3. HEALTH EFFECTS

3.1 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 guthion. 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.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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 first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure 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 end point 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 end points. 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.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. ATSDR considers an exposure level that elicits a 20– 59% inhibition of erythrocyte or brain AChE activity to be a less serious LOAEL. An exposure level that elicits an inhibition of erythrocyte or brain AChE activity of 60% or more is considered to represent a serious LOAEL (Chou and Williams-Johnson 1998). Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

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

3.2.1   Inhalation Exposure

The highest NOAEL values and all LOAEL values from each reliable study for appropriate end points in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Rats were exposed by inhalation for acute and intermediate, but not chronic, periods.

3.2.1.1   Death

No information was located regarding mortality in humans following inhalation exposure to guthion.

The 1-hour LC_{50} values and 95% confidence intervals in male and female rats were 69 (62–77) mg/m^3 and 79 (68–93) mg/m^3, respectively (EPA 1978a). There were no mortalities in male or female rats exposed to guthion aerosols at concentrations as high as 4.72 mg/m^3 for 6 hours/day, 5 days/week for 12 weeks (Kimmerle 1976).

Table 3-1 Levels of Significant Exposure to Guthion - Inhalation

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/m³)	Serious (mg/m³)		
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	1 hr				69 [b] M (LC50) 79 F (LC50)	EPA 1978a	
Neurological								
2	Rat (Sprague-Dawley)	1 hr			39 M (41% reduction in blood ChE)		EPA 1978a	
3	Rat (Wistar)	6 hr/d 5 d/wk 2 wk		1.24 [c] M	4.72 M (25% reduction in erythrocyte ChE activity)		Kimmerle 1976	
INTERMEDIATE EXPOSURE								
Systemic								
4	Rat (Wistar)	6 hr/d 5 d/wk 12 wk	Bd Wt	1.24 M	4.72 M (19.7% reduction in body weight gain)		Kimmerle 1976	

Table 3-1 Levels of Significant Exposure to Guthion - Inhalation   **(continued)**

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL				Reference Chemical Form	Comments
					Less Serious (mg/m³)		Serious (mg/m³)			
5	Rat (Wistar)	6 hr/d 5 d/wk 12 wk	Resp	4.72					Kimmerle 1976	No treatment-related effects on weight or morphology in thyroid, adrenals, heart, lung, liver, gonads, or kidneys.
			Cardio	4.72						
			Hemato	4.72						
			Hepatic	4.72						
			Renal	4.72						
			Endocr	4.72						
Immuno/ Lymphoret										
6	Rat (Wistar)	6 hr/d 5 d/wk 12 wk		4.72					Kimmerle 1976	No treatment-related effects on weight or morphology in thymus or spleen.
Neurological										
7	Rat (Wistar)	6 hr/d 5 d/wk 12 wk		1.24[d]	4.72	(29-48% reduction in erythrocyte ChE activity for males; 26-39% for females)			Kimmerle 1976	

a The number corresponds to entries in Figure 3-1.

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Used to derive an acute-duration inhalation minimal risk level (MRL) of 0.02 mg/m3; the MRL was derived by dividing the NOAEL[HEC] of 0.50 mg/m3 by an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability).

d Used to derive an intermediate-duration and chronic-duration inhalation minimal risk level (MRL) of 0.01 mg/m3; the MRL was derived by dividing the NOAEL[HEC] of 0.37 mg/m3 by an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; ChE = cholinesterase; d = day(s); Endocr = endocrine; F = Female; hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 3-1 Levels of Significant Exposure to Guthion - Inhalation

Acute (≤14 days)
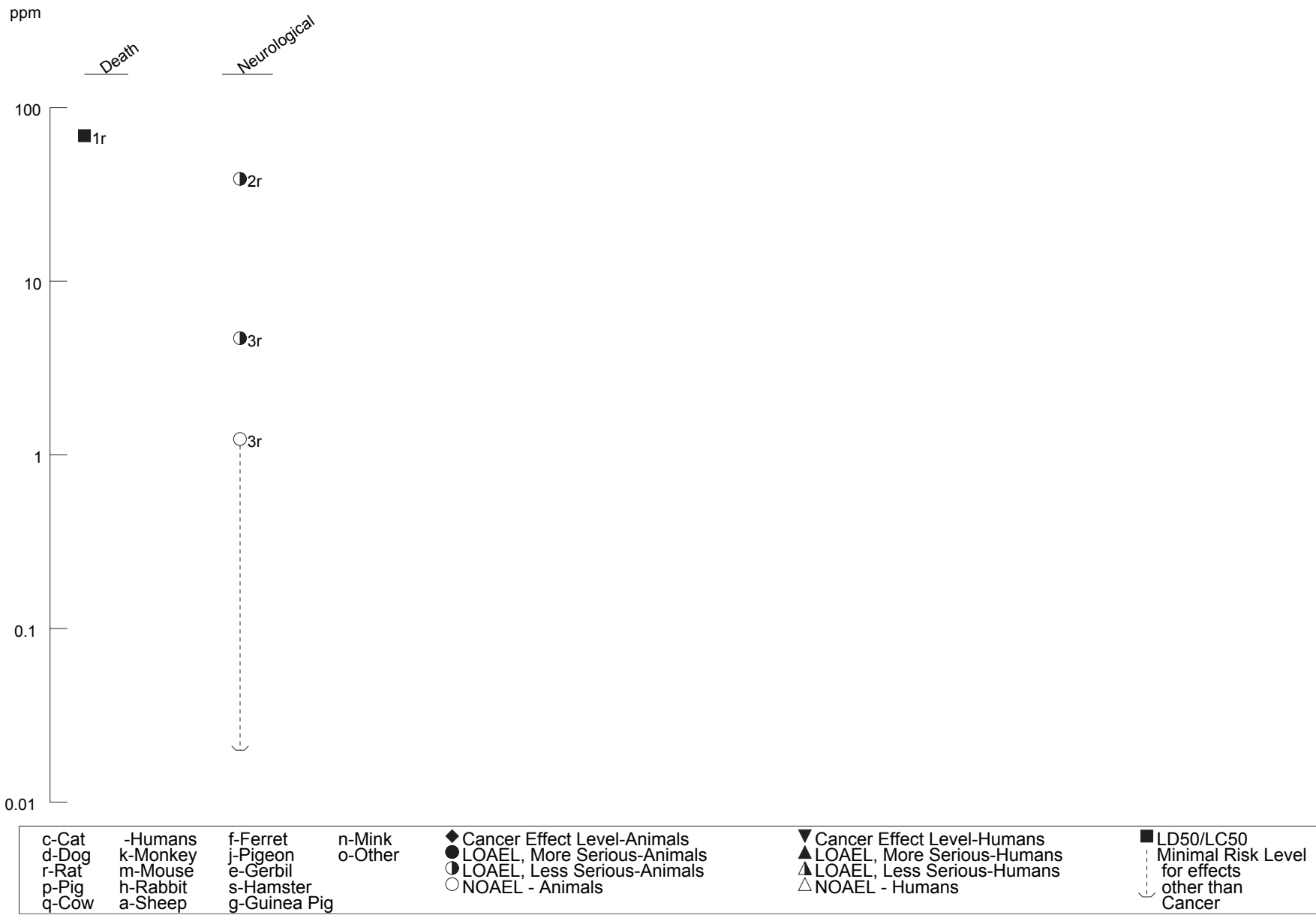
ppm

Death

Neurological

100

■1r

◑2r

10

◑3r

1

○3r

0.1

0.01

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	▲ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-1 Levels of Significant Exposure to Guthion - Inhalation *(Continued)*
Intermediate (15-364 days)

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◐ LOAEL, Less Serious-Animals	△ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

3.2.1.2 Systemic Effects

There were no significant changes in the absolute or relative weights of the thyroid, adrenals, gonads, heart, lungs, liver, or kidneys of Wistar rats exposed by inhalation to guthion at 4.72 mg/m^3, 6 hours/day, 5 days/week for 12 weeks (Kimmerle 1976). These organs also did not show morphological changes associated with exposure to guthion. No significant changes in hemoglobin concentration, red blood cell concentration, thrombocyte concentration, percent packed cell volume, or leucocyte differentials were observed in Wistar rats exposed by inhalation to guthion 4.72 mg/m^3, 6 hours/day, 5 days/week for 12 weeks (Kimmerle 1976). No information was located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, endocrine, dermal, or ocular effects in humans or animals after acute or chronic inhalation exposure to guthion.

Body Weight Effects.   A 19.7% reduction in body weight gain was observed in male, but not female, Wistar rats exposed by inhalation to guthion at 4.72 mg/m^3, 6 hours/day, 5 days/week for 12 weeks (Kimmerle 1976). Weight gain was not affected at 1.24 mg/m^3.

3.2.1.3 Immunological and Lymphoreticular Effects

There were no significant changes in the absolute or relative weights of the thymus or spleen of Wistar rats exposed by inhalation to guthion at 4.72 mg/m^3, 6 hours/day, 5 days/week for 12 weeks (Kimmerle 1976). These organs also did not show morphological changes associated with exposure to guthion.

No information was located regarding immunological or lymphoreticular effects in humans or animals following acute or chronic inhalation exposure to guthion.

3.2.1.4 Neurological Effects

No information was located that demonstrated an association between neurological effects in humans and inhalation exposure to guthion.

EPA (1978a) reported a 41% (range 27–59%) reduction in blood cholinesterase (ChE) activity in rats exposed to guthion aerosols at 39 mg/m^3 for 1 hour. Erythrocyte ChE (also known as acetylcholinesterase; AChE) activity was reduced by 25 and 18% in male and female rats, respectively, exposed to guthion aerosols at 4.72 mg/m^3, 6 hours/day, 5 days/week, for 2 weeks (Kimmerle 1976).

There were no biologically significant changes in erythrocyte AChE activity at $\leq$1.24 mg/m^3. Erythrocyte AChE activity was reduced by 26–48% in male and female rats exposed to 4.72 mg/m^3, 6 hours/day, 5 days/week, for 12 weeks but not at 1.24 mg/m^3. The reductions in AChE activity observed by Kimmerle (1976) were not associated with changes in appearance or behavior of the exposed animals. The study investigators noted that brain cholinesterase activity was not reduced at any of the concentrations tested (Kimmerle 1976).

No information was located regarding the following effects in humans or animals after inhalation exposure to guthion:

3.2.1.5 Reproductive Effects

3.2.1.6 Developmental Effects

3.2.1.7 Cancer

3.2.2   Oral Exposure

The highest NOAEL values and all LOAEL values from each reliable study for appropriate end points in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Animals were exposed orally for acute (rats and mice), intermediate (rats and dogs), and chronic (rats and dogs) periods. Mortality and systemic, reproductive, and developmental parameters were assessed.

3.2.2.1 Death

No information was located regarding mortality in humans following oral exposure to guthion. A number of studies have examined the acute lethality of guthion in laboratory animals. Single-dose, oral toxicity studies with guthion administered to male or female rats reported LD$_{50}$ values in the range of 11–26 mg/kg (Gaines 1960; EPA 1978a; Pasquet et al. 1976). These studies suggest that male and female rats have similar susceptibilities to the acute lethal toxicity of guthion administered orally.

Single or repeated oral doses of guthion at $\geq$8 mg/kg/day killed all treated virgin female mice or rats and pregnant mice (Kavlock et al. 1985; Short et al. 1980). Elevated mortality rates in the 15–62% range were also observed in pregnant rats administered guthion at $\geq$4.9 mg/kg/day (Holzum 1990; Short et al. 1980). No significant increases in mortality were observed in male or female mice or rats after acute-, intermediate-, or chronic-duration oral exposures to $\leq$4 mg/kg/day (Allen et al. 1990; Holzum 1990; Schmidt and Chevalier 1984; Short et al. 1980).

Table 3-2 Levels of Significant Exposure to Guthion - Oral

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg)	Serious (mg/kg)		
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	Once (G)				16 M [b] (14 day LD50) 18 F (14 day LD50)	EPA 1978a	
2	Rat (Sherman)	Once (GO)				13 M (14 day LD50) 11 F [b] (14 day LD50)	Gaines 1960	
3	Rat (CD)	Once (G)				26 M (10 day LD50) 24 F [b] (10 day LD50)	Pasquet et al. 1976	
4	Rat (CD)	35 d 1 x/d (GO)				8 F (100% mortality)	Short et al. 1980	
5	Mouse (CD-1)	Once Gd 8 (GO)				20 F (21/40 maternal death)	Kavlock et al. 1985	
6	Mouse (CD)	10 d 1 x/d (GO)				8 F (100% mortality)	Short et al. 1980	
Systemic								
7	Rat (Sprague-Dawley)	Gd 6-15 (GO)	Bd Wt	2 F			Astroff and Young 1998	

Table 3-2 Levels of Significant Exposure to Guthion - Oral   **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
8	Rat (CD)	Gd 6-15 (GO)	Bd Wt	2.5 F		5 F (50% reduction in maternal weight gain)	Short et al. 1980	
9	Mouse (CD-1)	Once Gd 8 (GO)	Bd Wt	16 F	20 F (19% reduction in maternal weight gain)		Kavlock et al. 1985	
10	Mouse (CD-1)	Gd 6-15 (GO)	Bd Wt	5 F			Short et al. 1980	
Neurological								
11	Rat (Sprague-Dawley)	Gd 6-15 (GO)		1 F [c]	2 F (40% reduction in maternal brain ChE activity on gestation day 16)	2 F (75% reduction in maternal erythrocyte ChE activity on gestation day 16)	Astroff and Young 1998	
12	Rat (Sprague-Dawley)	Once (G)				16 M (signs of cholinergic poisoning: salivation, lacrimation, exophthalmus, defecation, urination, and muscle fasciculations)	EPA 1978a	
13	Rat (CD)	Once (G)			2 F (21-24% reduction in erythrocyte and brain ChE activity)	18 F (65-82% reduction in brain and erythrocyte ChE activity)	Pasquet et al. 1976	

Table 3-2 Levels of Significant Exposure to Guthion - Oral   **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
14	Rat (CD)	35 d 1 x/d (GO)		4 F		8 F (salivation, urination, lacrimation, and tremors)	Short et al. 1980	
15	Rat (Holtzman)	1 wk (F)		2.8 F		5.7 F (78.2% reduction in brain ChE activity)	Su et al. 1971	
16	Mouse (CD-1)	10 d 1 x/d (GO)		4 F		8 F (salivation, urination, lacrimation, and tremors)	Short et al. 1980	
17	Mouse (CD-1)	Gd 6-15 (GO)		2.5 F		5 F (tremors, salivation, and urination observed in some pregnant mice)	Short et al. 1980	
Reproductive								
18	Rat (Sprague-Dawley)	Gd 6-15 (GO)		2 F			Astroff and Young 1998	
19	Mouse (CD-1)	Once Gd 8 (GO)		16 F	20 F (reduced incidence of viable litters)		Kavlock et al. 1985	
Developmental								
20	Rat (Sprague-Dawley)	Gd 6-15 (GO)		2			Astroff and Young 1998	

Table 3-2 Levels of Significant Exposure to Guthion - Oral   (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
21	Mouse (CD-1)	Once Gd 8 (GO)		16	20 (11% reduction in fetal body weight)		Kavlock et al. 1985	This dose level was associated with an increase in maternal mortality.
22	Mouse (CD-1)	Once Gd 8 (GO)			16 (increased incidence of supernumerary ribs)		Kavlock et al. 1985	
23	Mouse (CD-1)	Gd 6-15 (GO)		2.5	5 (increased incidence of malaligned sternebrae in fetuses)		Short et al. 1980	

INTERMEDIATE EXPOSURE

Death

24	Rat (Wistar)	14 wk before mating to ppd 5 or 28 (F)				4.9 F (7/46 rats died or were moribund and sacrificed)	Holzum 1990	
25	Rat (CD)	Gd 6-ppd 21 (GO)				5 F (62% mortality in dams)	Short et al. 1980	
26	Rat (Wistar)	3 wk (F)				11.5 M (increased mortality, incidence not provided)	Vos et al. 1983	

Table 3-2 Levels of Significant Exposure to Guthion - Oral   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
27	Dog Cocker spaniel	26 wk (F)		3.8[b] M 4.3 F			Allen et al. 1990	
Systemic								
28	Rat (Wistar)	8 wk (F)	Other	0.75 M	2.3 M (15/60 increased incidence of alopecia)		Schmidt and Chevalier 1984	
29	Rat (Fischer- 344)	13 wk (F)	Bd Wt	2.8[b] M 3.2 F	7.9 M (unspecified reduction in terminal body weight)		Sheets et al. 1997	
30	Rat (Wistar)	3 wk (F)	Bd Wt	2.3 M	11.5 M (decreased terminal body weight, magnitude not provided)		Vos et al. 1983	
31	Rat (Wistar)	3 wk (F)	Endocr	2.3 M	11.5 M (decreased relative pituitary weight; unspecified histopathologic findings in the pituitary and adrenals; quantitative results not provided)		Vos et al. 1983	

Table 3-2 Levels of Significant Exposure to Guthion - Oral   **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
32	Dog Cocker spaniel	8 wk (F)	Gastro	0.15[b] M 0.78 F	0.69[b] M (increased incidence of mucoid diarrhea and emesis) 4.3 F (increased incidence of mucoid diarrhea and emesis)		Allen et al. 1990	
33	Dog Cocker spaniel	26 wk (F)	Ocular	3.8[b] M 4.3 F			Allen et al. 1990	
34	Dog Cocker spaniel	26 wk (F)	Hemato	3.8[b] M 4.3 F			Allen et al. 1990	
Immuno/ Lymphoret								
35	Rat (Wistar)	3 wk (F)		2.3 M	11.5 M (decreased relative spleen and mesenteric lymph node weights, as well histopathologic findings in the thymus; quantitative results not provided)		Vos et al. 1983	

Table 3-2 Levels of Significant Exposure to Guthion - Oral   **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
Neurological								
36	Rat (Wistar)	14 wk before mating to ppd 5 or 28 (F)			0.55 F (25 and 47% reductions in erythrocyte ChE activity on lactation days 5 and 28, respectively)	1.5 F (75 and 84% reductions in erythrocyte ChE activity on lactation days 5 and 28, respectively)	Holzum 1990	
37	Rat (Fischer- 344)	13 wk (F)			0.91 M (37% reduction in erythrocyte ChE activity on week 13)	2.8 M (84% reduction in erythrocyte ChE activity on week 13)	Sheets et al. 1997	
38	Rat (Fischer- 344)	13 wk (F)		1.1 F		3.2 F (tremors, incoordinated gait, and perianal staining)	Sheets et al. 1997	
39	Rat (CD)	Gd 6-ppd 21 (GO)		2.5 F		5 F (tremors, salivation, and urination were observed in some pregnant CD rats)	Short et al. 1980	
40	Dog Cocker spaniel	26 wk (F)		0.15 M [d]	0.69 M (22-40% reduction in erythrocyte ChE activity)	3.8 M (66-88% reduction in erythrocyte ChE activity; 37-58% reduction in plasma ChE activity; 27% reduction in brain ChE)	Allen et al. 1990	

Table 3-2 Levels of Significant Exposure to Guthion - Oral   **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
41	Rat (Wistar)	14 wk before mating to ppd 5 or 28 (F)		3.7 [b] M 4.9 F			Holzum 1990	Insemination, fertility, or gestation indices or duration of gestation were not affected.
42	Rat (Wistar)	3 wk (F)		2.3 M	11.5 M (unspecified histopathologic findings in the testes)		Vos et al. 1983	
Developmental								
43	Rat (Wistar)	14 wk before mating to ppd 5 or 28 (F)		0.43 [b] M 0.55 F	1.3 [b] M (statistically significant reduction in viability of pups on ppd 5) 1.5 F (statistically significant reduction in viability of pups on ppd 5)		Holzum 1990	
44	Rat (Wistar)	14 wk before mating to ppd 5 or 28 (F)		1.5 F	4.9 F (significantly lower (19-25%) pup weight, relative to controls, on ppd 14 and 21)		Holzum 1990	
45	Rat (Wistar)	14 wk before mating to ppd 5 or 28 (F)		3.8 M			Holzum 1990	No reduction in viability when treated males were mated with untreated females.

Table 3-2 Levels of Significant Exposure to Guthion - Oral **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
46	Rat (Wistar)	14 wk before mating to ppd 5 or 28 (F)		1.5 F	4.9 F (in pups: significant (19%) reduction in brain weight on ppd 5 and 46% reduction in brain ChE activity on ppd 28)		Holzum 1990	
47	Rat (CD)	Gd 6-ppd 21 (GO)		2.5		5 (34% reduction in pup weight; 85% reduction in pup survival)	Short et al. 1980	This exposure level was also associated with an increase in maternal mortality.
48	Rat (CD)	Gd 6-ppd 21 (GO)		2.5	5 (in pups in the surviving litter: rear legs were stiff, at right angles to the body; pups lacked neuromuscular coordination of hind legs; muscle tremors in the tail and upturned snouts)		Short et al. 1980	The 5 mg/kg/day dose was associated with an increase in maternal mortality.

CHRONIC EXPOSURE

Death

| 49 | Rat (Wistar) | 2 yr (F) | | 2.3 [b] M

3.1 F | | | Schmidt and Chevalier 1984 | |

Systemic

| 50 | Rat (Wistar) | 2 yr (F) | Bd Wt | 0.75 [b] M

3.11 F | 2.33 M (10% reduction in body weight) | | Schmidt and Chevalier 1984 | |

Table 3-2 Levels of Significant Exposure to Guthion - Oral   **(continued)**

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
51	Rat (Wistar)	2 yr (F)	Dermal	0.75 M	2.3 M (15/60 increased incidence of alopecia)		Schmidt and Chevalier 1984	
52	Rat (Wistar)	2 yr (F)	Ocular	2.3[b] M 3.1 F			Schmidt and Chevalier 1984	
53	Rat (Wistar)	2 yr (F)	Hemato	2.3 M 0.96[b] F	3.1 F (thrombocyte values significantly elevated by 20-25%)		Schmidt and Chevalier 1984	
54	Rat (Wistar)	2 yr (F)	Hepatic	2.3 M 0.96[b] F			Schmidt and Chevalier 1984	
55	Rat (Wistar)	2 yr (F)	Renal	2.3[b] M 3.1 F			Schmidt and Chevalier 1984	

Table 3-2 Levels of Significant Exposure to Guthion - Oral   (continued)

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
56	Dog Cocker spaniel	52 wk (F)	Gastro	0.15[b] M 0.78 F	0.69[b] M (increased incidence of mucoid diarrhea and emesis) 4.3 F (increased incidence of mucoid diarrhea and emesis)		Allen et al. 1990	
57	Dog Cocker spaniel	52 wk (F)	Ocular	3.8[b] M 4.3 F			Allen et al. 1990	
58	Dog Cocker spaniel	52 wk (F)	Hemato	3.8[b] M 4.3 F			Allen et al. 1990	
59	Dog Cocker spaniel	52 wk (F)	Hepatic	0.69[b] M 0.78 F			Allen et al. 1990	
60	Dog Cocker spaniel	52 wk (F)	Bd Wt	0.69 M	3.8 M (12% decrease in terminal body weight)		Allen et al. 1990	

Table 3-2 Levels of Significant Exposure to Guthion - Oral   **(continued)**

Key to Figure [a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
61	Dog Cocker spaniel	52 wk (F)	Renal	3.8 M [b] 4.3 F			Allen et al. 1990	
Neurological								
62	Rat (Wistar)	2 yr (F)		0.25 M	2.3 M (38-49% reduction in plasma ChE activity; 32% reduction in brain ChE activity; 7-11% increase in relative brain weight) 0.75 M (10-22% reduction in erythrocyte ChE activity)		Schmidt and Chevalier 1984	
63	Dog Cocker spaniel	52 wk (F)		0.15 M [e]	0.69 M (27% reduction in erythrocyte ChE activity)	3.8 M (86% reduction in erythrocyte ChE activity)	Allen et al. 1990	

a The number corresponds to entries in Figure 3-2.

b Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

c Used to derive an acute-duration oral minimal risk level (MRL) of 0.01 mg/kg/day; the MRLs were derived by dividing the BMDL of 1.04 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 to protect sensitive subpopulations).

d Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.003 mg/kg/day; the MRL was derived by dividing the BMDL of 0.29 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 to protect sensitive subpopulations).

e Used to derive a chronic-duration oral minimal risk level (MRL) of 0.003 mg/kg/day; the MRL was derived by dividing the BMDL of 0.30 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 to protect sensitive subpopulations).

ChE = cholinesterase; Bd Wt = body weight; d = day(s); (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; ppd = post-parturition day; x = time(s); wk = week(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Guthion - Oral

Acute (≤14 days)

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◐ LOAEL, Less Serious-Animals	▲ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-2 Levels of Significant Exposure to Guthion - Oral *(Continued)*
Intermediate (15-364 days)

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	△ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-2 Levels of Significant Exposure to Guthion - Oral *(Continued)*
Intermediate (15-364 days)

mg/kg/day

Immuno/Lymphor   Neurological   Reproductive   Developmental

100

10   ◑35r   ◑42r

                        ●39r   ◑44r   ◑46r   ●47r   ◑48r
            ●40d   ●38r   ◐45r
            ●37r   ○39r   ○41r   ○47r   ○48r
            ○35r   ○42r

            ●36r   ○44r   ○46r
            ◐38r   ◑43r
            ◐37r
            ◐40d
            ◑36r   ○43r

1

            ○40d

0.1

0.01

0.001

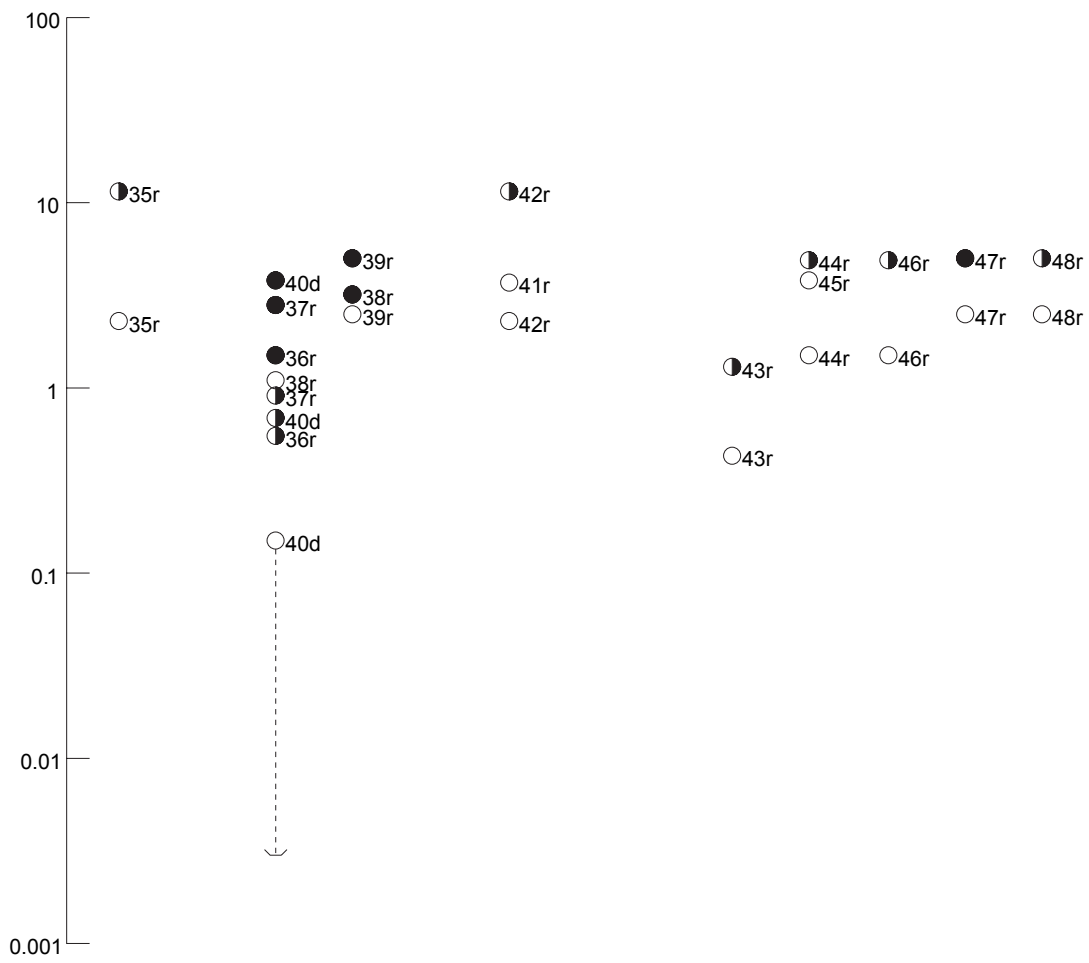| c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	┊ Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	△ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	↓ other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-2 Levels of Significant Exposure to Guthion - Oral *(Continued)*
Chronic (≥365 days)

3.2.2.2 Systemic Effects

No information was located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, or metabolic effects in humans. No information was located regarding respiratory, cardiovascular, musculoskeletal, hepatic, dermal, or metabolic effects in animals following oral exposure to guthion.

Gastrointestinal Effects.   An increased incidence (relative to control animals) of mucoid diarrhea was reported in male dogs administered guthion in the diet at 3.8 mg/kg/day for up to 1 year (Allen et al. 1990). Male dogs administered 0.69 mg/kg/day showed a higher incidence of mucoid diarrhea than that observed in males at 3.8 mg/kg/day. An increase in the incidence of diarrhea was observed in female dogs administered 4.3 mg/kg/day (Allen et al. 1990).

Hematological Effects.   Thrombocyte values became significantly elevated (20–25%) in female rats after 12 months of exposure to guthion at 3.1 mg/kg/day in the diet, but not in female rats exposed to 0.96 mg/kg/day (Schmidt and Chevalier 1984). Effects on thrombocyte values were not observed in male rats administered up to 2.3 mg/kg/day. No treatment-related hematological effects were observed in male or female dogs administered guthion in the diet at 3.8 or 4.3 mg/kg/day, respectively, for up to 52 weeks (Allen et al. 1990).

Renal Effects.   There were no dose-related changes in urinalysis parameters in male or female rats administered up to 2.3 or 3.1 mg/kg/day, respectively, for up to 2 years (Schmidt and Chevalier 1984) or in male or female dogs administered guthion at 3.8 or 4.3 mg/kg/day, respectively, for 52 weeks (Allen et al. 1990).

Endocrine Effects.   Vos et al. (1983) reported decreased relative pituitary weight as well as unspecified histopathologic findings in the pituitary and adrenals in male Wistar rats exposed to 11.5 mg/kg/day guthion (85% active ingredient [a.i.]) in the diet for 3 weeks. Quantitative results were not provided. No effects on the endocrine system were observed at 2.3 mg/kg/day.

Ocular Effects.   No treatment-related ocular effects were observed in male or female rats administered guthion in the diet at up to 2.33 mg/kg/day and 3.1 mg/kg/day, respectively, for up to 2 years (Schmidt and Chevalier 1984). No treatment-related effects were observed in opthalmoscopic examinations

conducted in male and female dogs administered guthion in the diet at 3.8 or 4.3 mg/kg/day, respectively (Allen et al. 1990).

Body Weight Effects.   Reductions in body weight gain or terminal body weights have been observed following acute, intermediate, or chronic exposure. In gestational exposure studies, a 19% decrease in maternal body weight gain was observed in mice following a single gavage dose of 20 mg/kg/day on gestational day 8 (Kavlock et al. 1985) and a 50% reduction in body weight gain was observed in rats administered gavage doses of 5 mg/kg/day on gestational days 6–15 (Short et al. 1980). However, maternal body weight was not adversely affected in rats or mice following gavage exposure to 2 or 2.5 mg/kg/day on gestational days 6–15 (Astroff and Young 1998; Short et al. 1980). In the Short et al. (1980) study, a concomitant decrease in food consumption (24%) and clinical signs of cholinesterase inhibition (tremors and salivation) were also observed in pregnant rats at 5 mg/kg/day. An unspecified decrease in body weight (investigators noted that most body weight changes observed in this study of several compounds were 5–15%) was observed in male rats following a 13 weeks exposure to 7.9 mg/kg/day, but not after exposure to 2.8 mg/kg/day (Sheets et al. 1997); decreases in body weight and food consumption were observed in females at 7 mg/kg/day but not at 3.2 mg/kg/day. Following chronic-duration exposure, 10–12% decreases in terminal body weight were observed in dogs exposed to 3.8 mg/kg/day in the diet for 52 weeks (Allen et al. 1990) and rats exposed to 2.33 mg/kg/day in the diet for 2 years (Schmidt and Chevalier 1984).

Other Systemic Effects.   An increased incidence of alopecia (relative to control animals) was observed in male and female rats administered guthion in the diet at 2.3 and 3.1 mg/kg/day, respectively, for 8 weeks to 2 years (Schmidt and Chevalier 1984). There were no dose-related changes in clinical chemistry parameters in male or female rats administered up to 2.3 or 3.1 mg/kg/day, respectively, for up to 2 years (Schmidt and Chevalier 1984). Clinical chemistry tests showed that albumin and albumin/globulin values were significantly reduced in male dogs administered guthion in the feed at 3.8 mg/kg/day (Allen et al. 1990). The observed reductions in albumin and albumin/globulin in male dogs ranged from 7 to 13% and from 17 to 20%, respectively, from weeks 13 to 52 (Allen et al. 1990). No effect on hearing was evident in male or female dogs administered guthion in the diet at 3.8 or 4.3 mg/kg/day, respectively, for up to 52 weeks (Allen et al. 1990). A 39% increase in cytochrome P-450 activity was observed in male dogs administered 3.8 mg/kg/day guthion in the diet for 52 weeks; a 15% increase in P-450 activity was observed in female dogs administered guthion in the diet at 4.3 mg/kg/day for 52 weeks (Allen et al. 1990). A 34% and 30% increase in N-demethylase activity was observed in male dogs at 3.8 mg/kg/day and in female dogs at 4.3 mg/kg/day, respectively.

3.2.2.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological and lymphoreticular effects in humans following oral exposure to guthion. Vos et al. (1983) reported decreased relative spleen and mesenteric lymph node weights, as well as unspecified histopathologic findings in the thymus in male Wistar rats exposed to guthion (85% a.i.) in the diet at 11.5 mg/kg/day for 3 weeks; no effects were observed at 2.3 mg/kg/day.

3.2.2.4 Neurological Effects

There is a paucity of data regarding the effects of guthion in humans; however, limited data are available in humans which indicate that no significant changes in plasma or erythrocyte ChE activity were observed in a group of five subjects receiving guthion orally on a daily basis at up to 0.29 mg/kg/day for 4 weeks (Rider and Puletti 1969; Rider et al. 1970, 1971, 1972).

The most commonly observed neurological effects in laboratory animals treated orally with guthion are reduced erythrocyte, plasma, or brain ChE activity and clinical signs of cholinesterase inhibition (Allen et al. 1990; Astroff and Young 1998; Holzum 1990; Pasquet et al. 1976; Schmidt and Chevalier 1984, Sheets et al. 1997; Short et al. 1980; Su et al. 1971). Reduction in erythrocyte AChE activity is generally the most sensitive end point. Reductions in brain and plasma ChE are observed at somewhat higher doses than those affecting erythrocyte AChE. Clinical signs are only evident in animals at doses several times higher than those eliciting reductions in erythrocyte, brain, or plasma ChE activity. Clinical signs such as hypercholinergy and nicotinic effects, salivation, lacrimation, exophthalmus, defecation, urination, and muscle fasciculations have been observed in rats or mice administered lethal oral doses of guthion (EPA 1978a; Pasquet et al. 1976; Short et al. 1980) and in rats and mice administered doses of approximately $\geq$3.2 mg/kg/day (Sheets et al. 1997; Short et al. 1980). Reductions in erythrocyte AChE activity of $\geq$75% have been observed in rats or dogs after acute, intermediate, or chronic oral exposures to guthion of $\geq$2 mg/kg/day (Allen et al. 1990; Astroff and Young 1998; Pasquet et al. 1976; Sheets et al. 1997) and reductions in the range of 20–50% have been observed in rats or dogs after acute-to-chronic oral exposure to guthion at 0.55–2 mg/kg/day (Allen et al. 1990; Holzum 1990; Pasquet et al. 1976; Schmidt and Chevalier 1984; Sheets et al. 1997). There was no reduction in erythrocyte AChE activity observed in rats exposed to 0.43 mg/kg/day for at least 14 weeks (Holzum 1990), dogs exposed to 0.15– 0.16 mg/kg/day for 52 weeks (Allen et al. 1990), or in rats exposed to 0.25–0.31 mg/kg/day for 2 years (Schmidt and Chevalier 1984).

Brain AChE activity was reduced by 20–78% in rats or dogs administered acute or chronic oral doses of guthion of approximately 0.96–5.7 mg/kg/day (Allen et al. 1990; Astroff and Young 1998; Pasquet et al. 1976; Schmidt and Chevalier 1984; Su et al. 1971). Doses of 3.2–18 mg/kg/day elicited reductions in brain AChE activity of ≥72% (Pasquet et al. 1976; Sheets et al. 1997; Su et al. 1971). There was no reduction on brain AChE activity in rats or dogs administered ≤1 mg/kg/day (Allen et al. 1990; Astroff and Young 1998). Reductions of 35–58% in plasma ChE activity were observed in rats or dogs exposed chronically to guthion at 0.96–4.3 mg/kg/day. No reduction in activity was observed at 0.15 mg/kg/day in dogs after 52 weeks (Allen et al. 1990; Schmidt and Chevalier 1984).

3.2.2.5 Reproductive Effects

No information was located regarding reproductive effects in humans following oral exposure to guthion.

Insemination, fertility, or gestation indices or duration of gestation were not affected in male and female rats administered guthion at 0.43 to 4.9 mg/kg/day in the diet for 14 weeks before mating and continuously through gestation (Holzum 1990). A significant reduction in the incidence of viable litters was observed in pregnant mice exposed to 20 mg/kg, but not 16 mg/kg, on gestation day 8 (Kavlock et al. 1985). Unspecified histopathologic findings were observed in the testes of Wistar rats administered 11.5 mg/kg/day in the diet for 3 weeks; no effects were observed at 2.3 mg/kg/day (Vos et al. 1983).

3.2.2.6 Developmental Effects

No information was located regarding developmental effects in humans following oral exposure to guthion.

An 11% reduction in fetal weight was observed in the offspring of pregnant mice administered technical-grade guthion at 20 mg/kg by gavage on gestation day 8; this dose level was also associated with a 53% increase in maternal mortality (Kavlock et al. 1985). No effect on fetal body weights was observed in the offspring of mice exposed to 16 mg/kg on gestation day 8 (Kavlock et al. 1985), rats exposed to 2.0 mg/kg/day on gestation days 6–15 (Astroff and Young 1998), or rats or mice administered 5.0 mg/kg/day on gestation days 6–15 (Short et al. 1980). However, exposure of rats to 5.0 mg/kg/day on gestational days 6 through postnatal day 21 resulted in a 34% reduction in pup weight and an 85% reduction in pup survival (Short et al. 1980). This exposure was also associated with a 62% increase in maternal mortality. Thus, the possibility that some of the effects in the offspring could be secondary to maternal toxicity, perhaps augmented by the potential exposure to guthion during lactation, cannot be

excluded. No information was available regarding concentrations of guthion in maternal milk. A statistically significant reduction in survival was observed in the 5-day old offspring of male and female rats administered 1.3 and 1.5 mg guthion/kg/day in the diet starting 14 weeks before mating and until postparturition day 5 (Holzum 1990). In male and female rats, guthion doses of 1.3 and 1.5 mg/kg/day were associated with 69% and 75% reductions in erythrocyte AChE (Holzum 1990). Survival of 5-day old pups was not affected when only male rats were administered up to 3.8 mg/kg/day (Holzum 1990).

Guthion exposure did not elicit external, visceral, or skeletal malformations or variations in offspring of rats administered guthion at 2.0 mg/kg/day on gestation days 6–15 (Astroff and Young 1998) or skeletal anomalies in pups from mice administered guthion at 2.5 mg/kg/day on gestation days 6–15 (Short et al. 1980); however, pups showed a dose-related increase in malaligned sternbrae at 5 mg/kg/day (Short et al. 1980). A marked increase in the incidence of supernumerary ribs was observed in the offspring of pregnant mice administered 16 or 20 mg/kg guthion by gavage on gestation day 8 (Kavlock et al. 1985). The incidence of supernumerary ribs was 3% in the control group, and approximately 24 and 58% in the 16 and 20 mg/kg groups, respectively; however, the authors reported an inverse correlation between maternal weight gain and the incidence of supernumerary ribs and suggested that there was an association between nonspecific adverse health effects in the dams and the development of supernumerary ribs in fetuses.

Neurological effects were observed in offspring from pregnant rats administered guthion at 5 mg/kg/day from gestation day 6 to postparturition day 21 by gavage (Short et al. 1980). One day after weaning, pups in the surviving litter presented stiff rear legs at right angles to the body and lack of neuromuscular coordination in the use of the hind legs, as well as muscle tremors in the tail and upturned snouts (Short et al. 1980). These effects were not observed at 2.5 mg/kg/day. Fetal brain cholinesterase activity on gestation day 20 was unaffected in pups from Sprague-Dawley rats administered guthion (87.7% a.i.) at 2 mg/kg/day on gestation days 6–15 (Astroff and Young 1998).

3.2.2.7 Cancer

No studies were located regarding cancer in humans following oral exposure to guthion.

A significant increase in the combined incidence of islet cell carcinoma or carcinomas of the pancreas, as compared to group controls, was observed in male Osborne-Mendel rats exposed to 10.9 mg/kg/day guthion in the diet for 80 weeks followed by a 35-week observation period (NCI 1978). However, it was

concluded that the incidence in the treated males cannot be clearly attributed to treatment with guthion given the high spontaneous incidence of this lesion (0–22% with a mean of 2%) in male Osborne-Mendel rats in this laboratory (NCI 1978). The significant increases in the incidence of benign thyroid tumors, malignant thyroid tumors, or combined follicular cell tumors were observed in male rats exposed to 5.5 or 10.9 mg/kg/day (NCI 1978). It was noted, however, that the spontaneous incidence of these neoplasms in male Osborne-Mendel rats in this laboratory ranges from 0 to 43% with a mean of 7%, and it was concluded that the incidence of the observed lesions could not be clearly ascribed to treatment with guthion (NCI 1978). There was no evidence of the occurrence of treatment-related tumors in female Osborne-Mendel rats (NCI 1978). No changes in the incidence of neoplastic lesions were observed in Wistar rats exposed to doses as high as 3.11 mg/kg/day in the diet for 2 years (Schmidt and Chevalier 1984).

Benign and malignant neoplasms were observed among dosed and control B6C3F1 mice (NCI 1978); however, in previous studies, each type has been observed as spontaneous lesions (NCI 1978). The incidence of hepatocellular adenomas (2/8, 11/49, and 19/50 in the 0, 5.4, and 10.7 mg/kg/day groups, respectively) in male mice provide equivocal evidence of an association between these lesions and guthion exposure. There were no statistically significant associations between tumor incidence and guthion exposure in female mice (NCI 1978).

Under the conditions of the bioassay, NCI (1978) concluded that guthion was not carcinogenic in male or female B6C3F1 mice or female Osborne-Mendel rats. The incidences of neoplasms of the pancreatic islets and of the follicular cells of the thyroid in male rats provide suggestive but insufficient evidence of the carcinogenic potential of guthion in male rats. The NTP concluded that, in a chronic feeding study, guthion was not carcinogenic in mice of either sex or in female rats, but there was equivocal evidence of pancreatic islet cell adenoma or carcinoma and thyroid gland follicular cell adenoma or carcinoma in male rats. In 1993, EPA concluded that there was a lack of evidence of carcinogenicity of guthion in male and female mice and rats. Currently, the EPA has no carcinogenicity classification for guthion (IRIS 2006). IARC has not classified guthion as to its carcinogenicity (IARC 2006).

3.2.3   Dermal Exposure

The highest NOAEL values and all LOAEL values from each reliable study for appropriate end points in each species and duration category are recorded in Table 3-3.

Table 3-3 Levels of Significant Exposure to Guthion - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	Less Serious	Serious	Reference Chemical Form	Comments
ACUTE EXPOSURE							
Death							
Rat (Sprague-Dawley)	Once				455 M mg/kg (14 day LD50)	EPA 1978a	
					222 F mg/kg (14 day LD50)		
Rat (Sherman)	Once				220 M mg/kg (14 day LD50)	Gaines 1960	
					220 F mg/kg (14 day LD50)		
Rat (CD)	Once				90 F mg/kg (10 day LD50)	Pasquet et al. 1976	
Mouse (Swiss-Webster)	Once				6000 M mg/kg (24 hour LD50)	Skinner and Kilgore 1982	
Immuno/ Lymphoret							
Human	Once		1 %volume			Lisi et al. 1987	Patch test with 1% guthion solution.
Human	Once			1 F %volume (allergic reaction to guthion in 1/64 fruit harvest workers)		Sartorelli et al. 1999	

Table 3-3 Levels of Significant Exposure to Guthion - Dermal   **(continued)**

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Neurological							
Human	Once		0.0007 mg/kg			Franklin et al. 1981	Erythrocyte ChE activity.
Human	1 x/d		0.46 M mg/kg/day			Schneider et al. 1994	Reductions in erythrocyte ChE activity were 16% or less.
Rat (Sprague-Dawley)	Once				222 F mg/kg (signs of cholinergic poisoning: salivation, lacrimation, exophthalmus, defecation, urination, muscle fasciculations)	EPA 1978a	
Mouse (Swiss-Webster)	Once			600 M mg/kg (24 hour ED50 for erythrocyte ChE activity)		Skinner and Kilgore 1982	

ChE = cholinesterase; d = day(s); ED50 = median effective dose, 50% effect in population; F = Female; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; x = time(s)

3.2.3.1 Death

No information was located regarding mortality in humans following dermal exposure to guthion.

A number of laboratory studies with animals have demonstrated the lethal toxicity of guthion applied on the skin. There is, however, a large variation in the lethal toxicity of guthion applied dermally. For instance, Pasquet et al. (1976) calculated an LD_{50} of 90 mg/kg in female rats administered guthion (>95% a.i.) once. The treated areas in these animals were washed after 24 hours and the animals were observed for 10 days (Pasquet et al. 1976). Gaines (1960) reported LD_{50} values of 220 mg/kg in male and female Sherman rats, suggesting that there was no sex-related difference in susceptibility to guthion lethal toxicity. In contrast, EPA (1978a) reported LD_{50} values of 455 and 222 mg/kg in male and female Sprague-Dawley rats, respectively, treated once with guthion. The highest reported dermal LD_{50} was 6,000 mg/kg reported by Skinner and Kilgore (1982) after a single dose of guthion was applied to the hind feet of male Swiss Webster mice.

3.2.3.2 Systemic Effects

No information was located regarding systemic effects in humans following dermal exposure to guthion. No information was located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, endocrine, dermal, ocular, or metabolic effects in animals following dermal exposure to guthion.

Hematological Effects. Male and female rabbits were treated with daily dermal applications of technical-grade guthion (94.1% a.i.) at 0, 2, or 20 mg/kg, 5 days/week, for 21 days (EPA 1999b). A 10% reduction in erythrocyte counts was observed in male rabbits administered 20 mg/kg/day, but not 2 mg/kg/day, dermally 5 days/week for 21 days (EPA 1999b).

Renal Effects. Male and female rabbits were treated with daily dermal applications of technical-grade guthion (94.1% a.i.) at 0, 2, or 20 mg/kg, 5 days/week, for 21 days (EPA 1999b). An increase in kidney weight and in the incidence of inflammatory changes in kidney were observed in male rabbits administered 20 mg/kg/day, but not 2 mg/kg/day, dermally 5 days/week for 21 days (EPA 1999b).

Body Weight Effects. Male and female rabbits were treated with daily dermal applications of technical-grade guthion (94.1% a.i.) at 0, 2, or 20 mg/kg, 5 days/week, for 21 days (EPA 1999b). A 40–

70% reduction in body weight gain was observed in female rabbits administered 20 mg/kg/day, but not
2 mg/kg/day, dermally 5 days/week for 21 days (EPA 1999b).

3.2.3.3 Immunological and Lymphoreticular Effects

Patch tests were administered to 64 female workers (aged 17–59 years; mean, age 35 years) involved for
an average of 11 years in the harvesting of cherries, peaches, olives, and grapes in Italy (Sartorelli et al.
1999). Only one subject, who was without symptoms, showed a positive allergic reaction to guthion. In
another study of 180 agricultural workers, 43 former agricultural workers, and 429 patients admitted to
the clinic for nonallergic skin disorders, none of the subjects showed allergic or irritant reactions to 1%
guthion patches applied to the upper back (Lisi et al. 1987).

3.2.3.4 Neurological Effects

Blood AChE activity was determined in approximately 34 peach harvest workers in California in
1991 (Schneider et al. 1994). Workers were classified as "harvesters" (approximately 10) or "sorters"
(approximately 24). Harvesters (all were male) entered orchards to pick fruit 51 days after treatment with
guthion (50% active ingredient at 1.5 pounds active ingredient per 100 gallons of water per acre) and
worked for 10 of the next 17 days, while sorters (males and females) went through fruit bins removing
culls or fruit that was too green. The latter group was considered to have minimal exposure to foliar
residues and served as a control group. There were no differences among harvesters or sorters in their
whole blood AChE before workers entered the orchards; however, 14 and 23 days after entering the field,
significant differences in AChE levels among these two groups were evident. The largest reduction in
AChE observed in harvesters 14 days after entering the orchard was of approximately 16%. Similar
reductions were reported 23 days after exposure, but conflicting data were offered by two separate
laboratories. During the study period, there were no statistically significant ($p > 0.05$) reductions in AChE
in sorters, whereas two of four measurements showed significant ($p < 0.05$) reductions in AChE in
harvesters. No symptoms of organophosphorous poisoning were reported by any of the workers.

A study was conducted with 17 orchardists who applied a single treatment of guthion in a wettable
powder formulation (50% a.i.) in the South Okanagan Valley, British Columbia (Franklin et al. 1981).
The amounts of guthion applied in this study ranged from approximately 1 to 5 kg. Respirators were
worn by applicators. Based on analysis of guthion residues on patches, dermal exposure was estimated to
range from 9 to 43 µg guthion/kg applied. A mean dermal exposure dose of 0.7 µg/kg was estimated
based on anatomical regional deposition of guthion on the bodies of subjects, surface area estimates of

these anatomical regions, and a reference body weight of 70 kg. Postexposure erythrocyte cholinesterase activity appeared to be reduced 15% in the exposed workers; however, these alterations did not exceed the variation observed in the group of unexposed individuals (n=10) in the control group (Franklin et al. 1981).

A study was conducted of 21 male agricultural workers (ages 21–63; mean age 35.5 years) exposed to foliage-borne residues of guthion during peach-thinning operations in California (Kraus et al. 1977). Workers entered the peach orchards 14 days after they had been treated with a 50% wettable powder of guthion (50% a.i.) at a rate of 2 pounds a.i. per 100 gallons of water per acre. Mean whole blood ChE activity levels during the 5-day exposure period ranged from 90.1 to 95.6% of mean baseline (3-day preexposure) levels (Kraus et al. 1977). Eryhrocyte AChE activity was not measured. Although postexposure examinations indicated a reduction in upper body reflex activity, it seems likely that the observation was due to fatigue from work-related exertion during thinning. There was no reduction in reflexes in the lower extremities (Kraus et al. 1977).

Reductions in erythrocyte AChE activity were observed in a group of 20 agricultural workers (ages 18–58; median age 28.5 years) who entered California peach orchards 30 days after they had been treated with guthion (1.5 pounds a.i. per acre) (McCurdy et al. 1994). Three days after entering the treated fields, erythrocyte AChE activity was 7% lower than baseline levels in the same workers. After 44 days of fieldwork, erythrocyte AChE activity had decreased 19% from baseline levels (McCurdy et al. 1994). No clinical signs were reported by the authors.

EPA (1978a) reported signs of cholinergic poisoning, such as salivation, lacrimation, exophthalmus, defecation, urination, and muscle fasciculations in male and female Sprague-Dawley rats administered lethal doses of guthion dermally. Although the precise doses at which these effects were observed were not provided, it was reported that the 14-day dermal LD_{50} values in male and female rats were 455 (95% confidence interval [CI]: 301–687) mg/kg and 222 (181–271) mg/kg, respectively. Skinner and Kilgore (1982) estimated that a single, dermal exposure to 600 mg/kg would elicit a 50% reduction in erythrocyte AChE activity in male Swiss-Webster mice.

Male and female rabbits were treated with daily dermal applications of technical-grade guthion (94.1% a.i.) at 0, 2, or 20 mg/kg, 5 days/week, for 21 days (EPA 1999b). A 24–38% reduction in erythrocyte AChE activity was observed in male and female rabbits administered 20 mg/kg/day dermally 5 days/week after 10 and 15 days (EPA 1999b).

Male rats were treated dermally with a 35% wettable powder formulation of guthion at doses equivalent to 0.056, 0.56, or 5.6 mg (a.i.)/kg. The rats were treated for 1, 4, 10, 24, 72, or 168 hours. A 16–17% reduction in erythrocyte AChE activity (relative to control animals) was observed within 10–24 hours in the 5.6 mg/kg group (EPA 1999b). There was no effect on erythrocyte AChE activity in rats in the 0.56 mg/kg group and there was no effect on plasma ChE activity at any dose level (EPA 1999b).

3.2.3.5 Reproductive Effects

No information was located regarding reproductive effects in humans or animals following dermal exposure to guthion.

3.2.3.6 Developmental Effects

García et al. (1998) studied the incidence of congenital malformations (nervous system defects, cardiovascular defects, oral clefts, epispadia or hypospadia, and musculoskeletal defects) in children born of fathers with occupational exposures to pesticides. Exposure was assessed via questionnaire. The odds ratio for the occurrence of birth defects in fathers (6 cases and 8 referent cases) exposed to guthion was 0.71 (0.23–2.25), indicating that there was no evident association between the occurrence of birth defects and paternal exposure to guthion.

3.2.3.7 Cancer

No information was located regarding cancer in human or animals following dermal exposure to guthion.

3.3 GENOTOXICITY

A limited number of studies of the genotoxicity of guthion have been conducted. The results of all *in vivo* and *in vitro* tests that were located are presented in Tables 3-4 and 3-5, respectively. *In vivo* evaluations of genotoxicity in humans were not located. In the only *in vivo* studies that were located, negative results were reported in a study of recessive lethality in *Drosophila* and two studies of micronuclei formation and dominant lethality in mice (Waters et al. 1982). The available *in vitro* genotoxicity data suggest that guthion is not genotoxic to prokaryotic organisms (Carere et al. 1978; Hrelia et al. 1990; Waters et al. 1982; Zeiger et al. 1987). Six of the 11 *in vitro* studies with eukaryotic organisms (fungi and mammalian cells) that were located showed positive results for genotoxic effects (Alam and Kasatiya 1976; Alam et

Table 3-4. Genotoxicity of Guthion *In Vivo*

Species (test system)	End point	Results	Reference
Drosophila melanogaster	Recessive lethality	–	Waters et al. 1982
Mammalian cells			
Mouse	Micronuclei formation	–	Waters et al. 1982
Mouse	Dominant lethal	–	Waters et al. 1982

– = negative result

Table 3-5. Genotoxicity of Guthion *In Vitro*

Species (test system)	End point	Results	Reference
Prokaryotic organisms			
Salmonella typhimurium (TA1535, TA1536, TA1537, TA1538)	Reverse mutation	–	Carere et al. 1978
S. typhimurium ((TA98, TA100, TA1535, TA1537, TA1538)	Reverse mutation	– (with or without metabolic activation)	Waters et al. 1982
S. typhimurium	Reverse mutation	– (with or without metabolic activation)	Hrelia et al. 1990
S. typhimurium (TA98, TA100, TA1535, TA1537)	Reverse mutation	+ (weakly mutagenic in TA98; negative in others)	Zeiger et al. 1987
Streptomyces coelicolor	Forward mutation	–	Carere et al. 1978
Escherichia coli	Reverse mutation	– (with and without metabolic activation)	Waters et al. 1982
Eukaryotic organisms			
Fungi			
Saccharomyces cerevisiae	Enhanced mitotic recombination	+ (with and without metabolic activation)	Waters et al. 1982
S. cerevisiae	Gene conversion; crossing over	– (with and without metabolic activation)	Waters et al. 1982
S. cerevisiae	Enhanced mitotic crossing over	+ (with metabolic activation)	Hrelia et al. 1990
Mammalian cells			
Human cell lines WI–38 and HEp-2	Chromosome breaks	+	Alam and Kasatiya 1976
Human lymphocytes	Micronucleus formation	+	Bianchi-Santamaria et al. 1997
Chinese hamster ovary cells (KI cell line)	Chromosome breaks	+	Alam et al. 1974
Chinese hamster ovary cells	Sister chromatid exchange	– (with and without metabolic activation)	Waters et al. 1982

Table 3-5. Genotoxicity of Guthion *In Vitro*

Species (test system)	End point	Results	Reference
Chinese hamster ovary cells (V79 line)	Sister chromatid exchange	– (without metabolic activation)	Chen et al. 1982a
Chinese hamster ovary cells (V79 line)	Sister chromatid exchange	– (with metabolic activation)	Chen et al. 1982b
Mouse lymphoma cells	Forward mutation	+ (with and without metabolic activation)	Waters et al. 1982
Human fetal lung fibroblasts	Unscheduled DNA synthesis	– (with and without metabolic activation)	Waters et al. 1982

– = negative result; + = positive result

al. 1974; Bianchi-Santamaria et al. 1997; Waters et al. 1982), but the remaining studies did not (Chen et al. 1982a, 1982b; Waters et al. 1982).

3.4   TOXICOKINETICS

3.4.1   Absorption

No information is available for any route of exposure as to whether absorption of guthion is different between children and adults or between juvenile and adult animals.

3.4.1.1 Inhalation Exposure

Absorption of guthion via the inhalation pathway can be inferred from a study demonstrating reductions in erythrocyte AChE activity in rats exposed to guthion aerosols at 4.72 mg/m^3 for 2 weeks (Kimmerle 1976). Absorption via the inhalation pathway appears to be rapid. Whole blood ChE activity was reduced by an average of 41% in male Sprague-Dawley rats 1 hour after exposure to 39 mg/m^3 (EPA 1978a).

3.4.1.2 Oral Exposure

There are no available human data to estimate the absorption of guthion in humans after oral exposure. Animal studies suggest that absorption of guthion after oral exposure is rapid. More than 90% of an 8 mg/kg dose of radiolabeled guthion was detected as radiolabeled residues in the internal organs, urine, feces, and exhaled air (as CO_2) of rats 6 hours after guthion was administered by gavage (Fakhr et al. 1996).

3.4.1.3 Dermal Exposure

Guthion can be absorbed through the skin in humans as was demonstrated by the urinary excretion of radiolabeled metabolites of guthion after the application of 4 µg guthion/cm^2 to the forearms of six volunteers (Feldmann and Maibach 1974). The radiolabeled metabolites could be detected in the urine ≤4 hours after application and approximately 16% of the dose was excreted within the 120-hour urinary sampling period (Feldmann and Maibach 1974).

Studies designed to quantify the percutaneous absorption of guthion have shown that approximately 60% of the guthion dose (100–400 µg/rat) applied to a shaved area (2.6 cm^2) of the dorsal skin of male

Sprague-Dawley rats was recovered in urine as the guthion metabolite dimethyl thiophosphate (DMTP) (Franklin et al. 1983). The authors speculated that the calculation of dermal absorption of guthion based on the detection of DMTP in urine may lead to underestimates of absorption given that DMTP constitutes only about 30% of the total alkyl phosphates excreted in urine after exposure to guthion (Franklin et al. 1983).

Dermal absorption of guthion was demonstrated in a study where a 35% wettable powder formulation of guthion was applied dermally to rats at 0.056, 0.56, or 5.6 mg (a.i.)/kg (EPA 1999b). Dermal absorption of guthion after 1 hour of exposure was 9.4, 3.7, and 0.5% of the applied doses of 0.056, 0.56, and 5.6 mg/kg, respectively (Zendzian 2003). After 10 hours, the treated skin sites were wiped with a moistened gauze pad and dermal absorption was determined 24, 72, or 168 hours after dosing. The highest absorption, observed after 168 hours of exposure, was 41.7, 21.9, and 18.3% of the applied dose for the 0.056, 0.56, and 5.6 mg/kg dose groups, respectively (Zendzian 2003).

3.4.2 Distribution

No studies are available on the distribution of guthion in exposed humans.

3.4.2.1 Inhalation Exposure

No information was located on the distribution of guthion in animals following inhalation exposure.

3.4.2.2 Oral Exposure

A study was conducted on the distribution and elimination of guthion after a single oral dose of radiolabeled guthion (labeled at the two methyl groups) to rats at 8 mg/kg (Fakhr et al. 1996). Six hours after dosing, 54.2% of the detected radiolabeled residues was found in the muscle tissue of rats; 22% of the residues was found in expired air (as CO_2), 10% was found in urine and feces, and approximately 6% were found in the blood and internal organs. After 24 hours the fraction in muscle had decreased to 7% and after 48 hours there were no detectable radiolabeled metabolites in muscle or any of the internal organs. At that time, radiolabeled residues were only detected in expired air (71% of the total amount found), feces (13%), and urine (6%) (Fakhr et al. 1996).

3.4.2.3 Dermal Exposure

Feldmann and Maibach (1974) conducted a study of the urinary excretion of radiolabeled metabolites of guthion after application of 4 μg guthion/cm^2 to the ventral forearm in six volunteers. These data were used to develop a toxicokinetic model for guthion (Carrier and Brunet 1999). The model, which does not include physiological details, predicted that the maximum body burdens of guthion after a single, 5-hour exposure or after repeated daily exposures for 9 consecutive days were 73 and 208%, respectively, of the absorbed daily dose (Carrier and Brunet 1999). The maximum body burden after a single exposure was predicted to occur 17 hours after the dose was administered. In the case of repeated doses, body burdens increased at an initially rapid rate, which decreased until it reached steady-state after approximately nine daily doses (Carrier and Brunet 1999).

3.4.3 Metabolism

The bioactivation of guthion to gutoxon proceeds via a cytochrome P450-mediated desulfuration (Figure 3-3). The oxidative activation of guthion was inhibited by 46% when piperonyl butoxide (an inhibitor of microsomal mixed function oxidases) was added to mouse liver homogenates amended with nicotinamide adenine dinucleotide phosphate (NADP) (1.3 mM), glucose 6-phosphate (G-6-P) (3.3 mM), and ethylenediamine tetraacetic acid (EDTA) (2 mM) (Levine and Murphy 1977). Recent work suggests that the desulfuration of guthion to gutoxon by cytochromes in human liver microsomes proceeds via two steps, each characterized by high and low affinities; that more than one cytochrome may be involved in the desulfuration process; and that the role of different cythochromes in desulfuration may be dependent on the guthion concentration (Buratti et al. 2003). For instance, a high degree of correlation (p≤0.05) was observed between gutoxon formation in human liver microsomes and activities of CYP1A2, CYP3A4, and CYP2B6 when guthion was added at 10 μM to the assay medium. In contrast, good correlations were observed only with CYP3A4 and CYP2B6 activity when guthion was added at 250 μM (Buratti et al. 2003). Immunoinhibition studies using CYP antibodies, added singly or in combination, confirmed that gutoxon formation proceeds under the influence of CYP1A2, CYP3A4, and CYP2B6 and suggested that CYP3A4 is an important isoform in the low-affinity phase of guthion desulfuration (Buratti et al. 2003). These findings might be relevant in establishing the activation of guthion at low, environmentally relevant exposures.

The efficient activation of guthion to gutoxon in whole liver homogenates of rat, mouse, or guinea pig requires NAD or NADP + G-6-P (Hitchcock and Murphy 1971). The amounts of gutoxon equivalents

Figure 3-3. Proposed Metabolism of Guthion
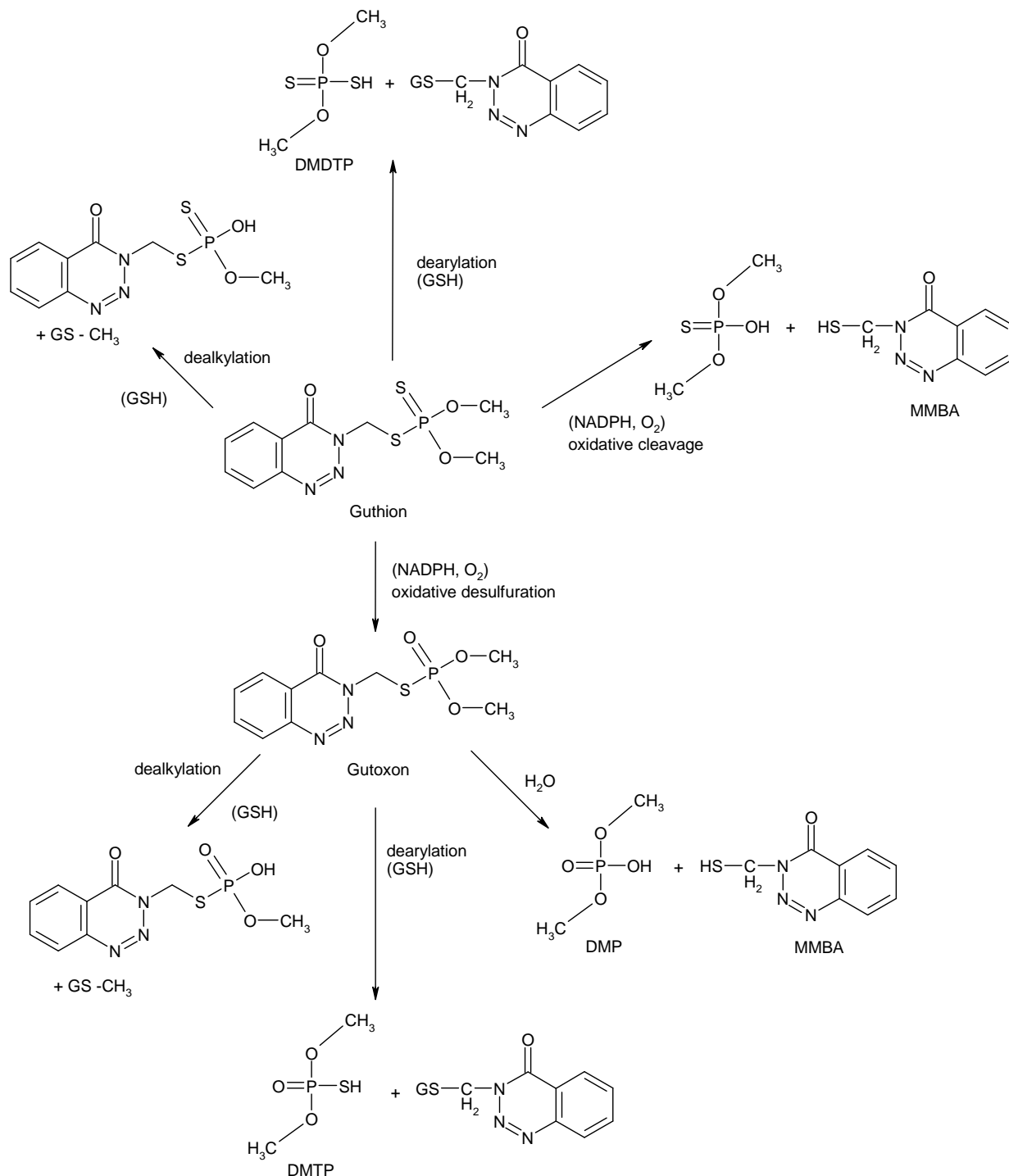
DMDTP = dimethyl phosphorodithioic acid; DMP = dimethylphosphate; DMTP = dimethylthiophosphate; MMBA = mercaptomethyl benzazimide

Sources: adapted from Fakhr et al. 1996; Levine and Murphy 1977; Motoyama and Dauterman 1972

formed in whole liver homogenates (amended with NADP and G-6-P) of rats, mice, and guinea pigs were 0.69, 0.59, and 0.66 nanomoles/10 mg liver tissue, respectively, in 15 minutes (Hitchcock and Murphy 1971), indicating that these three species showed only small differences in their guthion activation efficiency.

Although activation of guthion to the oxon form is necessary for the manifestation of its anticholinesterase activity, it is important to keep in mind that activation and degradation of guthion may occur concomitantly, as shown in liver homogenates and slices from a number of mammalian species. Moreover, the different activation or detoxication pathways may be more or less important under different biochemical conditions. For instance, gutoxon formation as well as guthion degradation (with the formation of dimethyl phosphoric acid and dimethyl phosphorothioic acid) were observed in the microsomal fraction of mouse liver (Motoyama and Dauterman 1972) (Figure 3-3). It was deduced that dimethyl phosphoric acid and dimethyl phosphorothioic acid were formed via the oxidative dearylation of gutoxon and guthion, respectively (Motoyama and Dauterman 1972). *In vitro* studies showed that guthion activation proceeded more rapidly and gutoxon degradation was markedly reduced when fluoride (0.01 M) was added to rat liver microsomes amended with cofactors and either guthion or gutoxon (Dahm et al. 1962). It has been postulated that fluoride interferes with the activity of phosphatases (Murphy and Dubois 1957). These studies indicate that alterations in the balance between the activation of guthion and the degradation of guthion and gutoxon can be elicited *in vitro*. It may reasonably be expected that these alterations might also affect the anticholinesterase activity of gutoxon *in vivo*.

Glutathione has been implicated in the detoxication of guthion in mammals (Motoyama and Dauterman 1972; Sultatos and Woods 1988); however, some studies contradict this role (Sultatos and Woods 1988). Support for the role of glutathione in detoxication comes from the observations that, in mice and rats, the depletion of glutathione, such as by pretreatment with methyl iodide or diethyl maleate, potentiates the toxicity of many dimethyl-substituted organothiophosphate insecticides and that the administration of large doses of certain dimethyl-substituted organothiophosphates has been shown to elicit decreases in hepatic glutathione content (Sultatos and Woods 1988); however, although depletion of hepatic glutathione in the mouse by pretreatment with diethyl maleate potentiated the acute toxicity of guthion, depletion of hepatic glutathione by pretreatment with buthionine sulfoximine did not (Sultatos and Woods 1988). Thus, normal levels of glutathione did not appear to be required for the detoxication of guthion in the mouse.

Under some circumstances, glutathione might be involved in the metabolism of guthion. For instance, incubation of guthion in mouse liver homogenates reduced glutathione levels by 25% (Levine and Murphy 1977), but when the oxidative cofactors NADP and G-6-P were added to the medium, glutathione levels remained at control levels during the 90 minutes of incubation. However, when guthion and the oxidative cofactors were added to liver homogenates from mice that were treated with piperonyl butoxide (an inhibitor of microsomal mixed function oxidases), levels of glutathione were reduced to approximately 80% of control values. These data suggest that glutathione is significantly involved in the detoxication of guthion when oxidative metabolism is inhibited (Levine and Murphy 1977). *In vitro* studies with gutoxon showed that liver glutathione levels were unaffected in mouse liver homogenates with or without NADP and G-6-P, suggesting that glutathione-dependent detoxication mechanisms are not active on gutoxon molecules.

Paraoxonase (PON1; serum A-esterase), an enzyme found in humans and other mammals, can hydrolyze the oxygen analogues of some organophosphate insecticides such as paraoxon, chlorpyrifos oxon, and diazinon oxon and in this manner, reduce their toxicity (Costa et al. 1999). In humans, serum PON1 is a polymorphic enzyme that shows low, intermediate, or high activity based on the hydrolysis of paraoxon (Akgür et al. 1999); however, PON1 is not involved in the hydrolysis of gutoxon. Thus, there was no difference in the inhibition of brain cholinesterase among homozygous wild (*Pon1* +/+) or knockout (*Pon1* -/-) mice treated with guthion (Costa et al. 1999).

Labeled and unlabeled urinary metabolites of guthion were detected in the urine of rats 6 hours after being administered a single oral dose (by gavage) of radiolabeled guthion at 8 mg/kg (Fakhr et al. 1996). The metabolites that were identified include dimethyl phosphorodithioic acid (DMDTP) and DMTP. Based on the urinary metabolites detected in rat urine, Fakhr et al. (1996) suggested that in the rat, guthion could be degraded by (1) cleavage of the P-S-C bond (mainly mediated by cytochrome P-450) to *O*,*O*-DMTP and mercaptomethyl benzazimide which may undergo further transformation or (2) by cleavage of the P-O-CH$_3$ bond to yield mono-demethylated guthion (Figure 3-3), which may be further dealkylated to di-demethylated guthion, the latter process being mediated by GSH-transferase, which is further metabolized perhaps even to CO$_2$ (Fakhr et al. 1996).

3.4.4 Elimination and Excretion

The urinary metabolites, DMDTP, DMTP, and dimethylphosphate (DMP), were detected in the urine of individuals (88 men, 11 women; ages 16–59 years) who resided near an area were guthion was used but

who were not known to be exposed occupationally to guthion (Aprea et al. 1994). The total excretion of DMDTP + DMTP + DMP had a geometric mean and standard deviation of 145 and 2.3 nmol/g creatinine, respectively, with a range of values of 5.5–884.5 nmol/g creatinine (Aprea et al. 1994); however, these metabolites are formed by, but are not specific to, guthion.

3.4.4.1 Inhalation Exposure

No information was located on the elimination and excretion of guthion in human or animals following inhalation exposure.

3.4.4.2 Oral Exposure

In rats given a single oral dose (8 mg/kg) of radiolabeled guthion (labeled at the two methyl groups), 22% of the labeled residues was found in expired air (as CO_2), 10% was found in urine and feces, 54.2% was found in the muscle tissue of rats, and approximately 6% was found in the blood and internal organs 6 hours after dosing (Fakhr et al. 1996). After 24 hours, the fractions in expired air, feces, and urine had increased to approximately 63, 11, and 5%, respectively. After 48 hours, there were no detectable radiolabeled metabolites in muscle or any other of the internal organs and radiolabeled residues were only detected in expired air (71% of the total amount found), feces (13%), and urine (6%). Seven radiolabeled and six nonlabeled metabolites were detected in the urine of treated rats, but guthion or its oxon metabolite were not detected by chromatographic analysis in the urine (Fakhr et al. 1996).

3.4.4.3 Dermal Exposure

Urinary excretion of radiolabeled metabolites of guthion was detected after application of 4 µg guthion/cm^2 to the ventral forearm of six volunteers (Feldmann and Maibach 1974). The treated areas of the forearms were not protected and the subjects were asked not to wash the area for 24 hours. Radiolabeled metabolites could be detected in the urine ≤4 hours after application of the insecticide. The urinary excretion rate of guthion metabolites increased from 0.04% dose/hour in the first 4 hours after dosing to a maximum of 0.29% dose/hour at 8–12 hours after the dose had been applied (Feldmann and Maibach 1974). After that time, the excretion rate decreased until it reached 0.04% dose/hour 96–120 hours after the dose had been applied. Approximately 16% of the dose was excreted within the 120-hour urinary sampling period (Feldmann and Maibach 1974). The urinary excretion values were corrected for guthion absorption efficiency as determined in a preliminary study where the subjects were administered a single, intravenous dose of 1 µCi of radiolabeled guthion (Feldmann and Maibach 1974).

The latter study showed that approximately 70% of the intravenous dose was excreted within 120 hours, with a half-life of 30 hours. Urinary excretion of the radiolabeled residues of intravenously-administered guthion was faster than observed with the dermally-applied insecticide, the former reaching 1.6% dose/hour 8–12 hours after administration (Feldmann and Maibach 1974).

The data provided by Feldmann and Maibach (1974) were used to develop a toxicokinetic model of the elimination of guthion based on the urinary elimination of alkylphosphate metabolites (Carrier and Brunet 1999). The model, which does not include details of the physiological mechanisms, was used to estimate that 76% of the administered dose of guthion is excreted in the urine within 20 days after a single, 5-hour exposure. Based on the toxicokinetic model, it was estimated that the rate of urinary excretion of guthion metabolites would reach steady state after approximately 9 days. The rate of urinary excretion of metabolites after repeated doses was 3 times higher than after a single dose. This follows from the fact that the estimated maximum body burdens after single or repeated exposures were 73 and 208%, respectively, of the absorbed daily dose and that the excretion rate was assumed to be (based on first-order kinetics) proportional to instantaneous body burden (Carrier and Brunet 1999).

Approximately 60% of the guthion doses (100–400 μg/rat) applied to a shaved area (2.6 cm^2) of the dorsal skin of male Sprague-Dawley rats was recovered in urine as the guthion metabolite DMTP (Franklin et al. 1983). The authors speculated that the calculation of dermal absorption of guthion based on the detection of DMTP in urine may lead to underestimates of absorption given that DMTP constitutes only about 30% of the total alkyl phosphates excreted in urine after exposure to guthion (Franklin et al. 1983). A linear relationship (r=0.943) between guthion doses and total DMTP output suggests that the capacity of the metabolic pathways was not exceeded at the doses administered (Franklin et al. 1983). Franklin et al. (1986) briefly presented the results of a study with human subjects (two subjects per dose) who were administered guthion at 500–6,000 μg/person (approximately 7–86 μg/kg) topically on the forehead. After 72 hours, the urinary excretion of DMTP ranged from 5 to 17% of the administered dose. In general, increasing cumulative excretion was observed with increasing doses (Franklin et al. 1986).

3.4.4.4 Other Routes of Exposure

The urinary output of radiolabeled guthion metabolites after a 1 μCi intramuscular dose in rats showed two peaks in urinary excretion of the administered dose, one 4 hours after the dose (approximately 13% of the dose) and a higher peak showing recovery of approximately 20% of the dose, after 24 hours, which was followed by a rapid decrease in output to very low levels after 120 hours (Franklin et al. 1983). The

urinary recovery of metabolites observed in a study with human subjects administered a single intravenous dose of 1 µCi radiolabeled guthion also showed an initial peak (1.5% dose/hour) 0–4 hours after the dose was administered which was followed by a drop in excretion and a second peak (1.6% dose/hour) 812 hours after the dose was administered (Feldmann and Maibach 1974).

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

Physiologically based pharmacokinetic (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 end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations

provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

A PBPK model for guthion was not located. Feldmann and Maibach (1974) conducted a study of the urinary excretion of radiolabeled metabolites of guthion after application of 4 µg guthion/cm^2 to the ventral forearm of six volunteers. These data were used to develop a toxicokinetic model of the elimination of guthion based on the urinary elimination of alkylphosphate metabolites (Carrier and Brunet 1999). The model, which does not include physiological details, predicted that the maximum body burdens of guthion after a single, 5-hour exposure or after repeated daily exposures for nine consecutive days were 73 and 208%, respectively, of the absorbed daily dose (Carrier and Brunet 1999). The maximum body burden after a single exposure was predicted to occur 17 hours after the dose was administered. In the case of repeated doses, the body burden increased at an initially rapid rate and continued to increase until it reached steady-state after approximately nine daily doses (Carrier and Brunet 1999). The model was also used to estimate that 76% of the administered dose of guthion is excreted in the urine within 20 days after a single, 5-hour exposure. It was estimated that after repeated daily doses, the rate of urinary excretion of guthion metabolites would reach steady state after approximately 9 days. The rate of urinary excretion of metabolites after repeated doses was 3 times higher than after a single dose. This follows from the fact that the excretion rate was assumed to be (based on first-order kinetics) proportional to instantaneous body burden (Carrier and Brunet 1999).

3. HEALTH EFFECTS

**Figure 3-4. Conceptual Representation of a Physiologically Based
Pharmacokinetic (PBPK) Model for a
Hypothetical Chemical Substance**
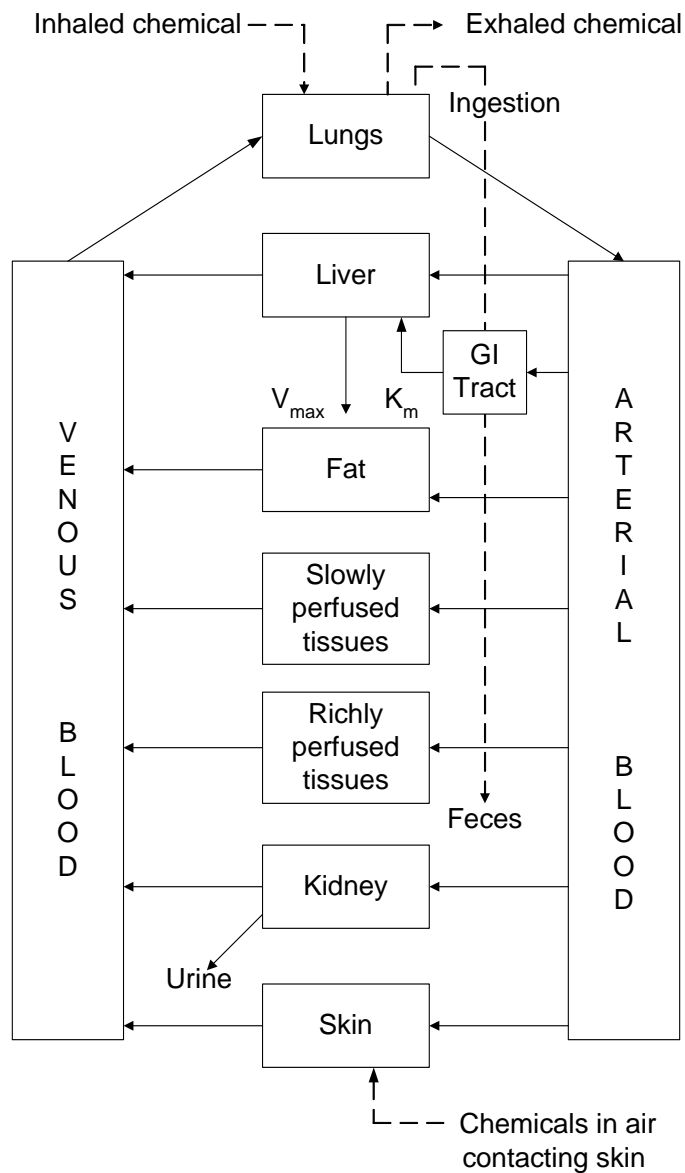
Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a
hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by
ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan et al. 1994

3.5   MECHANISMS OF ACTION

3.5.1   Pharmacokinetic Mechanisms

The detection of guthion metabolites in urine and the observed reductions in erythrocyte AChE activity shortly after exposure to guthion indicate that guthion is absorbed in humans and animals via the inhalation, oral, or dermal exposure routes. The extent of oral absorption of guthion in rats was >90% after a single oral dose of radiolabeled guthion at 8 mg/kg (Fakhr et al. 1996). Six hours after dosing, 54.2% of the radiolabeled guthion residues was found in the muscle tissue of rats; 22% of the residues was found in expired air (as CO_2), 10% was found in urine and feces, and approximately 6% was found in the blood and internal organs. After 48 hours, there were no detectable radiolabeled metabolites in muscle or any other of the internal organs and radiolabeled residues were only detected in expired air (71% of the total amount found), feces (13%), and urine (6%). Dermal absorption of guthion was demonstrated in a study where a 35% wettable powder formulation of guthion was applied dermally to rats (EPA 1999b). Dermal absorption 1-hour after the application of guthion at 0.056, 0.56, and 5.6 mg (a.i.)/kg was 9.4, 3.7, and 0.5% of the applied doses, respectively (Zendzian 2003). After 10 hours, dermal absorption of guthion applied at 0.056, 0.56, and 5.6 mg/kg had increased to 22.7, 15.2, and 2.9%, respectively (Zendzian 2003). The treated skin areas were wiped with a moistened gauze pad 10 hours after the application of guthion and dermal absorption was determined 24, 72, or 168 hours after dosing. The highest absorption, observed after 168 hours of exposure, was 41.7, 21.9, and 18.3% of the applied dose for the 0.056, 0.56, and 5.6 mg/kg dose groups, respectively (Zendzian 2003), indicating that dermal absorption of guthion continued to increase after the removable residues were wiped off after 10 hours and that the efficiency of guthion dermal absorption in rats decreased with increasing dose.

Dermal absorption of guthion was observed in volunteers who were administered 4 μg/cm^2 of radiolabeled guthion (Feldmann and Maibach 1974). Radiolabeled metabolites could be detected in the urine ≤4 hours after application and approximately 16% of the dose was excreted within the 120-hour urinary sampling period (Feldmann and Maibach 1974).

3.5.2   Mechanisms of Toxicity

The most salient systemic effects of exposure to guthion are related to its direct effect on the nervous system and the secondary effects that result from it. The direct manner in which guthion exerts its systemic effects is through inhibition of cholinesterases (ChE), specifically acetylcholinesterase (AChE)

in the central and peripheral nervous system. AChE is also present in erythrocytes. Thus, inhibition of erythrocyte AChE is commonly used as a surrogate indicator of the extent of inhibition of neural AChE. In addition, cholinesterases can be found in plasma. In humans, plasma ChE is almost exclusively composed of butyrylcholinesterase. Although butyrylcholinesterase is capable of hydrolyzing acetylcholine and butyrylcholine *in vitro*, the *in vivo* substrate of plasma ChE is unknown. Guthion is bioactivated *in vivo* and *in vitro* to its oxygen analog form, variably referred to as gutoxon or Gutoxon (Buratti et al. 2003; Hitchcock and Murphy 1971; Sultatos and Woods 1988). Gutoxon reacts with a serine hydroxyl group at the active site of AChE, rendering it largely inhibited and unreactive. Under normal circumstances, AChE rapidly and efficiently degrades the neurotransmitter acetylcholine following its release at the nerve synapse or at a neuromuscular junction; however, the inhibited AChE enzyme cannot degrade acetylcholine and the neurotransmitter accumulates at the ending of cholinergic nerves with the ensuing continual stimulation of electrical activity (Carrier and Brunet 1999). Cholinergic nerves play an important role in the normal function of the neuromuscular, central nervous, endocrine, immunological, and respiratory systems (Carrier and Brunet 1999). Thus, the inhibition of the enzyme AChE by gutoxon may have profound and wide-ranging systemic effects. Acetylcholine can be found in the autonomic nervous system, the somatic motor nervous system, and in the central nervous system. In the autonomic nervous system, accumulation of acetylcholine would lead to the overstimulation of the muscarinic receptors of the parasympathetic nervous system, which would lead to effects on the exocrine glands (increased salivation, perspiration, lacrimation), eyes (miosis, blurred vision), gastrointestinal tract (nausea, vomiting, diarrhea), respiratory system (excessive bronchial secretions, wheezing, and tightness of chest), and cardiovascular system (bradychardia, decrease in blood pressure) (Ecobichon 1995). Stimulation of the nictonic receptors in the parasympathetic or sympathetic nervous system of the autonomic nervous system would also lead to effects on the cardiovascular system such as tachycardia, pallor, and increased blood pressure. In the somatic nervous system, nerve fibers innervate the skeletal muscles motor end-plates. Accumulation of acetylcholine in the somatic nervous system would affect skeletal muscle and would manifest itself as muscle fasciculations, cramps, paralysis, and flaccid or rigid tone, among other signs and symptoms. Overstimulation of the nerves in the central nervous system, specifically the acetylcholine receptors of the brain, by the accumulation of acetylcholine may result in lethargy, drowsiness, and mental confusion among other effects. More severe effects on the central nervous system include a state of coma without reflexes, depression of the respiratory centers, and cyanosis (Ecobichon 1995). It has been recognized that, after repeated exposures to organophosphate insecticides, humans and other animal species may develop tolerance to the appearance of cholinergic signs (Costa et al. 1982). It has been proposed that this tolerance to the effect of excess acetylcholine develops by the down-regulation of postsynaptic cholinergic receptors. This reduces the apparent

cholinergic symptoms even in the presence of marked reductions in erythrocyte AChE activity (Sultatos 1994).

Other esterases, such as carboxylesterase, may be involved in the toxicity of organophosphate insecticides. For instance, malaoxon, the oxon form of malathion, is hydrolyzed by a carboxylesterase. When the carboxylesterase is inhibited, the acute toxicity of malaoxon increases (Agency for Toxic Substances and Disease Registry 2003); however, no data were located that indicate what role carboxylesterases may play in the toxicity of guthion.

3.5.3   Animal-to-Human Extrapolations

No studies were located that directly studied the comparative toxicokinetics of guthion in animals and humans. Nevertheless, the available studies suggest that the toxicokinetics of guthion in animals and humans are generally similar. Recent work suggests that the desulfuration of guthion to gutoxon in human liver microsomes is largely effected by at least three cytochromes (CYP1A2, CYP3A4, and CYP2B6), which show different affinities for the substrate (Buratti et al. 2003). If the spectrum of activities of these cytochromes in animals varies markedly from that in humans, notable differences in animals and humans might be expected. No data are available to suggest that such differences do or do not exist.

3.6   TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as endocrine disruptors. However, appropriate terminology to describe such effects remains controversial. The terminology endocrine disruptors, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning endocrine disruptors. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as hormonally active agents. The terminology endocrine modulators has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to

the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Although no studies were located regarding endocrine disruption in humans or animals after exposure to guthion, the studies discussed in this toxicological profile (Holzum 1990; Kavlock et al. 1985; NCI 1978; Short et al. 1980; Vos et al. 1983) do not suggest that guthion exerts consistent, clinically-evident effects on the neuroendocrine axis.

3.7   CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. 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. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age

(Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

There are no human data to determine whether children differ from adults in their susceptibility to the adverse health effects of guthion. However, developmental toxicity studies in rats and rabbits have shown no evidence of increased sensitivity of fetuses as compared to maternal animals following *in utero* exposure. Furthermore, a one- and a two-generation reproductive toxicity study in rats showed no

increased susceptibility in pups when compared to adults (EPA 1999b). Additional, relevant information from other organophosphorous pesticides is presented below in order to draw inferences as the data allow. Acute dermal, inhalation, and oral exposures to the organophosphorous pesticide methyl parathion has resulted in typical signs of organophosphate poisoning including reductions in plasma and erythrocyte AChE activity, alterations in the function of nervous, cardiac, pulmonary, and gastrointestinal systems, and deaths in adults (Fazekas 1971; Fazekas and Rengei 1964) as well as in children (Dean et al. 1984). These findings suggest that adults and children share similar targets of toxicity from exposure to methyl parathion. These findings might apply to guthion given the similarities in the mode of action between the two pesticides; however, it should be noted that there are no reported poisonings of children exposed to guthion. The neurotoxicity of guthion is dependent on its bioactivation via a cytochrome P450 mediated desulfuration to the oxon form (Buratti et al. 2003). Recent work suggests that the desulfuration of guthion to the oxon form by cytochromes in human liver microsomes proceeds via two steps, each characterized by high and low affinities; that more than one cytochrome may be involved in the desulfuration process; and that the role of different cythochromes in desulfuration may be dependent on the guthion concentration (Buratti et al. 2003). Some P450 isozymes are regulated differently during development than during adulthood (Leeder and Kearns 1997), but information specific to guthion is not available. Nevertheless, it is conceivable that developmental differences in the regulation of P450 isozymes could lead to differences in the susceptibility of children to guthion toxicity; however, the available data are insufficient to determine if this is, in fact, the case. It is known that acetylcholine, acetylcholinesterase, and butyrylcholinesterase are involved in the development of the nervous system (Brimijoin and Koeningsberger 1999; Layer 1990; Layer and Willbold 1994) and that some of this development is not completed until adulthood. Thus, it is plausible that by interfering with the normal function of the cholinesterases, guthion might elicit adverse developmental effects. Garcia-Lopez and Monteoliva (1988) showed that erythrocyte AChE activity increases with increasing age, starting at birth and until >60 years of age. It is not known whether these changes in AChE activity might elicit different responses to guthion among children and adults.

Although some studies have reported reductions in pup weight and survival, reduced brain weight and ChE activity, and increased incidence of supernumerary ribs and malaligned sternebrae in offspring of pregnant mice or rats (Holzum 1990; Kavlock et al. 1985; Short et al. 1980), most of the time, these effects have occurred at maternally toxic doses.

3.8   BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to guthion are discussed in Section 3.8.1.

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 effects caused by guthion are discussed in Section 3.8.2.

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 3.10, Populations That Are Unusually Susceptible.

3.8.1   Biomarkers Used to Identify or Quantify Exposure to Guthion

The ideal biomarker for the quantification of exposure to guthion would be specific to the chemical of interest and would probably be the insecticide itself or a metabolite that could only be detected after exposure to guthion. It has been shown that DMDTP, DMTP, and DMP are metabolic products of the *in vivo* degradation of guthion (Carrier and Brunet 1999) and have been detected in urine in humans under field and experimental conditions after dermal or otherwise unspecified exposure routes. For instance, Franklin et al. (1986) detected DMTP in the urine of volunteers 72 hours after they were administered guthion at 500–6,000 µg/person (approximately 7–86 µg/kg) on the forehead. Urinary excretion of the metabolites DMDTP, DMTP, and DMP was detected in a group (n=99) of individuals not known to be exposed occupationally to guthion (Aprea et al. 1994). These individuals may have been exposed to guthion in the diet but exposure estimates were not provided. The total excretion of DMDTP + DMTP + DMP had a geometric mean and standard deviation of 145 and 2.3 nmol/g creatinine, respectively, with a range of values of 5.5–884.5 nmol/g creatinine (Aprea et al. 1994). Unfortunately, these metabolites can be detected after exposure to guthion or other organophosphate insecticides and thus, under most circumstances, are of limited use as biomarkers of exposure. Neither guthion nor gutoxon were detected in urine collected during 48 hours from rats administered a single oral dose of guthion at 8 mg/kg (Fakhr et al. 1996). No studies were located that detect guthion or gutoxon in blood of exposed animals or humans.

3.8.2   Biomarkers Used to Characterize Effects Caused by Guthion

Monitoring erythrocyte or plasma ChE activity may assist in confirming a diagnosis and perhaps preventing the signs and symptoms of organophosphate poisoning; however, reductions in plasma or erythrocyte ChE activity can be affected not only by all organophosphate insecticides, but also by carbamate ester insecticides and, thus, reductions in ChE activity are not specific to exposure to guthion. In addition, the large degree of variability in ChE activity in human populations suggests that caution should be exercised when comparing ChE activities from exposed populations, such as agricultural workers, and reference populations. For example, activity levels at the upper limit of the normal range may be 200% higher than those at the lowest level (Maroni et al. 2000). Long-term sequential monitoring of ChE activity in populations of interest may allow a more accurate confirmation of enzyme inhibition (Coye et al. 1987).

Organophosphate poisoning may be categorized as mild, moderate, or severe based on the clinical signs and symptoms of poisoning and the measured reductions in ChE activity. Mild cases of poisoning, in which the patient retains the ability to move, may occur when plasma ChE activity levels are 20–50% of normal; moderate cases of poisoning in which the patient has lost the ability to walk can be seen with activity levels 10–20% of normal; and severe poisoning with respiratory distress and unconsciousness may be seen when plasma ChE activity levels are reduced to <10% of normal values (Tafuri and Roberts 1987). Thus, clinical signs and symptoms of intoxication with anticholinesterase insecticides may occur when plasma ChE activity levels drop to below 50% of the normal values. Methods for measuring erythrocyte and plasma cholinesterase are presented in Chapter 7.

3.9   INTERACTIONS WITH OTHER CHEMICALS

Chemicals that alter the metabolism of guthion, particularly its activation to gutoxon and the degradation of guthion or gutoxon, can be expected to alter the toxicity of guthion. Piperonyl butoxide, an inhibitor of microsomal mixed function oxidases, inhibited the activation of guthion to gutoxon *in vitro* (Levine and Murphy 1977). Although the activation and detoxication of guthion *in vivo* interact in complex ways, it would be expected that inhibition of the activation of guthion to its oxygen analog would result in a reduction of the anticholinesterase toxicity of guthion.

Given that guthion shares essential aspects of its mechanism of toxic action with many other organophosphate (and carbamate ester) insecticides, it is reasonable to expect that the toxicity of guthion and other organophosphate insecticides would show at least additive effects under concurrent exposure conditions. Dose additivity for anticholinesterase effect was observed *in vitro* when rat brain AChE was incubated with the guthion oxygen analog and chlorpyrifos-oxon simultaneously (Richardson et al. 2001). The anticholinesterase effect was nonlinear when the two chemicals were added to serum ChE. Greater-than-additive effects were observed when the two bioactive chemicals were added sequentially at high concentrations to rat serum or brain incubation media. In 2002, the EPA completed a Revised OP Cumulative Risk Assessment (EPA 2002) to address the risk of cumulative risk from exposure to organophosphate insecticides in food, water, and domestic applications. The reader should refer to that document, available on-line, for an in-depth discussion of the issue of cumulative risk from exposure to organophosphate insecticides.

Pyridostigmine is an anticholinesterase drug used in the treatment of symptoms of myasthenia gravis (Taylor 2001). Individuals who are undergoing medical treatment with pyridostigmine or other anti-ChE drugs on an ongoing basis and are concurrently exposed to guthion might experience an additional inhibition of AChE elicited by guthion; however, the extent of the additional reduction in AChE activity elicited by guthion and the clinical neurotoxic effects, if any, of this additional reduction in AChE activity are uncertain. Pyridostigmine was also used in 1990 during the Persian Gulf War to protect troops from poisoning with the nerve agent Soman (Taylor 2001). However, when administered prophylactically to U.S. troops, treatment with pyridostigmine would be discontinued upon exposure to Soman and the exposed personnel would be treated immediately with the antidotes atropine and pralidoxime.

The antagonistic effect of some drugs on the anticholinesterase action of organophosphates has been applied to great advantage in the emergency treatment of acute organophosphate intoxications in humans. Atropine, for instance, is a potent blocker of the activity of acetylcholine at muscarinic nerve receptors. In that manner, atropine reduces the clinical effects associated with the stimulation of the parasympathetic nervous system by excess acetylcholine. The antidote pralidoxime (2-PAM), can not only reverse the effect of cholinergic nicotinic overstimulation (such as skeletal muscle fasciculation, muscle weakness, and paralysis of respiratory muscles), but can also reactivate phosphorylated cholinesterase (Tafuri and Roberts 1987).

In vitro studies showed that guthion activation proceeded more rapidly and gutoxon degradation was markedly reduced when fluoride (0.01 M) was added to rat liver microsomes amended with cofactors and either guthion or gutoxon (Dahm et al. 1962). It has been postulated that fluoride interferes with the activity of phosphatases (Murphy and Dubois 1957). These studies indicate that alterations in the balance between the activation of guthion and the degradation of guthion and gutoxon can be elicited *in vitro*. It may reasonably be expected that these alterations might also affect the anticholinesterase activity of gutoxon *in vivo*.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to guthion than will most persons exposed to the same level of guthion in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of guthion or compromised function of organs

affected by guthion. Populations who are at greater risk due to their unusually high exposure to guthion are discussed in Section 6.7, Populations with Potentially High Exposures.

No information was located regarding differences in susceptibility among different populations exposed to guthion. However, individuals who respond to the anticholinesterase effects of organophosphates more rapidly and with greater reductions in ChE activity might be expected to be more susceptible to the neurotoxic effects of guthion. These responses may be genetic in origin or may be due to differences in development or life style factors, such as nutrition or behavior, or to preexisting disease states. Individuals with hereditary low plasma ChE levels (Kalow 1956; Lehmann and Ryan 1956) and those with unusually low levels of erythrocyte acetylcholinesterase, such as individuals with paroxysmal nocturnal hemoglobinuria (Auditore and Hartmann 1959), would have increased susceptibility to the effects of anticholinesterase agents such as guthion. During pregnancy, women have exhibited significantly decreased plasma ChE activity levels (De Peyster et al. 1993; Evans and Wroe 1980; Evans et al. 1988; Howard et al. 1978; Sanz et al. 1991; Venkataraman et al. 1990) and significantly increased erythrocyte AChE levels (De Peyster et al. 1993; Sanz et al. 1991; Venkataraman et al. 1990), but it is not known whether these differences might make pregnant women more susceptible to guthion toxicity. As was pointed out in a previous section, the *in vivo* substrate of plasma ChE is unknown. Thus, the role of reduced plasma ChE activity on guthion toxicity is uncertain.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to guthion. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to guthion. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to organophosphate pesticides:

Carlton FB, Simpson WM, Haddad LM. 1998. The organophosphates and other insecticides. In: Haddad LM, Shannon MW, Winchester JF, eds. Clinical management of poisoning and drug overdose. 3rd ed. Philadelphia, PA: W B Saunders Company, 836-845.

Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds. 1998. Goldfrank's toxicologic emergencies. 6th ed. Stamford, CT: Appleton and Lange.

Osmundson M. 1998. Insecticides and pesticides. In: Viccellio P, ed. Emergency toxicology. 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers, 401-413.

3.11.1 Reducing Peak Absorption Following Exposure

The information presented below was obtained from the books listed above. Since information specific to guthion was not located the information below is related to organophosphates in general. Respiratory distress is a common effect of poisoning after inhalation of organophosphates and its treatment is mostly supportive. Under some circumstances intubation may be necessary to facilitate control of secretions. Washing the skin with copious amounts of soap and water is recommended in cases of dermal contamination with organophosphates. This first wash may be followed by a second washing with ethyl alcohol. Exposure of the eyes should be immediately treated by copious irrigation of the eye with normal saline or lactated Ringer's solution (Aaron and Howland 1998). Contaminated clothing including leather garments should be destroyed. Activated charcoal is recommended for many organophosphates after oral exposure; however, Carlton et al. (1998) pointed out that this treatment may lack efficiency with some organophosphates. Ipecac should not be used for organophosphate poisoning (Osmundsen 1998). Cathartics may be unnecessary as intestinal motility is greatly increased. Gastric lavage may be performed with care, as organic solvent vehicles may cause pneumonitis if inhaled during the procedure.

3.11.2 Reducing Body Burden

No information was located regarding the reduction of the body burden of guthion. However, it should be pointed out that the body burden of guthion is expected to be rapidly reduced upon cessation of exposure to the insecticide. There were no detectable guthion metabolites in muscle or internal organs in rats 48 hours after being administered an 8 mg/kg dose of radiolabeled guthion by gavage (Fakhr et al. 1996).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Information on the interference with the mechanism of action for toxic effects of guthion was not located. Thus, information pertinent to organophosphate pesticides in general was extracted from Carlton et al. (1998), Goldfrank et al. (1998), and Osmundson (1998) and is presented in this section. Organophosphate poisoning is commonly treated by administration of atropine and pralidoxime (2-PAM). Atropine is a competitive antagonist at muscarinic receptor sites and is helpful in drying excessive secretions, especially from the tracheobronchial tree. Although atropine crosses the blood-brain barrier and thus also treats the central nervous system effects, it does not antagonize nicotinic effects. Initial doses of 1–2 mg for an adult and 0.05 mg/kg for children, preferably by the intravenous route, have been recommended. Treatment may be repeated every 15–30 minutes until signs of atropinization occur. Glycopyrrolate, a quaternary ammonium compound, has also been used instead of atropine (Bardin and

Van Eeden 1990). Glycopyrrolate does not cross the blood-brain barrier and has fewer central nervous system effects than atropine. Nicotinic effects such as muscle weakness and respiratory depression from organophosphate poisoning are commonly treated by administration of 2-PAM. 2-PAM is a quaternary amine oxime that can restore enzymatic activity by reversing the phosphorylation of acetylcholinesterase. 2-PAM and other oximes function by nucleophilic attack on the phosphorylated enzyme; the oxime-phosphonate is then split off, leaving the regenerated enzyme. Moreover, 2-PAM has an anticholinergic effect and may prevent continued toxicity by detoxifying the organophosphate molecule (Carlton et al. 1998). 2-PAM should be administered as soon as a diagnosis of poisoning is made. The initial dose is 1– 2 g for adults and 25–50 mg/kg for children administered intravenously over 30–60 minutes. The dose can be repeated in 1 hour and then every 8–12 hours until clinical signs have diminished and the patient does not require atropine. Since enzyme regeneration depends on plasma levels of the organophosphate, some patients may require multiple doses. A 2-PAM serum level of 4 μg/L is suggested as the minimum therapeutic threshold. 2-PAM is considered a safe drug with few side effects; however, high doses of 2-PAM can cause neuromuscular blockade and inhibition of AChE, although these effects are minimal at the recommended antidotal doses (Taylor 2001). An intravenous administration rate of 2-PAM >500 mg/minute can result in mild weakness, blurred vision, diplopia, dizziness, headache, nausea, and tachycardia (Taylor 2001).

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of guthion is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of guthion.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1   Existing Information on Health Effects of Guthion

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to guthion are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of guthion. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Human and animal studies suggest that the inhibition of AChE activity is the most sensitive end point of guthion toxicity. Inhibition of AChE activity has been observed after inhalation, oral, and dermal exposures to guthion. The inhibition of AChE activity by guthion is dose-related, but is not strongly influenced by duration of exposure. The inhibition of nervous system AChE leads to the accumulation of the neurotransmitter acetylcholine at the ending of cholinergic nerves with the ensuing continual stimulation of electrical activity (Carrier and Brunet 1999). Erythrocyte AChE is analogous to nervous system AChE and inhibition of the former is correlated with clinical toxicity in the nervous system (Carrier and Brunet 1999). In humans and animals, significant inhibition of erythrocyte AChE activity occurs at doses that are several times lower than those that elicit clinical signs and symptoms.

In humans, mild, moderate, and severe poisoning with organophosphate insecticides corresponds to ChE activity reductions to 20–50, 10–20, and <10% of normal levels, respectively (Aaron and Howland 1998). Despite these general guidelines, it should be kept in mind that a single ChE activity measurement cannot confirm or exclude exposure to organophosphate insecticides given the large variation in the normal levels of ChE activity in the general population.

There is a paucity of controlled studies of humans exposed to guthion. The only controlled studies that were located of humans exposed orally to guthion (Rider and Puletti, 1969; Rider et al. 1970, 1971, 1972) remain unpublished, and limited information from them is available only in abstracts; however, a small number of dermal absorption and dermatologic studies in humans and studies of agricultural workers exposed to guthion during application are available.

3. HEALTH EFFECTS

Figure 3-5. Existing Information on Health Effects of Guthion

The Human chart shows exposure routes (Inhalation, Oral, Dermal) against health effect categories (Death, Acute, Intermediate, Chronic [Systemic], Immunologic/Lymphoretic, Neurologic, Reproductive, Developmental, Genotoxic, Cancer).

Human:
- Inhalation: Immunologic/Lymphoretic ●, Developmental ●
- Oral: Immunologic/Lymphoretic ●
- Dermal: Acute ●, Intermediate ●, Chronic ●, Immunologic/Lymphoretic ●, Reproductive ●, Developmental ●

Human

Animal:
- Inhalation: Death ●, Acute ●, Intermediate ●, Chronic ●, Immunologic/Lymphoretic ●, Developmental ●
- Oral: Death ●, Acute ●, Intermediate ●, Chronic ●, Immunologic/Lymphoretic ●, Neurologic ●, Reproductive ●, Developmental ●, Genotoxic ●, Cancer ●
- Dermal: Death ●, Immunologic/Lymphoretic ●, Developmental ●

Animal

● Existing Studies

Neurological, systemic, reproductive, and developmental effects have been evaluated in dogs, rats, or mice after acute-, intermediate-, and chronic-duration exposures to guthion by inhalation, oral, and dermal routes. The potential carcinogenicity of guthion has also been evaluated.

3.12.2 Identification of Data Needs

Acute-Duration Exposure.   No controlled, acute toxicity studies in humans exposed to guthion orally or by inhalation were available. Studies of agricultural workers exposed to guthion were located (Franklin et al. 1981; Kraus et al. 1977; Schneider et al. 1994) as were studies of the dermal absorption of guthion in volunteers (Feldmann and Maibach 1974). Guthion is absorbed when applied dermally in humans as was demonstrated by the urinary excretion of radiolabeled metabolites of guthion after a single application of 4 µg guthion/cm^2 to the forearms of six volunteers (Feldmann and Maibach 1974). Feldmann and Maibach (1974) examined the excretion of radiolabeled guthion metabolites but the study was not designed to identify toxic end points. Acute-duration studies in rats and mice have evaluated the neurotoxic, systemic, reproductive, and developmental effects of guthion administered by inhalation, orally, or dermally (Astroff and Young 1998; EPA 1978a; Gaines 1960; Kavlock et al. 1985; Kimmerle 1976; Pasquet et al. 1976; Short et al. 1980; Skinner and Kilgore 1982; Su et al. 1971). ATSDR has derived an acute-duration inhalation MRL of 0.02 mg/m^3 based on the study by Kimmerle (1976). The study by Kimmerle (1976) is the only available acute-duration inhalation study with guthion in which activity levels of erythrocyte AChE were determined. Clinical signs at lethal doses were reported after a 1-hour exposure of rats to guthion, but erythrocyte AChE activity was not determined (EPA 1978a). An additional acute-duration inhalation study in mice or rats conducted at doses ranging from the low doses in Kimmerle (1976) to the higher doses used in EPA (1978a) would be useful to confirm the results of Kimmerle (1976) and to allow a better understanding of the dose-response curve for reductions in erythrocyte AChE activity and the onset of clinical signs of neurotoxicity. ATSDR has derived an acute-duration oral MRL of 0.01 mg/kg/day based on the study of Astroff and Young (1998). Additional acute-duration studies of the oral toxicity of guthion are not deemed to be necessary at this time. The large variation in dermal LD$_{50}$ values (EPA 1978a; Gaines 1960; Pasquet et al. 1976; Skinner and Kilgore 1982) could be due to differences in absorption of experimental method. An additional acute-duration dermal study in mice or rats would be useful in allowing a better understanding of the dose response curve for reductions in erythrocyte AChE activity and the onset of clinical signs of neurotoxicity.

Intermediate-Duration Exposure. Limited data are available regarding the effect on ChE activity of guthion taken orally by volunteers for 4 weeks (Rider and Puletti 1969; Rider et al. 1970, 1971, 1972). There was no effect on erythrocyte or plasma ChE activity at the doses tested. No intermediate-duration inhalation or dermal studies were located of guthion toxicity in humans. No intermediate-duration dermal studies were located of guthion toxicity in animals; however, the effects elicited by exposure to guthion are not expected to be route-dependent and the effects from dermal exposure are expected to be similar to those observed after oral or inhalation exposure. Intermediate-duration studies have evaluated the neurotoxic, systemic, reproductive, and developmental effects of guthion administered orally or by inhalation to rats and dogs (Allen et al. 1990; Holzum 1990; Kimmerle 1976; Schmidt and Chevalier 1984; Sheets et al. 1997; Short et al. 1980; Vos et al. 1983). A number of studies have demonstrated that neurotoxicity, exhibited as significant reductions in erythrocyte AChE or clinical signs of neurotoxicity, is the most sensitive end point related with intermediate-duration exposures to guthion. Increased mortality was observed in rats administered guthion by gavage (Short et al. 1980) or in the diet (Holzum 1990). The available experimental data suggest that developmental and reproductive effects are evident mostly at doses that are maternally toxic or that elicit significant reductions in parental erythrocyte AChE. ATSDR has derived an intermediate-duration inhalation MRL of 0.01 mg/m^3 based on the study by Kimmerle (1976) and an intermediate-duration oral MRL of 0.003 mg/kg/day based on the study by Allen et al. (1990).

Chronic-Duration Exposure and Cancer.   No controlled studies were located of the inhalation or dermal chronic-duration exposure to guthion in humans or animals. No studies of chronic, oral exposure to guthion in humans were located. Information from chronic toxicity studies is important because people working with guthion might be exposed to this pesticide for many years. The study by Weinbaum et al. (1997) suggests that dermal, and perhaps inhalation, exposures of workers to guthion may lead to adverse health effects. An increased association was observed between the occurrence of systemic illness (defined as an acute illness following pesticide exposure, with symptoms and signs not restricted to the eyes or skin) in workers and agricultural use of guthion (Weinbaum et al. 1997). Chronic-duration studies in dogs and rats have evaluated the systemic and neurological effects of guthion administered in the diet for up to 2 years (Allen et al. 1990; Schmidt and Chevalier 1984). ATSDR has derived a chronic-duration inhalation MRL of 0.01 mg/m^3 based on Kimmerle (1976) and a chronic-duration oral MRL of 0.003 mg/kg/day based on Allen et al. (1990). A study of the long-term neurological effects of exposure to guthion is warranted.

No studies were located regarding cancer in humans following oral exposure to guthion. A 2-year carcinogenicity study in rats and mice showed an increased combined incidence of islet cell carcinoma or carcinomas of the pancreas in male rats exposed to 10.9 mg/kg/day guthion in the diet for 80 weeks followed by a 35-week observation period (NCI 1978). However, this lesion occurs at a high spontaneous incidence in the animals used in this study and the increased incidence in the treated males could not be unequivocally attributed to treatment with guthion (NCI 1978). Similarly, the increases in the incidence of benign thyroid tumors, malignant thyroid tumors, or combined follicular cell tumors observed in male rats exposed to 5.5 or 10.9 mg/kg/day (NCI 1978) could not be attributed to treatment with guthion due to the historically high spontaneous incidence of these neoplasms in male rats in this laboratory (NCI 1978). There was no evidence of the occurrence of treatment-related tumors in female rats in this study or in another study of male and female Wistar rats exposed to 0.25–3.11 mg/kg/day for 2 years (Schmidt and Chevalier 1984). Benign and malignant neoplasms were observed among dosed and control B6C3F1 mice, but these lesions appear to occur spontaneously in mice in this laboratory and the effect could not be attributed to guthion (NCI 1978). The incidences of neoplasms of the pancreatic islets and of the follicular cells of the thyroid in male rats provide suggestive but insufficient evidence of a carcinogenic potential of guthion in male rats (NCI 1978). There was no significant increase in the incidence of tumors in female rats. The results of these studies led NCI (1978) to conclude that, under the conditions of this bioassay, guthion was not carcinogenic in male or female mice or female rats. There was suggestive but insufficient evidence to conclude that guthion was carcinogenic in male rats. Additional carcinogenicity studies with guthion are not needed at this time.

Genotoxicity.   No *in vivo* studies of genotoxic effects in humans were located. Six of the 11 *in vitro* studies with eukarytotic organisms (fungi and mammalian cells) that were located showed positive results for genotoxic effects (Alam and Kasatiya 1976; Alam et al. 1974; Bianchi–Santamaria et al. 1997; Hrelia et al. 1990; Waters et al. 1982; Zeiger et al. 1987), but the remaining studies (Carere et al. 1978; Hrelia et al. 1990; Waters et al. 1982) did not. An *in vivo* genotoxicity evaluation of persons exposed to guthion, particularly agricultural workers, would provide data that could assist in establishing the genotoxic potential of this insecticide in humans.

Reproductive Toxicity.   No studies are available on the reproductive toxicity of guthion in humans through any route of exposure or in animals exposed dermally or by inhalation. The reproductive toxicity of guthion has been evaluated in mice and rats administered guthion orally. Reductions in the incidence of viable litters were observed in the offspring of pregnant mice administered 20 mg/kg guthion orally once on gestation day 8 (Kavlock et al. 1985). Astroff and Young (1998) did not observe reproductive

effects in pregnant rats administered guthion at 2 mg/kg/day on gestation days 6–15. Insemination, fertility, or gestation indices or duration of gestation were not affected in male and female rats administered guthion at 0.43 to 4.9 mg/kg/day in the diet for 14 weeks before mating and continuously through gestation (Holzum 1990). The available evidence suggests that adverse reproductive effects are observed at doses that are higher than those that elicit maternal toxicity. Thus, an additional study of the reproductive toxicity of guthion in animals after intermediate-duration exposure is not needed at this time.

Developmental Toxicity.   No controlled studies are available on the developmental toxicity of guthion in humans by any route of exposure. No association was observed between occupational exposure to guthion and the occurrence of congenital malformations in a study of male agricultural workers conducted in Spain during 1993 and 1994 (García et al. 1998). An increased incidence of supernumerary ribs and reduced fetal body weight gain were observed in the offspring of pregnant mice administered a single oral dose of guthion at 16 and 20 mg/kg, respectively (Kavlock et al. 1985). An increased incidence of malaligned sternbrae and reduced body weight gain, brain weight, brain AChE activity, and survival were observed in the pups of pregnant rats administered 1.3–5 mg/kg/day during gestation (Holzum 1990; Short et al. 1980). The available experimental data suggest that in most studies developmental effects are evident only at doses that are maternally toxic. Thus, additional studies of the *in utero* developmental toxicity of guthion do not seem necessary at this time; however, information is lacking regarding the developmental effects of exposures of juvenile animals or children to guthion and a study to fill this data gap is warranted.

Immunotoxicity.   No studies were located on the immune toxicity in humans exposed to guthion by inhalation or oral exposure. Two studies examined the incidence of allergic responses in volunteers who were applied patches containing guthion on the skin. In one of these studies guthion did not elicit a dermal immune response (Lisi et al. 1987), while in the other study, 1 of 63 workers showed an allergic reaction to guthion (Sartorelli et al. 1999). Vos et al. (1983) reported decreased relative spleen and mesenteric lymph node weights, as well as unspecified histopathologic findings in the thymus in male Wistar rats exposed to guthion (85% a.i.) in the diet at 11.5 mg/kg/day for 3 weeks. An increase in mortality (rate not provided) was also observed at 11.5 mg/kg/day; no effects were observed at 2.3 mg/kg/day (Vos et al. 1983). Thymus and spleen morphology were not affected in rats exposed to guthion by inhalation for up to 12 weeks (Kimmerle 1976). The available evidence suggests that guthion elicits an unspecified immune response only at levels that also increase mortality. Thus, additional immunotoxicity studies are not warranted at this time.

Neurotoxicity.   The available studies strongly suggest that adverse effects on the nervous system are the most sensitive end points of guthion toxicity and these effects are characterized well in these studies. Although no significant changes in plasma or erythrocyte ChE activity were observed in a small group of subjects who took guthion orally at 0.057–0.086 mg/kg/day for 4 weeks (Rider and Puletti, 1969; Rider et al. 1970, 1971, 1972), studies of agricultural workers have demonstrated 10–20% reductions in erythrocyte or whole blood ChE activity after a single air-blast application of guthion (Franklin et al. 1981) or after entering field treated with guthion (Kraus et al. 1977; McCurdy et al. 1994; Schneider et al. 1994). Despite the reductions in erythrocyte AChE activity, workers did not exhibit clinical signs of neurotoxicity. A number of animal studies have demonstrated marked reductions in erythrocyte, brain, plasma, or whole blood ChE activity as well as clinical signs of neurotoxicity in mice, rats, or dogs after acute-, intermediate-, or chronic-duration exposures to guthion by inhalation (Kimmerle 1976), orally (Allen et al. 1990; Astroff and Young 1998; EPA 1978a; Holzum 1990; Pasquet et al. 1976; Schmidt and Chevalier 1984; Sheets et al. 1997; Short et al. 1980; Su et al. 1971), or dermally (EPA 1978a; Skinner and Kilgore 1982). No data are currently available to address the possibility of long-term neurological effects of repeated exposure to guthion. Thus, it is recommended that a battery of tests designed to detect subtle neurological effects be conducted among workers involved in the application of guthion or who enter fields treated with guthion.

Epidemiological and Human Dosimetry Studies.   Agricultural workers face the highest risk of exposure to guthion. Studies of agricultural workers who applied guthion (Franklin et al. 1981) or entered fields treated with guthion (Kraus et al. 1977; McCurdy et al. 1994; Schneider et al. 1994) showed reductions in erythrocyte or whole blood ChE activity, but did not exhibit clinical signs of neurotoxicity. These studies have examined changes in erythrocyte AChE activity over brief exposure durations and have generally not addressed systemic effects. Thus, an epidemiological study of agricultural workers exposed chronically to guthion would help evaluate the suggested association between the incidence of systemic illness and agricultural use of guthion (Weinbaum et al. 1997). An accurate quantification of exposure to guthion would be necessary to derive useful data from such a study.

Biomarkers of Exposure and Effect.

Exposure. The ideal biomarker for the quantification of exposure to guthion would be specific to the chemical of interest and would probably be the insecticide itself or a metabolite that could only be detected after exposure to guthion. It has been shown that DMDTP, DMTP, and DMP are metabolic products of the *in vivo* degradation of guthion (Carrier and Brunet 1999) and have been detected in urine

in humans under field and experimental conditions after dermal or otherwise unspecified exposure routes (Aprea et al. 1994; Franklin et al. 1986); however, these metabolites are not specific to guthion, but indicate potential exposure to several organophosphate pesticides. Direct monitoring data of guthion in humans is rare since its biological half-life is short. No studies were located that detected guthion or gutoxon in blood of exposed animals or humans. Reductions in plasma ChE and erythrocyte AChE activity and clinical symptoms of neurotoxicity are reliable biomarkers of exposure to guthion; however, it is currently not possible to use these biomarkers to distinguish exposure to guthion from that to other organophosphorus insecticides. Development of a biomarker of exposure specific to guthion would be useful in conducting exposure assessments and epidemiological studies.

Effect. Cholinergic symptoms of neurotoxicity and reductions in erythrocyte AChE activity (a surrogate for nervous system AChE activity) provide reliable biomarkers for the effect of guthion. Monitoring erythrocyte or plasma ChE activity may assist in confirming a diagnosis of organophosphate poisoning; however, reductions in plasma or erythrocyte ChE activity can be affected not only by all organophosphate insecticides, but also by carbamate ester insecticides. Thus, reductions in ChE activity are not specific to exposure to guthion. In addition, the large degree of variability in ChE activity in human populations suggests that caution should be exercised when comparing ChE activities from exposed populations, such as agricultural workers, and reference populations (Coye et al. 1987; Maroni et al. 2000). Development of a biomarker of effect specific to guthion would be useful in conducting exposure assessments and epidemiological studies.

Absorption, Distribution, Metabolism, and Excretion.   Animal studies have demonstrated that guthion is absorbed via the inhalation pathway, as can be inferred from the observed reductions in erythrocyte (Kimmerle 1976) and whole blood (EPA 1978a) ChE activity in acute-and intermediate-duration studies of rats exposed to guthion aerosols. There are no available human data to estimate the absorption of guthion in humans after oral exposure, but animal studies suggest that guthion is rapidly absorbed after oral exposure (Fakhr et al. 1996). The detection of urinary metabolites has demonstrated the dermal absorption of guthion in humans (Feldmann and Maibach 1974) and rats (Franklin et al. 1983).

No studies are available on the distribution of guthion in exposed humans or in animals following inhalation exposure; however, the distribution of guthion in exposed animals or humans is not expected to be route-dependent. Thus, studies to address this data need are not deemed necessary at this time. A study on the distribution of guthion administered orally to rats was located (Fakhr et al. 1996). The bioactivation of guthion to gutoxon and the detoxication of guthion is understood (Dahm et al. 1962;

3. HEALTH EFFECTS

Hitchcock and Murphy 1971; Levine and Murphy 1977; Motoyama and Dauterman 1972; Sultatos and Woods 1988). Studies suggest that the role of different cythochromes in the bioactivation process may be dependent on the guthion concentration (Buratti et al. 2003).

Urinary excretion of guthion metabolites has been demonstrated in humans (Aprea et al. 1994); however, the detected metabolites are not unique to guthion. No information was located on the elimination and excretion of guthion in human or animals following inhalation exposure. Elimination of guthion is not expected to be route dependent. Thus, additional studies of the elimination of guthion after inhalation exposure is not deemed to be necessary at this time. Radiolabeled guthion metabolites were eliminated largely in expired air, and feces of rats after a single oral dose (Fakhr et al. 1996). Guthion or its oxon metabolite were not detected by chromatographic analysis in the urine (Fakhr et al. 1996). Urinary excretion of radiolabeled metabolites of guthion was detected after application of guthion to the forearm of volunteers (Feldmann and Maibach 1974). A study with human subjects who were administered guthion at 500–6,000 µg/person (approximately 7–86 µg/kg) topically on the forehead showed urinary excretion of DMTP after 72 hours (Franklin et al. 1986). Approximately 60% of the guthion doses (100–400 µg/rat) applied to a shaved area (2.6 cm^2) of the dorsal skin of male Sprague-Dawley rats was recovered in urine as the guthion metabolite DMTP (Franklin et al. 1983). The urinary output of radiolabeled guthion metabolites after a 1 µCi intramuscular dose in rats showed two peaks in urinary excretion of the administered dose, one 4 hours after the dose (approximately 13% of the dose) and a higher peak showing recovery of approximately 20% of the dose, after 24 hours, which was followed by a rapid decrease in output to very low levels after 120 hours (Franklin et al. 1983). The urinary recovery of metabolites observed in a study with human subjects administered a single intravenous dose of 1 µCi radiolabeled guthion also showed an initial peak (1.5% dose/hour) 0–4 hours after the dose was administered which was followed by a drop in excretion and a second peak (1.6% dose/hour) 812 hours after the dose was administered (Feldmann and Maibach 1974).

Comparative Toxicokinetics. No studies were located that directly evaluated the comparative toxicokinetics of guthion in animals and humans. Nevertheless, available studies suggest that the toxicokinetics of guthion in animals and humans are generally similar (EPA 1999b, Feldmann and Maibach 1974, Zendzian 2003) and that neural AChE is the target organ of guthion toxicity in animals and humans (Buratti et al. 2003; Hitchcock and Murphy 1971). Recent work suggests that the desulfuration of guthion to gutoxon in human liver microsomes is largely effected by at least three cytochromes (CYP1A2, CYP3A4, and CYP2B6), which show different affinities for the substrate (Buratti et al. 2003). If the spectrum of activities of these cytochromes in animals varies markedly from that in

humans, notable differences in animals and humans might be expected. No data are available to determine whether such differences do or do not exist. A study of the comparative toxicokinetics of guthion in animals and humans may be warranted.

Methods for Reducing Toxic Effects.   Guthion exerts its systemic effects through inhibition of AChE in the central and peripheral nervous system. Guthion is bioactivated *in vivo* and *in vitro* to its oxygen analog form, variably referred to as gutoxon or guthion oxon (Buratti et al. 2003; Hitchcock and Murphy 1971; Sultatos and Woods 1988). Gutoxon reacts with a serine hydroxyl group at the active site of AChE, rendering it largely inhibited and unreactive. The inhibited AChE enzyme cannot degrade acetylcholine and the neurotransmitter accumulates at the ending of cholinergic nerves with the ensuing continual stimulation of electrical activity (Carrier and Brunet 1999). Intoxications with guthion are managed as are intoxications caused by other organophosphate insecticides, namely, by administering respiratory support, atropine treatment, and reactivation of neural AChE with 2-PAM (Carlton et al. 1998; Tafuri and Roberts 1987). The mechanism of inhalation, oral, or dermal absorption of guthion is not known. Research is needed to develop an understanding of the mechanisms of absorption of guthion via the inhalation, oral, or dermal routes. Currently, no methods exist to promote the excretion of guthion or its active metabolite, gutoxon. Research is needed to develop methods to promote the excretion of guthion and the active metabolite gutoxon.

Children's Susceptibility.   Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

No information is available for any route of exposure as to whether absorption of guthion is different between children and adults or between juvenile and adult animals. No cases of children poisoned by exposure to guthion were located. Nevertheless, the critical targets of guthion toxicity can be expected to be similar in children and adults. No animal studies comparing the effects or toxicokinetics of guthion in juvenile and adult animals were located. Comparative studies of the toxicity and toxicokinetics of guthion in juvenile and adult animals are needed.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

One ongoing study pertaining to the health effects of guthion has been identified in the Federal Research in Progress (FEDRIP) database. J.E. Chambers, J.S. Boone, and R.L. Carr of the College of Veterinary Medicine at Mississippi State University are conducting an investigation of the biochemical and physiological factors contributing to the age-related differences in responses of mammals to insecticides (FEDRIP 2006).