 1 hour for both sexes. These results indicate that glyphosate reaching the blood was rapidly eliminated and that the small fraction reaching bone marrow was rapidly eliminated. Anadón et al. (2009) reported a half-time of 9.99 hours for elimination of

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glyphosate from the blood of male Wistar rats administered glyphosate (95% purity) via intravenous injection at 100 mg/kg.

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 glyphosate were not located.

3.1.6 Animal-to-Human Extrapolations

No information was located to suggest significant differences between animals and humans regarding the toxicokinetics of glyphosate.

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.

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Populations at risk of exposure to glyphosate at unusually high levels are discussed in Section 5.7, Populations with Potentially High Exposures.

Limited information was located regarding possible age- or gender-related differences in susceptibility to toxic effects from glyphosate technical or glyphosate formulations. Mao et al. (2018) added glyphosate or Roundup Bioflow® to the drinking water of rat dams from GD 6 through lactation and to their offspring up to postpartum day 125 at a concentration resulting in a dose of 1.25 mg glyphosate/kg/day. Microbiome profiling of the gut resulted in significant changes in overall bacterial composition in the pups only (particularly apparent prior to puberty); this effect was noted for glyphosate and for Roundup Bioflow®. Romano et al. (2010) employed Roundup Transorb® as a test substance and found decreased serum testosterone in young male rats gavaged at a dose as low as 5 mg/kg/day; however, the effect may have been caused, at least in part, by other ingredients in the glyphosate formulation.

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 glyphosate 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 glyphosate 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

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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 formed by covalent bonding of a chemical to DNA, the formation of which can induce abnormal replication, mutation, and/or prevent proper DNA repair). Biomarkers of effect caused by glyphosate 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

Glyphosate and the metabolite, AMPA, have been measured in blood and urine (e.g., Connolly et al. 2018; Conrad et al. 2017; Mills et al. 2017; Soukup et al. 2020; Zhang et al. 2020; Zoller et al. 2020; Zouaoui et al. 2013). However, most absorbed glyphosate is rapidly excreted as parent compound, and urine is generally considered to be a stronger biomarker of exposure given that the level of detection (LOD) for glyphosate in urine is much lower than the LOD for glyphosate in blood (see Table 5-4). Meaningful quantification of exposure would require analysis of blood and/or urine within hours following exposure, though a case study on glyphosate poisoning reported plasma glyphosate levels over the course of 2 to 3 days, rather than hours (Talbot et al. 1991).

3.3.2 Biomarkers of Effect

No information was located regarding biomarkers of effect specific to glyphosate toxicity.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Information on the toxicological effects of glyphosate interacting with other chemicals such as inert ingredients is limited. Inert ingredients include other substances that alter the physico-chemical properties to improve plant absorption or stability of the active ingredient(s) (Defarge et al. 2016). Inert ingredients include surfactants. Surfactants such as polyethoxylated tallow amine (POEA) in glyphosate-containing products might enhance the toxicity of glyphosate; results from one study indicate that the surfactant may be more acutely toxic than glyphosate or the combination of glyphosate and POEA (e.g., Adam et al. 1997). In an *in vitro* study using three human embryonic cell lines (kidney, liver, and placenta), glyphosate

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formulations and formulation additives were found to be more toxic than glyphosate technical alone. Based on measured cytotoxicity evaluated via mitochondrial respiration, membrane disruption, and caspases 3/7 activity, the glyphosate formulations were more toxic than glyphosate alone, and the adjuvants POE-15 and Genamin were more toxic than either the glyphosate formulations or glyphosate alone (Mesnage et al. 2013).

Increased toxicity of glyphosate formulations compared to glyphosate technical was also observed in another *in vitro* study. Defarge et al. (2016) observed *in vitro* that co-formulants including polyethoxylated tallow amine (POEA), alkyl polyglucoside (APG), polyoxyethylenealkyl ether phosphate (POE-APE), quaternary ammonium compound (QAC) and glyphosate formulations (inert and glyphosate combined) to exert toxic effects far greater than glyphosate alone. Cytotoxicity was determined by measuring mitochondrial respiration and endocrine disrupting activity was measured by aromatase activity inhibition. Although, Defarge et al. did not find that glyphosate inhibited mitochondrial respiration, disturbed cell membranes, or disrupted endocrine activity, co-formulants APG and POAE were up to 18 times and 200 times more cytotoxic than glyphosate, respectively, and co-formulants and glyphosate-based herbicide formulations were cytotoxic at concentration 18-2000 times and 8-141 times lower, than the recommended agricultural dilution of 1%, respectively. In short, co-formulants POEA, APG, POE-APE, QAC and glyphosate formulation including R classic were found to be more toxic than the active ingredient glyphosate.