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

3.1 TOXICOKINETICS

- Tribufos is readily absorbed following oral or dermal exposure. Inhaled tribufos is absorbed through the lung, although quantitative data are not available.
- Absorbed tribufos is widely distributed.
- Tribufos undergoes oxidation, hydrolysis, and conjugation reactions in mammals. A reactive sulfoxide intermediate is involved when enzymes and a reducing agent are present.
- Tribufos and its metabolites are rapidly excreted, predominantly in urine.

No information was located regarding the toxicokinetics of tribufos in humans. CalEPA (2004) reviewed both publicly-available and unpublished animal studies that assessed the toxicokinetics of tribufos. The following information was summarized using results from publicly-available studies (Hur et al. 1992; Sahali et al. 1994; Wing et al. 1984), EPA DERs (EPA 2000b), and the CalEPA (2004) review.

3.1.1 Absorption

No studies were located regarding the extent of absorption following inhalation of tribufos. However, findings of decreased RBC and brain AChE activity and clinical signs of neurotoxicity in rats following nose-only or head-only exposure to tribufos aerosol is confirmation that inhaled tribufos is absorbed from the lung (EPA 1991a, 1992a, 1992b). Absorption is rapid and extensive following oral exposure to tribufos. Among rats administered ^{14}C-tribufos by gavage once at 5 or 100 mg/kg or for 14 days at 5 mg/kg/day, approximately 55–80% of the administered radioactivity was recovered in the urine within 24 hours postdosing, indicating that extensive absorption from the gastrointestinal tract had occurred (CalEPA 2004).

The extent of absorption following dermal exposure to tribufos is species- and dose-dependent. Following dermal application of ^{14}C-tribufos to rats for 10 hours at doses of 1.93, 12.4, or 100 μg/cm^2, radioactivity excreted in the 7-day urine accounted for approximately 26% (high-dose) and 36% (low-dose) of the administered dose; the feces accounted for 3.2–3.6% of the administered dose (CalEPA 2004). Mean dermal absorption rates of 47.5, 47.9, and 33.9% were calculated for low-, mid-, and high-dose groups, respectively. Following a single 8-hour dermal application of ^{14}C-tribufos to male rhesus monkeys at 3.5 μg/cm^2, the mean absorbed dose was reported to be 6.96% of the administered dose; a total of 6.24% of the administered radioactivity was recovered in the urine (mostly within 72 hours postadministration); and 0.72% was recovered in the feces (CalEPA 2004; EPA 2000b).

3.1.2 Distribution

No information was located regarding distribution following inhalation exposure of humans or animals to tribufos. However, findings of decreased RBC and brain AChE activity and clinical signs of neurotoxicity in rats following nose-only or head-only exposure to tribufos aerosol (EPA 1991a, 1992a, 1992b) is confirmation of absorption and distribution.

Oral administration of tribufos has been demonstrated to result in rapid distribution and elimination in rats. Following gavage administration of ^{14}C-tribufos to rats for 3 days at 5 or 100 mg/kg/day, <3% of the administered radioactivity was detected in the tissue and carcass at 72 hours postadministration; the liver contained the highest amount, followed by fat, lung, kidney, blood, gastrointestinal tract, spleen, bone, heart, gonads, muscle, and brain (CalEPA 2004). Following 3 consecutive daily administrations of encapsulated ^{14}C-tribufos to a lactating goat at 0.82 mg/kg/day (approximately 25 times the maximum tribufos residue level anticipated in animal feed), radioactivity was detected in liver (3.45 ppm), kidney (0.35 ppm), fat (0.19 ppm), muscle, (0.06 ppm), and milk (0.12 ppm), indicating relatively widespread distribution (Sahali et al. 1994).

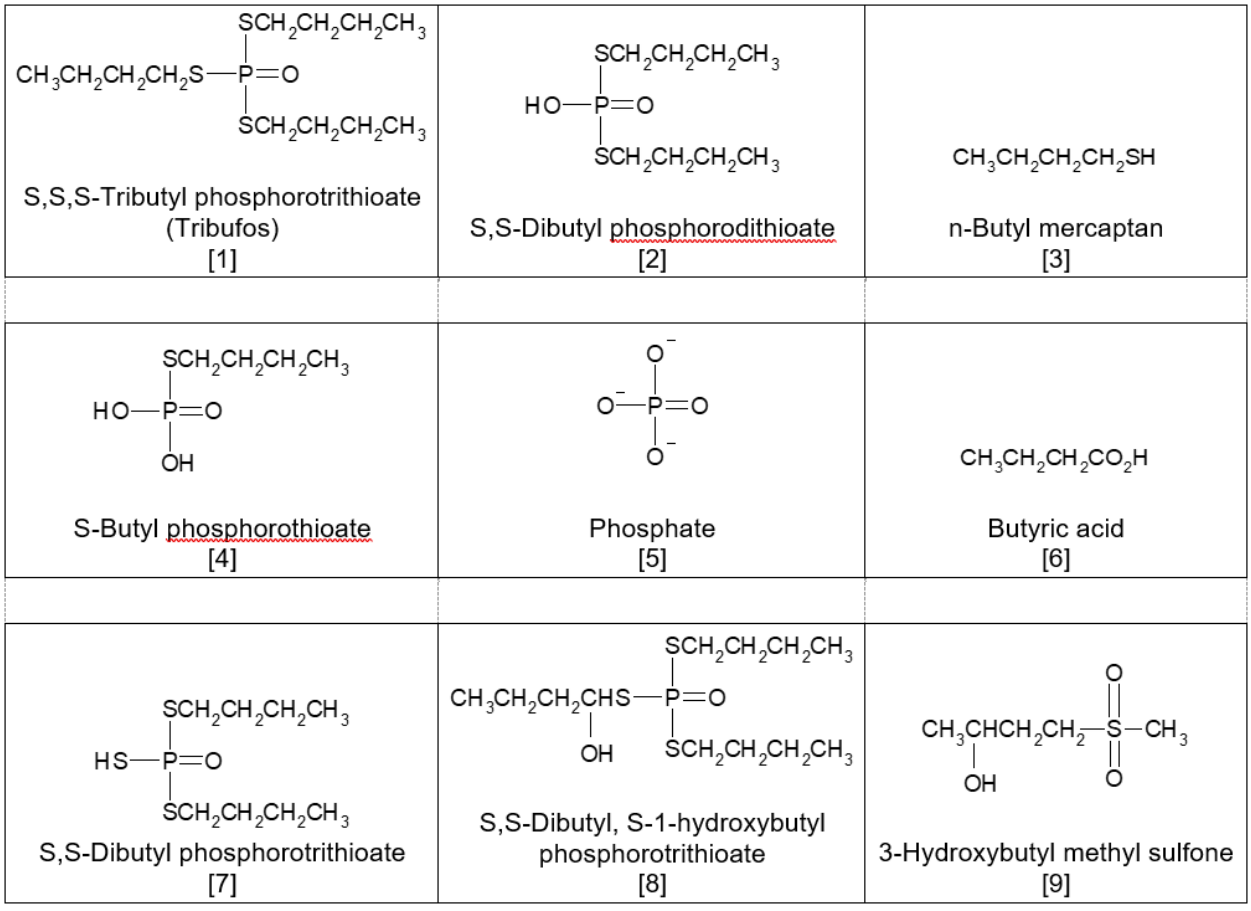
No information was located regarding distribution following dermal exposure of humans to tribufos. However, detection of radioactivity in the urine and feces of rats and monkeys following dermal application of ^{14}C-tribufos is confirmation of absorption and distribution (CalEPA 2004; EPA 2000b).

3.1.3 Metabolism

Metabolism of tribufos in animal systems has been studied both *in vivo* (Abou-Donia 1979; CalEPA 2004; Fujioka and Casida 2007; Hur et al. 1992; Sahali et al. 1994) and *in vitro* (Fujioka and Casida 2007; Hur et al. 1992; Levi and Hodgson 1985; Wing et al. 1983, 1984). Chemical structures for tribufos and selected metabolites (identified or proposed) are depicted in Figure 3-1. Numbers for each chemical are identified by bracketed numbers in the figure and following text; proposed metabolites are presented in brackets. Tribufos [1] can undergo hydrolysis at one of its SulfurPhosphorus (SP) bonds to form S,S-dibutyl phosphorodithioate [2] and n-butyl mercaptan [3]. This step may involve initial oxidation to an active sulfoxide intermediate. S,S-Dibutyl phosphorodithioate [2] can undergo hydrolysis at one of its SP bonds to form S-butyl phosphorothioate [4] and n-butyl mercaptan [3]. S-Butyl phosphorothioate [4]

can be further hydrolyzed to form phosphate [5] and n-butyl mercaptan [3]. S,S-Dibutyl phosphoro-dithioate [2] and its glutathione conjugate have been detected in liver extracts from mice following intraperitoneal injection of tribufos (Fujioka and Casida 2007). S,S-Dibutyl phosphorodithioate [2] was a major metabolite in urine from rats following intraperitoneal injection of tribufos; S,S-dibutyl phosphoro-dithioate [2] was also a product of *in vitro* incubation of tribufos with mouse liver microsomes (Hur et al. 1992). S,S-Dibutyl phosphorodithioate [2] and S-butyl phosphorothioate [3] were detected as minor urinary metabolites following oral administration of tribufos to a lactating goat (Sahali et al. 1994). Although n-butyl mercaptan [3] has not been detected *in vivo* as a tribufos metabolite in mammals, its glutathione conjugate was identified in liver extracts from tribufos-treated mice (Fujioka and Casida 2007) and in the urine from a tribufos-treated goat (Sahali et al. 1994). n-Butyl mercaptan [3] was also detected in the excreta of hens administered an oral dose of tribufos (Abou-Donia 1979). Phosphate [5] was found as the major phosphorus compound in the urine of tribufos-treated rats (Hur et al. 1992).

Figure 3-1. Chemical Structures for Tribufos and Selected Metabolites



n-Butyl mercaptan [3] can be converted to butyric acid [6], which undergoes fatty acid catabolism to form other fatty acids, lipids, and amino acids. n-Butyl mercaptan [3] can also react with other endogenous substances such as proteins, cysteine, and other endogenous thiols. Tribufos is extensively metabolized, as demonstrated by the detection of 17 unidentified metabolites in the urine of tribufos-treated rats (CalEPA 2004) and numerous mainly unidentified metabolites in the liver, urine, tissue, and milk from a tribufos-treated goat (Sahali et al. 1994).

Other tribufos metabolites have been identified. S,S-Dibutyl phosphorotrithioate [7] was detected as a minor metabolite in liver extracts from tribufos-treated mice (Fujioka and Casida 2007), a major metabolite in urine from tribufos-treated rats (Hur et al. 1992), and a major metabolite of tribufos oxidative metabolism in a mouse liver microsome-NADPH system *in vitro* (Hur et al. 1992). It was suggested that S,S-dibutyl phosphorotrithioate [7] may form via mixed function oxidase-mediated oxidation of tribufos to a reactive intermediate such as S,S-dibutyl, S-1 hydroxybutyl phosphoro-trithioate [8] and its subsequent conversion (Hur et al. 1992). Sahali et al. (1994) also identified 3-hydroxybutylmethyl sulfone [9] as a major metabolite in tissue, milk, and urine; its glucuronide conjugate in urine; and its sulfate conjugate in urine and kidney from a tribufos-treated lactating goat.

Findings that tribufos-induced AChE inhibition *in vitro* could be dramatically increased in the presence of microsomal oxidase activation systems and NADPH (Levi and Hodgson 1985; Wing et al. 1984) suggest that an initial step in tribufos metabolism *in vivo* may be its oxidation to a more reactive sulfoxide. Hur et al. (1992) and Fujioka and Casida (2007) proposed such a step based on results obtained from rats; Sahali et al. (1994) proposed a similar step based on results from a lactating goat.

Merphos (tributyl phosphorotrithioite) is a plant defoliant that is readily transformed in the environment to tribufos (tributyl phosphorotrithioate). Therefore, workers who use merphos would likely be exposed to tribufos as well.

3.1.4 Excretion

No information was located regarding the extent of elimination and excretion following inhalation exposure to tribufos. Following single oral dosing of rats with ^{14}C-tribufos at 5 mg/kg, as much as 95–98% of the radioactivity was recovered in the urine and feces during 72 hours postdosing (CalEPA 2004). Recoveries in the urine were 55% for males and 66% for females; recoveries in the feces were 42% for males and 30% for females. Relatively similar results were obtained following single gavage dosing at

100 mg/kg. Repeated gavage dosing at 5 mg/kg/day for 14 consecutive days resulted in a higher percentage of radioactivity in the urine (73% for males and 80% for females) and a lower percentage of radioactivity in the feces (24% for males and 15% for females). Only 1% of the administered radioactivity was recovered in expired air.

As stated previously in Section 3.1.1, during 7 days following a 10-hour dermal application of ^{14}C-tribufos to rats, the urine and feces accounted for 26–36 and 3.2–3.6%, respectively, of the administered radioactivity (CalEPA 2004). Following 8-hour dermal application of ^{14}C-tribufos to rhesus monkeys, the urine and feces accounted for 6.24 and 0.72%, respectively, of the administered radioactivity, mostly recovered within 72 hours postadministration (CalEPA 2004; EPA 2000b).

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 are available for tribufos.

3.1.6 Animal-to-Human Extrapolations

The general pharmacokinetic behavior of tribufos is expected to be similar in humans and laboratory animals. Following oral exposure, tribufos is rapidly absorbed, widely distributed, and metabolized to at least one reactive intermediate and other metabolites, which are primarily quickly eliminated in the urine (see Section 3.1.4). Although animals and humans share these similarities, potential differences in pharmacokinetic behavior and biotransformation in blood and target tissues, particularly at toxic levels, have not been extensively studied. Mice and rats are generally more resistant than humans to toxicity of organophosphorus compounds such as tribufos, in part because mice and rats have relatively higher levels

of circulating carboxylesterases (enzymes that metabolize organophosphorus compounds) (Pereira et al. 2014). Therefore, extrapolation from animals to humans includes an appreciable degree of uncertainty.

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

Although data exist regarding age-related susceptibility to selected organophosphorus compounds, no information was located regarding potential age-related differences in susceptibility to tribufos toxicity in humans. Results from acute-duration oral studies in rats indicate that neonates may be more sensitive than adults to tribufos neurotoxicity, as assessed by clinical signs. Single gavage dosing of 11-day-old Sprague-Dawley rat pups resulted in decreased movement at 10 mg/kg and additional clinical signs (unsteadiness, incoordination, and/or body tremors) at 40–50 mg/kg (EPA 2012a, 2012b, 2012d). Repeated dosing at 5 mg/kg resulted in decreased movement, unsteadiness, and prostration, as well as 20–21% decreased brain AChE activity (EPA 2012e). Repeated dosing at ≥10 mg/kg/day resulted in decreased movement, unsteadiness, and hind limb splay (EPA 2012a). No cageside signs of neurotoxicity were seen in young adult female Sprague-Dawley rats administered tribufos by gavage once at up to 80 mg/kg (EPA 2012c, 2012d) or for 11 days at up to 5 mg/kg/day (EPA 2012e). There was no effect on brain AChE activity among the young adult female rats dosed for 11 days at 5 mg/kg/day (EPA 2012e)

Studies on experimental animals showed that starvation depressed liver microsomal enzyme (P-450) activity due to actual loss of the enzyme protein (Boyd and Carsky 1969). Thus, dietary deficiency could potentially alter tribufos toxicity by diminishing its metabolism in the liver. Hereditary factors may also contribute to population sensitivity to tribufos. A small percentage of the population is affected by plasma cholinesterase (ChE) deficiency, an inherited condition in which plasma ChE (also known as butyrylcholinesterase [BuChE] or pseudocholinesterase) activity is lower than normal. Plasma ChE is a nonspecific cholinesterase enzyme that hydrolyzes many different choline-based esters. ChE deficiency results in delayed metabolism of selected xenobiotics (e.g., succinylcholine, mivacurium, procaine, heroin, cocaine). Since plasma ChE is strongly inhibited by tribufos (Astroff et al. 1998; EPA 1990b, 1992c), it is expected that individuals with ChE deficiency will be unusually sensitive to these xenobiotics. Congenital low plasma ChE activity may also increase subpopulation sensitivity to tribufos exposure. In ChE-deficient individuals, less tribufos would be bound in the blood and more unbound tribufos would be circulated to targets of tribufos toxicity. Ueyama et al. (2007) demonstrated significantly increased plasma ChE and RBC and brain AChE inhibition in streptozotocin-induced diabetic rats compared to normal rats, an indication that diabetics may be more susceptible to organophosphate-induced neurotoxicity.

Gender-specific differences in sensitivity to treatment-related cognitive deficits have been observed in rats, mice, and guinea pigs exposed to the organophosphorus pesticide, chlorpyrifos (Aldridge et al. 2005; Johnson et al. 2009; Levin et al. 2001; Mamczarz et al. 2016). Although similar gender-specific differences have not been reported for tribufos, it is one of a number of organophosphorus pesticides that share similarities in toxicity profiles.

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 tribufos 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 tribufos 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 tribufos 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

There are no known biomarkers of exposure specific to tribufos. Results from a rat study indicate that orally-administered tribufos is rapidly distributed, highly metabolized, and rapidly eliminated mainly as numerous mostly unidentified metabolites in the urine, and to a lesser extent, in the feces (CalEPA 2004). Some 18 radioactive tribufos metabolites were detected in urine of rats treated with radiolabeled tribufos; however, only butyl-gamma-glutamylcysteinylglycine disulfide was identifiable (CalEPA 2004). It is not likely that tribufos metabolites would serve as reliable indicators of exposure to tribufos.

3.3.2 Biomarkers of Effect

Exposure to very high levels of tribufos could result in excessive sweating, constricted pupils, unconsciousness, and difficulty with breathing. However, these effects are common to many organophosphorus compounds and carbamate pesticides and are not specific to tribufos. Decreased

activities of the enzymes BuChE, AChE, and/or NTE in blood serve as biomarkers of effect from exposure to substances (including tribufos) that inhibit these enzymes. However, decreased activity of these enzymes is not a biomarker specific to tribufos. Due to high interindividual variability in "normal" BuChE activity in the blood, repeat measurements of BuChE activity may be necessary to determine whether activity increases over time postexposure.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Tribufos is one of many organophosphorus compounds that inhibit AChE. Significant occupational exposure to tribufos could occur in workers who are exposed to other similarly-acting compounds. Neurotoxic effects in such individuals would be the result of a variety of factors, including cumulative dose, relative potency of each individual compound, and potential synergistic and/or antagonistic interactions.

Although no studies were located that specifically assessed dermal absorption of tribufos in the presence of other chemicals, it is reasonable to assume that some substances (e.g., solvents, etc.) might influence the rate and extent of absorption of AChE-inhibiting organophosphorus compounds (such as tribufos) upon dermal contact.

A variety of chemicals may interfere with the toxicity of tribufos indirectly by influencing its metabolism through their actions on drug metabolizing enzymes involved in hydrolysis, reduction, oxidation, and/or conjugation of xenobiotics (Parkinson and Ogilvie 2008). The duration and intensity of action of tribufos are largely determined by the speed at which it is metabolized in the body by oxidative and hydrolytic liver enzymes. Numerous drugs, insecticides, carcinogens, and other chemicals are known to induce the activity of liver microsomal drug-metabolizing enzymes. Thus, exposure to enzyme inducers concurrent with or after exposure to tribufos may result in accelerated bioactivation to a potentially more potent anticholinesterase metabolite. The extent of toxicity mediated by this phenomenon would depend on the rate at which tribufos and/or a potentially more potent metabolite would be hydrolyzed to less toxic metabolites, a process that is also accelerated by enzyme induction. Similarly, concurrent exposure to tribufos and mixed-function oxidase (MFO) enzyme-inhibiting substances may increase the toxicity of tribufos by decreasing the rate of hydrolytic dealkylation and hydrolysis. The balance between activation and detoxification determines the biological significance of these chemical interactions with tribufos.