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 RDX. 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. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of RDX are indicated in Table 3-1 and Figure 3-1. Because cancer effects could occur at lower exposure levels, Figure 3-1 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

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

3.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to RDX. Death attributed to impairment of the respiratory system was observed in rabbits and guinea pigs exposed to an unspecified concentration of RDX (Sunderman 1944).

3.2.1.2 Systemic Effects

Four studies were located regarding systemic effects in humans after inhalation exposure to RDX alone. The available studies have reported adverse gastrointestinal, hematological, hepatic, and renal effects in workers exposed to C-4 (an explosive composed of 91% RDX) or RDX dusts via inhalation. Since the exposure concentration and/or duration were not described for these studies, they are not presented in tables or figures. No studies were located regarding respiratory, cardiovascular, musculoskeletal, dermal, ocular, or other systemic effects in humans after inhalation exposure to RDX. Case reports are available regarding systemic effects in workers exposed to unknown levels of RDX via the inhalation or oral routes

(Ketel and Hughes 1972). These studies are also discussed in Section 3.2.2.2. Only one study is available regarding systemic effects in animals after inhalation exposure to RDX (Sunderman 1944). This study is limited by insufficient numbers of animals tested, no controls, and no data on exposure levels. No studies were located regarding gastrointestinal, hepatic, or dermal effects in animals.

Respiratory Effects. Three of 6 rabbits died from bronchopneumonia; death of 7 of 18 guinea pigs was attributed to pneumonia and pulmonary congestion (Sunderman 1944).

Cardiovascular Effects. Histopathology revealed the absence of striations in the cardiac muscle of guinea pigs exposed to unspecified levels of RDX for 4–67 days (Sunderman 1944).

Gastrointestinal Effects. Soldiers who were exposed to an unspecified amount of C-4 (91% RDX) as a cooking fuel for an unknown duration experienced nausea and vomiting (Hollander and Colbach 1969; Ketel and Hughes 1972); the soldiers were exposed to RDX via the inhalation and/or oral routes.

Hematological Effects. Two studies of workers exposed to RDX dusts are available, but neither revealed any adverse hematological effects. In one study, workers who were presumably exposed acutely to unknown levels of RDX dusts had normal blood counts (Kaplan et al. 1965). In the other study, workers exposed to an average of 0.28 mg/m^3 of RDX dusts in the workplace, presumably for a chronic period, showed no hematological changes compared to controls (Hathaway and Buck 1977). Transient elevation of the white blood count was frequently observed in individuals exposed to C-4 (91% RDX). Normal red blood count, leukocytes, and hemoglobin were reported in rats following intermediate exposure to RDX. However, in the same study, hemoglobin counts were decreased in guinea pigs (Sunderman 1944).

Hepatic Effects. No liver toxicity was revealed by blood or urine analyses of workers exposed to RDX in the air; the duration of exposure was not reported (Hathaway and Buck 1977).

Renal Effects. Blood and urine analyses of workers exposed to RDX in the air for acute (Kaplan et al. 1965) or chronic durations (Hathaway and Buck 1977) did not reveal any kidney toxicity. Although no renal toxicity was observed after exposure to RDX dust, there were some manifestations of renal damage after possible inhalation exposure to C-4 (91% RDX): transient oliguria and proteinuria in two patients and acute renal failure in one case (Ketel and Hughes 1972).

There was no kidney pathology in rats or guinea pigs exposed to RDX, but degeneration of the kidneys was found in rabbits exposed to unspecified levels of RDX for an intermediate period (Sunderman 1944). This study is limited in that no controls were used, and details of the study were not specified.

3.2.1.3 Immunological and Lymphoreticular Effects

Workers at an Army ammunition plant who were exposed to an average of 0.28 mg/m^3 of RDX dusts for an unknown period of time showed no significant differences in a test for antinuclear antibodies as compared to nonexposed workers. The results of this test provide no evidence of autoimmune disease (Hathaway and Buck 1977). No other immunological function tests were performed.

No studies were located regarding immunological effects in animals after inhalation exposure to RDX.

3.2.1.4 Neurological Effects

Convulsions and unconsciousness, accompanied by headache, dizziness, and vomiting, were noted in 5 out of 26 workers who were exposed to unknown levels of RDX dust in the air (Kaplan et al. 1965). Similar findings, such as convulsions, muscle twitching, and confusion, have been reported in five case studies of men exposed to C-4 fumes (91% RDX) when it was used as a cooking fuel (Hollander and Colbach 1969), and in a worker hand-sieving RDX (Testud et al. 1996a). The workers recovered a few days after they were removed from the source of exposure. Testud et al. (1996a) noted that CT scan and MRI (performed 1 week after exposure) were normal and electroencephalogram only showed signs of the administered anticonvulsant therapy; in the other studies, tests of neurological function were not performed. In a study of workers at an RDX facility, no increases in the occurrence of subjective symptoms were reported (Ma and Li 1993). Significant differences in performance on tests of memory retention and block design were found in workers exposed to 0.407 or 0.672 mg/m^3, as compared to controls; however, no differences were found between the two exposed groups. No significant alterations in performance on tests of reaction time were noted.

No studies were located regarding neurological effects in animals after inhalation exposure to RDX.

No studies were located regarding the following effects in humans or animals after inhalation exposure to RDX:

3.2.1.5 Reproductive Effects

3.2.1.6 Developmental Effects

3.2.1.7 Cancer

3.2.2 Oral Exposure

3.2.2.1 Death

No studies were located regarding death in humans after oral exposure to RDX.

Deaths were reported in animals following acute, intermediate, and chronic exposures to RDX. Three out of 12 rats died during induced seizures following acute exposure to 50 mg/kg RDX, which was administered by gavage (Burdette et al. 1988). LD_{50} values for single gavage doses were 71–118 mg/kg in rats (U.S. Army 1978b, 1980b), 86–97 mg/kg in mice (U.S. Army 1978b, 1980b), and 136–319 mg/kg in deer mice (Smith 2007). Apparent age-related differences in LD_{50} values were found in deer mice; the LD_{50} values were 136, 319, and 158 mg/kg in 21-, 50-, and 200-day-old mice (Smith et al. 2007). Miniature swine died (2/10) following single gavage doses of 100 mg/kg (Schneider et al. 1977). Rat dams that were fed 20 mg/kg/day of RDX during gestation had mortality rates of 24% (U.S. Army 1980b, 1986d).

In 90-day feeding studies, levels as low as 25 mg/kg/day (von Oettingen et al. 1949) and 100 mg/kg/day, produced deaths in rats (Levine et al. 1990), and levels of 320 mg/kg/day produced deaths in mice (U.S. Army 1980b). Increased mortality (25%) was observed in rats administered via gavage 10 mg/kg/day (U.S. Army 2006); however, no deaths were observed in dogs (U.S. Navy 1974a) or monkeys (U.S. Navy 1974b) also administered 10 mg/kg/day. In chronic-duration studies, an excessive number of deaths was observed in rats exposed to 40 mg/kg/day for 1–2 years compared to controls (U.S. Army 1983a). However, an excessive number of deaths was not observed in rats administered 10 mg/kg/day of RDX (U.S. Navy 1976). The LD_{50} values and all reliable LOAEL values for death are recorded in Table 3-1 and plotted in Figure 3-1.

Table 3-1 Levels of Significant Exposure to RDX - Oral

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
ACUTE EXPOSURE								
Death								
1	Rat (Long- Evans)	once (GW)				50 M (3/12 died during seizures)	Burdette et al. 1988	
2	Rat (Sprague-Dawley)	once (GW)				50 (2/10 died)	Schneider et al. 1977	
3	Rat (Sprague-Dawley)	once (GO)				71 M (LD50) 75 F (9/10 rats died)	U.S. Army 1978b	
4	Rat (Fischer 344)	Gd 6-19 (GW)				20 F (6/25 died)	U.S. Army 1980b	
5	Rat (Fischer 344)	once (G)				119 (LD50)	U.S. Army 1980b	
6	Rat (Sprague-Dawley)	Gd 6-15 (GW)				20 F (31% died)	U.S. Army 1986d	
7	Rat (Sprague-Dawley)	7 d/wk 14 d (GW)				25.5 (75% mortality)	U.S. Army 2006	
8	Rat (NS)	once (GW)				100 (40% mortality)	von Oettingen et al. 1949	

Table 3-1 Levels of Significant Exposure to RDX - 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
9	Mouse (Swiss-Webster)	once (GO)				86 M (LD50) 75 F (5/10 mice died)	U.S. Army 1978b	
10	Mouse (B6C3F1)	once (G)				97 M (LD50) 59 F (LD50)	U.S. Army 1980b	
11	Pig (NS)	once (GW)				100 F (2/10 died)	Schneider et al. 1977	
Systemic								
12	Rat (Fischer 344)	Gd 6-19 (GW)	Bd Wt		20 F (12% decrease in maternal body weight)		U.S. Army 1980b	
13	Rat (Sprague-Dawley)	7 d/wk 14 d (GW)	Hemato	17			U.S. Army 2006	
14	Mouse (Peromyscus leucopus)	daily 14 days (F)	Hepatic	68 F			EPA 1999	
			Renal	68 F				
			Bd Wt	68 F				
Neurological								
15	Human	once				357 M (seizures)	Stone et al. 1969	
16	Rat (Long- Evans)	once (GW)		12.5 M		25 M (seizures)	Burdette et al. 1988	

Table 3-1 Levels of Significant Exposure to RDX - 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
17	Rat (Sprague-Dawley)	once (GO)				87 F (convulsions in 2/2 rats)	Meyer et al. 2005	
18	Rat (Sprague-Dawley)	once (GW)				50 (convulsions)	Schneider et al. 1977	
19	Rat (Fischer 344)	Gd 6-19 (GW)		2 F		20 F (convulsions and hyperactivity in dams)	U.S. Army 1980b	
20	Rat (Sprague-Dawley)	once (GW)			12.5 (decreases in motor activity, taste aversion, learning, and auditory startle response amplitude)		U.S. Army 1985b	
21	Rat (Sprague-Dawley)	Gd 6-15 (GW)		6 F		20 F (convulsions, prostration in dams)	U.S. Army 1986d	
22	Rat (Sprague-Dawley)	7 d/wk 14 d (GW)		8.5[b]		17 (tremors and convulsions)	U.S. Army 2006	
23	Pig (NS)	once (GW)				100 F (convulsions)	Schneider et al. 1977	

Table 3-1 Levels of Significant Exposure to RDX - 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
Developmental								
24	Rat (Fischer 344)	Gd 6-19 (GW)		2 F			U.S. Army 1980b	
25	Rat (Sprague-Dawley)	Gd 6-15 (GW)		6 F	20 F (9% decrease in fetal weight and 5% decrease in fetal length)		U.S. Army 1986d	
INTERMEDIATE EXPOSURE								
Death								
26	Rat (Fischer 344)	13 wk (F)				100 (65% mortality)	Levine et al. 1981	
27	Rat (Fischer 344)	13 wk (F)				100 (13/20 died)	Levine et al. 1990	
28	Rat (Fischer- 344)	7 d/wk 90 d (GW)				10 (25% mortality)	U.S. Army 2006	
29	Rat (NS)	90 d (F)				25 (8/20 died)	von Oettingen et al. 1949	
30	Rat (NS)	10 wk (F)				50 (60% mortality)	von Oettingen et al. 1949	
31	Mouse (B6C3F1)	90 d (F)				320 M (4/10 died)	U.S. Army 1980b	

Table 3-1 Levels of Significant Exposure to RDX - 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
Systemic								
32	Monkey (Cynomolgus)	90 d 7 d/wk (GW)	Resp	10			U.S. Navy 1974b	
			Cardio	10				
			Gastro	1	10 (vomiting in 5/6 animals)			
			Hemato	1	10 (necrotic and degenerate megakaryocytes in bone marrow)			
			Hepatic	10				
			Renal	10				
			Endocr	10				
			Ocular	10				
33	Rat (Fischer 344)	13 wk (F)	Resp	100			Levine et al. 1981	
			Cardio	100				
			Gastro	100				
			Hemato		10 F (increased leukocyte counts)			
			Hepatic	10	30 (10-14% decrease in serum triglycerides)			
			Renal	100				
			Bd Wt	30	100 M (17% weight loss)			

Table 3-1 Levels of Significant Exposure to RDX - 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
34	Rat (Fischer 344)	3-13 wk (F)	Resp	100			Levine et al. 1990	
			Cardio	100				
			Gastro	100				
			Hemato	100				
			Hepatic		30 (decr serum triglyceride levels)			
			Renal	100				
			Bd Wt		30 M (13% decrease in body weight gain)	100 M (29% decr body weight gain)		

Table 3-1 Levels of Significant Exposure to RDX - 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
35	Rat (Fischer 344)	90 d (F)	Resp	40			U.S. Army 1980b	
			Cardio	28 F	40 F (decreased absolute heart weight, myocardial degeneration)			
			Gastro	40				
			Hemato	40				
			Musc/skel	40				
			Hepatic	40				
			Renal	40				
			Endocr	40				
			Dermal	40				
			Ocular	40				
			Bd Wt	40				

Table 3-1 Levels of Significant Exposure to RDX - 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
36	Rat (Fischer 344)	6 mo (F)	Resp	40				U.S. Army 1983a	
			Cardio	40					
			Gastro	40					
			Hemato	8	40	(decreased hemoglobin and erythrocyte levels)			
			Musc/skel	40					
			Hepatic	8	40	(decreased serum triglyceride and cholesterol levels)			
			Renal	40					
			Endocr	40					
			Dermal	40					
			Ocular	40					
			Bd Wt	8	40	(17% decrease in body weight gain)			
			Metab	8	40	(decreased blood glucose levels)			

Table 3-1 Levels of Significant Exposure to RDX - 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
37	Rat (Fischer- 344)	7 d/wk 90 d (GW)	Resp	15			U.S. Army 2006	
			Cardio	15				
			Gastro	15				
			Hemato	15				
			Hepatic	4 F	8 M (decreased serum cholesterol levels)			
			Renal	15				
			Ocular	15				
			Bd Wt	15				
38	Rat (NS)	90 d (F)	Bd Wt	15		25 (weight loss)	von Oettingen et al. 1949	
39	Rat (NS)	10 wk (F)	Bd Wt	15		50 (weight loss)	von Oettingen et al. 1949	

Table 3-1 Levels of Significant Exposure to RDX - 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
40	Mouse (B6C3F1)	90 d (F)	Resp	320			U.S. Army 1980b	
			Cardio	160 M	320 M (slight myocardial degeneration)			
			Gastro	320				
			Hemato	80 M	160 M (12% decrease in erythrocyte count and 7% decrease in hemoglobin concentration)			
			Hepatic	160 M	320 M (hepatocellular vacuolization)			
			Renal	160 M	320 M (mild tubular nephrosis)			
			Endocr	160 M	320 F (mild focal subscapular fibroplasia in adrenal gland)			
			Bd Wt	320				

Table 3-1 Levels of Significant Exposure to RDX - 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
41	Mouse (B6C3F1)	6 mo (F)	Resp	100			U.S. Army 1984c	
			Cardio	100				
			Gastro	100				
			Hemato	100				
			Musc/skel	100				
			Hepatic	100				
			Renal	100				
			Endocr	100				
			Ocular	100				
42	Dog (NS)	90 d (F)	Resp	10			U.S. Navy 1974a	
			Cardio	10				
			Hemato	10				
			Hepatic	10				
			Renal	10				
			Endocr	10				
			Ocular	10				

Table 3-1 Levels of Significant Exposure to RDX - 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
43	Dog (NS)	6 wk 6 d/wk (C)	Resp	50 F			von Oettingen et al. 1949	
			Cardio	50 F				
			Hemato	50 F				
			Hepatic	50 F				
			Renal	50 F				
			Endocr	50 F				
			Bd Wt			50 F (unspecified weight loss)		
Immuno/ Lymphoret								
44	Rat (Fischer- 344)	7 d/wk 90 d (GW)		15			U.S. Army 2006	
Neurological								
45	Monkey (Cynomolgus)	90 d 7 d/wk (GW)		1		10 (convulsions and seizures)	U.S. Navy 1974b	
46	Rat Fischer 344	13 wk (F)		30	100 (hyperreactive to approach)		Levine et al. 1981	
47	Rat (Fischer 344)	10 wk (F)		30	100 (hyperreactive to approach)		Levine et al. 1990	

Table 3-1 Levels of Significant Exposure to RDX - 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
48	Rat (Fischer 344)	25 wk (F)		8		40 (tremor, convulsions, hyperreactive)	U.S. Army 1983a	
49	Rat (Sprague-Dawley)	30 d (GW)		10 M			U.S. Army 1985b	testing conducted 24 hours after dose administration
50	Rat (Fischer- 344)	7 d/wk 90 d (GW)		4[c]		8 (tremors and convulsions)	U.S. Army 2006	
51	Rat (NS)	90 d (F)		15		25 (convulsions, hyperirritability and fighting)	von Oettingen et al. 1949	
52	Rat (NS)	10 wk (F)		15		50 (hyperirritability and convulsions)	von Oettingen et al. 1949	
53	Mouse (B6C3F1)	90 d (F)		160 M	320 M (hyperactivity and/or nervousness)		U.S. Army 1980b	
54	Dog (NS)	6 wk 6 d/wk (C)				50 F (hyperirritability and convulsions)	von Oettingen et al. 1949	

Table 3-1 Levels of Significant Exposure to RDX - 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
Reproductive								
55	Rat (Fischer 344)	3-13 wk (F)		100			Levine et al. 1990	
56	Rat (Fischer 344)	2 generation; 13 wk pre-mating, mating, gestation, & lactat. (F)		50			U.S. Army 1980b	
57	Rat (Fischer 344)	15 wk (F)		16 M	50 M (decreased fertility)		U.S. Army 1980b	Decreased fertility may be due to RDX-effect on general well-being of males
58	Rat (Fischer 344)	6 mo (F)		8 M 40 F	40 M (spermatic granuloma in prostate)		U.S. Army 1983a	
Developmental								
59	Rat (Fischer 344)	2 generation; 13 wk pre-mating, mating, gestation, & lactat. (F)		5	16 (decrease in F2 pup body weight)	50 (increase in number of stillbirths; decrease in pup survival in F1)	U.S. Army 1980b	
60	Rabbit (NS)	Gd 7-29 (GW)		20			U.S. Army 1980b	

Table 3-1 Levels of Significant Exposure to RDX - 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
CHRONIC EXPOSURE								
Death								
61	Rat (Fischer 344)	2 yr (F)				40 M (88% died)	U.S. Army 1983a	
Systemic								
62	Rat (Fischer 344)	1 & 2 yr (F)	Resp	40			U.S. Army 1983a	
			Cardio	40				
			Gastro	40				
			Hemato	8	40 (decreases in hematocrit, hemoglobin, and erythrocyte levels; splenic extramedullary hematopoiesis)			
			Musc/skel	40				
			Hepatic	8	40 (hepatomegaly, decreased serum trigylcerides and cholesterol)			
			Renal	8	40 (renal papillary necrosis with increased BUN)			
			Endocr	40				
			Ocular	8 F	40 F (cataracts)			
			Bd Wt	8		40 M (20-30% decrease in body weight gain)		

Table 3-1 Levels of Significant Exposure to RDX - 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
63	Rat (Sprague-Dawley)	2 yr (F)	Resp	10			U.S. Navy 1976	
			Cardio	10				
			Gastro	10				
			Hemato	10				
			Hepatic	10				
			Renal	10				
			Endocr	10				

Table 3-1 Levels of Significant Exposure to RDX - 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
64	Mouse (B6C3F1)	1 & 2 yr (F)	Resp	100			U.S. Army 1984c	
			Cardio	35	100 (increased relative heart weight)			
			Gastro	100				
			Hemato	100				
			Musc/skel	100				
			Hepatic	7	35 F (increased serum cholesterol levels)			
			Renal	35	100 (increased relative kidney weights and reversible cytoplasmic vacuolization)			
			Ocular	100				
			Bd Wt	100				
Neurological								
65	Rat (Fischer 344)	1 & 2 yr (F)		8[d]		40 (tremors, convulsions; hyperresponsive to stimuli)	U.S. Army 1983a	
Reproductive								
66	Rat (Fischer 344)	1 & 2 yr (F)		8 M 40 F	40 M (testicular degeneration)		U.S. Army 1983a	

Table 3-1 Levels of Significant Exposure to RDX - 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
67	Mouse (B6C3F1)	1 & 2 yr (F)		100 M 100 F			U.S. Army 1984c	
Cancer								
68	Mouse (B6C3F1)	1 & 2 yr (F)				35 F (CEL: hepatocellular carcinomas and adenomas)	U.S. Army 1984c	

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

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.2 mg/kg/day based on a PBPK model predicted internal dose metric (peak brain RDX concentration) of the NOAEL dose; a human equivalent dose (HED) of the NOAEL was also estimated using a PBPK model. The NOAELHED of 6.45 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to human with dosimetric adjustments and 10 for human variability).

c Used to derive an intermediate-duration oral MRL of 0.1 mg/kg/day based on a BMDL10 estimated using using a PBPK model predicted internal dose metric (peak brain RDX concentration); a human equivalent dose of the BMDL10 was also predicted using a PBPK model. The BMDLHED of 4.1308 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to human with dosimetric adjustment and 10 for human variability).

d Used to derive a chronic-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on a PBPK model predicted internal dose metric (peak brain RDX concentration) of the NOAEL dose; a human equivalent dose (HED) of the NOAEL was also estimated using a PBPK model. The NOAELHED of 4.223 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to human with dosimetric adjustments and 10 for human variability).

Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; min = minute(s); Metab = metabolic; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; occup = occupational; Resp = respiratory; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to RDX - Oral

Acute (≤14 days)

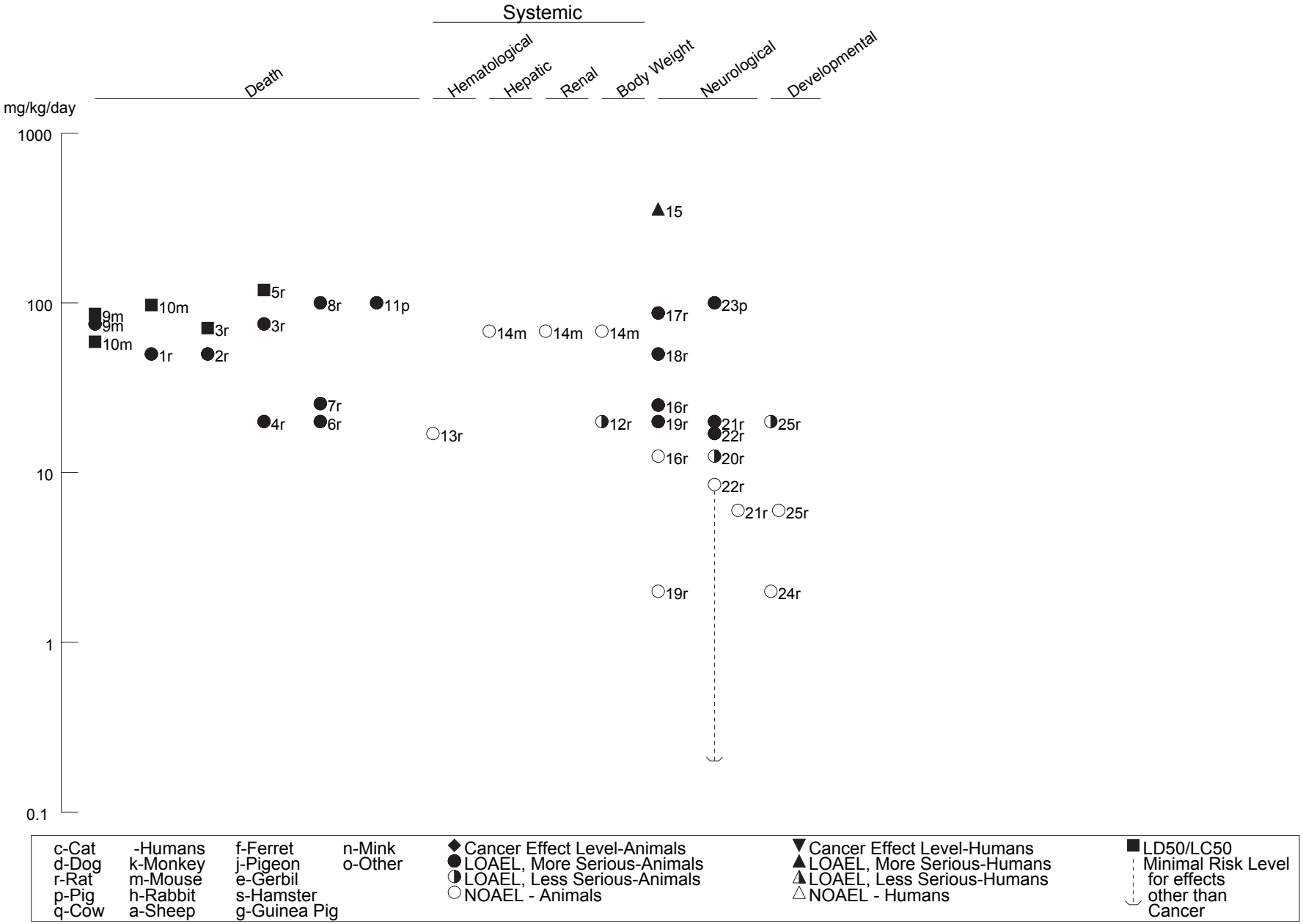


Figure 3-1 Levels of Significant Exposure to RDX - Oral *(Continued)*

Intermediate (15-364 days)

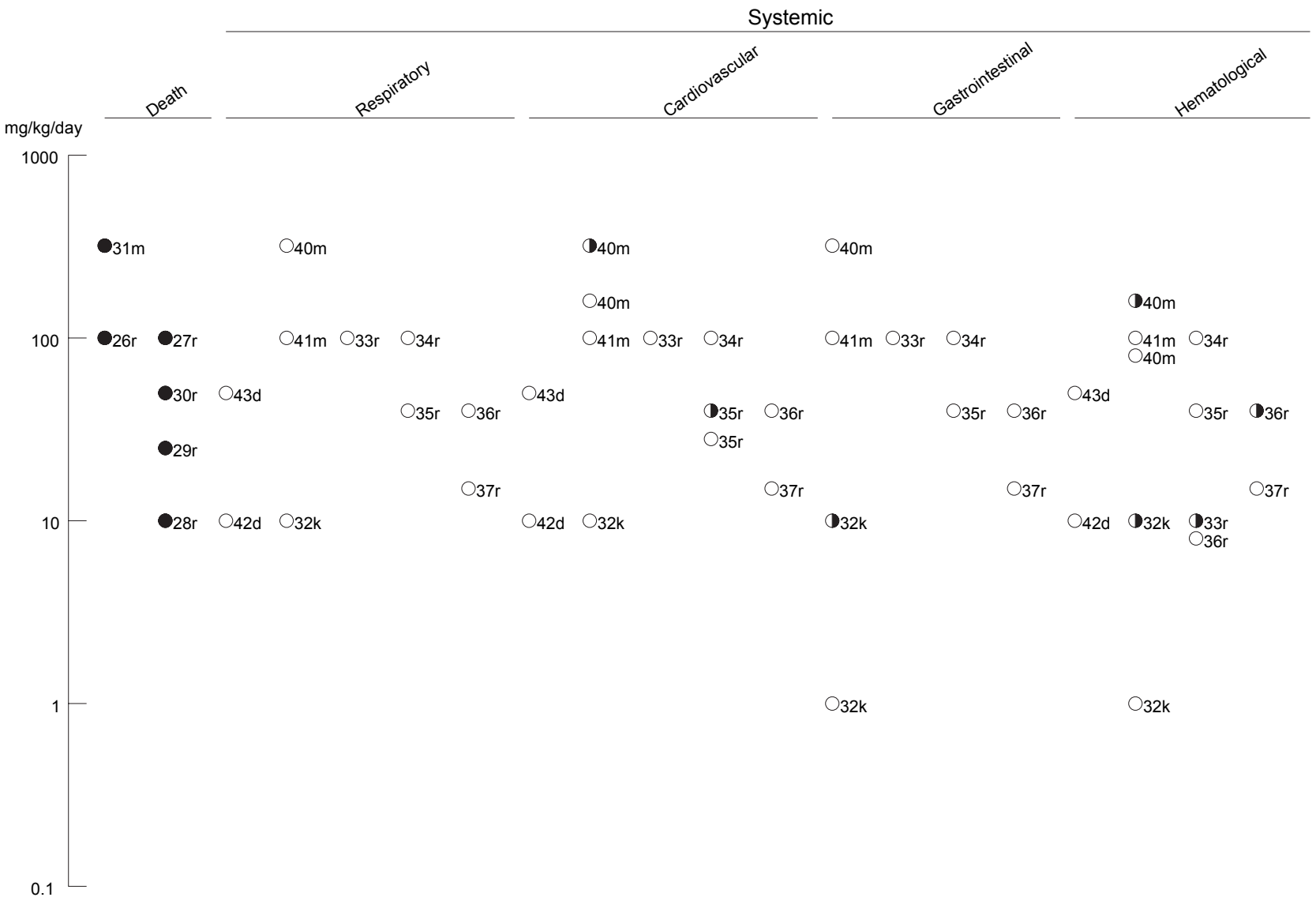


Figure 3-1 Levels of Significant Exposure to RDX - Oral *(Continued)*

Intermediate (15-364 days)

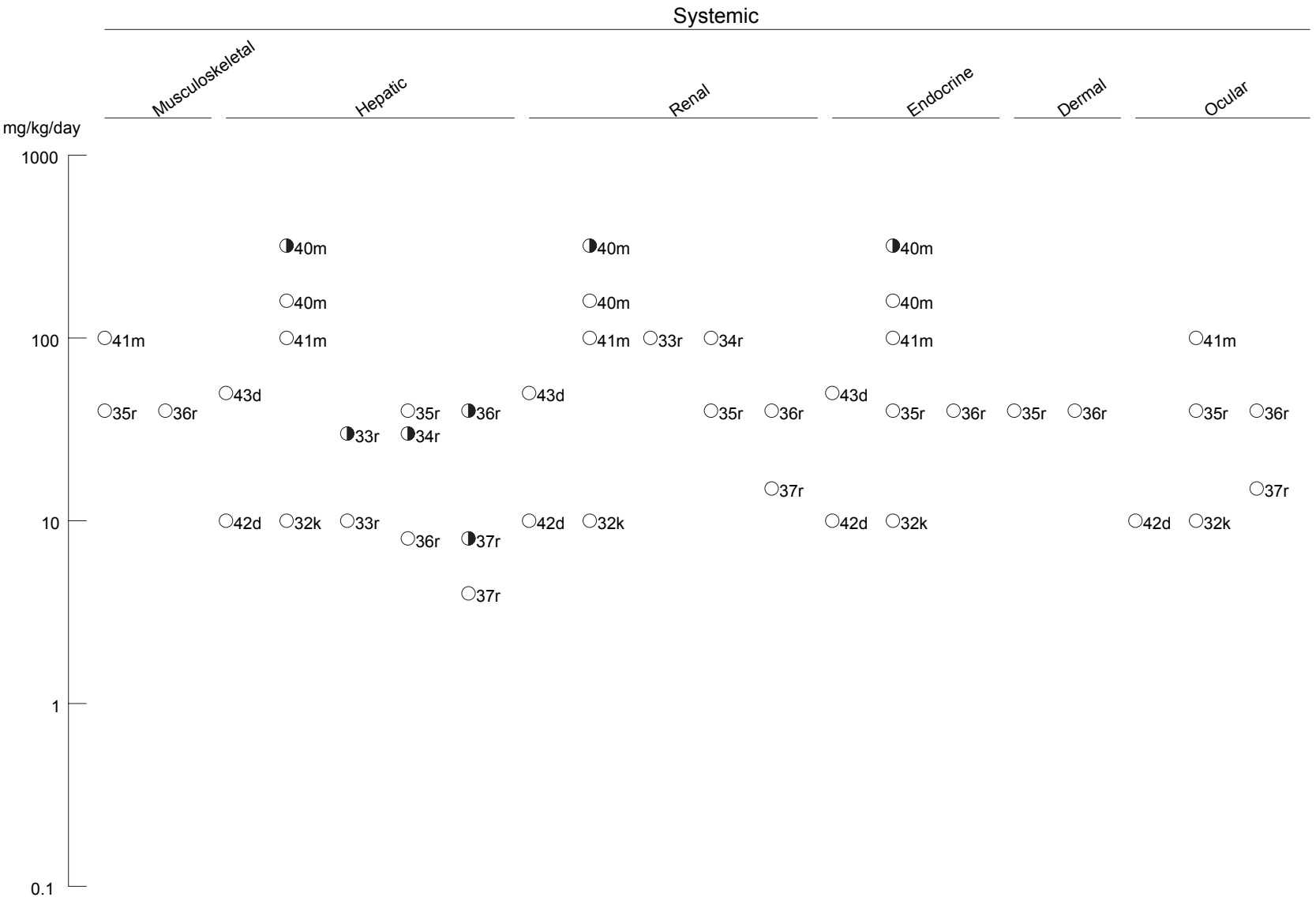


Figure 3-1 Levels of Significant Exposure to RDX - Oral *(Continued)*

Intermediate (15-364 days)

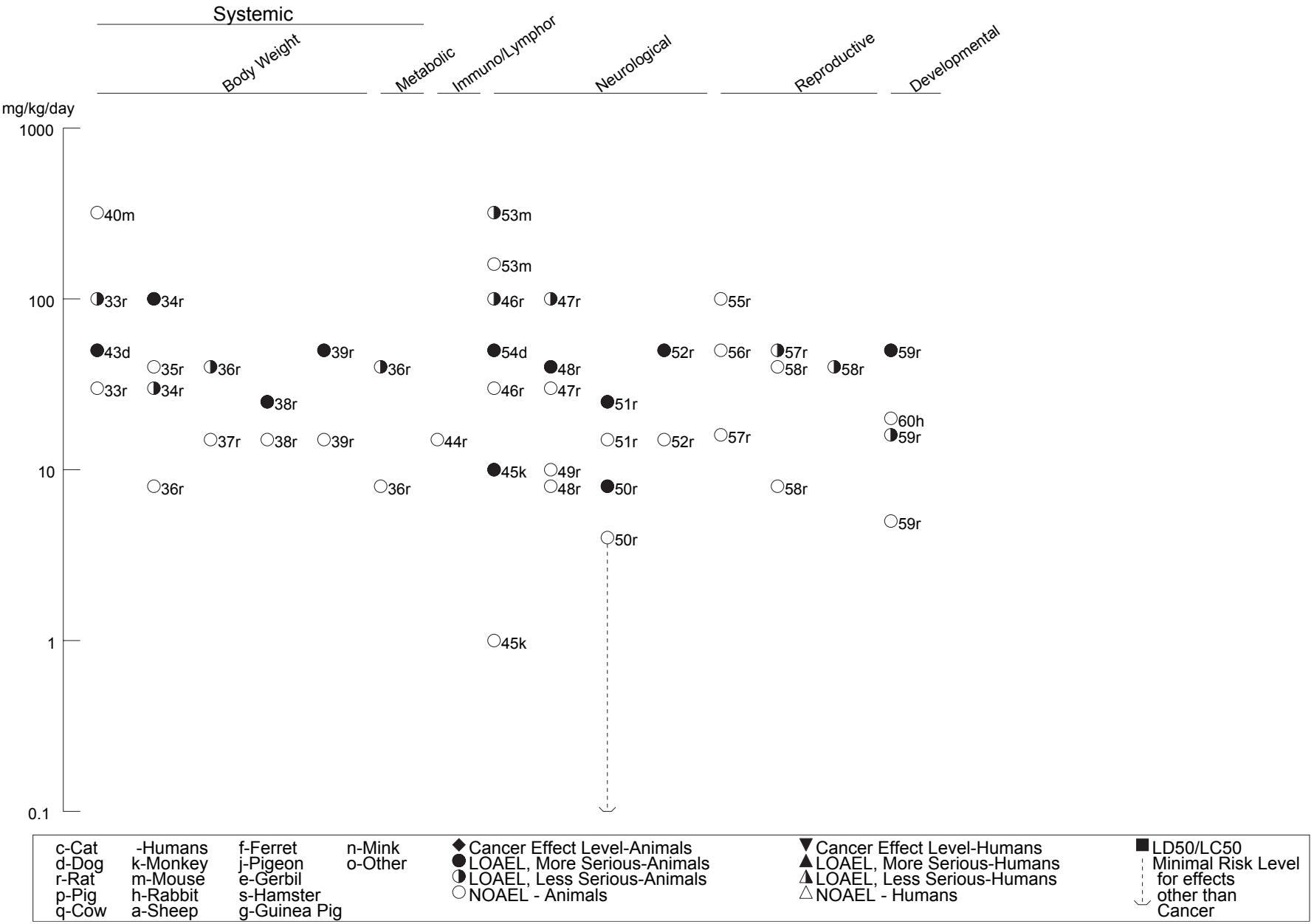
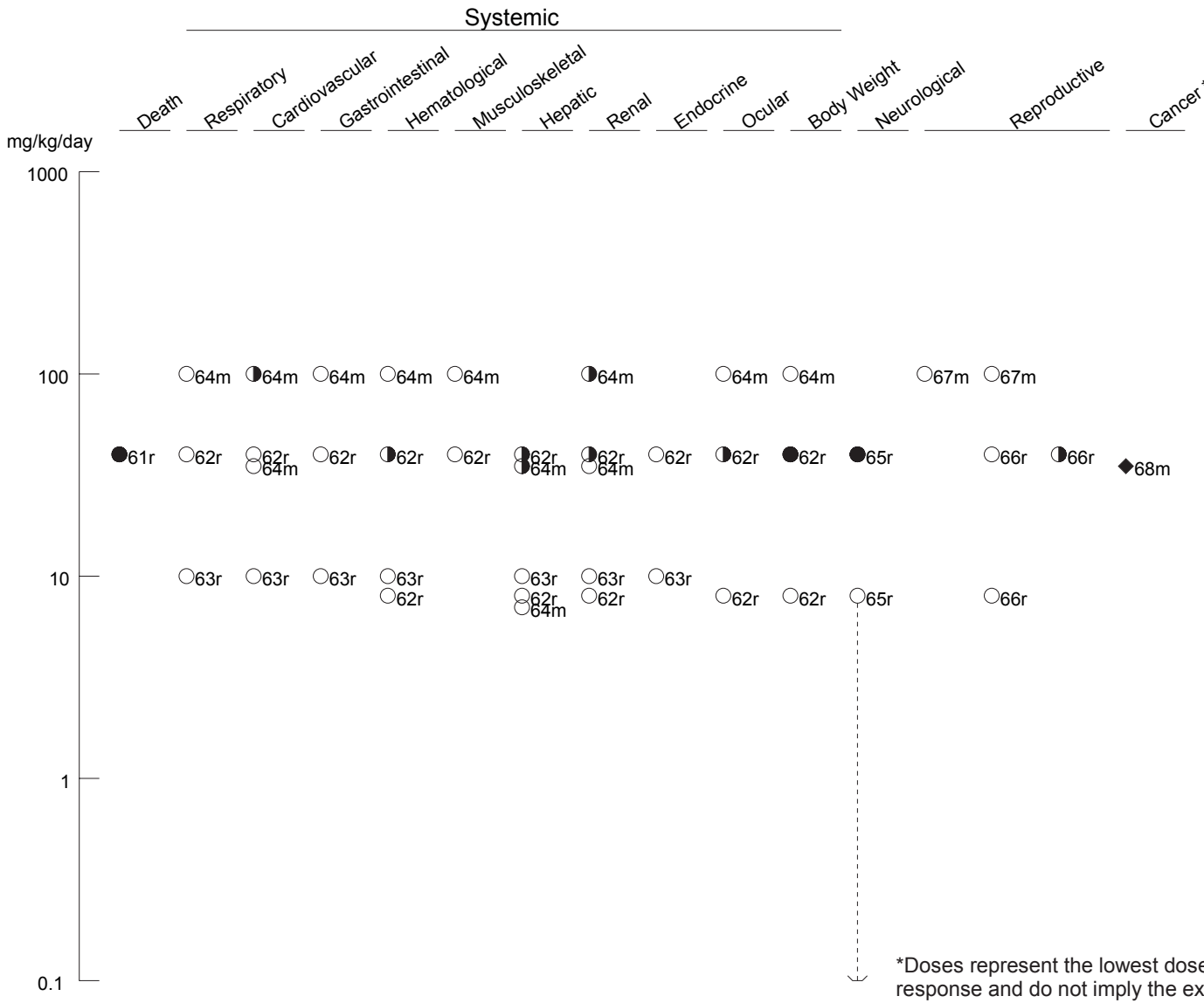


Figure 3-1 Levels of Significant Exposure to RDX - Oral *(Continued)*

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

3.2.2.2 Systemic Effects

No studies were located regarding respiratory, musculoskeletal, dermal, or ocular effects in humans after acute oral exposure to RDX. No studies were located regarding systemic effects in humans after intermediate or chronic oral exposure to RDX. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Adverse respiratory effects were not observed in animals following acute, intermediate, or chronic exposure. An acute-duration study in 3 anesthetized dogs showed no significant changes in breathing rate when 15 mg/kg RDX was administered by gavage (von Oettingen et al. 1949). No histopathology was seen in the lungs, trachea, or bronchi of rats exposed for 3–13 weeks to 100 mg/kg/day of RDX in the diet (Levine et al. 1990), 40 mg/kg/day RDX in the diet for 90 days (U.S. Army 1980b), or 15 mg/kg/day via gavage for 90 days (U.S. Army 2006). Similarly, no histopathological alterations were observed in the respiratory system of mice exposed to 100 or 320 mg/kg/day in the diet for 3 or 6 months (U.S. Army 1980b, 1984c), dogs exposed to 10 mg/kg/day in the diet for 90 days (U.S. Navy 1974a) or 50 mg/kg via capsules 6 days/week for 6 weeks (von Oettingen et al. 1949), or monkeys administered 10 mg/kg/day via gavage for 90 days (U.S. Navy 1974b). Chronic-duration studies also revealed no histopathology in rats (U.S. Army 1983a; U.S. Navy 1976) or mice (U.S. Army 1984c).

Cardiovascular Effects. Sinusoidal tachycardia was observed in five men who accidentally ingested 37–250 mg/kg RDX (Küçükardalĭ et al. 2003).

Few, if any, changes were observed in cardiovascular parameters measured in animals exposed to RDX. An acute-duration study in 3 anesthetized dogs showed no significant changes in heart rate when 15 mg/kg RDX was administered by gavage (von Oettingen et al. 1949). Intermediate-duration studies revealed no histopathology in the heart of rats exposed to 15–100 mg/kg/day of RDX (Levine et al. 1981; U.S. Army 2006). Slight myocardial degeneration was observed in rats exposed to 40 mg/kg/day and mice exposed to 320 mg/kg/day in the diet for 90 days (U.S. Army 1980b). No pathology was seen in the hearts of dogs (U.S. Navy 1974a; von Oettingen et al. 1949) or monkeys (U.S. Navy 1974b) exposed to RDX for intermediate periods. Hyaline degeneration of the heart muscles was observed in rats following intermediate exposure to 50 mg/kg/day of RDX (Sunderman 1944). Chronic exposure produced no cardiac histopathology in rats (U.S. Army 1983a; U.S. Navy 1976), but it increased relative heart weights in mice (U.S. Army 1984c).

Gastrointestinal Effects. Humans who accidentally or intentionally consumed unknown levels of RDX for an acute period had nausea, vomiting, and abdominal pain (Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĭ et al. 2003). In three of five cases in which men accidentally ingested 37–250 mg/kg, an endoscopic examination conducted 3 days after exposure revealed erosive gastroduodenitis (Küçükardalĭ et al. 2003).

Vomiting was reported in dogs acutely exposed to 100 and 300 mg/kg/day RDX (Sunderman 1944). Vomiting was also observed in five of six monkeys administered via gavage 10 mg/kg/day for 90 days, compared to one of six in the control group (U.S. Navy 1974b). There were 15 episodes of vomiting (excluding vomiting, which occurred during the gavage procedure) in this group compared to 1 episode in the control group. In monkeys administered 1 mg/kg/day, two of six animals (three episodes) vomited; one other animal in this group vomited only during the gavage procedure. Following intermediate exposure of rats to 50 mg/kg/day RDX, mild congestion of the intestines was reported (Sunderman 1944). No histopathology was seen in the stomachs or intestines of rats (Levine et al. 1981, 1990; U.S. Army 1980b, 1983a), mice (U.S. Army 1980b, 1984c), dogs (U.S. Navy 1974a; von Oettingen et al. 1949), or monkeys (U.S. Navy 1974b). Chronic exposure also did not produce histopathological alterations in rats (U.S. Army 1983a; U.S. Navy 1976) or mice (U.S. Army 1984c).

Hematological Effects. Humans who accidentally consumed unknown levels of RDX for an acute duration generally had normal blood counts (Ketel and Hughes 1972; Woody et al. 1986). Temporary decreased hematocrit and leukocytosis were reported in a study of six men who consumed C-4 containing RDX (Stone et al. 1969). Similarly, leukocytosis and methemoglobinemia were noted in a report of five men accidentally ingesting 37–250 mg/kg RDX (Küçükardalĭ et al. 2003).

Decreased hemoglobin and erythrocyte levels, increased platelet counts, and splenic extramedullary hematopoiesis were observed in male rats exposed to 40 mg/kg/day RDX in the diet for 6 months (U.S. Army 1983a). However, oral doses of 15 mg/kg/day (administered via gavage) (U.S. Army 2006) or 40 mg/kg/day (administered via the diet) (U.S. Army 1980b) for 13 weeks did not result in significant hematological effects. Similarly, decreased hemoglobin and erythrocyte levels were observed in mice exposed to 160 mg/kg/day for 90 days (U.S. Army 1980b). No significant hematological effects were found in mice exposed to 100 mg/kg/day for 6 months (U.S. Army 1984c) and dogs exposed to 50 mg/kg/day for 6 weeks (von Oettingen et al. 1949). Species differences in hematological responses to RDX may relate to differences in their activity of erythrocyte methemoglobin reductase (Rockwood et al.

2003; Smith and Beutler 1966). Slight, but statistically significant, increases in the number of leukocytes were observed in rats exposed to ≥10 mg/kg/day for 13 weeks (Levine et al. 1981). Necrotic and degenerative megakaryocytes were observed in the bone marrow of monkeys given 10 mg/kg/day of RDX for 90 days (U.S. Navy 1974b). Chronic administration of 40 mg/kg/day of RDX in the diet for 1–2 years produced decreased hematocrit, hemoglobin, and erythrocytes in rats; the effects were not considered biologically significant and there were no compensatory responses (U.S. Army 1983a). Significant increases in platelet levels were also observed at 40 mg/kg/day (U.S. Army 1983a). No significant hematological effects were observed in mice chronically exposed to 100 mg/kg/day (U.S. Army 1984c).

Musculoskeletal Effects. No histopathological alterations were observed in muscle or skeletal tissue of rats (Levine et al. 1981, 1990; U.S. Army 1980b, 1983a), mice (U.S. Army 1980b, 1984c), or dogs (U.S. Navy 1974a) exposed for intermediate periods. Muscles and bones were also normal in rats (U.S. Army 1983a; U.S. Navy 1976) and mice (U.S. Army 1984c) exposed for chronic periods.

Hepatic Effects. Slightly elevated serum aspartate aminotransferase and/or alanine aminotransferase (Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969) were observed in humans ingesting unknown levels of RDX after using C-4 cooking fuel for an acute duration; other studies reported normal liver enzyme levels (Ketel and Hughes 1972). Liver biopsies were normal (Stone et al. 1969).

Minor adverse hepatic effects have been noted in some animal studies. Slight decreases in alanine aminotransferase levels were observed in rats exposed to 40 mg/kg/day for 26 weeks (U.S. Army 1983a). Decreases in serum triglyceride levels were noted in rats exposed to ≥10 mg/kg/day RDX for 13 weeks (Levine et al. 1981, 1990), decreases in serum cholesterol were observed in male rats administered ≥8 mg/kg/day for 90 days (U.S. Army 2006), and decreases in serum triglycerides and cholesterol were observed in rats exposed to 40 mg/kg/day for 6 months to 2 years (U.S. Army 1983a). Increases in serum cholesterol were also observed in female mice exposed to 35 or 100 mg/kg/day for 1–2 years (U.S. Army 1984c). Increases in liver weight have been observed in rats exposed to 30 or 100 mg/kg/day (Levine et al. 1981, 1990) and mice exposed to 100 or 320 mg/kg/day (U.S. Army 1980b, 1984c); hepatomegaly was observed in rats exposed to 40 mg/kg/day (U.S. Army 1983a). However, most studies did not find histological alterations in the livers of rats (Levine et al. 1981; U.S. Army 1983a), mice (U.S. Army 1984c), dogs (U.S. Navy 1974a; von Oettingen et al. 1949), white-footed mice (U.S. Army 1999), or monkeys (U.S. Navy 1974b). Two studies did find histological effects; hepatocellular vacuolization was observed in mice exposed to 320 mg/kg/day for 90 days (U.S. Army 1980b) and fatty degeneration was

observed in rats exposed to 50 mg/kg/day for 78 days (Sunderman 1944). Although the alterations in serum clinical chemistry parameters may be indicative of minor changes in liver function, the lack of histological damage at similar or higher doses suggests that the liver may not be a sensitive target of RDX toxicity and the alterations may not be biologically significant.

Renal Effects. Humans who accidentally consumed unknown levels of RDX for an acute duration showed no (Woody et al. 1986) or only slight (Ketel and Hughes 1972; Stone et al. 1969) changes in renal function parameters. Proteinuria and glucosuria were observed in men after accidental ingestion of RDX (Küçükardalĭ et al. 2003; Merrill 1968).

Few adverse renal effects were reported in animals. No histopathological alterations were observed in the kidneys from white-footed mice following 14-day dietary exposure (U.S. Army 1999) or from rats following intermediate exposure periods (Levine et al. 1981, 1990; U.S. Army 1980b, 1983a, 2006). Normal kidney parameters were also observed in dogs (U.S. Navy 1974a; von Oettingen et al. 1949) and monkeys (U.S. Navy 1974b). In contrast, mild tubular nephrosis was reported in mice given high doses (320 mg/kg/day) in the food for 13 weeks, but was not seen at lower doses (160 mg/kg/day) (U.S. Army 1980b). Following chronic exposure to 40 mg/kg/day of RDX in food, renal papillary necrosis and elevated blood urea nitrogen levels were observed in rats (U.S. Army 1983a); these effects were not observed at 8 mg/kg/day. Other studies showed normal renal parameters in rats at lower levels (10 mg/kg/day) (U.S. Navy 1976). Increased kidney weights, but no other signs of kidney toxicity, were observed in mice chronically exposed to 100 mg/kg/day (U.S. Army 1984c).

Endocrine Effects. No histopathological alterations were observed in the adrenal glands of rats (U.S. Army 1980b, 1983a; U.S. Navy 1976), mice (U.S. Army 1984c), dogs (U.S. Navy 1974a), or monkeys (U.S. Navy 1974b) exposed for intermediate periods. One study (U.S. Army 1980b) observed mild focal subscapular fibroplasia in the adrenal glands of female mice exposed to 320 mg/kg/day RDX for 90 days.

Dermal Effects. No significant skin lesions were seen in rats (U.S. Army 1980b, 1983a) exposed for intermediate periods to RDX in the food.

Ocular Effects. No significant ophthalmologic alterations were observed in rats administered 15 mg/kg/day via gavage for 90 days (U.S. Army 2006). Female rats exposed to 40 mg/kg/day of RDX in their food for 2 years had cataracts (U.S. Army 1983a), but this was not seen in the male rats in this

study, in male or female rats exposed to 40 mg/kg/day in another study (U.S. Army 1980b), or in mice exposed to a higher level (100 mg/kg/day) (U.S. Army 1984c).

Body Weight Effects. Weight loss or lack of weight gain of >10% was seen in rats fed 25–40 mg/kg/day (Levine et al. 1981, 1990; von Oettingen et al. 1949) and dogs fed 50 mg/kg/day (von Oettingen et al. 1949) for an intermediate duration, and in rats receiving 40 mg/kg/day RDX (U.S. Army 1983a) and mice receiving 100 mg/kg/day (U.S. Army 1984c) for a chronic period.

Metabolic Effects. Hyperglycemia, hypokalemia, and metabolic acidosis with anion gap were observed in men accidentally ingesting 37–250 mg/kg RDX (Küçükardalĭ et al. 2003). In rats exposed to 40 mg/kg/day RDX in the diet for 13–78 weeks, significant decreases in blood glucose levels were observed (U.S. Army 1983a).

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after oral exposure to RDX.

No studies were located regarding immunological effects in animals after acute oral exposure to RDX. Studies of intermediate duration (6–13 weeks) failed to reveal any marked pathological alterations in the spleen, thymus, and/or lymph nodes in rats (Levine et al. 1990; U.S. Army 1980b), mice (U.S. Army 1980b), dogs (U.S. Navy 1974a; von Oettingen et al. 1949), or monkeys (U.S. Navy 1974b). No significant alterations in spleen and thymus organ weights or cellularity, or in the proportion of cell surface markers were observed in rats exposed to 15 mg/kg/day for 90 days (U.S. Army 2006). The NOAEL value from the U.S. Army (2006) is recorded in Table 3-1 and plotted in Figure 3-1; NOAEL values for studies only examining potential histopathological alterations in lymphoreticular organs were not listed.

3.2.2.4 Neurological Effects

The available studies have identified the nervous system as a target system in humans following oral exposure to RDX. Numerous case reports are available that describe seizures in men (Hollander and Colbach 1969; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969) and in one child (Woody et al. 1986) after accidental consumption of unknown quantities of RDX for acute periods and in men intentionally chewing on C-4 (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995). The RDX was almost always mixed with other components in the form of the

explosive C-4, which is 91% RDX (mixed with polyisobutylene, motor oil, and di-(2-ethylhexyl) sebacate). In most of the cases, RDX intakes were not known; Stone et al. (1969) reported doses (357 and 2,571 mg/kg) for two cases. In a report of five cases (Küçükardalĩ et al. 2003), repetitive tonic-clonic convulsions were first observed 4–16 hours after RDX exposure; in one case, convulsions were observed for 3 days, although the frequency and duration gradually decreased with time. Most studies reported that recovery occurred within a few days or weeks. Accompanying complaints included disorientation, nausea, restlessness, muscle twitching, lethargy, and hyperactive deep tendon reflexes. In most cases, no other neurological evaluations were performed. Küçükardalĩ et al. (2003) noted abnormalities in electroencephalograms (EEG) in three of the five cases. In the case reported by Harrell-Bruder and Hutchins (1995), no EEG abnormalities were found. No intermediate- or chronic-duration exposure data have been reported for humans.

Animal studies have also shown that the nervous system is a target system following oral exposure to RDX. Seizures were observed in rats receiving a single gavage dose of ≥25 mg/kg (U.S. Army 2006; Burdette et al. 1988; Meyer et al. 2005; Schneider et al. 1977), deer mice receiving a gavage dose of 136 mg/kg (Smith et al. 2007), and miniature swine receiving a single gavage dose of ≥10 mg/kg (JHU/U.S. Army 2006; Schneider et al. 1977). Seizures were also observed in 20% of rats administered a single dose of 12.5 mg/kg/day; however, the incidence was not significantly different from controls (Burdette et al. 1988). Following administration of a single dose of ≥25 mg/kg RDX, seizures and convulsions typically occur within 3 hours (U.S. Army 2006; Burdette et al. 1988). Convulsions and hyperactivity were also noted in "several" surviving rat dams administered 20 mg/kg/day by gavage during gestation days 6–15 (U.S. Army 1986d); incidence data were not provided. Hyperactivity was observed in approximately 70% of rat dams administered 20 mg/kg/day RDX via gavage on gestation days 6–19 (U.S. Army 1980b). In a 14-day exposure study, tremors and convulsions were observed in rats receiving gavage doses of ≥17 mg/kg/day; no marked neurological effects were noted at 8.5 mg/kg/day (U.S. Army 2006). In neurobehavioral tests, decreases in motor activity, hindlimb splay, taste aversion to saccharine, response rate in scheduled-controlled behavior tests, auditory startle amplitude, and increases in startle latency were observed in rats receiving a single gavage dose of ≥12.5 mg/kg/day (U.S. Army 1985b).

Seizures and convulsions have also been observed in rats, monkeys, and dogs exposed to RDX for intermediate or chronic durations. Seizures and convulsions were observed in rats administered gavage doses ≥8 mg/kg/day for 90 days (U.S. Army 2006), in rats exposed to dietary RDX at doses of ≥25 mg/kg/day (Sunderman 1944; U.S. Army 1983a; von Oettingen et al. 1949), dogs exposed to 50 mg/kg/day via a capsule (von Oettingen et al. 1949), and monkeys administered 10 mg/kg/day via

gavage (U.S. Navy 1974b). Few studies have reported the time course of the convulsions/seizures. In the U.S. Army (2006) study, convulsions/seizures were observed at the beginning of the study in rats administered 12 or 15 mg/kg/day and were seen throughout the study; convulsions/seizures were also observed at 8 and 10 mg/kg/day, however, the investigators did not note when the convulsions/seizures first occurred for these dose levels. In a chronic dietary study, seizures and convulsions were first observed after 26 weeks of exposure to 40 mg/kg/day (U.S. Army 1983a). In monkeys, the effects were typically observed after 34–57 doses, although effects were also seen in some monkeys after the 2nd or 12th dose, and did not occur on a regular basis (U.S. Navy 1974b). Other overt neurological signs observed following intermediate or chronic exposure include hyperactivity, hyperreactivity, increased arousal, and increased fighting in rats exposed to gavage doses of ≥10 mg/kg/day (U.S. Army 2006), in rats exposed to ≥25 mg/kg/day in the diet (Levine et al. 1990; U.S. Army et al. 1983a; von Oettingen et al. 1949), and dogs administered capsules containing 50 mg/kg/day (von Oettingen et al. 1949). The highest NOAELs for overt neurological effects were 4 mg/kg/day in rats receiving gavage doses (U.S. Army 2006), 15 mg/kg/day in rats exposed via the diet (von Oettingen et al. 1949), and 1 mg/kg/day in monkeys receiving gavage doses (U.S. Navy 1974b). Neurobehavioral performance was assessed in rats receiving gavage doses of RDX for 30 or 90 days. No significant alterations were observed in motor activity, flavor aversion, scheduled-controlled behavior, or acoustic startle in rats administered 10 mg/kg/day for 15 or 30 days (U.S. Army 1985b). No RDX-related alterations in foot splay, front limb grip strength, or response to stimuli were found in rats administered 15 mg/kg/day for 90 days (U.S. Army 2006). No significant histological alterations have been found in the brain (U.S. Army 1983a, 2006).

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to RDX.

Toxicity studies lasting 13 weeks showed no pathological changes in the gonads or uteri of rats (Levine et al. 1981, 1990; U.S. Army 1980b, 1983a) or mice (U.S. Army 1980b, 1984c) exposed to RDX. No functional tests were performed. One study did report spermatic granulomas in the prostates of rats exposed to 40 mg/kg/day for 6 months (U.S. Army 1983a); this effect was not observed in rats exposed after 1 or 2 years of exposure (U.S. Army 1983a). This study (U.S. Army 1983a) also reported an

increase in the incidence of testicular degeneration in rats exposed to 40 mg/kg/day for 6 months (3/10, not statistically significant) or 1 year (4/10), but not after 2 years (0/4).

Histological examinations of rats exposed to ≥1.5 mg/kg/day in the feed for 2 years revealed suppurative inflammation in the prostate (U.S. Army 1983a). The prostate effects were predominantly observed in rats dying early and may have been secondary to a bacterial infection of the urinary tract. Urinary bladder distention and cystitis were observed in rats exposed to 40 mg/kg/day for 1 or 2 years. Testicular degeneration was observed in rats exposed to 40 mg/kg/day for 1 year (U.S. Army 1983a); a nonstatistically significant increase in testicular degeneration was also observed in mice exposed to ≥35 mg/kg/day for 1–2 years (U.S. Army 1984c). No significant histological alterations have been observed in the ovaries or uterus of rats (U.S. Army 1983a) or mice (U.S. Army 1984c) chronically exposed to RDX.

Two studies examined reproductive function. In a two-generation study, no significant alterations in reproduction were observed in the F_0 and F_1 rats exposed to 16 mg/kg/day in the diet (U.S. Army 1980b). At 50 mg/kg/day, nonstatistically significant decreases in fertility were observed in the F_0 generation. In a dominant lethality assay (U.S. Army 1980b), decreases in fertility were observed in male rats exposed to 50 mg/kg/day for 15 weeks prior to mating with unexposed females; however, the investigators noted that this effect may have been secondary to the impaired well-being of the males. The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to RDX.

There are two available developmental studies in rats (exposed for 9 or 13 days during gestation) that are inconclusive because of excessive maternal toxicity at the high dose (20 mg/kg/day). In one study, no excessive gross, visceral, or skeletal anomalies were found in fetuses when the dams were exposed to 2 mg/kg/day of RDX (U.S. Army 1980b). High maternal lethality, decreased maternal body weights, and adverse maternal neurological effects precluded judgment regarding fetal toxicity at 20 mg/kg/day. The other rat study (U.S. Army 1986d) also showed high maternal toxicity (increased mortality and seizures) at 20 mg/kg/day. These investigators also reported a significant decrease in fetal weights and lengths at ≥2 mg/kg/day when data were analyzed on an individual basis rather than a litter basis. However, it

appears that there was an overlap in the standard deviations for the fetal body weight and length values; when analyzed on a litter basis, decreases in fetal weights and lengths were only significant at the 20 mg/kg/day dose level. In contrast to rats, rabbits (exposed for 22 days during gestation) showed no adverse fetal or maternal effects at 20 mg/kg/day (U.S. Army 1980b). In a two-generation reproduction study, an increase in the number of stillbirths, a decrease in the number of pups per litter at birth, and a decrease in the number of live litters at weeks 7, 14, and 21 were observed in the F_1 offspring of rats exposed to 50 mg/kg/day (a dose that also resulted in increased maternal deaths and decreased feed consumption) (U.S. Army 1980b). In the F_2 generation, a decrease in terminal body weights and an increase in renal tubular epithelial-lined cysts were observed at 16 mg/kg/day. Similar cysts were observed in F_2 pups exposed to 0 or 5 mg/kg/day. The highest NOAEL values and all reliable LOAEL values for developmental effects for each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.2.7 Cancer

No studies were located regarding cancer in humans after oral exposure to RDX.

RDX was not found to be carcinogenic when fed to F344 rats (U.S. Army 1983a) or Sprague-Dawley rats (U.S. Navy 1976) for at least 1 year. Adequate doses, numbers of animals, and survival rates were achieved for both of these studies. Only female $B6C3F_1$ mice showed an increased incidence of combined hepatocellular adenomas and carcinomas when compared to concurrent or historical controls (U.S. Army 1984c). However, a re-evaluation of these data using revised diagnostic criteria resulted in a reclassification of several hepatocellular adenomas as foci of cytoplasmic alterations (Parker et al. 2006). As noted in the abstract of the Parker et al. (2006) paper, the combined incidence of hepatocellular adenomas and carcinomas was significantly increased (no information regarding statistical analysis was presented in the paper) in the 35 mg/kg/day group, but not in the 100 mg/kg/day group. The investigators noted that the combined incidence of hepatocellular adenomas and carcinomas in the 35 mg/kg/day group (10/64, 16%) was within the range of published historical control data (0–21%) and suggested that the study provided equivocal evidence of a carcinogenic effect. The 35 mg/kg/day dose is listed as a cancer effect level (CEL) in Table 3-1 and Figure 3-1. The lifetime average doses that would result in risk of $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, and $1x10^{-7}$ are 0.9, 0.09, 0.009, and 0.0009 mg/kg/day, respectively, as indicated in Figure 3-1.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to RDX.

Deaths were observed in rabbits receiving repeated dermal applications of 37.5 mg/kg/day RDX in cyclohexanone (1/6 deaths) or 27 mg/kg/day RDX in acetone (2/6) deaths; no gross pathological effects were seen (U.S. Army 1974). Because of the lack of data presented, it is difficult to determine whether RDX alone was responsible for the deaths reported in this study.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, or renal effects in humans after dermal exposure to RDX. Two older studies of dermal and ocular effects were located for humans following dermal exposure to RDX.

One animal study examined the potential systemic toxicity of RDX following a single dermal application (U.S. Army 1974); however, no details were provided regarding the "pathological examination"; thus, NOAELs for systemic effects were not presented for this study. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-2.

Respiratory Effects. No alterations were noted in the respiratory rates of dogs following single or multiple dermal exposures to RDX in dimethyl sulfoxide (DMSO) (U.S. Army 1974).

Cardiovascular Effects. No adverse effects were seen on blood pressure, heart rate, or electrocardiograms of dogs dermally exposed to a single application or repeated exposure (5 days/week for 4 weeks) of 289 mg/kg RDX in DMSO (U.S. Army 1974). No lesions were seen in the hearts of rabbits exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks (U.S. Army 1974).

Gastrointestinal Effects. Necropsy did not reveal any lesions in the intestines of rabbits exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks (U.S. Army 1974).

Table 3-2 Levels of Significant Exposure to RDX - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
ACUTE EXPOSURE							
Systemic							
Gn Pig (NS)	once or 3 times	Dermal	510 mg/kg/day	1000 mg/kg/day (erythema)		U.S. Army 1974	RDX in DMSO
Dog (NS)	once	Resp	289 mg/kg/day			U.S. Army 1974	RDX in DMSO
		Cardio	289 mg/kg/day				
Rabbit (NS)	once	Hemato	165 mg/kg			U.S. Army 1974	RDX in DMSO (165 mg/kg), RDX in cyclohexanone (37 mg/kg)
		Dermal		27 mg/kg (dermatitis)			
Neurological							
Dog (NS)	3 d 1x/day	Cardio	480 mg/kg/day			U.S. Army 1974	RDX in DMSO
INTERMEDIATE EXPOSURE							
Systemic							
Gn Pig (NS)	3 wk 3 d/wk	Dermal	165 mg			U.S. Army 1974	RDX in DMSO
		Ocular	165 mg				

Table 3-2 Levels of Significant Exposure to RDX - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Dog (NS)	4 wk 5 d/wk	Resp	289 mg/kg/day			U.S. Army 1974	RDX in DMSO
		Cardio	289 mg/kg/day				
		Ocular	289 mg/kg/day				
Rabbit (NS)	4 wk 5 d/wk	Resp	165 mg/kg/day			U.S. Army 1974	RDX in DMSO (165 mg/kg/day), RDX in cyclohexanone (37.5 mg/kg/day)
		Cardio	165 mg/kg/day				
		Gastro	165 mg/kg/day				
		Musc/skel	165 mg/kg/day				
		Hepatic	165 mg/kg/day				
		Renal	165 mg/kg/day				
		Dermal	37.5 mg/kg/day	165 mg/kg/day (dermatitis)			

Cardio = cardiovascular; d = day(s); Gastro = gastrointestinal; Gn Pig = guinea pig; LOAEL = lowest-observed-adverse-effect level; Metab = metabolic; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s)

Hematological Effects. Blood samples taken from rabbits after a single exposure to 165 mg/kg RDX in DMSO revealed no significant changes in hematological parameters (U.S. Army 1974).

Musculoskeletal Effects. Necropsy did not reveal pathology in the muscle or bone tissue of rabbits exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks (U.S. Army 1974).

Hepatic Effects. No alterations in serum clinical chemistry parameters were found in rabbits after acute or intermediate dermal exposure to RDX. Also, no pathological alterations were noted in the liver of rabbits exposed for 4 weeks (U.S. Army 1974).

Renal Effects. No histological alterations were noted in the kidneys of rabbits exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks (U.S. Army 1974).

Dermal Effects. One volunteer had a patch of skin covered with dry RDX for 2 days. No irritation was observed following removal of the gauze coverings (von Oettingen et al. 1949). An accurate dose could not be determined because of the lack of information provided in the study. Another study reported dermatitis in workers exposed to RDX fumes of unknown levels and for unknown duration (Sunderman 1944).

Dermatitis was observed in rabbits exposed once to 27 mg/kg RDX in acetone, 37.5 mg/kg RDX in cyclohexanone, or 165 mg/kg RDX in DMSO (U.S. Army 1974); the dermatitis persisted for at least 30 days and was most pronounced in the rabbits exposed to 165 mg/kg RDX in DMSO. Slight erythema was noted in guinea pigs exposed once to 1,000 mg/kg (U.S. Army 1974). Guinea pigs exposed once to an unspecified amount of RDX had exudative dermatitis with edema (Sunderman 1944). The lesions healed promptly after the guinea pigs were removed from the source of exposure.

In rabbits repeatedly exposed to 165 mg/kg RDX in DMSO 5 days/week for 4 weeks, dermatitis was observed after 14 and 30 days of exposure; no dermal effects were observed at 16.5 mg/kg RDX in DMSO or in rabbits administered lower RDX doses in cyclohexanone (37.5 mg/kg/day) or acetone (27 mg/kg/day) vehicles (U.S. Army 1974).

Ocular Effects. There are limited human data regarding the ocular toxicity of RDX. Conjunctivitis was reported by workers exposed to RDX fumes (Sunderman 1944); no information was provided regarding exposure levels or duration of exposure.

Cataracts were observed in guinea pigs exposed through cutaneous or intradermal applications of RDX in solvents. However, the incidence of cataracts did not appear to be greater than that found after exposure to the solvents alone. This suggests that RDX itself did not contribute to cataract formation (U.S. Army 1974).

Body Weight Effects. Decreased body weight classified as small and transient (no further details were provided) was reported in rabbits after a single dermal application of 2,000 mg/kg of RDX. However, by the end of the observation period, most of the surviving animals showed weight gain (U.S. Army 1984b).

No studies were located regarding the following effects in humans or animals after dermal exposure to RDX:

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

No studies were located regarding genotoxicity of RDX in humans following inhalation, oral, or dermal exposure to the chemical. One *in vitro* study was located in which human fibroblasts (WI-38 cells) were incubated in the presence of RDX and tritiated thymidine (3H-TdR) to measure unscheduled deoxyribonucleic acid (DNA) synthesis (U.S. Army 1978b) (see Table 3-3). RDX was tested in concentrations of up to 4,000 µg/mL both with and without metabolic activation. RDX was not found to significantly increase the rate of unscheduled DNA synthesis in the cells of any exposure group regardless of whether or not metabolic activators were present. Therefore, RDX was not observed to induce DNA damage in human fibroblasts under the conditions of the study (U.S. Army 1978b).

Table 3-3. Genotoxicity of RDX *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	−	−	U.S. Army 1980b
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	−	−	U.S. Army 1977b
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	−	−	Whong et al. 1980
S. typhimurium TA98, TA100	Gene mutation	−	−	Lachance et al. 1999
S. typhimurium TA98, TA100	Gene mutation	−	−	George et al. 2001
S. typhimurium TA98, TA100	Gene mutation	−	−	Pan et al. 2007
S. typhimurium TA97a	Gene mutation	±	−	Pan et al. 2007
Vibrio fischeri	Gene mutation	±	±	Arfsten et al. 1994
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae	Gene mutation	−	−	U.S. Army 1977b
Mammalian cells:				
Human fibroblasts	DNA damage	−	−	U.S. Army 1978b
Chinese hamster V79 lung cells	Gene mutation	−	−	Lachance et al. 1999
Mouse lymphoma L5178Y cells	Gene mutation	−	−	Reddy et al. 2005a

— = negative result; + = positive result; ± = weak or equivocal result; DNA = deoxyribonucleic acid

Only two *in vivo* animal studies were located and both provided negative evidence of mutagenicity. U.S. Army (1980b) investigated the effects of oral doses of RDX on dominant lethal mutations in rats. RDX was administered to the rats in the diet in doses of 0, 5, 16, or 50 mg/kg/day for 15 weeks. The males in each exposure group were then allowed to mate with untreated females for 2 weeks. There were no significant effects on the number of corpora lutea, implants, or live or dead embryos (U.S. Army 1980b); no dominant lethal mutations were observed. In the other *in vivo* study, administration of a single gavage dose of up to 250 mg RDX/kg to male mice did not significantly increase the incidence of micronuclei in bone marrow cells examined 24 hours after dosing (Reddy et al. 2005a).

The *in vitro* genotoxicity of RDX has been investigated in several assays (Table 3-3). Most of the results of reverse mutation assays with *Salmonella typhimurium* conducted by several investigators (George et al. 2001; Lachance et al. 1999; Pan et al. 2007; U.S. Army 1977b, 1980b; Whong et al. 1980), *Saccharomyces cerevisiae* (U.S. Army 1977b), or *Vibrio fischeri* (Arfsten et al. 1994) have been negative. A weakly positive result was found in one *S. typhimurium* strain (TA97a) (Pan et al. 2007). In mammalian cells, forward mutation assays in mouse lymphoma L5178Y cells (Reddy et al. 2005a) and hamster V79 lung cells (Lachance et al. 1999) were negative.

Although the results of *in vitro* assays have been negative for RDX, some studies of environmental biotransformation products of RDX have reported positive results. For example, George et al. (2001) reported that hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3-dinitroso-5-nitro-1,3,5,-triazine, were not mutagenic in *S. typhimurium* TA98 or TA100 with or without metabolic activation, but hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) was weakly genotoxic in strain TA100 but negative in strain TA98. Studies conducted by Pan et al. (2007) showed that in the presence of metabolic activation, both MNX and TNX were mutagenic in *S. typhimurium* TA97a, weakly mutagenic in strain TA102, and not mutagenic in strain TA98; in addition, TNX was weakly mutagenic in strain TA100 in the presence of metabolic activation. Pan et al. (2007) also reported that neither MNX nor TNX were mutagenic in *S. typhimurium* TA97a in the absence of metabolic activation.

Collectively, the available information suggests that RDX is not a mutagenic substance, but some of its environmental biotransformation products may be of concern, especially since they have been identified as metabolic products in mammals (Major and Reddy 2007).

3.4 TOXICOKINETICS

Very little is known regarding the toxicokinetics of RDX in humans, but reports of adverse effects following inhalation and oral exposure and measurements of RDX in blood from poisoned individuals indicate that RDX is absorbed through the lungs and the gastrointestinal tract. No information is available regarding the distribution and metabolism of RDX in humans. A single case study found RDX in the cerebrospinal fluid following oral exposure (Woody et al. 1986), suggesting possible distribution to the nervous system. RDX was almost completely absorbed in miniature pigs after a single oral dose (Major and Reddy 2007). In rats, mixing RDX with soil considerably reduced absorption compared to administration of neat RDX (Crouse et al. 2008). No preferential accumulation of RDX in specific tissues has been reported in animal studies (Schneider et al. 1977, 1978). Several metabolites were identified in the urine from miniature pigs dosed orally with RDX (Major and Reddy 2007). The urine was the main route of elimination of ^{14}C-RDX-derived radioactivity (Schneider et al. 1977).

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Neurological effects have been observed in humans following inhalation exposure, indicating that RDX can be absorbed through the lungs (Kaplan et al. 1965; Testud et al. 1996a). The extent of absorption through the lungs has not been determined. As described in an abstract, approximately 30% of an intratracheal dose was excreted in the urine and feces during a 6-day period (Reddy et al. 1989).

3.4.1.2 Oral Exposure

Adverse effects observed in humans following accidental or intentional ingestion of RDX indicate that it is absorbed through the gastrointestinal tract (Davies et al. 2007; Hollander and Colbach 1969; Harrell-Bruder and Hutchins 1995; Kaplan et al. 1965; Kasuske et al. 2009; Ketel and Hughes 1972; Merrill 1968; Stone et al. 1969). Measurements of RDX in blood provide direct evidence that gastrointestinal absorption occurs. A study of a child who ingested an unknown amount of RDX reported an apparent peak plasma concentration prior to 24 hours postingestion (Woody et al. 1986). At 24 hours (first time measurements were made), the serum concentration of RDX was 10.7 μg/mL and decreased gradually thereafter, but it was detectable in serum over a 120-hour period following the estimated time of ingestion. Using an estimated volume of distribution of 2.2 L/kg, Woody et al. (1986) estimated an ingested dose of 84.8 mg RDX/kg or 1.23 g for the 14.5 kg child. More recently, Küçükardalĭ et al.

(2003) reported five cases of accidental ingestion of RDX with estimated doses between 37 and 250 mg/kg (how the doses were estimated was not indicated). Three hours after ingestion, the serum concentrations of RDX ranged from 268 to 969 pg/mL. In two of the patients, blood levels 72 hours after ingestion were approximately 2-fold those measured 3 hours after ingestion, suggesting very slow absorption.

Administration of a single oral dose of ^{14}C-RDX in 0.5% carboxymethylcellulose in water to miniature pigs resulted in relatively low levels of radioactivity (6% of the dose in males and 2% in females) in the gastrointestinal contents and feces over a 24-hour period, suggesting nearly complete absorption (Major and Reddy 2007). Data shown for a single male pig (two males and two females were used in the study) indicate that Peak RDX concentration in plasma in miniature pigs administered single doses of RDX occurred 8–12 hours after dosing, indicating that although absorption may have been complete over a 24-hour period, it occurred at a relatively slow rate (JHU/U.S. Army 2006; Major and Reddy 2007). In contrast, a study in rats administered 3 or 18 mg/kg RDX via capsule reported Peak RDX levels in the blood 3.5 hours after exposure (Bannon et al. 2009a).

Crouse et al. (2008) studied the bioavailability of RDX from soil in rats. Rats were administered capsules containing neat RDX or RDX mixed with two types of soils. The results showed that administration of RDX mixed with soils resulted in peak blood levels of RDX 15–25% lower that when administered neat. However, the times to reach peak levels (4–6 hours postdosing) did not appear to differ significantly between neat doses of RDX and RDX mixed with soil.

3.4.1.3 Dermal Exposure

A study using excised human skin in flow-through diffusion cells showed poor absorption of RDX in this type of preparation (Reddy et al. 2008). ^{14}C-RDX was applied in acetone or in two soils differing in their carbon content (1.9 vs. 9.5%) to the epidermal surface and receptor fluid was collected for up to 24 hours. At this time, the RDX remaining on the skin was washed with soap and water and the radioactivity in the washing was counted. Dermal absorption was defined as the amount of radioactivity in the receptor fluid, the dermis, and the portion of the epidermis beneath the stratum corneum. A total of 2.5% of the dose applied in acetone diffused through the skin into the receptor fluid, whereas 5.7% of the applied dose was found in the combined receptor fluid and skin (stratum corneum, epidermis and dermis). Approximately

80% of the applied dose was recovered (receptor fluid plus skin plus washings). Application of RDX in soil resulted in even less absorption; 2.6% in the low-carbon soil and 1.4% in the high-carbon soil were recovered in the receptor fluid and skin in 24 hours.

A similar study using excised pig skin was conducted earlier by Reifenrath et al. (2002). The results also showed relatively poor absorption. Only 4% of the applied dose of RDX in acetone was absorbed over a 24-hour period. Application of RDX mixed with soil resulted in only 1–2% of the applied dose being absorbed.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were located in humans or animals regarding distribution following inhalation exposure.

3.4.2.2 Oral Exposure

Limited information is available from a study of child who ingested an unknown amount of RDX (Woody et al. 1986). RDX was found in the cerebrospinal fluid of the child at a concentration of 8.94 μg/mL 24 hours after ingestion (only time measured).

In rats given RDX by gavage, levels in the plasma and brain reached a steady state for 2–24 hours and then disappeared 3 days postexposure, but no other tissues were sampled (U.S. Army 1985b). In another single exposure study in rats (Bannon et al. 2009a), blood and brain RDX levels paralleled each other during the first 48 hours post-exposure; these data suggest that RDX did not accumulate in the brain. Miniature swine showed no preferential distribution of RDX to the brain, heart, liver, kidneys, or fat 24 hours following a single gavage dose of 100 mg RDX/kg (Schneider et al. 1977). Three hours after oral administration of RDX to juvenile miniature pigs, the highest levels of RDX were found in the hippocampus and cortex compared to the heart, kidney, liver, blood, lung, and muscle (JHU/U.S. Army 2006). Rats given RDX once by gavage showed the highest levels of RDX in the kidneys, with less in the brain and heart, and the least amount in the plasma and liver over a 24-hour observation period (Schneider et al. 1977). Tissue/plasma ratios during the first 24 hours varied between 0.15 and 10.46, indicating that RDX accumulated to some extent in the tissues examined. In mice administered radiolabelled RDX via stomach perfusion, the highest levels of radioactivity were found in the liver, followed by the kidney, muscle, lung, spleen, heart, and brain (Guo et al. 1985). Results from longer-term studies showed no

preferential distribution of RDX in rats given the chemical by gavage or in the drinking water for 90 days (Schneider et al. 1978). In a recent study of the effects RDX on gene expression in the brain of rats, administration of 3 or 18 mg RDX/kg in a capsule resulted in peak brain and blood concentrations of RDX approximately 3.5 hours after dosing, regardless of the dose (Bannon et al. 2009a). No RDX could be detected in the brain or blood from low-dose rats 24 hours after dosing or in blood or brain from high-dose rats 48 hours after dosing.

An unpublished study indicates that RDX was found in the brain of rat pups whose mothers were administered RDX from gestation day 6 through postnatal day 10 (U.S. Army 2007b). On postnatal days 0, 3, 5, and 10, dams and pups were tested for RDX in milk and brain, respectively. Significantly higher concentrations of RDX were found in the brain from pups sacrificed immediately after birth than in the brain of pups sacrificed on postnatal day 10. No explanation was offered for this finding by the investigators. It is plausible that the gastrointestinal tract of newborn pups did not absorb RDX, but RDX readily crossed the placenta. Alternatively, it could be that newborn pups have the ability to metabolize/excrete RDX that is not present in the fetus. In any case, transplacental exposure occurred. Since RDX was also found in the dam's milk, transfer of RDX to the offspring via the milk can also occur.

3.4.2.3 Dermal Exposure

No studies were located in humans or animals regarding distribution following dermal exposure.

3.4.3 Metabolism

There are no studies available regarding RDX metabolism in humans following inhalation, oral, or dermal exposure.

RDX was extensively metabolized in rats (Schneider et al. 1977). Administration of a single gavage dose of 50 mg ^{14}C-RDX/kg resulted in <0.6% of the dose in the carcass 4 days after dosing and only 3% was excreted unchanged, mostly in the urine. The metabolites were not characterized.

A study of the metabolism of RDX in miniature pigs showed that RDX is rapidly and extensively metabolized by loss of two nitro groups followed by ring cleavage (Major and Reddy 2007). Pigs were administered a single gavage dose (43 mg/kg) of ^{14}C-RDX combined with carboxymethylcellulose in water and blood and excreta were collected for up to 24 hours. Metabolites were characterized by liquid

chromatography/mass spectrometry (LC/MS) in selected samples of urine, plasma, and liver. Analysis of urine revealed two major metabolites, 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diazabutanamide. Using a more sensitive method of analysis, the investigators also identified MNX in both male and female urine and DNX in male urine. Analysis of plasma showed quantifiable amounts of RDX, and trace levels of MNX, DNX, and TNX. Analysis of liver extracts showed that most of the radioactivity was in the form of water-soluble, high-molecular-weight compounds rather than as RDX or any identifiable metabolites.

An *in vitro* study examining RDX metabolism (assessed by measuring loss of RDX) under low oxygen conditions determined that 46.6, 40.1, 34.6, 25.5, and 11.6% of the RDX was metabolized in human, rat, monkey, pig, and rabbit liver microsomes, respectively, following a 30-minute incubation period (U.S. Army 2008). After a 180-minute incubation period, 51.8, 47.2, 35.7, 33.7, and 18.0% of the RDX was metabolized, respectively. Under anaerobic conditions with nitrogen replacing oxygen, RDX was metabolized by several human recombinant cytochrome P450 isoforms (CYP1A1, CYP2B6, CYP2C8, CYP2C18, CYP2E1, CYP3A5); with the exception of CYP1A1, the RDX metabolite, MEDINA, was produced. In contrast, under aerobic conditions, no loss of RDX was detected in human liver microsomes, S9, hepatocytes, or a number of human recombinant cytochrome 450 isoforms (U.S. Army 2008).

RDX was metabolized *in vitro* by rabbit cytochrome CYP2B4 to 4-nitro-2,4-diazabutanal, nitrite, formaldehyde, and ammonia (Bhushan et al. 2003). This reaction was observed in a cell-free, isolated enzyme system; therefore, it's relevance to *in vivo* metabolism is unknown.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No relevant information was located from studies in humans. In rats receiving a single intratracheal dose of radiolabelled RDX, 23 and 3% of the label was excreted in the urine and feces, respectively, during the first 4 days; during the first 6 days, 26 and 5%, respectively, was excreted (Reddy et al. 1989; only available as an abstract).

3.4.4.2 Oral Exposure

Only one study is available that provides some data on excretion in humans after oral exposure. In a child who ingested an unknown amount of RDX, apparent peak concentration in urine occurred at

approximately 48 hours after ingestion and in feces 96 hours after ingestion (Woody et al. 1986). RDX could still be detected in feces 144 hours following ingestion.

Rats given a single radiolabeled gavage dose of RDX eliminated 43% of the radioactivity in exhaled air, 34% in the urine, and 3% in the feces within 4 days; about 10% remained in the carcass (Schneider et al. 1977). A longer-term study showed similar excretion patterns; during a continuous drinking water study, 50% was eliminated in the exhaled air, 34% in the urine, and 5% in the feces (Schneider et al. 1978). There was no evidence that RDX accumulated in the tissues during longer-term exposure. Following administration of radiolabelled RDX to mice via stomach perfusion, 38.18% of the dose was excreted in the urine and 26.64% was excreted in the feces on day 1. On days 2–9, 11.20% of the dose was excreted in the urine. Ten days after dosing, 75.25% of the dose was excreted in the urine and feces (Guo et al. 1985).

Urine was the major route of elimination of ^{14}C-RDX-derived radioactivity in miniature pigs given a single dose of the chemical (Major and Reddy 2007). Over a 24-hour period, 16–17% of the administered dose was recovered in the urine compared to ≤6% recovered in the gastrointestinal contents and feces.

3.4.4.3 Dermal Exposure

No relevant information was located from studies in humans or animals.

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.

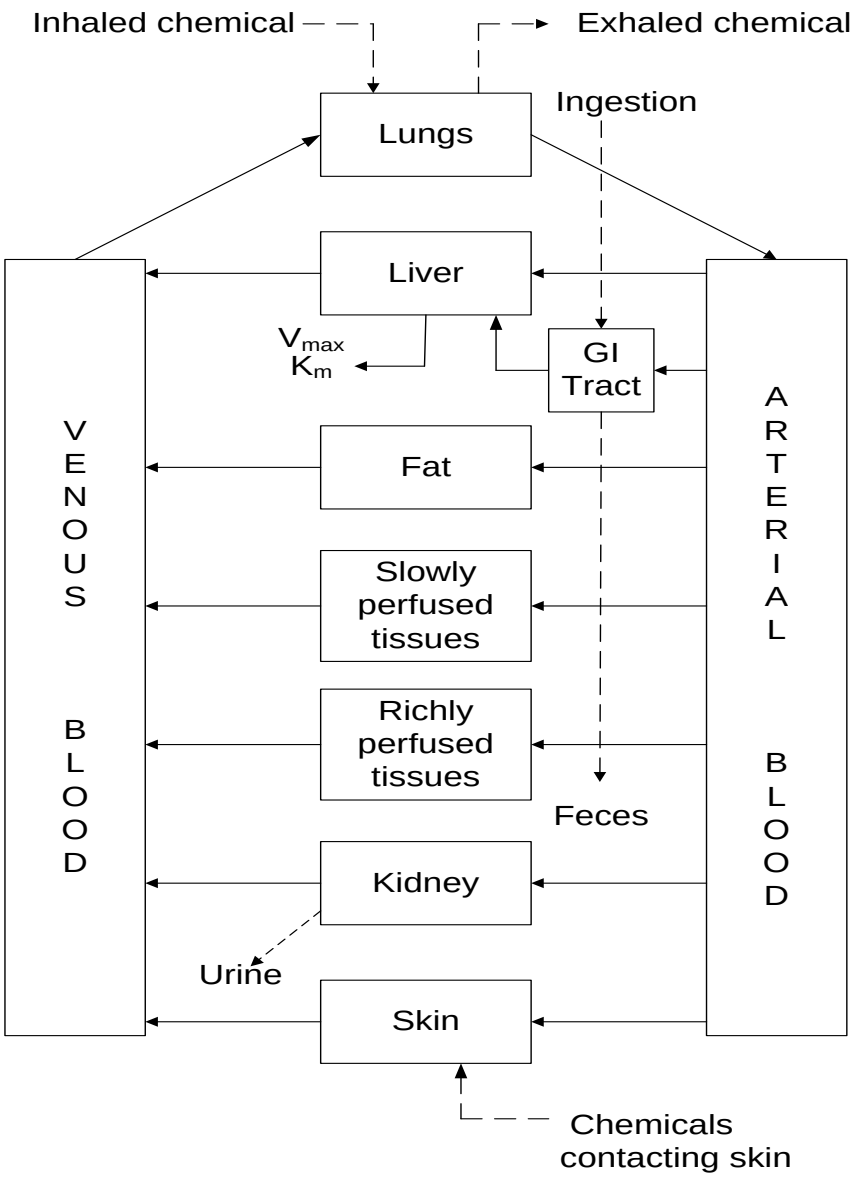
The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically 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 3-2 shows a conceptualized representation of a PBPK model.

If PBPK models for RDX exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Figure 3-2. 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 and Andersen 1994

Krishnan Model (Krishnan et al. 2009; U.S. Army 2007a; Sweeney et al. 2012)

Description of the Model. Krishnan et al. (2009; U.S. Army 2007a) developed a PBPK model for simulating kinetics of RDX in rats. The model was subsequently modified and extended to include simulations of human kinetics (Sweeney et al. 2012). The structure of the model is essentially identical to the generic model depicted in Figure 3-2, with the following tissue compartments: brain, fat, liver, richly perfused tissues (RPT), and slowly perfused tissues (SPT). Parameters and parameter values reported in Sweeney et al. (2012) are presented in Table 3-4.

The model simulates absorption of RDX from the gastrointestinal tract as first order transfers to liver from stomach (KAS, $hour^{-1}$) and duodenum (KAD, $hour^{-1}$), with first-order transfer from stomach contents to duodenum contents (KT, $hour^{-1}$). Since no other transfers from the gastrointestinal tract are simulated (i.e., transfer to lower gastrointestinal-tract or fecal excretion), 100% of the oral dose is eventually absorbed at infinite time after an oral dose. Distribution of RDX to tissues is simulated as flow-limited transfers in which instantaneous partitioning of RDX between tissue and blood is assumed, the tissue-venous blood concentration ratio is given by a tissue-blood partition coefficient, and blood-tissue clearance (L/hour) is assumed to be equivalent to tissue blood flow. Elimination of absorbed RDX is assumed to be entirely by metabolism, all of which is attributed to the liver, and is simulated as a first order processes (KfC, $kg^{0.33}/hour^{-1}$). Metabolites of RDX are not simulated in the model.

Sweeney et al. (2012) derived values for several parameters in the rat model, based on statistical optimization of predicted blood concentration kinetics against observations from rat studies not used for parameter estimation by Krishnan et al. (2009). A value for a single absorption rate constant in rats was estimated by Krishnan et al. (2009) based on model performance (visual inspection of fit to observations) in simulating blood RDX kinetics in rats that received a single oral dose of RDX (Schneider et al. 1977). Sweeney et al. (2012) derived alternative values for a two-compartment gastrointestinal model (stomach, duodenum) by optimization against observed blood RDX kinetics from various rat oral studies (Bannon et al. 2009a; Crouse et al. 2008; Krishnan et al. 2009).

Tissue:blood partition coefficients for RDX were estimated by Krishnan et al. (2009) based on a measured n-octanol:water partition coefficient for RDX, and reported water and lipid contents of specific tissues. The value for the metabolism rate constant was estimated by evaluating alternative values against

Table 3-4. Parameter Values for Sweeney et al.(2012) PBPK Model of RDX in Rats and Humans

Description	Value		Source
	Rat	Human	
Body weight (*BW*, kg)	0.3	70	Observed
Cardiac output (*KQC*, L/hour/kg$^{0.74}$)	15	14	Brown et al. 1997, as cited in Sweeney et al. 2012; Krishnan et al. 2009; Timchalk et al. 2002
Blood flow (K*Q*) fraction of cardiac output			
Liver (K*QL*)	0.25	0.175	Brown et al. 1997, as cited in Sweeney et al. 2012; Krishnan et al. 2009; Timchalk et al. 2002
Brain (*KQB*)	0.03	0.114	
Fat (*KQF)*	0.09	0.085	
Slowly perfused tissues (*KQS*)	0.20	0.2449	
Rapidly perfused tissues (*KQR*)	0.43	0.3811	1-(*KQL+KQB+KQF+KQS*)
Compartment volumes (V_i) fraction of body weight			
Liver (*KVL)*	0.04	0.026	Brown et al. 1997, as cited in Sweeney et al. 2012; Krishnan et al. 2009; Timchalk et al. 2002
Brain (*KVB*)	0.012	0.02	
Fat (*KVF)*	0.07	0.21	
Rapidly perfused tissues (*KVR*)	0.04	0.052	
Blood (*KVV*)	0.06	0.079	
Slowly perfused tissues (*KVS*)	0.688	0.523	0.91 – (*KVL+KVB+KVF+KVR+KVV*)
Tissue:blood partition coefficients			
Liver *(PL*)	1.2	1.3	Krishnan et al. 2009 (predicted from n-octanol:water partition coefficient)
Brain (*PB*)	1.4	1.6	
Rapidly perfused tissues (*PS*)	1.4	1.6	
Fat (*PF*)	5.57	5.57	Optimized—intravenous rat data[a]
Slowly perfused tissues (*PR*)	0.15	0.15	
Liver metabolism			
Metabolism (*KfC*, kg$^{0.33}$/hour)	2.6	11.2	Optimized—intravenous rat data[a] Optimized—oral human data[b]
Gastrointestinal absorption			
Absorption from stomach (*KAS*, hour^{-1})		0.033	Optimized—oral human data[b]
gavage (rat)	0.83	NA	Optimized—oral rat data[c]
capsule (rat)	0.12	NA	
coarse (rat)	0.005	NA	
Transfer to duodenum (*KT*, hour^{-1})		0	Optimized—oral human data[b]
gavage (rat)	1.37	NA	Optimized—oral rat data[c]
capsule (rat)	0	NA	
coarse (rat)	0	NA	
Absorption from duodenum (*KAD*, hour^{-1})		NA	Optimized—oral human data[b]
gavage (rat)	0.0258	NA	Optimized—oral rat data[a]
capsule (rat)	NA	NA	
coarse (rat)	NA	NA	

[a]Krishnan et al. 2009.
[b]Özhan et al. 2003; Woody et al. 1986.
[c]Bannon et al. 2009a; Crouse et al. 2008; Krishnan et al. 2009; Schneider et al. 1977.

Source: adapted from Sweeney et al. 2012

observed blood RDX kinetics following intravenous dosing of rats with RDX (Schneider et al. 1977). Sweeney et al. (2012) re-evaluated the values for the partition coefficients for adipose and slowly perfused tissue, and the metabolism rate constant, and simultaneously optimized all three parameters against blood RDX kinetics from a rat intravenous study conducted by Krishnan et al. (2009). The simultaneous optimization yielded values of 5.57 and 0.15 for the partition coefficients for adipose and slowly perfused tissue, respectively, whereas Krishnan et al. (2009) estimated values of 7.55 and 1. The optimized value for the metabolism rate constant was 2.6 $kg^{0.3}$/hour, whereas Krishnan et al. (2009) estimated the value to be 2.6 kg/hour. For a 0.4 kg rat (Krishnan et al. 2009), the corresponding values for the metabolism rates constants are 3.4 $hours^{-1}$, based on the Sweeney et al. (2012) estimate, and 5.5 $hours^{-1}$, based on the Krishnan et al. (2009) estimate.

Parameter values for the human model were scaled to body weight (e.g., flows scaled to $BW^{0.74}$ and volumes to BW^{1}), with the exception of the metabolism and absorption rate constants. Absorption and metabolism rate constants for humans were optimized against observations of blood RDX kinetics in cases of ingestion exposures in humans (Özhan et al. 2003; Woody et al. 1986). Because doses in the human cases were unknown, dose was also optimized for each case. The estimated parameter values based on simulation of plasma RDX concentrations in a 3-year old child who ingested an unknown amount of RDX (Woody et al. 1986) were: dose 58.9 mg/kg; KAS 0.060/hour; KfC 9.87 $kg^{0.33}$/hour. Corresponding values based on plasma RDX kinetics in adults were: dose 3.5 mg/kg; KAS 0.033/hour; KfC 11.2 $kg^{0.33}$/hour. Sweeney et al. (2012) also estimated the value for the first-order metabolism rate constant based on scaling of the rat value against the ratio of *in vitro* metabolism rates in rats and humans, adjusted for microsomal protein (Lipscomb and Poet 2008, as cited in Sweeney et al. 2012; U.S. Army 2008) as follows:

$$KfC_h = KfC_r \cdot MRR_{h/r} \cdot MSPR_{h/r} \cdot BWR_{h:r}^{0.33}$$

where KfC is the first-order metabolism rate constant in human or rat (human or rat, respectively), MRR is the *in vitro* metabolism rate ratio (human:rat), MSP is the microsomal protein yield ratio (human:rat), and BWR is the body weight ratio (human:rat). The estimated values for the metabolism rate constant in humans was 12.4 $kg^{0.33}$/hour, which was similar to the value for adults estimated by optimization against the Özhan et al. (2003) data.

Validation of the Model. Krishnan et al. (2009) estimated the metabolism rate constant based on blood RDX kinetics obtained from an intravenous study in rats (Schneider et al. 1977), and then evaluated model performance against data obtained from a different intravenous study conducted in rats (Krishnan et al. 2009). The same approach was used to evaluate the gastrointestinal absorption rate constant; data from a study in which rats received a single gavage dose of RDX (Krishnan et al. 2009) were used to estimate the parameter values, and the resulting model was evaluated against data from a different gavage rat study (Schneider et al. 1977). In both evaluations, the studies that were used to evaluate the model administered lower doses than the studies used to estimate parameter values.

As previously described, Sweeney et al. (2012) optimized the absorption and metabolism parameter values, and tissue:blood partition coefficients for adipose and SPT in the rat using data from intravenous and oral dosing studies reported in Krishnan et al. (2009). The results of optimization of the intravenous studies were compared to an independent data set (Schneider et al. 1977) and the results were summarized with the following conclusion: *"…the agreement between the model and the iv data of Schneier eta l. (1977) was very good (not shown)."* In the oral dosing studies, rats received a gavage dose of RDX dissolved in water. The absorption parameters were re-optimized to simulate blood RDX kinetics in studies in which RDX was administered as a granular RDX (coarse) or in a gelatin capsule, or as a suspension in water (Bannon et al. 2009a; Crouse et al. 2008; Schneider et al. 1977). One of the studies included measurements of brain RDX concentrations (Bannon et al. 2009a). Since these studies were used to calibrate the absorption kinetics parameters to account for the different dosing formulations, they do not represent a fully independent validation of the rat model for simulating oral dosing. However, these evaluations do allow validation of the simulations of distribution and elimination kinetics derived from intravenous studies. Blood RDX kinetics obtained for cases of human ingestion of RDX were used to estimate values for absorption and metabolism parameters in the human. The human model was not evaluated against observations independent of those used to estimate parameter values.

All of the model calibration and evaluation studies were conducted with data from single-dose studies. Although Sweeney et al. (2012) used a statistical procedure (maximum likelihood) to estimate parameter values, statistical comparisons of goodness of fit were not reported in Sweeney et al. (2012), and were not reported in Krishnan et al. (2009).

Risk Assessment. The RDX model predicts blood and brain levels of RDX that would occur in association with oral doses to RDX. These predictions are potentially useful for predicting internal doses of RDX in rats and/or humans (e.g., blood or brain concentrations), and for making extrapolations of

these internal dose metrics across species. The model has been used to extrapolate dosages in repeated oral dosing studies in rats to equivalent oral dosages in humans in a derivation of human equivalent external doses and candidate chronic oral reference doses (Sweeney et al. 2012). Several internal dose metrics were explored in the interspecies dosimetry extrapolation. Peak and average brain RDX concentrations were used for dosimetry of neurological end points observed in rats in intermediate gavage and chronic dietary studies (U.S. Army 1983a, 2006). The time-weighted average RDX concentration in richly perfused tissue and steady-state body weight-adjusted rate of metabolism were used for dosimetry of prostate inflammation observed in rats in a chronic dietary study (U.S. Army 1984c) and alterations in survival time, terminal body weight, and hematocrit and hemoglobin concentrations in rats in a chronic dietary study (U.S. Army 1984c). Sweeney et al. (2012) estimated human equivalent external doses ranging from 1.6 to 8 mg/kg/day.

Target Tissues. The RDX model was calibrated to predict blood RDX kinetics following oral exposures to RDX, although it also predicts concentrations in brain and other tissues. Sweeney et al. (2012) presented simulations of brain RDX concentration in comparison to measurements made in rats (Bannon et al. 2009a). The model has been used to predict concentrations of RDX in brain, which has been shown to be an important toxicity target tissue for RDX (Sweeney et al. 2012).

Species Extrapolation. Sweeney et al. (2012) scaled the rat model to humans using a combination of allometric scaling and optimization of selected model parameters (absorption and metabolism rate constants). The scaled human model has not been evaluated against independent observations not used to estimate model parameter values. The model has been used to extrapolate rat dosages to humans based on predicted internal dosimetry (Sweeney et al. 2012).

Interroute Extrapolation. The RDX model as it is currently configured simulates RDX kinetics associated with intravenous and oral dosing. Simulation of other potential routes of exposure (e.g., inhalation, dermal) would require development of models for the absorption of inhaled RDX, or RDX deposited on the skin.

Strengths and Limitations. Strengths of the model are that it simulates disposition and clearance of intravenously injected or ingested RDX in rodents and humans, including predicting levels of RDX in the brain, a target tissue for toxicity. However, limitations include: (1) all model calibration and evaluation studies were conducted with data from single-dose studies and confidence in simulating RDX kinetics of repeated dosing schedules has not been evaluated; (2) the human model was not evaluated against

observations independent of those used to estimate parameter values; and (3) the validity of predictions of brain levels of RDX in humans is based solely on performance of the model in predicting observed blood kinetics in humans who ingested unknown doses of RDX (dose was optimized to the blood RDX data).

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. The mechanism(s) of absorption of RDX is not known. There are no studies that calculated rates of absorption that could have provided some indication of a possible mechanism of absorption. In rats administered RDX in a capsule, peak blood concentrations were achieved 4–6 hours after dosing (Crouse et al. 2008). In a male miniature pig given a single gavage dose of RDX as a suspension in 0.5% carboxymethylcellulose in water, peak plasma concentration of RDX occurred at approximately 12 hours after dosing, which would suggest a relatively low rate of absorption. Studies with excised human and pig skin showed that mixing RDX with soil significantly reduced dermal absorption relative to RDX neat (Reddy et al. 2008; Reifenrath et al. 2002).

Distribution. No specific mechanism of distribution was apparent in the available studies. In rats, the distribution of RDX (single doses) seemed unaffected by the route of administration (parenteral vs. oral) or by the dose (Schneider et al. 1977). The concentration of RDX-derived radioactivity in most tissues was fairly stable between 2 and 24 hours after dosing except in the liver, where it fluctuated widely. High concentrations of radioactivity occurred in the liver at 2, 12, and 24 hours after dosing, which led Schneider et al. (1978) to suggest that there might be diurnal variations in the hepatic metabolism of RDX. In 90-day studies, RDX did not accumulate in any of the tissues examined (Schneider et al. 1978).

Metabolism. The metabolism of RDX has been studied in some detail in miniature pigs (Major and Reddy 2007) and there is some evidence suggesting that a cytochrome orthologue to the rabbit, CYP2B4, may be involved (Bhushan et al. 2003). The two major metabolites characterized were 4-nitro-2,4-diaza-butanal and 4-nitro-2,4-diazabutanamide. Trace amount of MNX, DNX, and TNX were also detected. Some studies have provided some information regarding the role of metabolism in the toxicity of RDX. In rats, administration of RDX intravenously resulted in convulsive activity within seconds after the injection, which suggested that the convulsions are produced by the parent compound (Schneider et al. 1977). In a 90-day gavage study in monkeys, convulsive events were associated with higher RDX concentrations in plasma (U.S. Navy 1974b), which would also support the idea of the parent compound being responsible for the convulsive activity. More recently, Meyer et al. (2005) reported that MNX and

RDX were equipotent in inducing convulsions and lethality in female Sprague-Dawley rats in single-dose gavage studies of 14-day duration; both DNX and TNX were less potent. In a study of age-dependent acute toxicity of RDX in deer mice, Smith et al. (2007) reported that, for all three age brackets tested, RDX was significantly more potent than MNX and TNX.

Excretion. The urine and exhaled CO_2 were the main routes of excretion of ^{14}C-RDX-derived radioactivity in rats following acute- or intermediate-duration exposure to RDX (Schneider et al. 1977, 1978). In the acute studies, only 3% of the administered radioactivity was recovered in the feces over a 4-day period (Schneider et al. 1977). The urine was also the main excretory route of radioactivity in miniature pigs following a single gavage dose of RDX (Major and Reddy 2007). No information was located regarding how the size of the dose might affect the distribution of metabolic products among excretory pathways.

3.5.2 Mechanisms of Toxicity

The main effect of high doses of RDX in humans and animals is the induction of hyperactivity manifested as convulsions or seizures. RDX has also induced other effects; however, because these effects have not been well characterized and/or have been seen inconsistently in animal studies, this section will focus mainly on the potential mechanisms of neurological effects. *In vitro* studies in primary human cells, including neurons, astrocytes, and microglia cells, have found minimal evidence of cytotoxicity (U.S. Army 2010), suggesting that the observed neurological effects of RDX are likely to be reversible effects on neurotransmission. Hyperactivity can result from a chemical acting centrally and/or on the peripheral nervous system. Chemicals such as organophosphorus pesticides or nerve agents, such as sarin and soman, act mainly by inhibiting cholinesterase activity in the brain (McDonough and Shih 1997), but limited information is available regarding possible effects of RDX on cholinesterase activity. Based purely on the chemical structure of RDX, it seems unlikely that it would possess potent anticholinesterase properties. In rats receiving a single intraperitoneal dose of RDX, small, but significant, decreases in brain cholinesterase levels were found 1.5, 3, or 6 hours after dosing. By 24 hours after dosing, the cholinesterase levels were similar to controls (Maryland University 1975). However, in rats receiving 2.5 or 6.5 mg/kg/day RDX administered intraperitoneally for 6 or 12 weeks, significant increases in brain cholinesterase levels were found. An *in vitro* study found a 53% decrease in cholinesterase activity in brain homogenates incubated with $4.5x10^{-3}$ M RDX (Maryland University 1975). In contrast, no alterations in frontal lobe or blood acetylcholinesterase activity were observed at the onset of seizures in rats administered a single gavage dose of 75 mg/kg RDX (Williams et al. 2011). The Maryland

University study (1975) also found significant increases in monoamine oxidase activity in rats receiving intraperitoneal doses of 2.5 or 6.5 mg/kg/day RDX for 6 or 12 weeks or 0.3 mg/kg/day for 12 weeks. However, following a single dose, a small, but not statistically significant, decrease in monoamine oxidase activity was observed 0.5, 1.5, 3, 6, or 24 hours after dosing. As with cholinesterase activity, RDX induced a dose-related decrease in monoamine oxidase activity *in vitro*.

Studies in rats by Burdette et al. (1988) suggested that limbic structures may be a primary target for RDX toxicity. The suggestion was based on observations of spontaneous seizure characteristics and an accelerated rate of amygdaloid kindling following administration of a subconvulsive dose of RDX. Recent studies provide evidence of the involvement of GABA (γ-aminobutyric acid) receptors in RDX-induced neurologic dysfunction. Antagonism of GABAergic neurons within the central nervous system leads to generalized nervous system stimulation. Binding of GABA to its receptor opens chloride-selective ion channels leading to influx of chloride into neurons through an electrochemical gradient resulting in hyperpolarization of the membrane and inhibition of cell firing. A reduced inhibitory drive results in uninhibited activity in effector neurons. Williams et al. (2011) found that RDX binds to the picrotoxin convulsant site on $GABA_A$ receptors, but did not bind to other neurotransmitter receptors that are targets of other known convulsants, including the glutamate family of receptors, nicotinic and muscarinic acetylcholine receptors, the glycine receptors, and the batrachotoxin site of the sodium channel. This finding is supported by 3-D modeling, which found that RDX does not appear to be a ligand for the N-methyl-D-aspartate-glutamate receptor in postsynaptic neurons (Ford-Green et al. 2011). *In vitro*, RDX reduced the frequency and amplitude of spontaneous $GABA_A$ receptor-mediated inhibitory postsynaptic currents and the amplitude of GABA-evoked postsynaptic currents in the rat basolateral amygdala. Williams et al. (2011) also found a significant negative correlation between the levels of RDX in the brain and the time to seizure onset in rats administered 75 mg/kg RDX via gavage. These findings suggest that the convulsions were due to parent compound rather than a metabolite. Similarly, a study of Northern bobwhite quail found 20 times higher brain RDX levels in birds with seizures, compared to birds exposed to the same dosage but did not develop seizures (Gust et al. 2009).

Some support for the hypothesis of an RDX-induced imbalance between inhibitory and excitatory systems is provided by a recent study of global gene expression in the brain of rats dosed with either 3 or 18 mg RDX/kg (Bannon et al. 2009a). Relative to low-dose rats, gene expression in the cerebral cortex of high-dose rats was significantly decreased, particularly for processes related to the generation, packaging, mobilization, and release of neurotransmitters. Significantly down-regulated was the glutamate signaling pathway, which could be a response to excessive excitation resulting from the removal of the inhibition

by GABAergic pathways, caused in turn by RDX. *In vitro* studies using human neuroblastoma cells found an RDX-induced transient increase in calcium levels (released from intracellular calcium stores) (Ehrich et al. 2009); this increase in calcium may mediate the release of glutamate. Genomic results from a study of Northern bobwhite quail found significant alterations in the differential expression of transcripts involved the electrophysiology and signal transduction of neurons (Gust et al. 2009). The investigators suggested that these alterations may result in an inhibition of neuronal cell repolarization postaction potential leading to heightened neuronal excitability and seizures. Zhang and Pan (2009) found significant alterations in the number of microRNAs (miRNAs) expressed and expression levels in the brains of mice exposed to low levels of RDX in the diet for 28 days. The most affected miRNA was MiR-206, which was significantly up-regulated in the brain. The brain-derived neurotrophic factors (BDNF) gene is a potential miRNA target. Zhang and Pan (2009) speculated that miR-206 may contribute to the neurological effects associated with RDX exposure through its reduction of BDNF gene expression. The results of this study should be interpreted cautiously; additional research is needed to evaluate the role of altered miRNA expression in RDX toxicity. Bannon et al. (2009b) noted that the significance of miRNA as a predictor of toxic insult or disease has not been demonstrated.

3.5.3 Animal-to-Human Extrapolations

Virtually all of the information regarding the effects of RDX is derived from cases of acute exposure to doses of RDX that induced frank effects. In both humans and animals, high doses of RDX affect primarily the nervous system. However, which experimental animal is the best model for human exposure is unknown, although the basic mechanism for seizure induction is probably the same in humans and animals. Studies in animals have provided enough information to establish approximate blood levels of RDX that are associated with convulsive activity (Burdette et al. 1988; Schneider et al. 1977, 1978; U.S. Navy 1974b). That information is lacking in humans. Only two of the numerous case reports available measured RDX in the blood of the patients, Woody et al. (1986) and Küçükardalĭ et al. (2003). Blood levels of RDX reported by Woody et al. (1986) appear to be consistent with what has been measured in animal studies administered doses similar to those estimated for the patient in Woody et al. (1986). However, blood levels of RDX reported by Küçükardalĭ et al. (2003) were at least 3 orders of magnitude lower, even though the doses that the investigators estimated the patients had consumed (37–250 mg RDX/kg) were in the range of that estimated by Woody et al. (1986) (84.8 mg RDX/kg). No explanation was offered by Küçükardalĭ et al. (2003) for this discrepancy. Although there are limited data on the toxicokinetics of RDX in humans, the Krishnan PBPK model (Krishnan et al. 2009; Sweeney et al. 2012; U.S. Army 2007a) allows for extrapolation of the results of animal studies to humans.

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).

There is no evidence suggesting that the reproductive and developmental effects reported in animals summarized in Sections 3.2.2.5 and 3.2.2.6, respectively, involve actions of RDX on the neuroendocrine axis. No *in vitro* studies were located regarding endocrine disruption of RDX.

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 limited data on the toxicity and toxicokinetic properties of RDX in children. Clonic-tonic convulsions were reported in a 3-year-old child ingesting RDX (Woody et al. 1986). As described in the case-report, the observed effects are similar to those observed in adults (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969). The lack of adequate exposure data in most of these cases precludes evaluating whether children are more susceptible to RDX toxicity. Age-specific differences in LD_{50} values were found in deer mice; 21-day-old mice were the most sensitive followed by 200- and 50-day-old animals (Smith et al. 2007). The LD_{50} in the 50-day-old mice was approximately twice as great as the value in 200-day-old mice. It is not known if similar differences would occur for other toxic effects.

As discussed in greater detail in Section 3.2.2.6, developmental effects (decreases in growth and survival) have been observed in the offspring of rats orally exposed to RDX (U.S. Army 1980b, 1986d). These effects were typically observed at doses associated with RDX-induced seizures in the dams and may not have been a direct effect on the fetus/pup. No developmental effects have been observed in rabbits (U.S. Army 1980b).

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).

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 RDX 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 RDX 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 RDX

RDX was detected in the serum, urine, and feces of a child who consumed unknown levels of RDX in the form of C-4 (91% RDX). RDX was measured in the serum for 120 hours and in the feces for 144 hours after the presumed time of ingestion (Woody et al. 1986). RDX was also measured in plasma from five

male cases described by Küçükardalĭ et al. (2003). Therefore, the chemical itself is a specific biomarker of exposure. Since the metabolism of RDX in humans has not been studied, it is not known whether single measurements of RDX in blood or urine could be used only as a biomarker of recent exposure or also as a biomarker of low-level prolonged exposure.

3.8.2 Biomarkers Used to Characterize Effects Caused by RDX

High oral doses of RDX are known to produce seizures in humans (Davies et al. 2007; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kaplan et al. 1965; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969; Testud et al. 1996a; Woody et al. 1986) and animals (Burdette et al. 1988; Schneider et al. 1977; U.S. Army 1983a; U.S. Navy 1974b; von Oettingen et al. 1949), but this effect is not specific to RDX. Thus, there are no known specific biomarkers to characterize effects caused by inhalation, oral, or dermal exposure to RDX.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Many of the human studies on the accidental inhalation or ingestion of RDX involved composition C-4, which was used for demolition by the U.S. Armed Forces during the Vietnam War. Composition C-4 was 91% RDX, with the other components consisting of polyisobutylene, motor oil, and 2-ethylhexyl sebacate. Minimal information is available on the toxicological properties of these components of C-4, and it is not known whether they may contribute to the effects seen from exposure to C-4. However, since RDX is the primary component of C-4, the assumption has been made that the major effects noted from C-4 are due to RDX. In addition, the human and animal reports of ingested RDX usually are not limited to pure RDX, but are almost always reports of RDX contaminated with octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) or other substances. There are no studies regarding the interactions of these substances. However, there are several studies in which the oral toxicity of trinitrotoluene (TNT) and RDX were investigated. In one study (Levine et al. 1990), TNT and RDX were co-administered in the feed of rats for 13 weeks. This co-administration potentiated the decrease in body weight gain as compared to RDX alone. TNT antagonized the lethal effects and the hypotriglyceridemia induced by RDX. RDX antagonized the hypercholesterolemia, splenomegaly, testicular atrophy, hepatocytomegaly, degeneration of the seminiferous tubules, and pigmentation of renal cortices induced by TNT.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to RDX than will most persons exposed to the same level of RDX 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 RDX, or compromised function of organs affected by RDX. Populations who are at greater risk due to their unusually high exposure to RDX are discussed in Section 6.7, Populations with Potentially High Exposures.

There are no known populations that would be unusually susceptible to RDX toxicity because of their genetic make-up, developmental stage, health status, nutritional status, or chemical exposure history.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to RDX. 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 RDX. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

3.11.1 Reducing Peak Absorption Following Exposure

A general recommendation for reducing absorption after inhalation exposure to RDX is to move the patient to fresh air (HSDB 2009). Emesis is not recommended following oral exposure because of the probability of developing seizures (HSDB 2009). Charcoal may be administered to reduce absorption following oral exposure. The only information located for reducing absorption following dermal exposure specifically of RDX is a study by Twibell et al. (1984) which reported that washing the hands immediately after handling RDX can remove approximately 90% of the residue. Information summarized by HSDB (2009) suggests removing contaminated clothing and washing the exposed area thoroughly with soap and water. In case of eye contact, irrigation of the exposed eyes with copious amounts of room temperature water for at least 15 minutes is recommended.

3.11.2 Reducing Body Burden

No information was located on specific methods for reducing the body burden of RDX. However, Küçükardalĭ et al. (2003) reported that hemodialysis was unsuccessful in reducing the serum levels of RDX in three cases of oral intoxication with the chemical when performed approximately 3 hours after ingestion.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The primary adverse effect of RDX is the induction of convulsive activity and seizures for which standard treatments are available. Intravenous administration of a benzodiazepine such as diazepam or lorazepam is recommended (HSDB 2009). If seizures recur after diazepam, phenobarbital or propofol should be considered (30 mg for adults or 10 mg for children older than 5 years).

3.12 ADEQUACY OF THE DATABASE

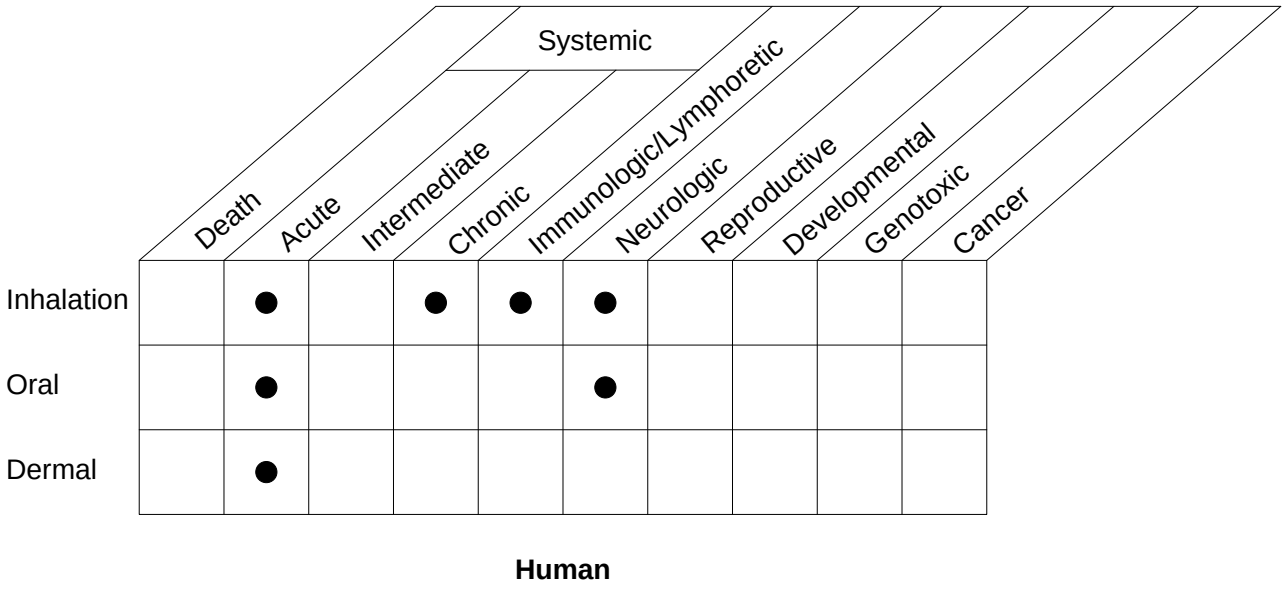
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of RDX is available. Where adequate information is not available, 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 RDX.

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.

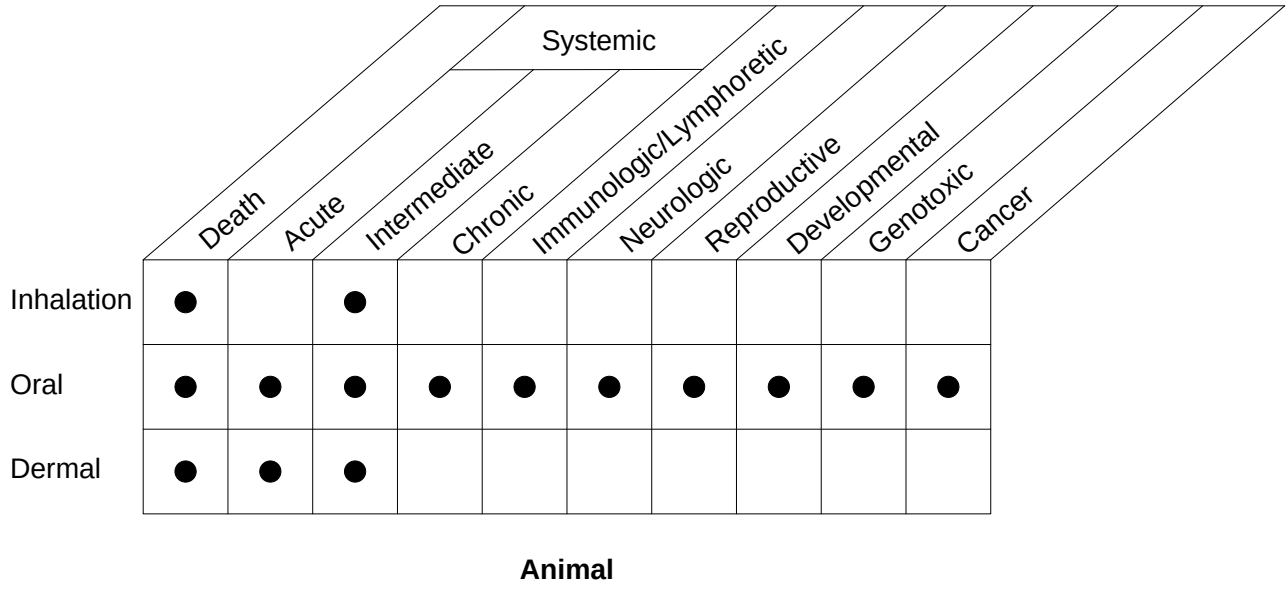
3.12.1 Existing Information on Health Effects of RDX

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to RDX are summarized in Figure 3-3. The purpose of this figure is to illustrate the existing information concerning the health effects of RDX. Each dot in the figure indicates that one or more studies provide

Figure 3-3. Existing Information on Health Effects of RDX



Human



Animal

● Existing Studies

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 1989d), 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.

Case studies are available regarding systemic effects in humans following acute exposures to RDX via all three routes. One study in the workplace provides information on immunological and neurological effects following inhalation exposure for chronic periods (Hathaway and Buck 1977). Neurological effects have also been described following acute oral exposures to RDX (Hollander and Colbach 1969; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986).

Animal data on inhalation exposure is limited to one study. Oral animal data are available for all exposure durations and for all end points. Dermal data on death and systemic effects are available for animals exposed to RDX for acute and intermediate exposure periods.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. The nervous system is one of the main targets for RDX toxicity in humans exposed by the inhalation (Hollander and Colbach 1969; Testud et al. 1996a) or oral (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kasuske et al. 2009; Ketel and Hughes 1972; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986) routes, and animal studies involving oral exposure support this finding (Burdette et al. 1988; Meyer et al. 2005; Schneider et al. 1977; U.S. Army 1985b, 2006). There is a small number of acute-duration animal studies and no studies that adequately examined potential systemic effects. Increases in occurrence of convulsions, tremors, and/or seizures were consistently observed in the available studies (Burdette et al. 1988; U.S. Army 1980b, 1985b, 1986d, 2006). In addition, decreases in growth were observed in the fetuses of rats exposed to lethal doses of RDX (U.S. Army 1986d). One animal study suggests that the skin is a target organ for RDX following dermal exposure (U.S. Army 1974). However, the use of solvents confounded the results. No acute inhalation MRLs could be derived because of the lack of human and animal studies with accurate exposure estimates. The available acute exposure data for

animals was adequate for the derivation of an MRL based on an increased incidence of convulsions/seizures/tremors (U.S. Army 2006). Further acute inhalation and oral studies on the developmental and neurological effects of RDX would be useful in determining levels that may cause harm to humans living near hazardous waste sites; these studies should also evaluate potential systemic effects.

Intermediate-Duration Exposure. No studies examining the toxicity in humans following intermediate-duration exposure to RDX were identified. No animal studies were identified examining RDX toxicity following inhalation exposure; thus, an intermediate-duration inhalation MRL could not be derived. Inhalation studies are needed to identify potential targets of toxicity and establish dose-response relationships; these studies would be useful in determining levels that may cause harm to humans who live near hazardous waste sites. The nervous system is the target organ for RDX toxicity in animals exposed by the oral route for intermediate periods (Levine et al. 1981, 1990; U.S. Army 1983a, 1985b, 2006; U.S. Navy 1974b; von Oettingen et al. 1949). The most consistently observed effect was convulsions, seizures, and tremors. Systemic effects (hematological and serum chemistry alterations), reproductive effects (testicular degeneration, possible decrease in male fertility), and developmental effects (decreases in growth and decreased viability) have also been observed. However, these effects have not been consistently observed across studies. Difference in the exposure route (dietary versus gavage) and RDX formulation (finely ground versus coarsely ground) may explain possible differences in the results; however, this has not been adequately assessed and additional oral exposure studies are needed to evaluate apparent study differences. An intermediate oral MRL based on an increased incidence of convulsions in rats was derived (U.S. Army 2006). Studies involving intermediate dermal exposure to RDX did not identify a target organ (U.S. Army 1974).

Chronic-Duration Exposure and Cancer. Only one human study was located for chronic-inhalation exposure. This study revealed no adverse health effects following chronic exposures to unknown levels of RDX in the air (Hathaway and Buck 1977). No animal studies concerning chronic inhalation exposure were located. No chronic inhalation MRLs could be derived because of the lack of human and animal studies with accurate exposure estimates. Therefore, further inhalation studies would be useful to identify target organs and define the potential for human health risks.

No human studies concerning chronic oral exposure were located. The most sensitive target organ for adverse effects in animals following chronic oral exposure is the nervous system; an increased occurrence of convulsions and seizures were observed in rats (U.S. Army 1983a). Mild adverse systemic effects

have also been observed in rats (U.S. Army 1983a) and mice (U.S. Army 1984c). A second chronic-duration study in rats (U.S. Navy 1976) did not find any adverse effects. An increased incidence of prostate gland inflammation was observed in rats exposed to RDX for 2 years (U.S. Army 1983a). The inflammation was observed at the lowest adverse effect level; it may have been secondary to a bacterial infection. Because the second rat study (U.S. Navy 1976) did not examine the prostate, the prostate effect could not be confirmed. Additional studies are needed to further evaluate the prostate as a potential target of RDX toxicity. These studies should include end points addressing immunotoxicity of chronic exposure to RDX. A chronic-duration oral MRL was derived for RDX based on the neurological effects observed in the U.S. Army (1983a) study. Only one human study was located for chronic dermal exposure (Sunderman 1944). This study reported dermatitis in workers exposed to RDX, but no dose levels were reported. No animal studies concerning chronic dermal exposure were located. Additional chronic oral and dermal studies would be useful to better define dose levels that may cause a risk to humans.

No studies are available regarding cancer in humans following any route of exposure. Increased incidences of combined hepatocellular adenomas and carcinomas were found in female mice orally exposed to RDX (U.S. Army 1984c). A re-evaluation of the histopathology slides from this study resulted in a re-classification of several of the tumors as nonneoplastic alterations (Parker et al. 2006). No increases in neoplastic lesions were observed in rat oral exposure studies (U.S. Army 1983a; U.S. Navy 1976). The risk of developing cancer by the inhalation or dermal routes has not been investigated. Further inhalation, oral, or dermal carcinogenicity studies would be useful to determine whether RDX poses a risk of cancer for humans.

Genotoxicity. Data from microbial mutagenicity studies using *S. typhimurium* and *S. cerevisiae* have consistently produced negative results (George et al. 2001; Lachance et al. 1999; Pan et al. 2007; U.S. Army 1977b, 1980b; Whong et al. 1980). Therefore, at this time, additional studies with RDX would probably not provide any new key information. Studies involving humans and mammalian species are few. The three mammalian studies available were negative for DNA damage in human fibroblasts (U.S. Army 1978b), dominant lethal mutations in rats (U.S. Army 1980b), and induction of micronuclei in bone marrow cells from mice (Reddy et al. 2005a). Additional studies of N-nitroso metabolites of RDX, such as MNX and TNX, would be valuable since N-nitroso compounds often yield genotoxicity. Research employing toxicogenomics or a combination of genetics, molecular biology, and bioinformatics may be able to uncover the molecular targets of RDX.

Reproductive Toxicity. No data are available on the reproductive toxicity of RDX in humans via inhalation, oral, or dermal routes of exposure. No inhalation or dermal studies are available for animals. An oral study in mice (U.S. Army 1984c) and one in rats (U.S. Navy 1976) revealed no histopathology in the ovaries, testes, or uterus. One oral study (U.S. Army 1983a) did reveal spermatic granulomas in the prostate of rats after 6 months of exposure and testicular degeneration in rats exposed for 1 year. This study also reported an increased incidence of suppurative inflammation of the prostate in rats exposed for 2 years; however, the inflammation was primarily observed in rats dying early and there is concern that the inflammation may be secondary to a bacterial infection rather than a primary effect of RDX. No pharmacokinetic data are available that can be used to determine whether the reproductive system is likely to be a target for RDX toxicity. Therefore, further studies to determine whether the prostate is indeed the most sensitive organ are important. A two-generation reproductive study in rats (U.S. Army 1980b) reported nonsignificant decreases in F_0 male fertility when the exposed males were mated with unexposed females or exposed females; additional studies are needed to confirm this effect.

Developmental Toxicity. No human studies on developmental effects are available for exposure to RDX via inhalation, oral, or dermal routes. No inhalation or dermal studies are available for animals. Two acute duration oral studies examined the potential developmental toxicity of RDX. Maternal deaths were observed in both studies at the highest dose tested (U.S. Army 1980b, 1986d). No increases in the occurrence of fetal malformations were observed (U.S. Army 1980b). One study reported a decrease in fetal weight and length at the dose level associated with maternal deaths and neurotoxicity. In a two-generation study, increases in the occurrence of stillbirths and decreases in pup survival were observed in the F_1 offspring of dams exposed to lethal doses; a decrease in pup body weights and increase in the incidence of renal cysts were observed in the F_2 pups (U.S. Army 1980b). The one available oral study in rabbits revealed no fetotoxicity (U.S. Army 1980b). No pharmacokinetic data are available that can be used to determine whether the developmental system is likely to be a target organ. Further developmental studies via the oral route are important to determine whether humans exposed to RDX at or near hazardous waste sites are at risk of experiencing adverse developmental effects.

Immunotoxicity. The only available immunological study in humans reveals no changes in the antinuclear antibodies of workers exposed to RDX in the air (Hathaway and Buck 1977). No other functional tests were performed. No histopathological alterations were found in the spleen, thymus, or lymph nodes of rats (Levine et al. 1990; U.S. Army 1980b, 2006) or mice (U.S. Army 1980b), or in the spleens of dogs (U.S. Navy 1974a; von Oettingen et al. 1949) or monkeys (U.S. Navy 1974b), after intermediate exposure via the oral route. In addition, no alterations in the proportion of cell surface

markers were observed in rats (U.S. Army 2006). A study by Levine et al. (1981) demonstrated mild leukocytosis. Further oral studies examining immune function would be useful to determine whether RDX adversely affects the immune system. In addition, inhalation and dermal studies would help determine whether exposure to RDX at or near hazardous waste sites would affect the human immune system.

Neurotoxicity. The nervous system is a major target organ for RDX toxicity. Seizures have been reported in humans exposed for acute periods by inhalation (Kaplan et al. 1965; Testud et al. 1996a), ingestion (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995; Kasuske et al. 2009; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986), or a combination of the inhalation and oral routes (Hollander and Colbach 1969; Ketel and Hughes 1972). Oral studies in animals have supported this finding for acute (Burdette et al. 1988; Meyer et al. 2005; Schneider et al. 1977; U.S. Army 1980b, 1986d, 2006), intermediate (Sunderman 1944; U.S. Army 1983a, 2006; U.S. Navy 1974b; von Oettingen et al. 1949), and chronic (U.S. Army 1983a) exposure durations. Neurobehavioral alterations were observed in rats receiving a single gavage dose of RDX (U.S. Army 1985b), but not after repeated intermediate-duration exposure (U.S. Army 1985b, 2006). The conflicting results may be a reflection of when the behavioral tests were conducted, in relation to gavage dosing rather than a duration-related difference. Additional neurobehavioral function tests are needed to confirm the results observed in the acute study. More sensitive neurological tests in animals via inhalation, oral, or dermal routes would be helpful in establishing definite less serious LOAELs.

Epidemiological and Human Dosimetry Studies. There is one human study that tested blood chemistry and hematology in 70 workers exposed to an average of 0.28 mg/m^3 of RDX in the air (Hathaway and Buck 1977). All of the other human studies are case reports of individuals ingesting RDX (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Ketel and Hughes 1972; Küçükardalĩ et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986) or exposed to RDX dust (Kaplan et al. 1965; Testud et al. 1996a). No epidemiology studies are available for exposure in drinking water. If populations with appropriate exposures could be identified, it would be useful to conduct epidemiologic and human dosimetry studies to establish cause-and-effect relationships and to plan future monitoring of individuals living near hazardous waste sites.

Biomarkers of Exposure and Effect.

Exposure. Thus far, RDX in urine or blood is the only known biomarker of exposure to RDX. RDX has been measured in these media in cases of accidental ingestion of the chemical (Küçükardalĭ et al. 2003; Woody et al. 1986). There is no information regarding the metabolism of RDX in humans; therefore, monitoring of the blood and/or urine of RDX workers could help identify RDX-derived products that can be used as biomarkers in studies of populations living near sites where RDX has been found.

Effect. There is no known sensitive biomarker for the effects of RDX. The most prominent effects are seizures in humans (Davies et al. 2007; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kaplan et al. 1965; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969; Testud et al. 1996a; Woody et al. 1986) or animals (Burdette et al. 1988; Schneider et al. 1977; U.S. Army 1983a; U.S. Navy 1974b; von Oettingen et al. 1949), but seizures can be evoked by a large number of substances and disease states. As mentioned previously, two studies of accidental poisoning with RDX measured levels of RDX in blood from the patients. However, additional studies are necessary to establish: (1) levels of RDX in blood that are associated with adverse neurological effects and (2) levels of exposure that are associated with specific levels of RDX in blood.

Absorption, Distribution, Metabolism, and Excretion. No studies are available regarding the toxicokinetics of RDX in humans. However, pulmonary and gastrointestinal absorption of RDX in humans can be inferred from reports of adverse health effects following exposure by these routes (Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Kaplan et al. 1965; Ketel and Hughes 1972; Küçükardalĭ et al. 2003; Merrill 1968; Stone et al. 1969; Testud et al. 1996a; Woody et al. 1986) and from measurements of RDX in blood and urine after exposure in some studies (i.e., Küçükardalĭ et al. 2003; Woody et al. 1986). Relatively poor dermal absorption of RDX was reported in a study with excised human skin (Reddy et al. 2008); similar findings were reported in a study with excised pig skin (Reifenrath et al. 2002). No studies were located regarding the metabolism of RDX in humans. Analysis of blood and excreta from workers exposed to RDX could provide valuable information regarding the metabolism of RDX in humans. No inhalation toxicokinetic data were located in animals. Oral studies in rats indicate that mixing RDX with soil considerably reduces its bioavailability (Crouse et al. 2008). A recent study in miniature pigs showed that oral administration of RDX in carboxymethylcellulose and water results in almost complete absorption (Major and Reddy 2007). Earlier studies in rats provided information on some parameters of oral absorption, distribution, and elimination (Schneider et al. 1977, 1978). These studies did not show any preferential accumulation of RDX in tissues. RDX was found in

the brain of rat pups born to dams exposed to RDX during gestation (U.S. Army 2007b). Additional studies of the perinatal transfer of RDX in animals are needed, particularly to determine the relative contribution of gestational vs. lactational exposure. The metabolism of RDX has been studied in miniature pigs and the major metabolites have been characterized (Major and Reddy 2007). Since the main target for RDX appears to be the nervous system, additional studies of distribution of RDX and metabolites to different brain areas would be valuable. These studies should try to determine possible temporal correlations between the presence of RDX and/or metabolites in specific brain areas and the manifestation of clinical signs such as convulsive activity and seizures.

Comparative Toxicokinetics. The only comparative toxicokinetics data available are the results of dermal absorption studies in excised human and pig skin, which showed relatively poor absorption in both preparations (Reddy et al. 2008; Reifenrath et al. 2002). This suggests that pigs would probably be a good animal model for dermal absorption studies. As mentioned previously, analyses of blood and urine from subjects exposed to RDX during its manufacture or use or from individuals accidentally or intentionally exposed to high amounts of RDX could provide information on the metabolism of RDX in humans that can be compared with data collected from animals studies to establish which animal species serves as the best model for extrapolating results to humans. A PBPK model was developed that simulates disposition of RDX in the rat, swine, and humans (Krishnan et al. 2009; Sweeney et al. 2012; U.S. Army 2007a); this model was considered suitable for risk assessment.

Methods for Reducing Toxic Effects. There are no known mitigation measures specifically for RDX-induced toxicity, other than standard anticonvulsant therapy. Since gastrointestinal absorption of RDX seems to be quite slow (Küçükardalĭ et al. 2003), studies should focus on developing methods to accelerate its removal from the gastrointestinal tract to prevent RDX-induced adverse neurological effects. Washing the skin was reported to be effective in removing the chemical from the skin (Twibell et al. 1984). No data are available regarding adverse health effects of low-level, long-term exposure of humans to RDX; therefore, no specific mitigation studies can be proposed at this time for that exposure scenario.

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.

There are limited data on potential age-related differences in the toxicity and toxicokinetics of RDX. Neurological effects, similar to those observed in adults, have been observed in a child accidentally ingesting RDX (Woody et al. 1986). However, the lack of dose information precludes determining whether children are more susceptible than adults. A study in deer mice found age-related differences in lethality (Smith et al. 2007). Additional animal studies are needed to evaluate whether there are potential differences in RDX toxicity between adults and children. These studies should include a wide-range of ages from birth through old age to assess whether there are differences in susceptibility as the nervous system matures.

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

The U.S. Army is concurrently developing a swine PBPK model, which can be extrapolated to humans.

No ongoing studies pertaining to RDX were identified in the Federal Research in Progress database (FEDRIP 2009).