CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of tribufos. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to tribufos, but may not be inclusive of the entire body of literature.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2. Animal oral studies are presented in Table 2-2 and Figure 2-3. Animal dermal studies are presented in Table 2-3.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the

Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of tribufos are indicated in Table 2-2 and Figure 2-3.

A User's Guide has been provided at the end of this profile (see Appendix C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

As an organophosphorus compound, tribufos inhibits the action of acetylcholinesterase (AChE), resulting in muscarinic cholinergic effects (e.g., glandular secretions [salivation, lacrimation, rhinitis], miosis, bronchoconstriction, vasodilation, hypotension, diarrhea, nausea, vomiting, urinary incontinence, and bradycardia) and nicotinic cholinergic effects (e.g., tachycardia, mydriasis, fasciculations, cramping, twitching, muscle weakness, and muscle paralysis). Central nervous system toxicity includes respiratory depression, anxiety, insomnia, headache, apathy, drowsiness, dizziness, loss of concentration, confusion, tremors, convulsions, and coma. These effects usually occur within a few minutes to 24 hours after dosing.

In addition to its presence and function in central and peripheral nervous tissue, AChE is also expressed in RBCs (Silman and Sussman 2005). According to Chou and Williams-Johnson (1998), a 20–59% inhibition of neural or RBC AChE (i.e., 20–59% decrease in AChE activity) may be considered a less serious effect in the absence of more serious indicators of neurotoxicity. A ≥60% inhibition of neural or RBC AChE is considered a serious effect in the presence or absence of additional signs of neurotoxicity. However, the degree of RBC AChE inhibition does not always correlate with the severity of acute signs of organophosphorus toxicity, especially with respect to chronic exposure scenarios.

The health effects of tribufos have been evaluated mainly in unpublished animal studies. As illustrated in Figure 2-1, the oral exposure route was employed in the majority of animal studies. The most examined endpoints in animal studies were body weight (68% of the animal studies) and neurotoxicity (72% of the animal studies).

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Animal studies suggest that the nervous system is the most sensitive target of tribufos toxicity. Other relatively sensitive targets following oral exposure include the blood and gastrointestinal tract.

- **Neurological effects.** Inhalation exposure of experimental animals to tribufos aerosol has been associated with clinical signs of neurological effects (e.g., altered gait, decreased movement, constricted pupils, piloerection, aggressive behavior, sensitivity to touch, convulsions, salivation), decreased RBC and brain AChE activity, and depressed amplitude of a- and b-waves in electroretinographic tests. Decreased RBC and/or brain AChE activity has been associated with oral exposure as well.

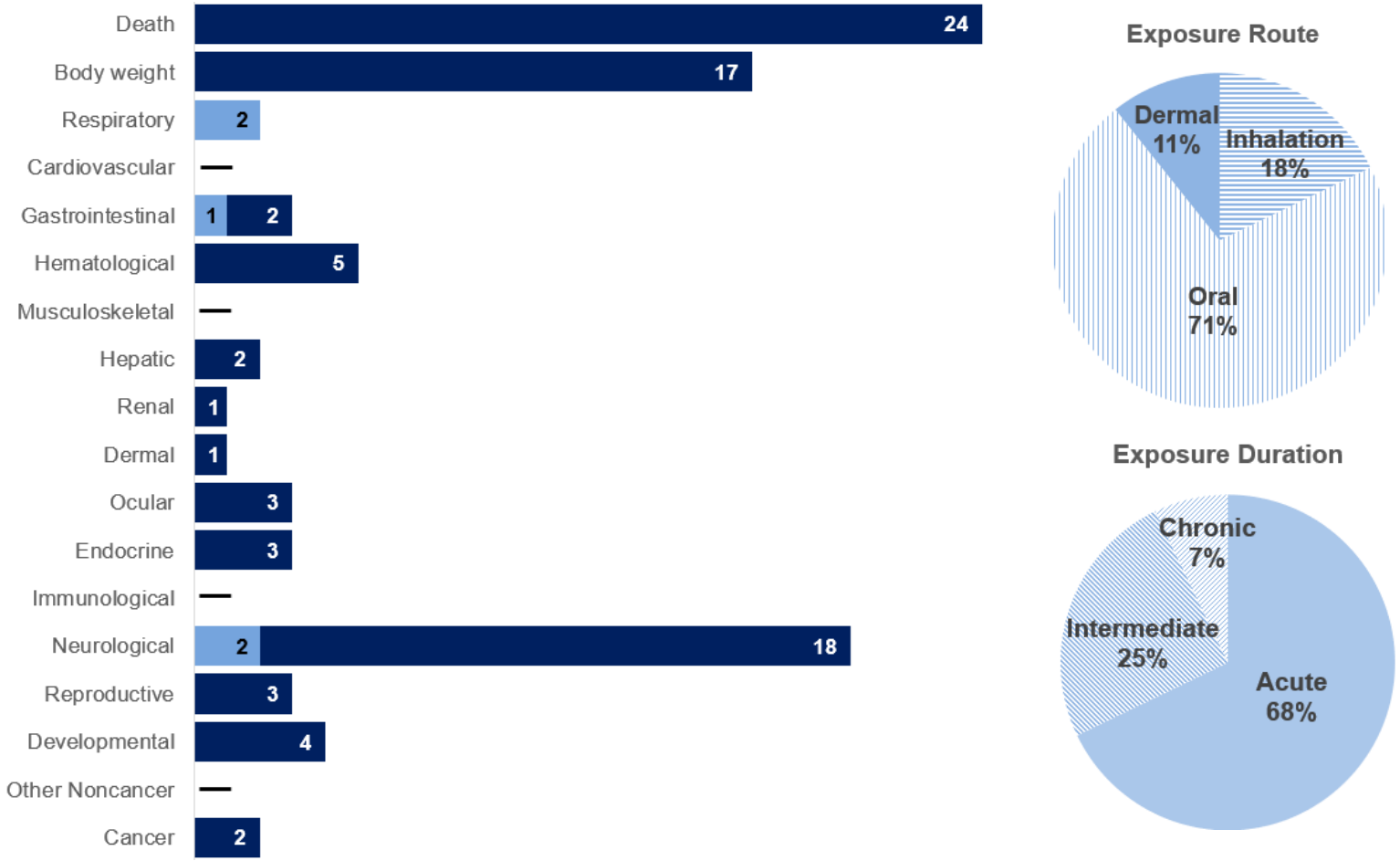
- **Hematological effects.** Decreases in RBC counts, hemoglobin, and hematocrit have been observed following oral exposure of experimental animals to tribufos.

- **Gastrointestinal effects.** Histopathologic gastrointestinal tract lesions have been associated with oral exposure of experimental animals to tribufos.

Figure 2-1. Overview of the Number of Studies Examining Tribufos Health Effects

Most studies examined the potential body weight and neurological effects of tribufos

Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 28 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to Tribufos – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effects
ACUTE EXPOSURE									
1	Rat (Sprague-Dawley) 6 M, 6 F	One 4-hour exposure (nose-only)	M: 0, 2,920, 5,690, 6,030 F: 0, 1,590, 2,920, 3,190	BW, CS, GN, LE	Death			4,650 M 2,460 F	4-hour LC_{50}
EPA 1991a, 1992a									
INTERMEDIATE EXPOSURE									
2	Rat (Wistar) 10 M, 10 F	13 weeks 5 days/week 6 hours/day (head-only)	0, 0.93, 2.43, 12.2, 59.5	BH, BW, CS, EA, GN, HE, HP, LE, OP, OW, UR	Bd wt	59.5			
					Hemato	59.5			
					Hepatic	59.5			
					Renal	59.5			
					Ocular	59.5			
					Endocr	12.2 M 59.5 F	59.5 M		Increased adrenal weight and cortical fat deposition
					Neuro	2.43[b]		12.2	Up to 65% decreased RBC AChE activity
EPA 1992b									

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 0.04 mg/m^3; based on a NOAEL of 2.43 mg/m^3, adjustment for intermittent exposure, conversion to a human equivalent concentration, and a total uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability); see Appendix A for more detailed information regarding the MRL.

AChE = acetyl cholinesterase; Bd wt or BW = body weight; BH = behavioral; CS = clinical signs; EA = enzyme activity; Endocr = endocrine; F = female(s); GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; LC_{50} = lethal concentration, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; OP = ophthalmology; OW = organ weight; RBC = red blood cell; UR = urinalysis

Figure 2-2. Levels of Significant Exposure to Tribufos – Inhalation

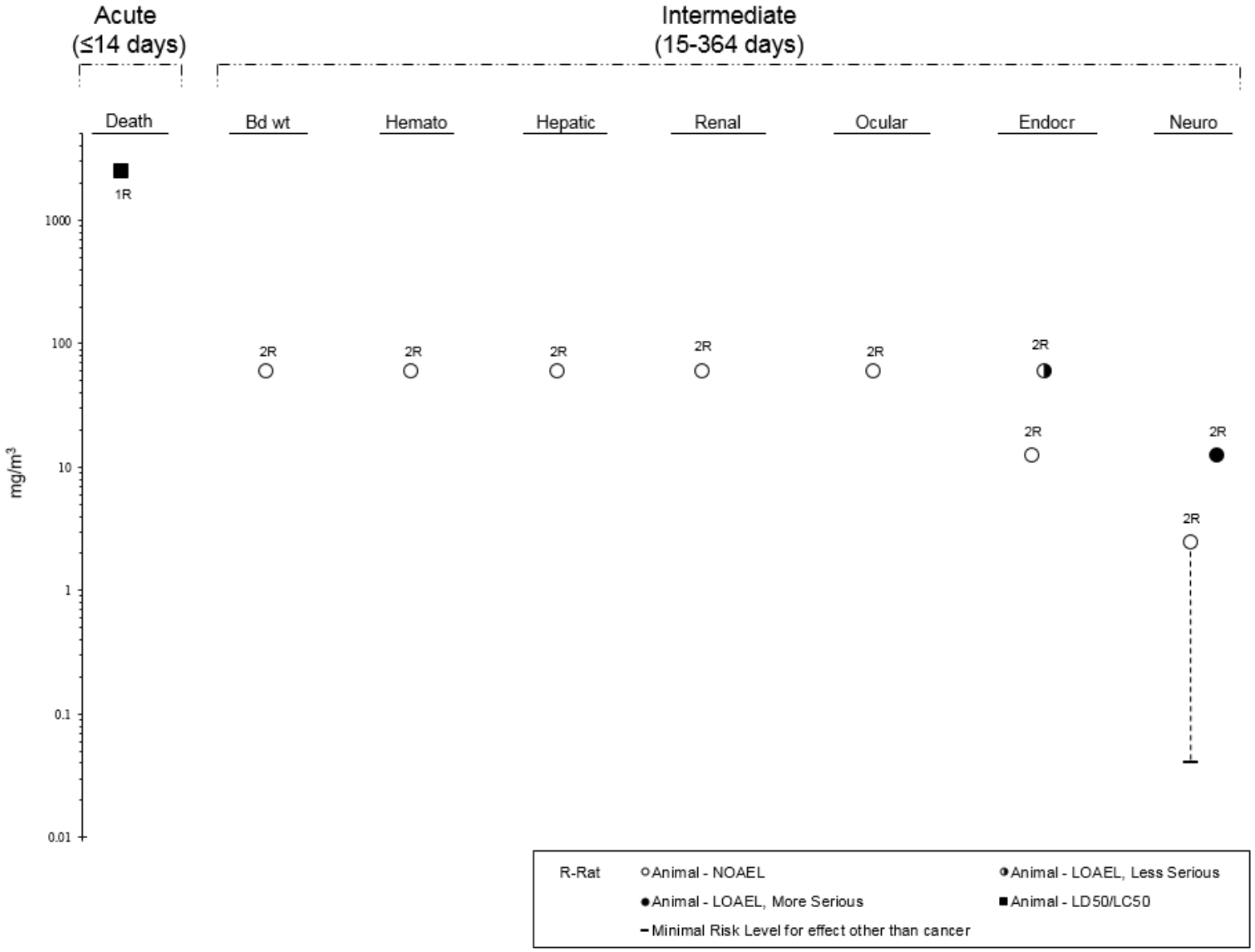


Table 2-2. Levels of Significant Exposure to Tribufos – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
1	Rat (Sprague-Dawley) 33 F	GDs 6–15 1 time/day (G)	0, 1, 7, 28	BW, CS, DX, EA, FI, FX, GN, LE, MX, OW, TG	Bd wt	28			
					Neuro	1		7	71% decreased RBC AChE activity in pregnant rats on GD 16
					Develop	28			
Astroff and Young 1998; EPA 1990b									
2	Rat (NS) 5 M, 5 F	Once (GO)	M: 294, 429, 552 F: 192, 235, 294	BW, CS, GN, LE	Death			435 M 234 F	LD_{50}
EPA 1993a									
3	Rat (Sprague-Dawley; 11-day-old pups) 3–4 M, 3–4 F	Once (GO)	0, 20, 40, 50	BW, CS, EA, GN, LE, OW	Neuro		20 M	20 F	M: 59% decreased RBC AChE activity F: 71% decreased RBC AChE activity
EPA 2012a									
4	Rat (Sprague-Dawley; 11-day-old pups) 3–4 M, 3–4 F	Up to 11 days 1 time/day (GO)	0, 5, 10, 15, 20	BW, CS, EA	Bd wt	5	10		>10% depressed mean body weight
					Neuro		5		36–49% decreased RBC AChE activity
EPA 2012a									

Table 2-2. Levels of Significant Exposure to Tribufos – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
5	Rat (Sprague-Dawley; 11-day-old pups) 2–4/sex at scheduled sacrifice 4, 6, 8, 24, or 48 hours postdosing	Once (GO)	0, 50	BW, CS, EA, GN, LE, OW	Bd wt			50	Body weight loss at 24 and 48 hours postdosing
					Neuro			50	79-92% decreased RBC AChE activity during 48 hours postdosing
EPA 2012b									
6	Rat (Sprague-Dawley; young adults) 24 F	Once (GO)	0, 80	BW, CS, EA, GN, LE, OW	Neuro			80	69–90% decreased RBC AChE activity at 8–48 hours postdosing
EPA 2012c									
7	Rat (Sprague-Dawley; 11-day-old pups) 8 M, 8 F	Once (GO)	0, 2, 10, 50	BW, CS, EA, GN, LE, OW	Neuro	2 M	10 M 2 F		M: 47% decreased RBC AChE activity F: 27% decreased RBC AChE activity
EPA 2012d									
8	Rat (Sprague-Dawley; young adults) 8 F	Once (GO)	0, 2, 10, 80	BW, CS, EA, GN, LE, OW	Neuro	10		80	74% decreased RBC AChE activity
EPA 2012d									

Table 2-2. Levels of Significant Exposure to Tribufos – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
9	Rat (Sprague-Dawley; 11-day-old pups) 8 M, 8 F	11 days 1 time/day (GO)	0, 0.1, 1, 5	BW, CS, EA, GN, LE, OW	Neuro	1		5	66–69% decreased RBC AChE activity
EPA 2012e									
10	Rat (Sprague-Dawley; young adults) 8 F	11 days 1 time/day (GO)	0, 0.1, 1, 5	BW, CS, EA, GN, LE, OW	Neuro	1		5	64% decreased RBC AChE activity at 24 hours postdosing
EPA 2012e									
11	Rat (Sprague-Dawley) 10 F	GDs 6–19 1 time/day (GO)	0, 0.3-0.8, 7, 28	BW, CS, DX, EA, FI, GN, LE	Bd wt	7		28	27% depressed mean maternal body weight gain
					Neuro	0.3		7	75% decreased maternal RBC AChE activity
					Develop	7	28		6% lower mean fetal body weight in male fetuses; concomitant 27% depressed mean body weight gain in dams
EPA 2012f									
12	Rat (Sherman); unspecified numbers/sex/ group	Once (GO)	NS	LE	Death			233 M 150 F	LD_{50}
Gaines 1969									

Table 2-2. Levels of Significant Exposure to Tribufos – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
13	Rabbit (American Dutch) 17 F	GDs 7–19 1 time/day (G)	0, 1, 3, 9	BW, CS, DX, EA, FI, FX, GN, LE, MX, OW, TG	Bd wt	3	9		No maternal body weight gain versus 5% body weight gain among controls
					Neuro			1	70% decreased maternal RBC AChE activity on GD 20
					Repro	9			
					Develop	9			
EPA 1990c									
INTERMEDIATE EXPOSURE									
14	Rat (Sprague-Dawley) 30 M, 30 F per generation	2 generations (F) 10 weeks premating, mating up to 28 days, 3 weeks of gestation, 3 weeks of lactation	M: 0, 0.28, 2.0–2.9, 17.6–20.63 F: 0, 0.27–0.81, 2.03–6.77, 18.07–49.61	BW, CS, DX, EA, FI, FX, GN, HP, LE, MX, TG	Bd wt	17.6 M 18.07 F			No effect at highest dietary level; calculated dose ranges for F0 and F1 parental rats; include separately-calculated doses to females for premating, gestation, and lactation phases
					Neuro	0.28[b] M 0.31 F	2 M 2.25 F		RBC AChE activity decreased by 35 and 37% in F0 males and females, respectively, in pre-mating phase
					Repro	17.6 M 18.07 F			Lowest dose in a particular range considered a NOAEL

Table 2-2. Levels of Significant Exposure to Tribufos – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Develop	2.0 M 2.03 F		17.6 M 18.07 F	Decreases in number of live pups born, litter size, pup viability, lactational pup body weights, number of live pups on LD 21; NOAEL, serious LOAEL values represented by lowest dose in range for F0 and F1 parental exposure
Astroff et al. 1998; EPA 1992c									
15	Rat (Fischer 344) 50 M, 50 F	Up to 2 years (F)	M: 0, 0.2, 1.8, 16.8 F: 0, 0.2, 2.3, 21.1	BC, BW, CS, EA, FI, GN, HE, HP, LE, OP, OW	Hemato	0.2 M 0.2 F	1.8 M 2.3 F		Decreases in RBC count, hemoglobin, and hematocrit in mid- and high-dose groups at 3- and 6-month evaluations
CalEPA 2004; EPA 1992d									
16	Rat (Wistar) 30 F	42 days GD 0–LD 21 (F)	Gestation: 0, 0.4, 3.4–3.5, 16.4–18.2 Lactation: 0, 0.6–1.0, 6.1–9.9, 33.5–55.4	BW, CS, DX, EA, FI, OF, OW	Bd wt	6.1	33.5		8–12% lower mean maternal body weight during lactation
					Neuro	0.4		3.4	76% decreased RBC AChE activity (lowest dose in range for gestation listed as serious LOAEL)
					Repro	16.4			Lowest dose in range for gestation considered a NOAEL

Table 2-2. Levels of Significant Exposure to Tribufos – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Develop	3.4		16.4	16–23% depressed lactational pup body weight, delays in preputial separation and development of righting reflex, decreased locomotor and motor activity at PND 13, increased motor activity at PND 17, decreased auditory startle amplitude at PND 22
EPA 2005a									
17	Rat (Han Wistar) 10 F	4 weeks (F)	0, 0.43, 4.32, 44.62	BW, CS, EA, FI, GN, LE, OF, OW, WI	Bd wt	4.32		44.62	80% depressed mean body weight gain during first 11 days, 29% less water intake, 16% less food intake during first week
					Hemato	4.32		44.62	23% increased mean relative spleen weight
					Immuno	44.62			In PFC assay, no effects on numbers of PFCs/spleen or PFC response to sheep RBCs
					Neuro	0.43		4.32	66% decreased RBC AChE activity
EPA 2013									

Table 2-2. Levels of Significant Exposure to Tribufos – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
18	Mouse (CD-1) 15 M, 15 F	8 weeks (F)	M: 0, 3.4, 9.4, 40, 140 F: 0, 5.6, 14.3, 54, 132	BW, CS, EA, FI, LE	Bd wt	140 M 132 F			
					Neuro	3.4 M 5.6 F	9.4 M 14.3 F		37 and 44% decreased RBC AChE activity (males and females, respectively)
CalEPA 2004									
19	Dog (beagle) 4 M, 4 F	364 days (F)	M: 0, 0.1, 0.4, 1.7 F: 0, 0.1, 0.4, 2.0	BC, BW, CS, EA, FI, HE, OP, UR	Bd wt	1.7 M 2.0 F			
					Ocular	1.7 M 2.0 F			
					Neuro	0.4 M 0.4 F	1.7 M 2.0 F		At treatment day 91, RBC AChE activity decreased by 24 and 29% in males and females, respectively
CalEPA 2004; EPA 1991b									
CHRONIC EXPOSURE									
20	Rat (Fischer 344) 50 M, 50 F	2 years (F)	M: 0, 0.2, 1.8, 16.8 F: 0, 0.2, 2.3, 21.1	BC, BW, CS, EA, FI, GN, HE, HP, LE, OP, OW	Bd wt	1.8 M 2.3 F	16.8 M 21.1 F		15% depressed mean body weight gain
					Gastro	0.2 M[c] 0.2 F	1.8 M 2.3 F		Vacuolar degeneration and hyperplasia in small intestines
					Hemato	0.2 M 0.2 F	1.8 M 2.3 F		Decreases in RBC count, hemoglobin, and hematocrit at 12 months (some values returned to normal by 18 and 24 months)

Table 2-2. Levels of Significant Exposure to Tribufos – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Endocr	1.8 M 2.3 F	16.8 M 21.1 F		Enlarged adrenals, increased adrenal weight, vacuolar degeneration
					Neuro	0.2 M 0.2 F	1.8 M 2.3 F		27–28% decreased RBC AChE activity
CalEPA 2004; EPA 1992d									
21	Mouse (CD-1) 50 M, 50 F	90 weeks (F)	M: 0, 1.5, 8.4, 48.1 F: 0, 2.0, 11.3, 63.1	BW, CS, EA, FI, GN, HE, HP, LE, OW	Death			48.1 M 63.1 F	M: 28% decreased survival F: 24% decreased survival
					Bd wt	48.1 M 63.1 F			
					Gastro	1.5 M 2.0 F	8.4 M 11.3 F		Vacuolar degeneration in small intestine
					Hemato	1.5 M 11.3 F	8.4 M 63.1 F		Splenic extramedullary hematopoiesis
					Hepatic	48.1 M 11.3 F	 63.1 F		Hepatocellular hypertrophy
					Endocr	8.4 M 11.3 F	48.1 M 63.1 F		Degeneration and pigmentation in adrenals
					Neuro	1.5 M 2.0 F	8.4 M 11.3 F		42 and 37% decreased RBC AChE activity in males and females, respectively

Table 2-2. Levels of Significant Exposure to Tribufos – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Cancer			48.1 M 63.1 F	CEL M: small intestine adenocarcinoma, hemangiosarcoma. CEL F: alveolar/bronchiolar adenoma

CalEPA 2004; EPA 1990a

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive an intermediate-duration oral MRL of 0.003 mg/kg/day based on tribufos-induced decreased RBC AChE activity. The NOAEL of 0.28 mg/kg/day was divided a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). Refer to Appendix A for more detailed information regarding derivation of the intermediate-duration oral MRL for tribufos.
[c]Study result used to derive a chronic-duration oral MRL of 0.0005 mg/kg/day based on tribufos-induced vacuolar degeneration in the small intestine. Benchmark dose analysis of incidence data for vacuolar degeneration resulted in a point of departure ($BMDL_{10}$) of 0.05 mg/kg/day; a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) was applied. Refer to Appendix A for more detailed information regarding derivation of the chronic-duration oral MRL for tribufos.

AChE = acetylcholinesterase; BC = serum (blood) chemistry; Bd wt or BW = body weight; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FX = fetal toxicity; (G) = gavage; (GO) = gavage in oil; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LD = lactation day(s); LD_{50} = dose estimated to cause death in 50% of treated animals; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; MX = maternal toxicity; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; PFC = plaque-forming cell; PND = postnatal day(s); Repro = reproductive; RBC = red blood cell; TG = teratogenicity; UR = urinalysis; WI = water intake

Figure 2-3. Levels of Significant Exposure to Tribufos – Oral
Acute (≤14 days)

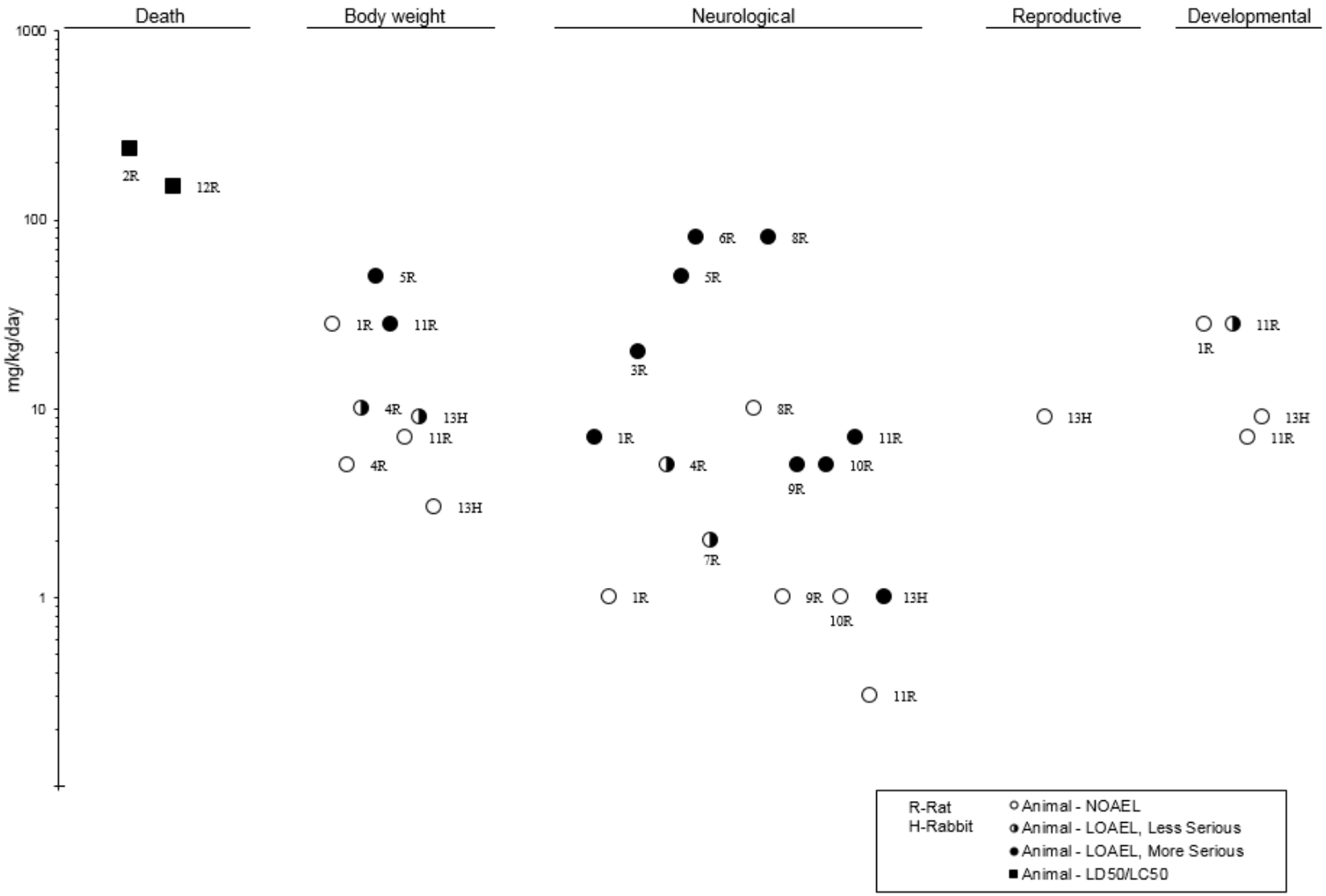


Figure 2-3. Levels of Significant Exposure to Tribufos – Oral
Intermediate (15-364 days)

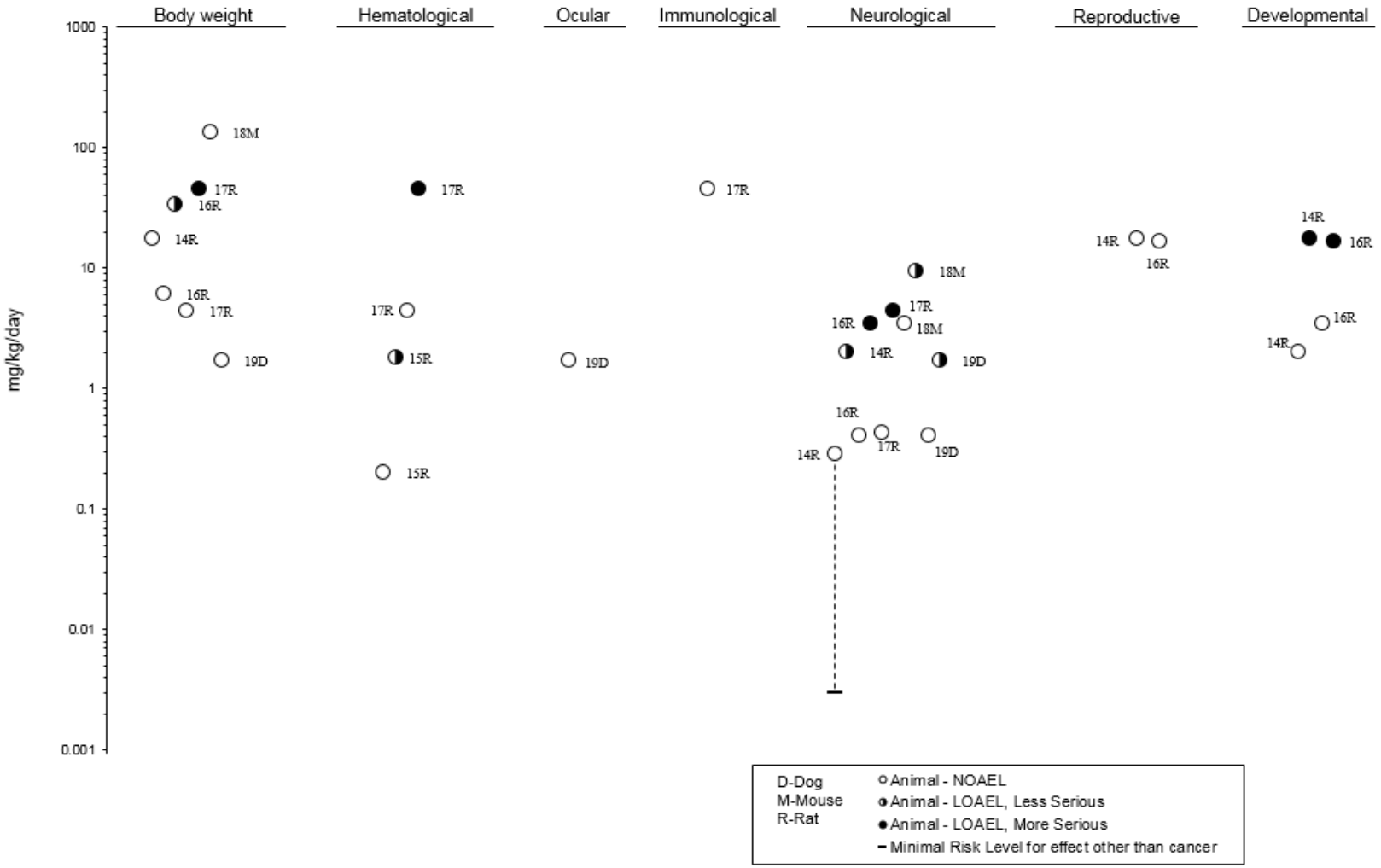


Figure 2-3. Levels of Significant Exposure to Tribufos – Oral
Chronic (≥365 days)

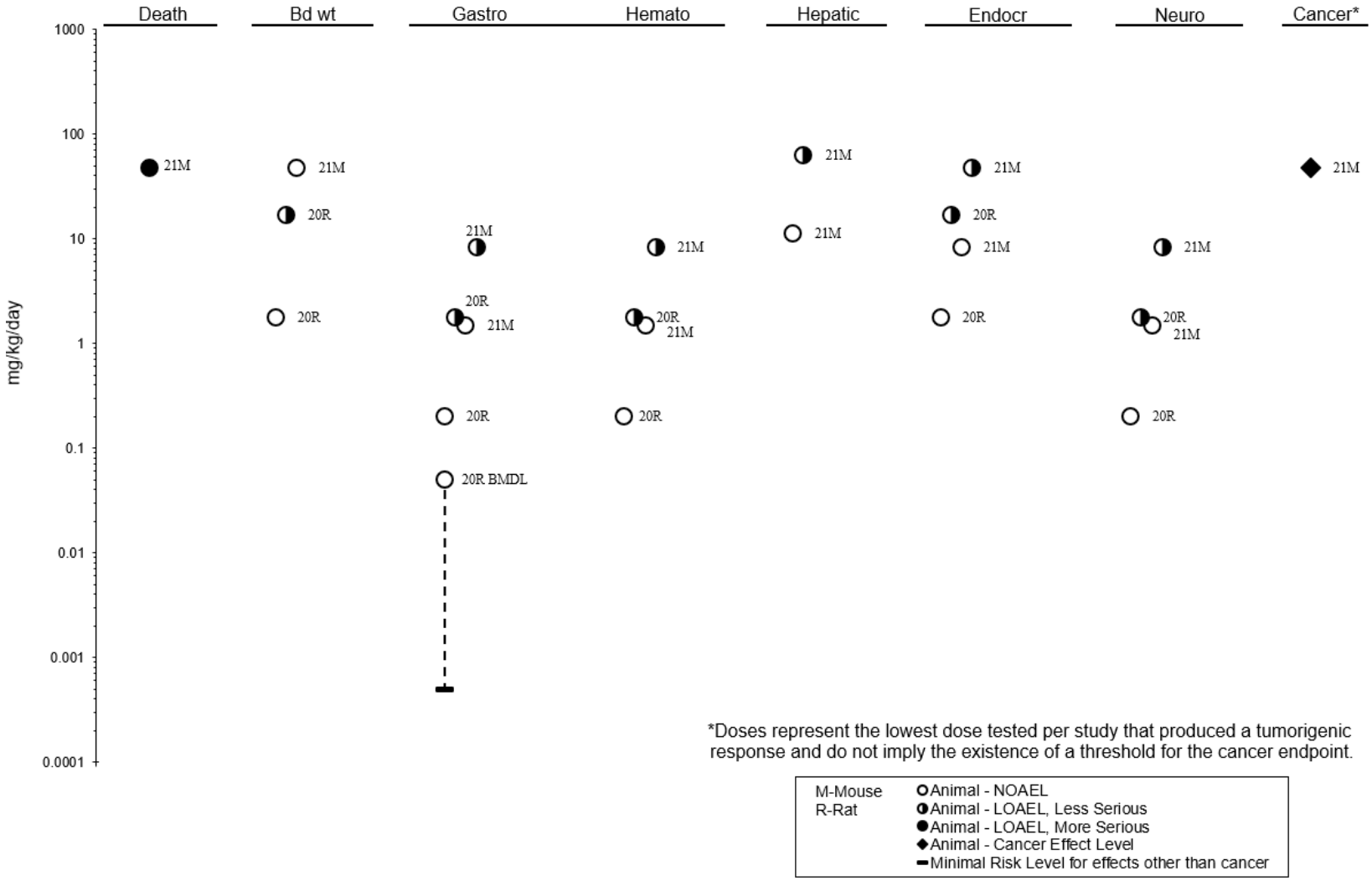


Table 2-3. Levels of Significant Exposure to Tribufos – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE								
Rat (Sherman) NS	Once for unspecified duration	NS	LE	Death			360 M 168 F	LD_{50}
Gaines 1969								
Rabbit (NS) 5/sex	One 24-hour exposure	500, 1,000, 2,000	BW, CS, GN, LE	Death			1,093	LD_{50} for combined sexes
EPA 1993b								
INTERMEDIATE EXPOSURE								
Rabbit (New Zealand) 5 M, 5 F	21 days 5 days/week 6 hours/day	0, 2, 11, 29	BC, BW, CS, EA, FI, GN, HE, LE, OP, OW	Death			29	1/5 males and 4/5 females died or were sacrificed *in extremis*
				Bd wt	29			
				Hemato	29			
				Dermal	2	11		Mild to moderate application site irritation
				Ocular	29			
				Neuro	2 M	2 F	11	20% decreased RBC AChE activity 11 mg/kg/day: 70% decreased RBC AChE activity, muscle fasciculations
EPA 1993d								

AChE = acetyl cholinesterase; BC = biochemistry; Bd wt or BW = body weight; CS = clinical signs; EA = enzyme activity; F = female(s); FI = food intake; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; LD_{50} = lethal concentration, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OP = ophthalmology; OW = organ weight; RBC = red blood cell

2.2 DEATH

No information was located regarding death in humans exposed to tribufos.

Limited information is publicly available regarding lethality in rats exposed to tribufos by inhalation. Calculated 4-hour LC_{50} values (exposure concentration associated with 50% mortality) were 4,650 and 2,460 mg/m^3 for male and female rats, respectively (EPA 1991a, 1992a).

Single-dose gavage treatment of rats with tribufos resulted in oral LD_{50} values in the range of 150–435 mg/kg/day (EPA 1993a; Gaines 1969); females were more sensitive than males. Decreased survival was observed among male and female mice administered tribufos in the diet for up to 90 weeks at concentrations resulting in estimated tribufos doses of 48.1 and 63.1 mg/kg/day, respectively (EPA 1990a).

An acute LD_{50} value of 1,093 mg/kg was reported for male and female rabbits (combined sexes) administered tribufos by single 24-hour occluded dermal application and observed for up to 14 days postadministration (EPA 1993b). Gaines (1969) reported respective acute dermal LD_{50} values of 360 and 168 mg/kg for male and female Sherman rats administered tribufos dermally at unspecified dose levels for an unspecified exposure duration and observed for up to 14 days postdosing. The lowest lethal doses to the males and females were 200 and 100 mg/kg, respectively. In a study of young adult New Zealand white rabbits receiving repeated 6-hour occluded dermal application of tribufos, 1/5 males and 4/5 females dosed at 29 mg/kg/day died or were sacrificed *in extremis* between days 12 and 19 (EPA 1993d). Most of the rabbits dosed at 29 mg/kg/day exhibited clinical signs of muscular fasciculations, tremors, and decreased movement.

2.3 BODY WEIGHT

No information was located regarding body weight in humans exposed to tribufos.

Available information regarding body weight effects in experimental animals following inhalation exposure is limited to results from a single study in which intermittent, head-only exposure of Wistar rats to tribufos aerosol at up to 59.5 mg/m^3 resulted in no apparent body weight effects (EPA 1992b).

Effects on body weight in experimental animals have been reported following acute-, intermediate-, and chronic-duration oral exposure to tribufos. Depressed body weight (12–27% less than controls) was noted among rats repeatedly exposed by gavage or receiving tribufos from the diet at doses in the range of 10–33.5 mg/kg/day (CalEPA 2004; EPA 1992d, 2005a, 2012a, 1012f). Pregnant rabbits dosed at 9 mg/kg/day during gestation days (GDs) 7–19 exhibited no body weight gain (EPA 1990c). Dietary treatment of female Han Wistar rats for 4 weeks at an estimated dose of 44.62 mg/kg/day resulted in 41% depressed mean body weight gain (EPA 2013).

Body weight was not affected by repeated dermal exposure of New Zealand rabbits to tribufos at 29 mg/kg/day (EPA 1993d).

2.4 RESPIRATORY

Limited information was located regarding the potential for tribufos-induced respiratory effects in humans. One study compared self-reported symptoms among 232 residents of three towns in cotton-growing areas during the 1987 cotton defoliation season (exposed group) with self-reported symptoms among 175 residents of non-cotton-growing agricultural communities (unexposed group) (Scarborough et al. 1989). Tribufos was one of the defoliants used at the time of the study. The exposed group was subdivided into a group with high likelihood of exposure (n=142) and a group with low likelihood of exposure (n=92) based on respondents' reports of whether or not nearby fields had been sprayed. The presence of tribufos in air was confirmed using monitoring data for tribufos collected near the centers of the three towns by the California Air Resources Board during the study period. Using the unexposed group as reference, a relative risk (RR) of 1.7 (95% CI 1.1, 2.5) was reported for cough among the group with low probability of exposure; a RR of 1.6 (95% CI 1.1, 2.3) for throat irritation was reported for the group with high probability of exposure. Limitations of the study include small numbers of subjects, self-reporting of symptoms, lack of quantitative tribufos exposure data, and lack of consideration of other airborne substances, including other defoliant and desiccant products used to defoliate cotton.

A subsequent study evaluated possible associations between cotton defoliation and respiratory cause mortality in communities surrounding cotton fields during and immediately following cotton defoliation (Ames and Gregson 1995). The study included cotton defoliation periods during the years 1970–1990. Mortality data for "all respiratory causes" of death and "all natural causes" were collected from the California Department of Health Services; the mortality data were divided into two groups: respiratory mortality in the San Joaquin Valley cotton-growing areas and respiratory mortality in the rest of the state.

The proportions of respiratory-caused mortality (number of deaths due to respiratory causes during the cotton defoliation period of each year divided by the respiratory deaths during the rest of that year in cotton-growing areas divided by a similar proportion of respiratory cause mortality in non-cotton-growing areas) ranged from 0.798 to 1.153 and exhibited a statistically significant ($p<0.05$) pattern of increases for 15 of the 21 years. However, the pattern of increases was not explained by amounts of defoliants (tribufos and folex) used. Limitations of this study include lack of quantitative tribufos exposure data and lack of accounting for other possible airborne contaminants, including unrelated particulates that may have been at increased levels during harvest seasons.

Nose-only exposure of Sprague-Dawley rats to tribufos aerosol for 4 hours resulted in respiratory effects that included clinical signs and gross pathology (dyspnea, nasal discharge, discolored lungs and nasal bones); however, publicly-available summaries of the unpublished study did not specify exposure concentration(s) causing these effects (EPA 1991a, 1992a). Unspecified changes in respiration were reported among Wistar rats repeatedly exposed (head-only) to tribufos aerosol at 59.5 mg/m^3 for 13 weeks (EPA 1992b). Minor changes in histology of nasal and paranasal cavities and lungs were attributed to vehicle (polyethylene glycol 400) rather than tribufos.

No information was located regarding respiratory effects in experimental animals following oral or dermal exposure to tribufos.

2.5 CARDIOVASCULAR

No information was located regarding cardiovascular effects in humans or experimental animals following inhalation, oral, or dermal exposure to tribufos.

2.6 GASTROINTESTINAL

Human data are limited to results from the study described in Section 2.4 (Respiratory Effects). The study results include RRs of 1.9 (95% CI 1.1, 3.2) for nausea and 2.0 (95% CI 1.1, 3.6) for diarrhea within a group (n=142) with high probability for exposure to tribufos during the 1987 cotton defoliation season (Scarborough et al. 1989). As noted earlier, limitations of the study include small numbers of subjects, self-reporting of symptoms, lack of quantitative tribufos exposure data, and lack of consideration of other airborne substances, including other defoliant and desiccant products used to defoliate cotton.

In a study of male and female CD-1 mice receiving tribufos from the diet for up to 90 weeks, significantly increased incidences of vacuolar degeneration in the small intestine were noted in males at 8.28 mg/kg/day (8/50 versus 0/50 controls) and females at 11.14 mg/kg/day (11/50 versus 0/50 controls) (EPA 1990a). Histopathologic lesions at a higher dose level (48.02 and 63.04 mg/kg/day for males and females, respectively) included vacuolar degeneration, dilation/distension, and mucosal hyperplasia of the small intestine; rectal lesions (inflammation, ulceration, and necrosis in males; ulceration in females); and edema in the caecum (females). CalEPA (2004) summarized results from an unpublished study in which Fischer 344 rats were administered tribufos in the diet at 0, 4, 40, or 320 ppm for up to 2 years; estimated tribufos doses were 0, 0.2, 1.8, and 16.8 mg/kg/day, respectively, for the males and 0.2, 2.3, and 21.1 mg/kg/day, respectively, for the females. Incidences of vacuolar degeneration of the small intestines for the 0, 4, 40, and 320 ppm groups were 0/20, 0/10, 7/10, and 18/20, respectively, for the males and 0/20, 0/10, 8/10 and 16/20, respectively, for the females at 12-month interim sacrifice and 0/50, 1/50, 24/50, and 37/50, respectively, for the males and 0/50, 0/50, 19/50, and 35/50, respectively, for the females at 24-month terminal sacrifice. In addition, CalEPA (2004) reported incidences of hyperplasia in the small intestines (0/50, 3/50, 23/50, and 34/50, respectively, for the males and 1/50, 0/50, 11/50, and 30/50, respectively, for the females) at 24-month terminal sacrifice.

No information was located regarding gastrointestinal effects in experimental animals following inhalation or dermal exposure to tribufos.

2.7 HEMATOLOGICAL

No information was located regarding hematological effects in humans exposed to tribufos.

There was no evidence of hematological effects in Wistar rats following intermittent head-only exposure to tribufos aerosol at up to 59.5 mg/m^3 for 13 weeks (EPA 1992b). Dietary exposure of Fischer 344 rats to tribufos at estimated doses ≥1.8 resulted in statistically significant decreases in RBC counts, hemoglobin, and hematocrit, but some of these values had returned to normal by 18 and 24 months (CalEPA 2004; EPA 1992d). At terminal sacrifice, significant increases in RBC count and hematocrit were noted in high-dose (16.8 mg/kg/day) males and significant increases in hemoglobin and hematocrit were observed in high-dose (21.1 mg/kg/day) females, indicating the possible involvement of some compensatory mechanism. In a 90-week dietary study of mice, significant decreases in RBC count, hemoglobin, and hematocrit (indicative of treatment-related anemia) were observed at estimated tribufos doses of 48.02 and 63.04 mg/kg/day for males and females, respectively (EPA 1990a). Available

secondary source summaries (CalEPA 2004; EPA 1990a, 1992d) of these unpublished studies did not include quantitative data regarding the magnitude of hematological changes; therefore, it is impossible to judge the seriousness of the changes.

In a 21-day repeated-dose dermal study, young adult New Zealand rabbits repeatedly exposed by occluded application at up to 29 mg/kg/day exhibited no signs of tribufos-induced effects on RBCs, white blood cells (WBCs), platelets, hemoglobin, or hematocrit (EPA 1993d).

2.8 MUSCULOSKELETAL

No information was located regarding musculoskeletal effects in humans or experimental animals following exposure to tribufos.

2.9 HEPATIC

No information was located regarding hepatic effects in humans exposed to tribufos.

There was no evidence of hepatotoxicity (serum liver enzymes, histopathology results) following intermittent head-only exposure of Wistar rats to tribufos aerosol at up to 59.5 mg/m^3 for 13 weeks (EPA 1992b). Significantly increased incidence of hepatocellular hypertrophy (6/50, severity 1.8 out of 5.0; versus 0/50 controls) was reported for female CD-1 mice receiving tribufos from the diet for up to 90 weeks at an estimated dose of 63.04 mg/kg/day (EPA 1990a). The toxicological significance of this finding is questionable in the absence of other indicators of tribufos-induced hepatotoxicity. No information was located regarding hepatic effects in experimental animals following dermal exposure to tribufos.

2.10 RENAL

No information was located regarding renal effects in humans exposed to tribufos.

Available information in experimental animals is limited to results from a single study. There was no evidence of renal toxicity (based on results of urinalysis and histopathological evaluations) following intermittent head-only exposure of Wistar rats to tribufos aerosol at up to 59.5 mg/m^3 for 13 weeks (EPA 1992b).

2.11 DERMAL

No information was located regarding dermal effects in humans exposed to tribufos.

Available information in experimental animals is restricted to a report of mild to moderate contact-site dermal irritation in both sexes of young adult New Zealand rabbits receiving repeated occluded dermal applications of tribufos for up to 3 weeks at dose levels ≥11 mg/kg/day (EPA 1993d).

2.12 OCULAR

No information was located regarding ocular effects in humans exposed to tribufos.

Exophthalmos (abnormal protrusion of the eyeballs) was observed in Wistar rats intermittently exposed to tribufos aerosol at up to 59.5 mg/m^3 for 13 weeks (EPA 1992b). There was no other evidence of ocular effects, as judged by ophthalmologic examinations. This effect was considered a result of direct ocular contact with airborne tribufos aerosol.

No signs of treatment-related ocular effects were observed during ophthalmological examinations of beagle dogs receiving tribufos from the diet for 364 days at estimated doses of 1.7–2.0 mg/kg/day (EPA 1991b). In a 2-year study of Fischer 344 rats, treatment-related ocular effects (cataracts, corneal opacity, corneal neovascularization, iritis, and/or uveitis) were observed in males and females receiving tribufos from the diet at estimated doses of 16.8–21.1 mg/kg/day (CalEPA 2004; EPA 1992d). According to the available secondary sources of information for the unpublished study, the study pathologist considered these effects to have been secondary to retinal atrophy (a neurological effect).

No signs of treatment-related adverse ocular effects were observed during ophthalmologic examinations performed on young adult New Zealand rabbits following repeated occluded dermal applications of tribufos for up to 3 weeks at dose levels as high as 29 mg/kg/day (EPA 1993d). There was no indication of treatment-related ocular irritation among male rabbits during 3–6 days of observation following instillation of 0.1 mL of tribufos into the conjunctival sac of one eye (EPA 1993c).

2.13 ENDOCRINE

No information was located regarding endocrine effects in humans exposed to tribufos.

Increased adrenal weight and increased incidence of cortical fat deposit in adrenals were observed in male (but not female) Wistar rats following intermittent head-only exposure to tribufos aerosol at 59.5 mg/m^3 for 13 weeks (EPA 1992b). Significantly increased incidences of degeneration/pigmentation in the adrenal glands (males: 39/50 versus 17/50 controls; females: 38/49 versus 18/50 controls) were reported in a study of CD-1 mice receiving tribufos from the diet for up to 90 weeks at estimated doses of 48.02 mg/kg/day (males) and 63.04 mg/kg/day (females) (EPA 1990a). CalEPA (2004) summarized results from an unpublished study in which Fischer 344 rats were administered tribufos in the diet at up to 320 ppm for up to 2 years; significantly increased incidences of vacuolar degeneration in adrenal glands were reported in the high-dose groups at 12-month interim sacrifice (estimated doses of 16.8 and 21.1 mg/kg/day to the males and females, respectively). No information was located regarding endocrine effects in experimental animals following dermal exposure to tribufos.

2.14 IMMUNOLOGICAL

No information was located regarding immunological effects in humans exposed to tribufos.

Available information in experimental animals is restricted to results from a single study in which female Han Wistar rats received tribufos from the diet for 4 weeks at estimated doses up to 44.62 mg/kg/day and intravenous injection of sheep red blood cells (SRBC) 4 days prior to terminal sacrifice to evaluate production of anti-SRBC IgM (plaque-forming cell [PFC] assay) (EPA 2013). There was no significant tribufos-induced effect on numbers of PFCs/spleen or the PFC response to SRBCs.

2.15 NEUROLOGICAL

Human data are limited. Evaluation of the results from the study described in Section 2.4 (Respiratory Effects) yielded a RR of 1.7 (95% CI 1.3, 2.4) for fatigue for a group (n=142) with high probability for exposure to tribufos during the 1987 cotton defoliation season (Scarborough et al. 1989). As noted earlier, limitations of the study include small numbers of subjects, self-reporting of symptoms, lack of quantitative tribufos exposure data, and lack of consideration of other airborne substances, including other defoliant and desiccant products used to defoliate cotton.

Lotti et al. (1983) reported a 50% decrease in the enzyme, neuropathy target esterase (NTE), in lymphocytes from seven workers repeatedly exposed (during 9–34 days) to tribufos and folex during mixing and/or aerial and ground application of the compounds during one season of cotton defoliation. NTE is the target enzyme in organophosphate-induced delayed neuropathy (OPIDN). Exposure was assessed by sampling air in the breathing zone; collection of material deposited on cloth patches attached to thighs, chest, upper arms, and neck; and collection of material rinsed from hands. The results implicated dermal deposition as the major route of exposure. There were no signs of exposure-related effects on peripheral nerve function or neuromuscular transmission, and no exposure-related effects on RBC AChE activity. Furthermore, there were no signs of OPIDN among the workers evaluated 3 weeks following cessation of tribufos and folex use.

Clinical signs of tribufos-induced neurotoxicity (e.g., abnormal posture, ataxia, hypoactivity, muscle tremors, excitability) were reported in Sprague-Dawley rats exposed nose-only to tribufos aerosol (mass median aerodynamic diameter [MMAD] 1.4–1.55 μm; 69–78% of particles <2 μm in diameter) for 4 hours; however, the available summary of the unpublished study did not specify tribufos concentrations (range 1,590–6,030 mg/m^3) or frequency of observed signs of neurotoxicity (EPA 1991a). Intermittent, head-only exposure of Wistar rats to tribufos aerosol at 59.5 mg/m^3 for 13 weeks resulted in clinical signs of neurological effects (e.g., altered gait, decreased movement, constricted pupils, piloerection, aggressive behavior, sensitivity to touch, convulsions, salivation), decreased brain AChE activity (40% less than that of controls), >60% decreased RBC AChE activity, and depressed amplitude of a- and b-waves in electroretinographic tests (considered a neurological effect rather than an ocular effect) (EPA 1992b). There were no indications of adverse electroretinographic effects or clinical signs of neurotoxicity at lower exposure levels (0.93, 2.43, or 12.2 mg/m3); however, the 12.2 mg/m^3 exposure level also resulted in >60% decreased RBC AChE activity, which ATSDR considers a serious adverse effect (Chou and Williams-Johnson 1998). In a 2-year study of Fischer 344 rats receiving tribufos from the diet at estimated doses of 16.8–21.1 mg/kg/day, treatment-related ocular effects (cataracts, corneal opacity and neovascularization, iritis, uveitis) were considered secondary to retinal atrophy (a neurological effect) (CalEPA 2004; EPA 1992d).

CalEPA (2004) summarized results from three unpublished studies designed to investigate the potential for inhaled tribufos to cause OPIDN and cholinergic signs in hens subjected to scenarios ranging from a single 4-hour exposure to intermittent exposures for 3 weeks. Following a single 4-hour exposure, the lowest-observed-effect level (LOEL) for cholinergic signs was on the order of 2-fold lower than the

LOEL for OPIDN (391 and 878 mg/m^3, respectively). However, following five consecutive 6-hour exposures, the LOEL for OPIDN was nearly 2-fold lower than the LOEL for cholinergic signs (145 and 246 mg/m^3, respectively). Although studies of hens are useful for hazard identification, applicability of the dose-response in hens to humans is uncertain. Therefore, hen study results are not included in Table 2-1 or Figure 2-2.

Table 2-4 summarizes results from studies that evaluated the effects of oral exposure to tribufos on indicators of neurological effects (e.g., RBC and brain AChE activity; clinical signs of neurotoxicity) in experimental animals. Single gavage dosing of rats at 20–80 mg/kg typically resulted in >60% decreased RBC and/or brain AChE activity (EPA 2012a, 2012b, 2012c, 2012d). Available study reports and Data Evaluation Reports (DERs) for acute-duration repeated-dose oral exposure of rats to tribufos identified NOAELs of 0.3–1.0 mg/kg/day and serious LOAELs of 1–15 mg/kg/day for >60% decreased RBC and/or brain AChE activity (Astroff and Young 1998; EPA 1990b, 2012a, 2012e, 2012f). The lowest LOAEL for clinical signs of neurotoxicity was 5 mg/kg/day for decreased movement, unsteadiness, and prostration among 11-day-old Sprague-Dawley rat pups gavaged once per day for 11 days (EPA 2012e). In intermediate-duration oral studies, the lowest less serious LOAELs were 1.7 mg/kg/day for 24% decreased RBC activity in beagle dogs and 2.25 mg/kg/day for 29% decreased brain AChE activity in Sprague-Dawley rats; serious LOAELs for >60% decreased RBC and/or brain AChE activity were as low as 3.4 mg/kg/day for 76% decreased RBC activity in Wistar rats (EPA 2005a) and 16.4 mg/kg/day for 74% decreased brain AChE activity in Wistar rats (EPA 2005a). The 16.4 mg/kg/day dose level in Wistar rats also resulted in slight tremors.

In chronic oral studies, rats appeared to be more sensitive to tribufos neurotoxicity. Male Fischer 344 rats exhibited 27% decreased RBC AChE activity at a dose level of 1.8 mg/kg/day and 60% brain AChE activity and atrophy of ocular nerves at 16.8 mg/kg/day (CalEPA 2004; EPA 1992d). Male CD-1 mice exhibited 42% decreased RBC AChE activity at 8.4 mg/kg/day and 37% decreased brain AChE activity at 48.1 mg/kg/day and atrophy of ocular nerves at 16.8 mg/kg/day in the absence of clinical signs of neurotoxicity (CalEPA 2004; EPA 1990a).

Abou-Donia et al. (1979) administered tribufos orally (in gelatin capsule) to groups of hens (5/group) once per day for up to 3 months at doses ranging from 0.1 to 80 mg tribufos/kg/day. The study included a group of vehicle controls. No treatment-related effects were observed in hens treated with 0.1 mg tribufos/kg/day. Dose-related increased incidence and severity and decreased onset of clinical signs of OPIDN (ataxia) were noted in all hens given 0.5–80 mg tribufos/kg/day, beginning as early as treatment

Table 2-4. NOAELs and LOAELs for Neurological Effects (RBC and/or Brain AChE Inhibition, Clinical Signs, Pathological Lesions) in Mammalian Species Orally Exposed to Tribufos

	Tribufos doses (in mg/kg or mg/kg/day) associated with NOAELs and LOAELs for RBC and brain AChE inhibition, clinical signs, and pathological lesions							
	RBC AChE (percent inhibition)			Brain AChE (percent inhibition)				
Study design (doses in mg/kg or mg/kg/day)	NOAEL[a]	LOAEL[b]	Serious LOAEL[c]	NOAEL[a]	LOAEL[b]	Serious LOAEL[c]	Clinical signs and/or pathological lesions	Reference
Acute-duration exposure								
Young adult female Sprague-Dawley rats GO 1 time (0, 80)	ND	ND	80 (>80%)	ND	80 (15-20%)	ND	80; no clinical signs	EPA 2012c
Young adult female Sprague-Dawley rats GO 1 time (0, 2, 10, 80)	10	ND	80 (74%)	80	ND	ND	80; no clinical signs	EPA 2012d
11-day-old Sprague-Dawley rat pups GO 1x (0, 50)	M: ND F: ND	M: ND F: ND	M: 50 (88%) F: 50 (87%)	ND	ND	M: 50 (74%) F: 50 (76%)	50; decreased movement	EPA 2012b
11-day-old Sprague-Dawley rat pups GO 1x (0, 20, 40, 50)	M: ND F: ND	M: 20 (59%) F: ND	M: 40 (76%) F: 20 (71%)	M: 20 F: ND	M: 40 (52%) F: 20 (34%)	M: ND F: 50 (60%)	40; decreased movement	EPA 2012a
11-day-old Sprague-Dawley rat pups GO 1 time (0, 2, 10, 50)	M: 2 F: ND	M: 10 (47%) F: 2 (27%)	M: 50 (86%) F: 50 (89%)	M: 10 F: 10	ND ND	M: 50 (76%) F: 50 (75%)	10; decreased movement: 50; decreased movement, incoordination, unsteadiness	EPA 2012d
Young adult female Sprague-Dawley rats GO 1 time/day, 11 days (0, 0.1, 1, 5)	1	ND	5 (64%)	5	ND	ND	5; no clinical signs, with exception of salivation in one mid-dose rat and one high-dose rat	EPA 2012e
11-day-old Sprague-Dawley rat pups GO 1 time/day, 11 days (0, 0.1, 1, 5)	M: 1 F: 1	M: ND F: ND	M: 5 (66%) F: 5 (69%)	M: 1 F: 1	M: 5 (20%) F: 5 (21%)	ND	5; decreased movement, unsteadiness, prostration	EPA 2012e
11-day-old Sprague-Dawley rat pups GO 1 time/day, 11 days (0, 5, 10, 15, 20)	M: ND F: ND	M: 5 (49%) F: 5 (36%)	M: 15 (83%) M: 15 (66%)	M: ND F: 5	M: 5 (23%) F: 10 (32%)	M: ND F: ND	10–15; decreased movement, unsteadiness, hind limb splay: 20; severe clinical signs	EPA 2012a
Pregnant Sprague-Dawley rats G 1 time/day, GDs 6–15 (0, 1, 7, 28)	1	ND	7 (71%)	7	28 (58%)	ND	28; no signs, with exception of salivation in two high-dose dams	Astroff and Young 1998; EPA 1990b
Pregnant Sprague-Dawley rats GO 1 time/day, GDs 6–19 (0, 0.3–0.8, 7, 28)	0.3[d]	ND	7 (75%)	0.3[d]	7 (22%)	28 (81%)	28; no clinical signs	EPA 2012f

Table 2-4. NOAELs and LOAELs for Neurological Effects (RBC and/or Brain AChE Inhibition, Clinical Signs, Pathological Lesions) in Mammalian Species Orally Exposed to Tribufos

	Tribufos doses (in mg/kg or mg/kg/day) associated with NOAELs and LOAELs for RBC and brain AChE inhibition, clinical signs, and pathological lesions							
	RBC AChE (percent inhibition)			Brain AChE (percent inhibition)				
Study design (doses in mg/kg or mg/kg/day)	NOAEL[a]	LOAEL[b]	Serious LOAEL[c]	NOAEL[a]	LOAEL[b]	Serious LOAEL[c]	Clinical signs and/or pathological lesions	Reference
Pregnant American Dutch rabbits G 1 time/day, GDs 7–19 (0, 1, 3, 9)	ND	ND	1 (70%)	9	ND	ND	9; no clinical signs	EPA 1990c
Intermediate-duration exposure								
Female Han Wistar rats, diet for 4 weeks (0, 0.43, 4.32, 44.62)	0.43	ND	4.32 (66%)	4.32	ND	44.62 (78%)	44.62; no clinical signs	EPA 2013
Wistar rat dams, diet GD 1–LD 21 GDs (0, 0.4, 3.4–3.5, 16.4–18.2) LDs (0, 0.6-1.0, 6.1-9.9, 33.5-55.4)	0.4[e]	NA	3.4 (76%)[e]	0.4[e]	3.4 (22%)[e]	16.4 (74%)[e]	16.4[e]; slight tremors in five dams on day of parturition	EPA 2005a
Sprague-Dawley rats, diet for 2 generations								Astroff et al. 1998; EPA 1992c
F0 M (0, 0.28, 2.00, 17.6)	0.28	2.00 (35%)	ND	2.00	17.6 (36%)	ND	7.6; no clinical signs	
F0 F (0, 0.31, 2.25, 20.04)	0.31	2.25 (37%)	ND	0.31	2.25 (29%)	20.04 (81%)	20.04; no clinical signs	
F1 M (0, 0.28, 2.09, 20.63)[f]	0.28	2.09 (26%)	ND	2.09	20.63 (33%)	ND	20.63; no clinical signs	
F1 F (0, 0.31, 2.40, 22.93)	0.31	2.40 (28%)	ND	0.31	2.40 (29%)	22.93 (81%)	22.93; no clinical signs	
CD-1 mice, diet for 8 weeks								CalEPA 2004
M: (0, 3.4, 9.4, 40, 140)	3.4	9.4 (37%)	40 (64%)	40	140 (26%)		140; no clinical signs	
F: (0, 5.6, 14.3, 54, 132)	5.6	14.3 (44%)	54 (64%)	54	132 (29%)		132; no clinical signs	
Beagle dogs, diet for up to 364 days								CalEPA 2004; EPA 1991b
M (0, 0.1, 0.4, 1.7)	0.4	1.7[g] (24%)	ND	NA[h]	NA[h]	NA[h]	1.7; no clinical signs	
F (0, 0.1, 0.4, 2.0)	0.4	2.0[g] (29%)	ND	NA[h]	NA[h]	NA[h]	2.0; no clinical signs	

Table 2-4. NOAELs and LOAELs for Neurological Effects (RBC and/or Brain AChE Inhibition, Clinical Signs, Pathological Lesions) in Mammalian Species Orally Exposed to Tribufos

	Tribufos doses (in mg/kg or mg/kg/day) associated with NOAELs and LOAELs for RBC and brain AChE inhibition, clinical signs, and pathological lesions							
	RBC AChE (percent inhibition)			Brain AChE (percent inhibition)				
Study design (doses in mg/kg or mg/kg/day)	NOAEL[a]	LOAEL[b]	Serious LOAEL[c]	NOAEL[a]	LOAEL[b]	Serious LOAEL[c]	Clinical signs and/or pathological lesions	Reference
Chronic-duration exposure								
CD-1 mice, diet for 90 weeks								CalEPA
M (0, 1.5, 8.4, 48.1)	1.5	8.4 (42%)	ND	8.4	48.1 (38%)	ND	48.1; no clinical signs	2004; EPA
F (0, 2.0, 11.3, 63.1)	2.0	11.3 (37%)	ND	11.3	63.1 (27%)	ND	63.1; no clinical signs	1990a
Fischer 344 rats, diet for 2 years								CalEPA
M (0, 0.2, 1.8, 16.8)	0.2	1.8 (27%)	ND	1.8	ND	16.8 (60%)	16.8; atrophy ocular nerves	2004; EPA
F (0, 0.2, 2.3, 21.1	0.2	2.3 (28%)	ND	2.3	ND	21.1 (68%)	21.1; atrophy ocular nerves	1992d

[a]<20% decrease in RBC and/or brain AChE represents a NOAEL.
[b]20–59% decrease in RBC and/or brain AChE activity represents a less serious adverse effect.
[c]≥60% decrease in RBC and/or brain AChE activity represents a serious adverse effect.
[d]Low test substance concentrations measured in the 1 mg/kg/day dose group resulted in estimated time-weighted average dosing in the range of 0.3–0.8 mg/kg/day; using a conservative approach, the lowest dose in the range is considered the NOAEL.
[e]The available study summary included only ranges of doses during gestation and lactation periods; using a conservative approach, the NOAELs and LOAELs are considered the low end of a given range for gestational exposure.
[f]F1 parental rats had been exposed *in utero* and lactationally as well.
[g]At treatment day 91.
[h]Brain AChE activity was only assessed at day 371 (i.e., 7 days following cessation of tribufos treatment).

AChE = acetylcholinesterase; d = day(s); F = female(s); F0 = first generation parental; F1 = second generation parental; G = gavage; GD = gestation day; GO = gavage in oil; LD = lactation day; LOAEL = lowest-observed-adverse-effect level; M = male(s); NA = not applicable; ND = not determined; NOAEL = no-observed-adverse-effect level; RBC = red blood cell

day 8 in the 80 mg/kg/day dose group. Signs of OPIDN persisted until death or terminal sacrifice during a 30-day observation period following cessation of tribufos dosing. Doses of 40 and 80 mg/kg/day also resulted in typical signs of cholinergic effects; hens in the 40 and 80 mg/kg/day dose groups were subsequently administered atropine sulfate in an attempt to counteract the cholinergic effects. However, after several days, the hens exhibited unsteadiness, followed by general weakness, malaise, loss of balance, tremors, paralysis, and death. Hens administered tribufos at 20 mg/kg/day developed similar (but milder) signs with recovery after 8–11 days. This effect was termed a "late acute" effect because it was not relieved by atropine sulfate and was not considered to be associated with AChE activity.

Francis et al. (1985) reported clinical signs of OPIDN as early as 11–28 days following the initiation of dosing in hens repeatedly administered tribufos orally (gelatin capsule, corn oil vehicle) at 21–30 mg/kg/day.

2.16 REPRODUCTIVE

No information was located regarding reproductive effects in humans exposed to tribufos.

No apparent reproductive effects were observed in studies that employed gavage dosing of tribufos to pregnant animals, including Sprague-Dawley rat dams treated during GDs 6–15 (Astroff and Young 1998; EPA 1990b) or GDs 6–19 (EPA 2012f) at doses as high as 28 mg/kg/day, or maternal American Dutch rabbits treated during GDs 7–19 at up to 9 mg/kg/day (EPA 1990c). No apparent reproductive effects were observed in a study of Wistar rat dams receiving tribufos from the diet throughout gestation and lactation at estimated doses up to 16.4–18.2 mg/kg/day (EPA 2005a). No reproductive effects were observed in a 2-generation study of Sprague-Dawley rats receiving tribufos from the diet for approximately 8–9 weeks prior to mating, and throughout mating, gestation, and lactation at estimated doses as high as 17.6–22.93 mg/kg/day during the premating phase (Astroff et al. 1998; EPA 1992c).

2.17 DEVELOPMENTAL

No information was located regarding developmental effects in humans exposed to tribufos.

There were no signs of treatment-related fetal effects in a study of Sprague-Dawley rat dams gavaged with tribufos during GDs 6–15 at doses as high as 28 mg/kg/day (Astroff and Young 1998; EPA 1990b) or a study of maternal American Dutch rabbits gavaged during GDs 7–19 at doses as high as 9 mg/kg/day

(EPA 1990c). In another study of Sprague-Dawley rat dams gavaged during GDs 6–19, there were no signs of treatment-related fetal effects, with the exception of significantly lower mean male fetal body weight (6% lower than that of controls) at 28 mg/kg/day (EPA 2012f).

Several indicators of treatment-related developmental effects were noted in a study of male and female Sprague-Dawley rats administered tribufos in the diet during 8–9 weeks premating and throughout mating, gestation, and lactation for 2 generations (Astroff et al. 1998; EPA 1992c). At estimated premating doses in the range of 17.6–22.93 mg/kg/day (high-dose groups), mean body weights of F1 and F2 pups during lactation ranged from 14 to 30% lower than controls; however, decreased food consumption and depressed mean maternal body weight among the high-dose F0 and F1 dams during lactation may have been at least partially responsible for the effects on pup body weights. Other significant indicators of tribufos-induced developmental effects in the high-dose groups from one or both generations included decreased numbers of live pups/number of pregnant females, decreased numbers of pups born/number of implantation sites, decreased pup viability, decreased numbers of live pups on lactation day 21, and decreased mean litter size. However, these effects occurred at maternally-toxic doses.

In another study, groups of Wistar rat dams received tribufos from the diet at estimated doses up to 16.4–18.2 mg/kg/day during gestation and 33.5–55.4 mg/kg/day during lactation (EPA 2005a). Maternal effects were noted in the high-dose maternal rats and included tremors and decreased body weight during lactation. Indicators of treatment-related developmental effects were noted in the high-dose group and included 16–23% depressed pup mean body weight during lactation, delayed preputial separation, delayed development of righting reflex, decreased motor activity at postnatal day (PND) 13 and increased motor activity at PND 17, and decreased auditory startle amplitude at PND 22. There were no apparent treatment-related effects on pup motor activity or auditory startle response at PNDs 38 or 60.

2.18 OTHER NONCANCER

Hypothermia was reported among Wistar rats intermittently exposed (head-only) to tribufos aerosol (MMAD 1.2–1.3 µm) for 13 weeks at an analytically-determined concentration of 59.5 mg/m^3 (EPA 1992b). A clinical sign of treatment-related hypothermia (i.e., cold to the touch) was reported as early as 4 hours postdosing in young Sprague-Dawley rat pups (11 days of age) administered tribufos by gavage for 11 days at doses ≥10 mg/kg/day (EPA 2012a); similar treatment by single gavage dose at 50 mg/kg resulted in the same effect (EPA 2012b).

Ray and coworkers (Little and Ray 1979; Ray 1980; Ray and Cunningham 1985) reported hypothermic responses in rats, mice, and guinea pigs (but not rabbits) administered tribufos via single intraperitoneal injection at doses in the range of 10–200 mg/kg; a dose-response relationship was noted and the effect lasted from several hours to several days at environmental temperatures below thermoneutrality (30–31°C). Based on findings of little effect on basal metabolism at thermoneutrality, lack of apparent effect on heat conservation mechanisms (peripheral vasoconstriction and piloerection), and normal adrenal catecholamine secretion in response to handling or acute cold exposure in tribufos-treated animals but marked reduction in the tribufos-induced hypothermic response upon injection of noradrenaline (but not atropine), the investigators suggested a selective action of tribufos (or a metabolite) on a central thermogenic control process.

2.19 CANCER

No information was located regarding tribufos-induced cancer in humans.

There were no indications of treatment-related increased incidences of malignant or benign tumors among male and female Fischer 344 rats receiving tribufos from the diet for 2 years at estimated doses as high as 16.8–21.1 mg/kg/day (CalEPA 2004; EPA 1992d) or male and female beagle dogs receiving tribufos from the diet for 364 days at estimated doses as high as 1.7–2.0 mg/kg/day (EPA 1991b). However, in a study of male and female CD-1 mice receiving tribufos from the diet for up to 90 weeks, significantly increased incidences of adenocarcinoma in the small intestine (9/50 versus 0/50 controls) and hemangiosarcoma in the liver (7/50 versus 1/50 controls) were observed in males at an estimated dose level of 48.02 mg/kg/day (EPA 1990a). High-dose (63.04 mg/kg/day) female mice exhibited significantly increased incidence of alveolar/bronchiolar adenoma (15/50 versus 5/50 controls) and nonsignificantly increased incidence of adenocarcinoma of the small intestine (4/50 versus 0/50 controls). It should be noted that adenocarcinoma of the small intestine is a rare tumor type in CD-1 mice.

A Health Effects Division Carcinogenicity Peer Review Committee for EPA's Office of Pesticide Programs evaluated the weight-of-evidence regarding the carcinogenic potential of tribufos (EPA 1997). The committee noted increased liver tumors in male mice, increased lung tumors in female mice, and increased small intestine tumors (rare tumors) in both sexes of mice at high oral doses (48.02 mg/kg/day in males and 63.04 mg/kg/day in females) (EPA 1990a). The committee also noted that the tribufos-related increases in mouse tumors occurred only at doses eliciting severe noncancer toxicity as well and

recommended a nonlinear (margin of exposure) approach for extrapolating to lower dose levels. The committee (EPA 1997) identified a lack of tribufos-induced tumors in a rat study (EPA 1992d), a lack of human data, no apparent concern for mutagenicity, no identified structural analogs of concern, and no mechanistic or mode-of-action data in its assessment. The committee concluded that tribufos should be considered unlikely to be carcinogenic at low doses, but likely to be carcinogenic at high doses. The EPA committee stated that human exposure to tribufos would not likely approach the dose level associated with tumors in the tribufos-treated mice.

The International Agency for Research on Cancer (IARC 2019) does not include a classification for tribufos. The National Toxicology Program 14th Report on Carcinogens (NTP 2016) does not include tribufos.

2.20 GENOTOXICITY

Limited publicly-available information was located. Tribufos did not induce sister chromatid exchanges in Chinese hamster V79 cells exposed for 32 hours or two cell cycles at doses in the range of 2.5–20 µg/mL either with (Chen et al. 1982b) or without (Chen et al. 1982a) exogenous metabolic activation (rat liver S9 mix). Results from several unpublished studies were evaluated in EPA's Human Health Risk Assessment for tribufos (EPA 2000a) and CalEPA's Risk Characterization Document for tribufos (CalEPA 2004); a summary of the results follows; exposure duration information was not presented in available secondary sources. Tribufos was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 at concentrations the range of 667–10,000 µg/plate either with or without exogenous metabolic activation. Tribufos did not induce chromosomal aberrations in Chinese hamster ovary cells at concentrations of 0.04, 0.007, 0.013, 0.025, or 0.05 µL/mL without exogenous metabolic activation (cytotoxicity noted at 0.025 and 0.05 µL/mL) or 0.007, 0.013, 0.025, 0.05, or 0.01 µL/mL with exogenous metabolic activation (cytotoxicity noted at 0.05 and 0.1 µL/mL). Tribufos did not induce sister chromatid exchanges in another study of Chinese hamster V79 cells exposed at up to 18.9 µg/mL in the absence of exogenous metabolic activation. Tribufos did not induce unscheduled deoxyribonucleic acid (DNA) synthesis in rat primary hepatocytes at concentrations in the range of 0.0001–0.03 µg/mL (cytotoxicity noted at concentrations >0.006 µg/mL).

2.21 MECHANISMS OF ACTION

No tribufos-specific information was located regarding mechanisms of toxicity. Tribufos (and other organophosphorus compounds) induce toxicity resulting predominantly from the inhibition of AChE in the central and peripheral nervous system. AChE is responsible for terminating the action of the neurotransmitter, acetylcholine, in cholinergic synapses. The action of acetylcholine does not persist long as it is hydrolyzed by AChE and rapidly removed. As an anticholinesterase organophosphate, tribufos inhibits AChE by reacting with the active site to form a stable phosphorylated complex incapable of destroying acetylcholine at the synaptic gutter between the pre- and postsynaptic nerve endings or neuromuscular junctions of skeletal muscles resulting in accumulation of acetylcholine at these sites. This leads to continuous or excessive stimulation of cholinergic fibers in the postganglionic parasympathetic nerve endings, neuromuscular junctions of the skeletal muscles, and cells of the central nervous system that results in hyperpolarization and receptor desensitization. These cholinergic actions involving end organs (heart, blood vessels, secretory glands) innervated by fibers in the postganglionic parasympathetic nerves result in muscarinic effects, which are manifested as miosis, excessive glandular secretions (salivation, lacrimation, rhinitis), nausea, urinary incontinence, vomiting, abdominal pain, diarrhea, bronchoconstriction or bronchospasm, increased bronchosecretion, vasodilation, bradycardia, and hypotension. Nicotinic effects are due to accumulation of acetylcholine at the skeletal muscle junctions and sympathetic preganglionic nerve endings. Nicotinic effects are manifested as muscular fasciculations, weakness, mydriasis, tachycardia, and hypertension. The central nervous system effects are due to accumulation of acetylcholine at various cortical, subcortical, and spinal levels (primarily in the cerebral cortex, hippocampus, and extrapyramidal motor system). The central nervous system effects are manifested as respiratory depression, anxiety, insomnia, headache, restlessness, tension, mental confusion, loss of concentration, apathy, drowsiness, ataxia, tremor, convulsion, and coma.

As noted previously, organophosphorus compounds such as tribufos inhibit RBC and brain AChE. However, the degree of RBC AChE inhibition does not always correlate with the severity of acute signs of organophosphorus toxicity, especially when individuals are chronically exposed to organophosphorus compounds. For example, RBC AChE activity was reduced by as much as 40–80% from baseline in farm workers who were chronically exposed to organophosphorus pesticides, but otherwise presented no overt clinical sign or symptom of organophosphorus intoxication (Ames et al. 1989; Farahat et al. 2011; Singleton et al. 2015). On the other hand, prenatal exposure to levels of organophosphorus pesticides not anticipated to induce substantial AChE inhibition was associated with abnormal neonatal reflexes, pervasive development disorder, cognitive deficits, and tremors in children ranging from 2 to 7 years of

age (Bouchard et al. 2011; Gunier et al. 2016; Marks et al. 2010; Rauh et al. 2012, 2015; Rosas and Eskenazi 2008; Stein et al. 2016). A meta-analysis of results from 14 studies published between 1960 and 2012 found a significant association between long-term exposure to low levels of organophosphorus pesticides and impairment of a number of neurological functions, including working memory, attention, psychomotor speed, executive function, and visuospatial ability (Ross et al. 2013).

Relatively high-dose inhalation, oral, or dermal exposure of hens to tribufos resulted in OPIDN (Abou-Donia et al. 1979; Francis et al. 1985). Husain (2014) reviewed possible mechanisms of OPIDN and concluded that the initial mechanism involves phosphorylation and subsequent aging of the enzyme, NTE; a second mechanism appears to involve disruption of calcium homeostasis. It was suggested that OPIDN results from loss of NTE's phospholipid activity, which causes malfunction of endoplasmic reticulum and perturbation of axonal transport and glial-axonal interactions. Although tribufos-induced OPIDN has been demonstrated in hens, no cases of OPIDN have been reported in humans exposed to tribufos.

Numerous studies have also provided evidence of non-enzymatic functions mediated by AChE that include axonal outgrowth (Bigbee et al. 2000), synaptogenesis (Sternfeld et al. 1998), cell adhesion (Bigbee and Sharma. 2004), and neuronal migration (Dori et al. 2005). These non-enzymatic actions of AChE appear to be especially critical for synaptic development (Silman and Sussman 2005).

AChE-unrelated mechanisms, which are likely to differ from one organophosphorus compound to another, have been proposed to explain the effects of long-term exposure to low levels. Organo-phosphorus compounds can directly interact with nicotinic and muscarinic receptors (Albuquerque et al. 1985; Bomser and Casida 2001; Jett et al. 1991) and structural proteins such as tubulin, kinesin, and dynein (Androutsopoulos et al. 2013; Terry 2012). These and other non-AChE mechanisms, including exacerbated oxidative stress (Garry 2004; Ray 1998), imbalanced intracellular Ca2+ homeostasis, increased signaling mediated by inflammatory mediators such as interleukins and cytokines, changes in cellular signaling mediated by neurotrophin receptors and protein kinases, and mitochondrial disruption, have been proposed to contribute to the toxicity of organophosphorus compounds (Androutsopoulos et al. 2013; Banks and Lein 2012; Terry 2012). However, no information was located to suggest that such non-AChE mechanisms are involved in tribufos toxicity.