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 DEET. 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.

Although normal use of products containing DEET involves predominantly dermal exposure, some inhalation and oral exposure may occur. It is also important to note that although most human exposures are to DEET and to other chemicals in the specific formulations, exposure to DEET is the common factor between the studies.

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 (e.g., death, systemic, immunological, neurological, reproductive, developmental, 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.

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 reports of deaths in humans following inhalation exposure to DEET were located in the literature.

Without providing additional information, Army (1979) reported 4-hour LC_{50} values for aerosolized DEET in male and female Sprague-Dawley rats of 6,000 and 5,860 mg/m^3, respectively. Army (1980a) reported that the LC_{50} for aerosolized DEET in Sprague-Dawley rats was 5,950 mg/m^3. Rats were exposed whole-body for 4 hours and were observed for 14 days. No gross lesions were reported following the 14-day observation period. In male Swiss albino mice exposed head-only for 4 hours to target aerosol concentrations between 35 and 2,000 mg/m^3, the LC_{50} was 1,369 mg/m^3 (Deb et al. 2010). All mice exposed to 2,000 mg/m^3 (n=4) died after approximately 2 hours; there were no deaths at lower concentrations. The investigators noted that the actual exposure concentrations were 50–60% of the theoretical concentrations with large variations. Because they did not provide the actual exposure concentrations, the actual LC_{50} might be more than twice the reported value and is unreliable.

3.2.1.2 Systemic Effects

No studies were located regarding hematological, musculoskeletal, hepatic, renal, endocrine, dermal, ocular, or body weight effects in humans following inhalation exposure to DEET.

Respiratory Effects. In a study of 20,764 human exposures involving insect repellents containing DEET that were reported to poison control centers from 1993 to 1997, 520 were identified as being exposed predominantly by inhalation (Bell et al. 2002). Of these, 70 exhibited respiratory effects that included coughing/choking, dyspnea, bronchospasm, respiratory depression, and pneumonitis. Two of those (a male infant and a male adult) experienced a major respiratory outcome.

Head-only exposure of albino rats to an aerosol of 85% DEET for single periods of 2–6 hours did not induce gross alterations in the lungs or trachea, but did induce unspecified minor microscopic changes in both tissues (Ambrose 1959). There was no indication that a control group was used in this study and the actual exposure concentrations were not provided. Head-only exposure of male Swiss albino mice to target concentrations of DEET aerosol between 35 and 950 mg/m^3 for 4 hours resulted in an increase in respiratory frequency at 135 mg/m^3 but not at lower or higher exposure concentrations (Deb et al. 2010). Other respiratory parameters that were measured including tidal volume (Vt), air flow at 0.5 Vt, time of inspiration, and time of expiration were not affected. Because the actual exposure concentrations were not specified and were 50–60% of the target concentrations, according to the investigators, the findings from this study are unreliable.

In an intermediate-duration study, exposure of albino rats to air saturated with DEET vapor (approximately 71 mg/m^3; 1 mL of DEET was carried over 14,000 L of air) 8 hour/day, 5 days/week for 7 weeks resulted in unspecified microscopic changes in the lungs and trachea (Ambrose 1959). There was no indication that a control group was used in the study. Whole-body intermittent exposure of male and female Sprague-Dawley rats to up to 1,511 mg/m^3 (the highest concentration tested) aerosolized DEET for 13 weeks did not cause gross or microscopic alterations in the respiratory tract, including nares and nasal passages (Army 1980a). In the same study, there were no differences in measurements of pulmonary compliance and resistance between control and exposed Beagle dogs, but there were only two dogs per exposure group, so the study is limited.

Cardiovascular Effects. Only 12 of the 520 human cases of exposure to DEET by inhalation and 6 cases by multiple routes studied by Bell et al. (2002) exhibited cardiovascular effects; these included tachycardia, hypertension, and hypotension.

In the Army (1980a) 13-week, intermittent exposure study in Sprague-Dawley rats mentioned above, examination of the heart and aorta did not show exposure-related gross or microscopic alterations.

Gastrointestinal Effects. In the study of Bell et al. (2002) mentioned above of the 520 human cases reported to the AAPCC, those involving gastrointestinal effects included 130 exposed by inhalation and 225 by multiple routes. The gastrointestinal effects included oral irritation, vomiting, and nausea.

Exposure of Sprague-Dawley rats to up to 1,511 mg/m^3 aerosolized DEET for 13 weeks had no significant effect on the gross or microscopic morphology of the gastrointestinal tract (Army 1980a).

Hematological Effects. Hematology tests conducted in Sprague-Dawley rats and Beagle dogs exposed to up to 1,511 mg/m^3 DEET aerosol intermittently for 13 weeks were within normal limits (Army 1980a). Because only two dogs per sex per group were tested, the results in this species are unreliable. No further information was located.

Musculoskeletal Effects. Exposure of Sprague-Dawley rats to up to 1,511 mg/m^3 aerosolized DEET for 13 weeks had no significant effect on the gross or microscopic morphology of skeletal muscle, femur, or sternum (Army 1980a).

Hepatic Effects. Intermediate-duration exposure of Sprague-Dawley rats to 253, 752, or 1,511 mg/m^3 aerosolized DEET induced a significant trend for increased relative liver weight in females after 7 weeks of exposure and in males and females after 13 weeks of exposure (Army 1980a). In the absence of morphological alterations in the liver, the increase in relative liver weight probably represents an adaptive effect.

Renal Effects. Exposure of Sprague-Dawley rats to 253, 752, or 1,511 mg/m^3 aerosolized DEET for 13 weeks resulted in a significant trend for increased relative kidneys weight in males (Army 1980a). Microscopic examination of the kidneys from exposed rats, however, did not reveal exposure-related alterations.

Endocrine Effects. Exposure of Sprague-Dawley rats to up to 1,511 mg/m^3 aerosolized DEET for 13 weeks had no significant effect on the gross or microscopic morphology of the adrenal, pituitary, or thyroid glands (Army 1980a).

Dermal Effects. Exposure of Sprague-Dawley rats to up to 1,511 mg/m^3 DEET aerosol for 13 weeks had no significant effect on gross or microscopic appearance of the skin (Army 1980a).

Ocular Effects. Exposure of Sprague-Dawley rats to up to 1,511 mg/m^3 DEET aerosol for 13 weeks had no significant effect on gross or microscopic appearance of the eye (Army 1980a).

Body Weight Effects. Exposure of male and female Sprague-Dawley rats to concentrations of up to 4,100 mg/m^3 DEET aerosol for 4 hours did not significantly affect body weight during a 14-day observation period (Army 1979). Head-only exposure of male Swiss albino mice to target concentrations of up to 950 mg/m^3 for 4 hours did not significantly affect body weight over a 14-day observation period (Deb et al. 2010). Exposure to Sprague-Dawley rats to up to 1,511 mg/m^3 DEET aerosol for 13 weeks had no significant effect on body weight (Army 1980a). No further relevant information was located.

Metabolic Effects. Values for serum electrolytes and glucose were within normal ranges in male and female Sprague-Dawley rats following intermittent exposure to up to 1,511 mg/m^3 aerosolized DEET for 13 weeks (Army 1980a).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans following inhalation exposure to DEET.

The only information in animals is that exposure of Sprague-Dawley rats to up to 1,511 mg/m^3 aerosolized DEET for 13 weeks had no significant effect on the gross or microscopic morphology of the spleen or thymus (Army 1980a).

The NOAEL values for immunological and lymphoreticular effects from the Army (1980a) study is presented in Table 3-1 and plotted in Figure 3-1.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Rat (Sprague-Dawley)	4 hr				5950 (LC50)	Army 1980a	
INTERMEDIATE EXPOSURE								
Systemic								
2	Rat (albino)	7 wk d/wk 8 hr/d	Resp		71 (unspecified alterations in lungs and trachea)		Ambrose 1959	
3	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d	Resp	1511			Army 1980a	
			Cardio	1511				
			Gastro	1511				
			Hemato	1511				
			Musc/skel	1511				
			Hepatic	1511				
			Renal	1511				
			Endocr	1511				
			Dermal	1511				
			Ocular	1511				
			Bd Wt	1511				
			Metab	1511				
Immuno/ Lymphoret								
4	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		1511			Army 1980a	NOAEL is for histopathology of spleen and thymus.

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
Neurological								
5	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		1511			Army 1980a	NOAEL is for histopathology of the brain and spinal cord.
Reproductive								
6	Rat (Sprague-Dawley)	13 wk 5 d/wk 6 hr/d		1511			Army 1980a	NOAEL is for histopathology of the sex organs.

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

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LC50 = lethal concentration, 50% kill; LOAEL = lowest-observed-adverse-effect level; Metab = metabolic; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

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

Acute (≤14 days)

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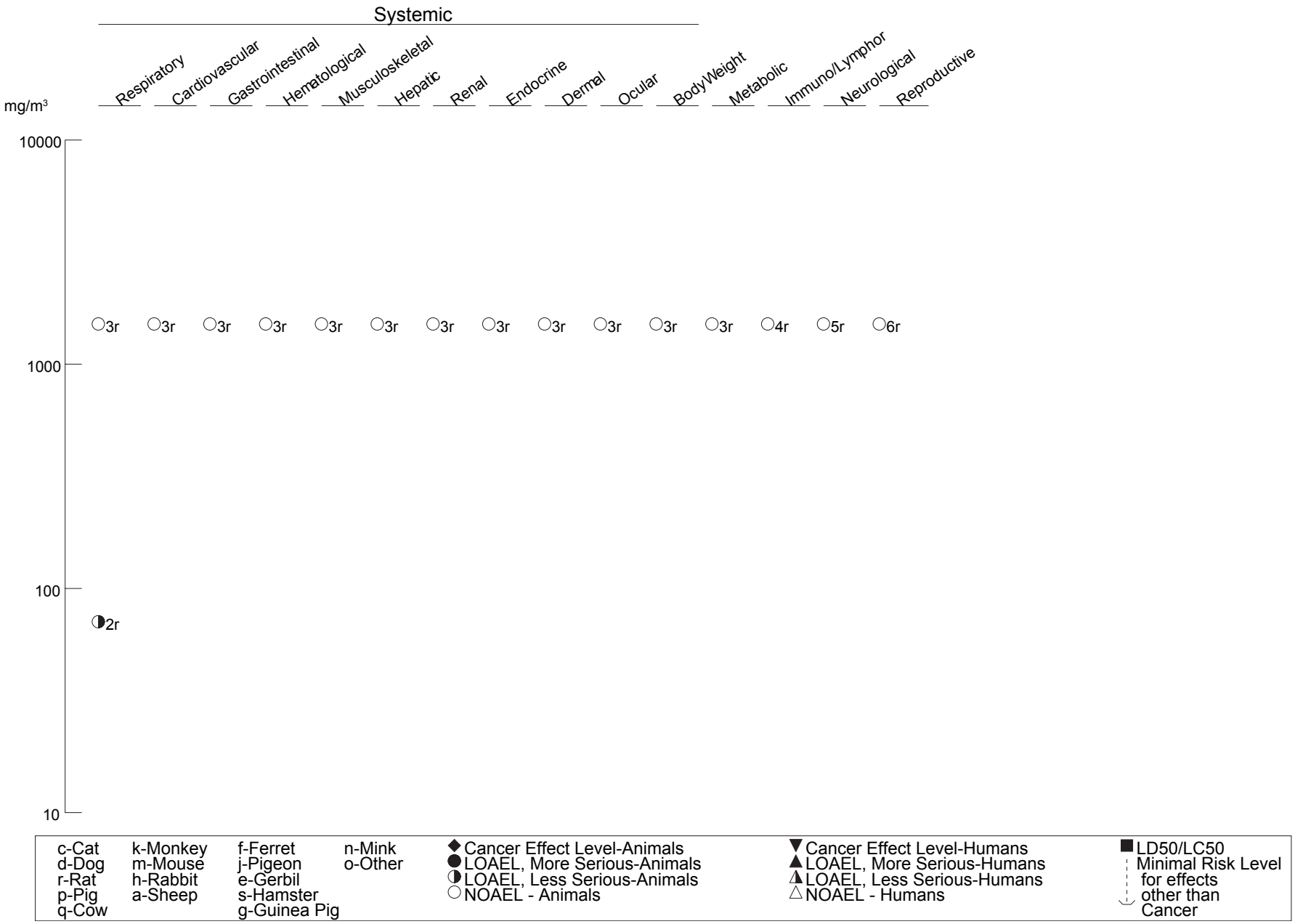
Figure 3-1 Levels of Significant Exposure to DEET - Inhalation *(Continued)*

Intermediate (15-364 days)



3.2.1.4 Neurological Effects

Fifty-seven of the 520 human cases in which inhalation was the leading route of exposure in the study by Bell et al. (2002) showed neurological effects. The most common symptoms were dizziness/vertigo, headache, and drowsiness/lethargy.

The only information available in animal studies is that from an acute-duration study in Sprague-Dawley rats (Army 1979). After a 4-hour exposure to 0, 2,300, 2,900, or 4,100 mg/m^3 DEET aerosol, the rats were examined for 15 common toxic neurological signs. In addition, seven behavioral tests were conducted within 50 minutes of termination of exposure. Toxic signs were restricted to the high-exposure group and consisted of shaking, prostration, and loss of balance in females and shaking in males. In general, performance in the various tests decreased as the exposure concentration increased. The test that seemed to show the clearest dose-response relationship and was affected at the lowest exposure concentration tested was the performance on a balance beam. Gross necropsy at the end of a 14-day observation period did not show treatment-related lesions. In an intermediate-duration study, exposure of Sprague-Dawley rats to up to 1,511 mg/m^3 aerosolized DEET for 13 weeks did not induce gross or microscopic alterations in the brain or spinal cord (Army 1980a).

The NOAEL values for neurological effects from the Army (1980a) study is presented in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

The only relevant information available is that from a 13-week inhalation study in which whole-body exposure of male and female Sprague-Dawley rats to up to 1,511 mg/m^3 aerosolized DEET for 13 weeks did not induce gross or microscopic alterations in the reproductive organs (Army 1980a).

The NOAEL values for reproductive effects from the Army (1980a) study is presented in Table 3-1 and plotted in Figure 3-1.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to DEET.

3.2.1.7 Cancer

No studies were located regarding cancer effects in humans or animals after inhalation exposure to DEET.

3.2.2 Oral Exposure

3.2.2.1 Death

Ingestion of DEET by humans has resulted in death; however, in two out of four cases other substances were ingested at the same time, so deaths could not be attributed solely to DEET in these two cases. Tenenbein (1987) reported two cases. The first case was a 33-year-old woman who intentionally ingested up to 50 mL of an insect repellent containing 95% DEET and 5% related toluamides along with presumably excessive amounts of prescription chlorpromazine hydrochloride and hydralazine hydrochloride. She was discovered approximately 1 hour after the ingestion and taken to a hospital where she was comatose with a pulse of 80 beats per minute and blood pressure of 80/55 mm Hg. After gastric lavage and treatment with activated charcoal, she was transferred to a tertiary care facility, arrived comatose and pulseless, was resuscitated and received aggressive care in the intensive care unit. During the first 24 hours, she experienced generalized seizure activity and died the second day of a massive generalized bowel infarction. DEET was measured prior to death in blood (16.8 mg/dL) and postmortally in blood and liver (11.2 and 17.7 mg/dL, respectively). The second case was a 26-year-old man who was found dead after ingesting up to 50 mL of an insect repellent containing 95% DEET and 5% related toluamides following a bout of drinking. DEET levels measured in blood, vitreous, and urine were 24, 15, and 10 mg/dL, respectively. Blood alcohol was 130 mg/dL and cannabinoids were present in the urine. The authors estimated that consumption of 50 mL of 100% DEET by an 8-year old child is potentially lethal, if the 2.0 g/kg median lethal dose for rats reported by Ambrose (1959) is applicable to humans (50 mL weighs approximately 50 g based on a specific gravity of almost 1 for DEET; therefore, 50 g/25 kg body weight for an 8-year-old boy yields a lethal dose of 2 g/kg). In their study of 9,086 exposures involving insect repellents containing DEET reported to Poison Control Centers from 1985 to 1989, Veltri et al. (1994) reported that one 33-year-old adult male died 9 days after intentionally ingesting 8 ounces of an insect repellent containing between 11–50% DEET. Clinical signs included transitory cardiorespiratory arrest shortly after poisoning followed by hyperglycemia on day 2, status epilepticus and disseminated intravascular coagulopathy, and ultimately cerebral edema. Recently, Wiles et al. (2014) described the case of a 37-year-old male who died 3 days after ingesting 6 ounces of an insect repellent containing 40% DEET (approximately 748 mg DEET/kg). Within minutes of ingesting the solution, the man suffered a seizure and was transported to a community emergency department and

later to a healthcare facility. On arrival to the latter, the patient was unresponsive, had metabolic acidosis, tachycardia and hypotension, and hypothermia. Physical examination showed the patient to be unresponsive, areflexic with unreactive dilated pupils, and having an altered electrocardiogram (ECG). Blood samples collected <1 hour after poisoning showed DEET concentrations ranging from 8.7 to 10.2 mg/dL; urine samples contained an average of 0.64 mg DEET/dL. Over the next 3 days, the patient remained unresponsive. On the 3rd day, tests revealed no cerebral blood flow, little brain electrical activity, cerebral edema, and transtentorial and tonsillar herniations, and the patient was declared brain dead.

WHO identified an LD_{50} of 2,000 mg/kg for male rats (WHO 1987). An early study determined oral LD_{50} values of 1.8–2.7 and 1.75–1.8 mL/kg (1,793–2,689 and 1,743–1,793 mg/kg based on a specific gravity of 0.996 for DEET) with a central range of 1.83–2.19 mL/kg for 90% DEET in cottonseed oil in male and female albino rats, respectively; the observation period was 7 days (Ambrose 1959). Clinical signs included hyperemia at the base of the ears, lacrimation, chromodacryorrhea, depression, prostration, tremors, and asphyxial convulsions. Respiratory failure usually preceded cardiac failure. Gross examination showed questionable degrees of hyperemia of the gastrointestinal tract. An oral LD_{50} of 3,664 mg/kg was estimated in male Sprague-Dawley rats administered technical-grade DEET in propylene glycol (McCain et al. 1997). Seven out of 10 rats administered 5,010 mg/kg, the highest dose tested, died during the 14-day observation period, whereas only one rat died following administration of 2,000 mg/kg, the lowest dose tested (McCain et al. 1997). EPA (1998c) reported that the LD_{50} in rats (strain not specified) varied from 2,170 to 3,664 mg/kg. A study that examined the effect of age on the acute toxicity of DEET reported oral LD_{50} values of 3,564 and 3,429 mg/kg in adult male and female Wistar rats, respectively (Verschoyle et al. 1992); the respective LD_{50} values in 11-day-old rats were 891 and 667 mg/kg, indicating a 4–5-fold increased sensitivity in the young rats relative to the older rats.

The LC_{50} in rats from the Army (1980a) study is presented in Table 3-1 and plotted in Figure 3-1.

3.2.2.2 Systemic Effects

No studies were located regarding respiratory, musculoskeletal, endocrine, dermal, ocular, or body weight effects in humans following oral exposure to DEET.

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

Table 3-2 Levels of Significant Exposure to DEET - 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 (albino)	once (GO)				1793 M (LD50) 1743 F (LD50)	Ambrose 1959	
2	Rat (Wistar)	once (G)				2669 M (LD50)	Carpenter et al. 1974	
3	Rat (Sprague-Dawley)	once (G)				3664 M (LD50)	McCain et al. 1997	
4	Rat (Wistar)	once (GO)				3564 M (LD50 in adults) 891 M (LD50 in 11-day-old) 3429 F (LD50 in adults) 667 F (LD50 in 11-day-old)	Verschoyle et al. 1992	
Systemic								
5	Rat (CD)	once (G)	Bd Wt	500			Schoenig et al. 1993	
6	Rat (CD)	10 d Gd 6-15 1 x/d (G)	Bd Wt	250 F		750 F (35% reduced weight gain)	Schoenig et al. 1994	
7	Rabbit (New Zealand)	13 d Gd 6-18 1 x/d (G)	Bd Wt	100 F		325 F (69% reduced weight gain)	Schoenig et al. 1994	

Table 3-2 Levels of Significant Exposure to DEET - 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
Neurological								
8	Rat (Sprague-Dawley)	once (G)		500			Hoy et al. 2000a	NOAEL is for locomotor activity.
9	Rat (Sprague-Dawley)	7 d 1 x/d (G)		200			Hoy et al. 2000b	NOAEL is for locomotor activity and thigmotaxis.
10	Rat (CD)	once (G)		500			Schoenig et al. 1993	NOAEL is for neurobehavioral effects.
11	Rat (CD)	10 d Gd 6-15 1 x/d (G)				750 F (hypoactivity, ataxia, decreased muscle tone)	Schoenig et al. 1994	Neurological signs occurred during dosing period.
12	Rat (Wistar)	once (GO)			1000 (vacuolization of myelin sheath in cerebellum)		Verschoyle et al. 1992	
Developmental								
13	Rat (CD)	10 d Gd 6-15 1 x/d (G)		250 F	750 F (6% reduced fetal weight)		Schoenig et al. 1994	Weight gain significantly reduced in dams.
14	Rabbit (New Zealand)	13 d Gd 6-18 1 x/d (G)		325 F			Schoenig et al. 1994	NOAEL is for fetotoxicity and teratogenicity.

Table 3-2 Levels of Significant Exposure to DEET - 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
INTERMEDIATE EXPOSURE								
Systemic								
15	Rat (albino)	200 d ad lib (F)	Resp	863 F			Ambrose 1959	NOAELs are for organ histopathology.
			Cardio	863 F				
			Gastro	863 F				
			Hepatic	863 F				
			Renal	863 F				
			Endocr	863 F				
			Bd Wt	397 F	863 F (11.1% reduced terminal body weight)			
16	Rat (Sprague-Dawley)	80 d ad lib (F)	Renal		25 M (hyaline nephropathy)		EPA 1989	
17	Rat (CD)	9 mo ad lib (F)	Bd Wt	200 M	500 M (14% reduced final body weight)		Schoenig et al. 1993	

Table 3-2 Levels of Significant Exposure to DEET - 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
18	Hamster (Golden Syrian)	90 d ad lib (F)	Resp	940			EPA 1990b	NOAELs are for organ histopathology.
			Cardio	940				
			Hemato	940				
			Hepatic	940				
			Renal	940				
			Endocr	940				
			Bd Wt	305	624 M (12.7% reduced terminal body weight)			
			Metab	624	940 (10-16% increased serum potassium)			

Table 3-2 Levels of Significant Exposure to DEET - 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
19	Dog (Beagle)	52 wk 2 x/d (C)	Resp	400			Schoenig et al. 1999	NOAELs are for organ and tissue histopathology.
			Cardio	400				
			Gastro	400				
			Hemato	100	400 F (decreased hemoglobin and hematocrit; increased platelets)			
			Musc/skel	400				
			Hepatic	100 M	400 M (decreased serum alkaline phosphatase; decreased cholesterol)			
			Renal	400				
			Endocr	400				
			Dermal	400				
			Ocular	400				
			Bd Wt	100	400 (>10% reduced terminal body weight)			
			Metab	100 M	400 M (23% increased serum potassium)			

Table 3-2 Levels of Significant Exposure to DEET - 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
20	Rabbit (New Zealand)	15 d 1 x/d (G)	Resp	528 M			Army 1980b	
			Cardio	528 M				
			Gastro	528 M				
			Musc/skel	528 M				
			Hepatic	264 M	528 M (fatty change in hepatocytes)			
			Renal	528 M				
			Endocr	528 M				
			Dermal	528 M				
			Ocular	528 M				
			Bd Wt	264 M		528 M (22% body weight loss)		
			Metab	264 M	528 M (14% decrease in serum calcium)			
Immuno/ Lymphoret								
21	Rat (albino)	200 d ad lib (F)		863 F			Ambrose 1959	NOAEL is for spleen histopathology.
22	Hamster (Golden Syrian)	90 d ad lib (F)		940			EPA 1990b	NOAEL is for histopathology of lymphoreticular organs.

Table 3-2 Levels of Significant Exposure to DEET - 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
23	Dog (Beagle)	52 wk 2 x/d (C)		400			Schoenig et al. 1999	NOAEL is for histopathology of lymphoreticular organs.
24	Rabbit (New Zealand)	15 d 1 x/d (G)		528 M			Army 1980b	NOAEL is for histopathology of lymphoreticular organs.
Neurological								
25	Rat (albino)	200 d ad lib (F)		863 F			Ambrose 1959	NOAEL is for brain histopathology.
26	Rat (CD)	9 mo ad lib (F)		200	500 (transient increase in motor activity)		Schoenig et al. 1993	Effects were considered of questionable biological significance.
27	Hamster (Golden Syrian)	90 d ad lib (F)		940			EPA 1990b	NOAEL is for histopathology of the brain.
28	Dog (Beagle)	52 wk 2 x/d (C)		400			Schoenig et al. 1999	1 in 8 dogs showed DEET-related tremors during the study.
29	Rabbit (New Zealand)	15 d 1 x/d (G)		528 M			Army 1980b	NOAEL is for histopathology of the brain.

Table 3-2 Levels of Significant Exposure to DEET - 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								
30	Rat (albino)	200 d ad lib (F)		701 M 863 F			Ambrose 1959	NOAEL is for histopathology of reproductive organs.
31	Rat (Sprague-Dawley)	80 d ad lib (F)		250			EPA 1989	NOAEL is for fertility.
32	Hamster (Golden Syrian)	90 d ad lib (F)		305 M		624 M (tubular degeneration in testes)	EPA 1990b	Fertility was not tested.
33	Dog (Beagle)	52 wk 2 x/d (C)		400			Schoenig et al. 1999	NOAEL is for histopathology of the reproductive organs.
34	Rabbit (New Zealand)	15 d 1 x/d (G)		528 M			Army 1980b	NOAEL is for histopathology of the testes.
Developmental								
35	Rat (Sprague-Dawley)	80 d ad lib (F)		100[b]	250 (reduced F1 and F2 pup weights during lactation)		EPA 1989	

Table 3-2 Levels of Significant Exposure to DEET - 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								
Systemic								
36	Rat (CD)	104 wk ad lib (F)	Resp	400 F			Schoenig et al. 1999	Highest dose in males was 100 mg/kg/day; no effects were reported in males.
			Cardio	400 F				
			Gastro	400 F				
			Hemato	400 F				
			Musc/skel	400 F				
			Hepatic	100	400 F (increased serum cholesterol)			
			Renal	400 F				
			Endocr	400 F				
			Dermal	400 F				
			Ocular	400 F				
			Bd Wt	100	400 F (>10% reduced terminal body weight)			
			Metab	400				

Table 3-2 Levels of Significant Exposure to DEET - 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	Mouse (CD-1)	78 wk ad lib (F)	Resp	1000			Schoenig et al. 1999	NOAELs are for tissues and organs histopathology.
			Cardio	1000				
			Gastro	1000				
			Hemato	1000				
			Musc/skel	1000				
			Hepatic	1000				
			Renal	1000				
			Endocr	1000				
			Dermal	1000				
			Bd Wt	500	1000 (>10% reduced terminal body weight)			
Immuno/ Lymphoret								
38	Rat (CD)	104 wk ad lib (F)		100 M 400 F			Schoenig et al. 1999	NOAELs are for histopathology of lymphoreticular organs.
39	Mouse (CD-1)	78 wk ad lib (F)		1000			Schoenig et al. 1999	NOAEL is for histopathology of lymphoreticular organs.

Table 3-2 Levels of Significant Exposure to DEET - 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
Neurological								
40	Rat (CD)	104 wk ad lib (F)		100 M 400 F			Schoenig et al. 1999	NOAELs are for histopathology of brain and sciatic nerve.
41	Mouse (CD-1)	78 wk ad lib (F)		1000			Schoenig et al. 1999	NOAEL is for histopathology of nervous system tissues.
Reproductive								
42	Rat (CD)	104 wk ad lib (F)		100 M 400 F			Schoenig et al. 1999	NOAELs are for histopathology of reproductive organs.
43	Mouse (CD-1)	78 wk ad lib (F)		1000			Schoenig et al. 1999	NOAEL is for histopathology of reproductive organs.

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

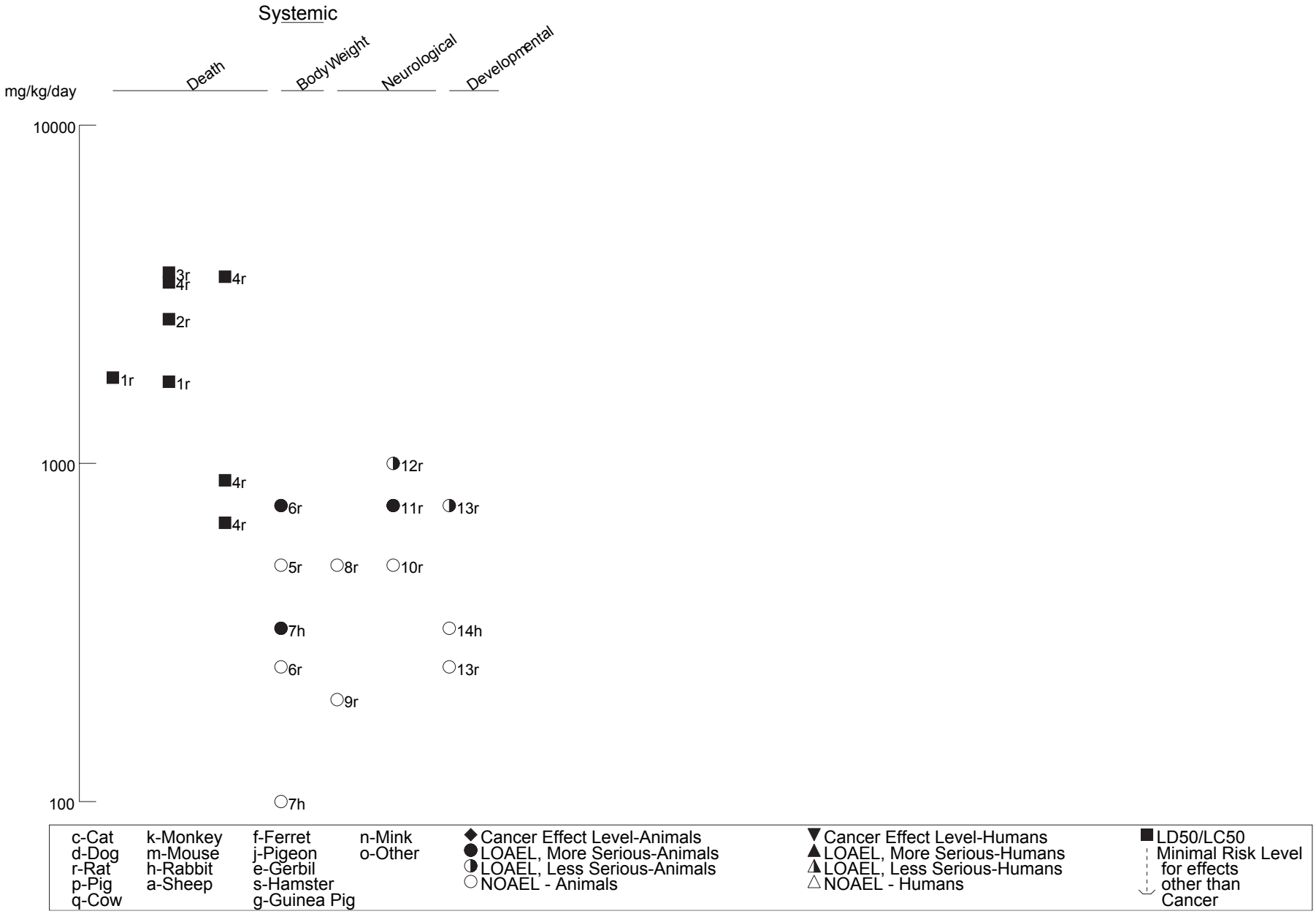
b Used to derive an intermediate-duration oral minimal risk level (MRL) of 1.0 mg/kg/day for DEET; the MRL was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; d = day(s); Endocr = endocrine; (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolic; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; x = time(s); wk = week(s)

3. HEALTH EFFECTS

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

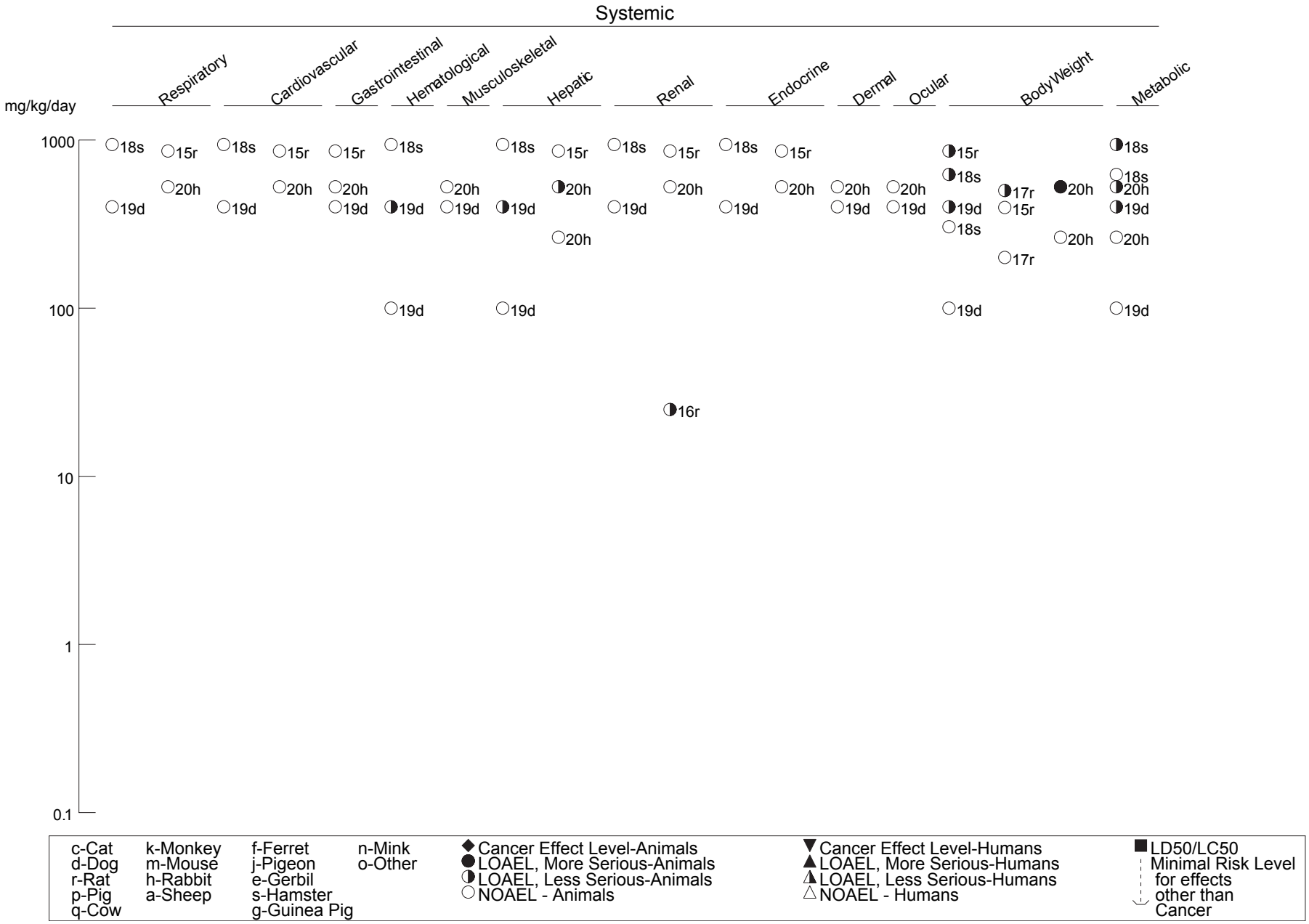
Acute (≤14 days)



3. HEALTH EFFECTS

Figure 3-2 Levels of Significant Exposure to DEET - Oral *(Continued)*

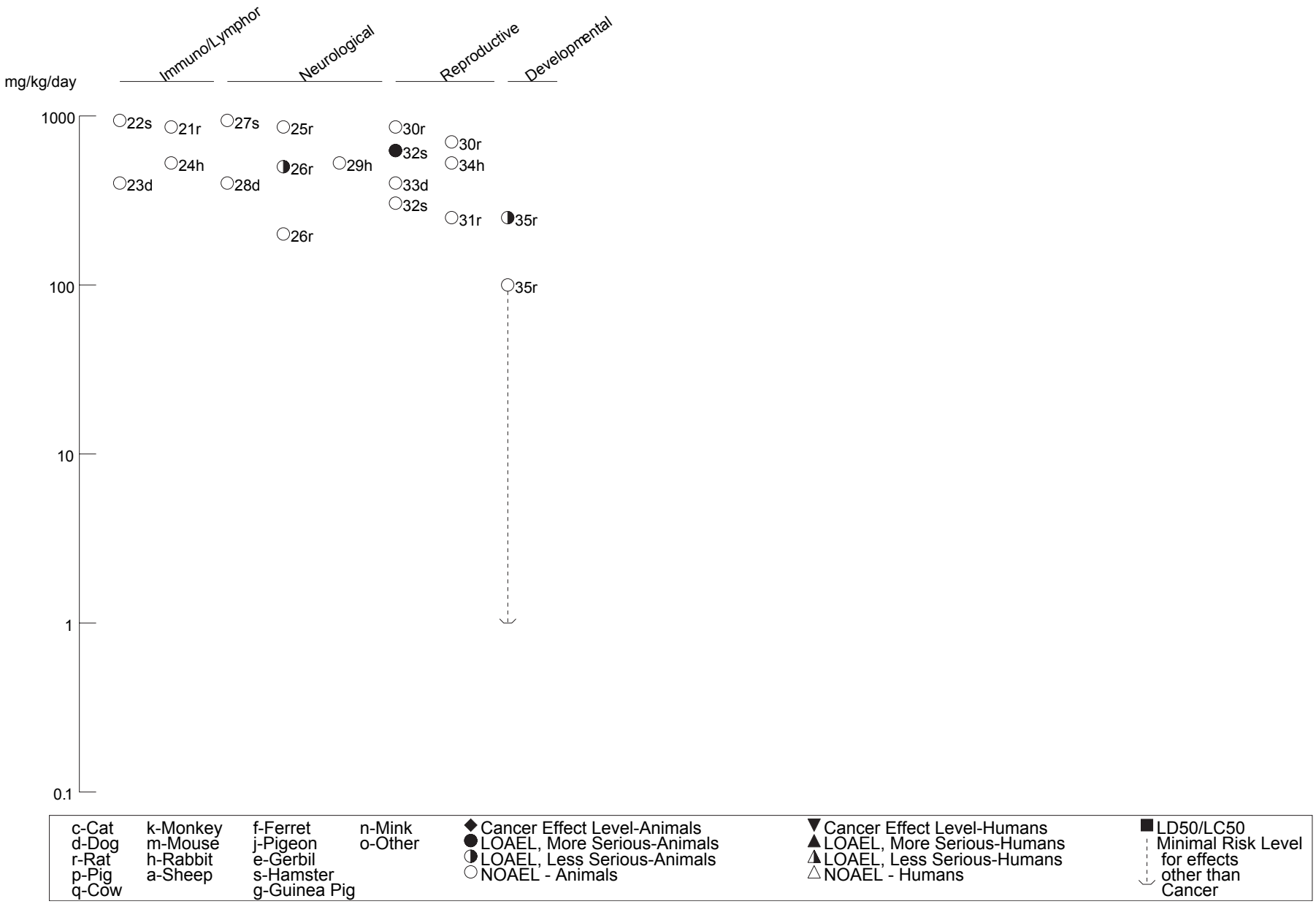
Intermediate (15-364 days)



3. HEALTH EFFECTS

Figure 3-2 Levels of Significant Exposure to DEET - Oral *(Continued)*

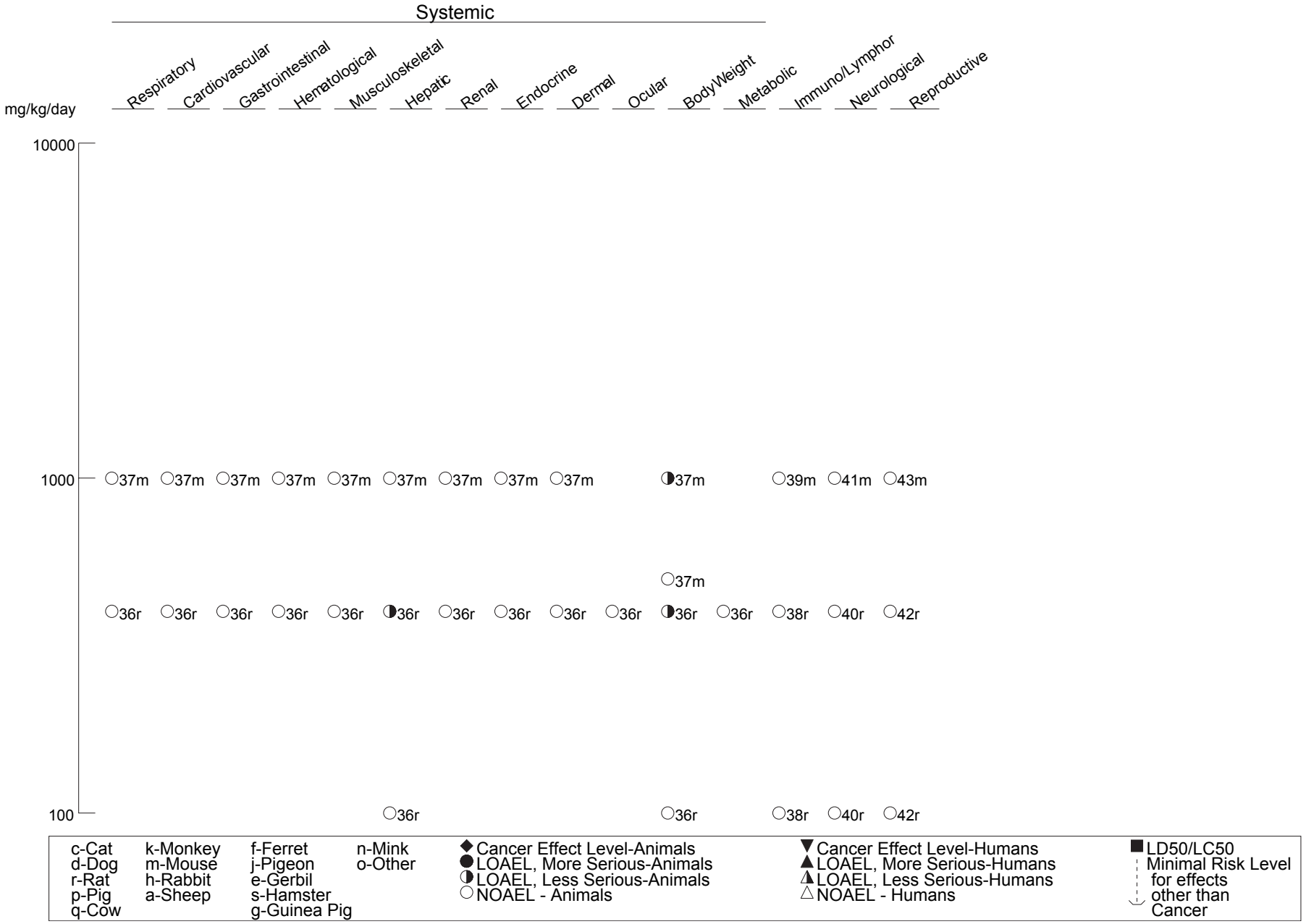
Intermediate (15-364 days)



3. HEALTH EFFECTS

Figure 3-2 Levels of Significant Exposure to DEET - Oral *(Continued)*

Chronic (≥365 days)



Respiratory Effects. No information was located regarding respiratory effects in humans following oral exposure to DEET.

Several intermediate-duration (i.e., 15 days to 52 weeks) and one chronic-duration (18 months to 2 years) oral study conducted gross and microscopic examinations of the lungs of animals (i.e., rats, mice, hamsters, dogs, rabbits) following oral exposure to DEET and did not find significant treatment-related alterations. Reported NOAELs for respiratory effects included 701 and 863 mg DEET/kg/day in male and female albino rats, respectively, treated for 200 days (Ambrose 1959), 940 mg DEET/kg/day in male and female Golden Syrian hamsters treated for 90 days (EPA 1990b), 400 mg DEET/kg/day in Beagle dogs treated for 52 weeks (Schoenig et al. 1999), and 528 mg/DEET/kg/day in New Zealand White rabbits treated for 15 days (Army 1980b).

In chronic-duration studies, the highest doses of DEET tested (100 mg/kg/day in male CD rats and 400 mg/kg/day in female CD rats treated for 2 years; 1,000 mg/kg/day in male and female CD-1 mice treated for 18 months) did not induce morphological alterations in the lungs (Schoenig et al. 1999).

Cardiovascular Effects. An abnormal ECG was reported in a 19-year-old woman 1 hour after ingesting 15–25 mL of an insect repellent containing 95% DEET (Fraser et al. 1995). The ECG indicated right and left atrial enlargement, diffuse ST-T abnormalities, and a normal QT interval. Within 24 hours, the ECG returned to baseline. An unremarkable ECG was described in a 3-year-old girl who ingested an estimated 800 mg of DEET (4 mL of a 20% solution) (Petrucci and Sardini 2000). Hypotension (blood pressure 80/50 mm Hg) was reported in a 33-year-old woman approximately 1 hour after ingesting an unknown amount of an insect repellent containing 95% DEET along with presumably excessive amounts of prescription chlorpromazine hydrochloride and hydralazine hydrochloride (Tenenbein 1987). The same investigator reported hypotension in a 16-year-old girl (blood pressure 90/50 mm Hg) and in a 14-year-old girl (systolic 60 mm Hg) following ingestion of DEET. Tachycardia, hypotension, and altered ECG were reported in a 37-year-old man who ingested 6 ounces of a repellent containing 40% DEET (approximately 748 mg DEET/kg) (Wiles et al. 2014). No further relevant information was located regarding cardiovascular effects in humans following oral exposure to DEET.

No treatment-related gross or microscopic alterations in the heart were reported in intermediate- and chronic-duration oral studies in albino or CD rats (Ambrose 1959; Schoenig et al.1999), Golden Syrian hamsters (EPA 1990b), beagle dogs (Schoenig et al. 1999), New Zealand White rabbits (Army 1980b),

and CD-1 mice (Schoenig et al. 1999). The NOAELs were the highest doses tested and were the same as indicated above for Respiratory Effects.

Gastrointestinal Effects. In a study of 20,764 human exposures involving insect repellents containing DEET that were reported to poison control centers from 1993 to 1997, 10,748 were identified as being exposed predominantly by ingestion (Bell et al. 2002). Of these, 770 exhibited gastrointestinal effects that included stomach irritation, vomiting, and nausea.

Albino rats treated with DEET in the diet (701 mg/kg/day in males, 863 mg/kg/day in females) for 200 days did not show treatment-related gross or microscopic alterations in the stomach or small intestine (Ambrose 1959). Similar findings were reported regarding the gastrointestinal tract of Beagle dogs dosed with 400 mg DEET/kg/day for 52 weeks (Schoenig et al. 1999), and New Zealand White rabbits dosed with 528 mg DEET/kg/day for 15 days (Army 1980b). The same was reported in CD rats dosed with DEET for 104 weeks (100 mg/kg/day in males, 400 mg/kg/day in females) and CD-1 mice dosed with 1,000 mg DEET/kg/day for 78 weeks (Schoenig et al. 1999).

Hematological Effects. The leukocyte count reported in a 3-year-old girl who ingested an estimated 800 mg DEET (4 mL of a 20% insect repellent solution) was within normal limits (Petrucci and Sardini 2000). No explicit statements regarding hematology tests were provided in other cases of acute intoxication with DEET that were reviewed.

Hematological tests conducted in Golden Syrian hamsters after 90 days of dosing with up to 940 mg DEET/kg/day showed sporadic inconsistent changes in certain parameters without dose-response and were not considered treatment-related by the investigators (EPA 1990b). A 52-week oral study in Beagle dogs reported that doses of 400 mg DEET/kg/day induced significant reductions in hemoglobin and hematocrit in males and females after 6 and 12 months of dosing and significant increases in platelets in females (Schoenig et al. 1999). Hematological tests were conducted in CD rats and CD-1 mice during chronic exposure to DEET (100 mg/kg/day in male rats; 400 mg/kg/day in female rats; 1,000 mg/kg/day in mice) and did not show report treatment-related hematological alterations (Schoenig et al. 1999).

Musculoskeletal Effects. Skeletal muscle and bone were examined in intermediate-duration studies in Beagle dogs (Schoenig et al. 1999) and New Zealand White rabbits (Army 1980b) and in chronic-duration studies in CD rats and CD-1 mice (Schoenig et al. 1999). None of these studies found treatment-related gross or microscopic alterations in bone or muscle. NOAELs for musculoskeletal effects were

400 mg/kg/day in dogs, 528 mg/kg/day in rabbits, 100 and 400 mg/kg/day in male and female rats, respectively, and 1,000 mg/kg/day in mice.

Hepatic Effects. Of the few reported cases of ingestion of DEET by humans, only a report by Petrucci and Sardini (2000) explicitly indicated that liver function studies were conducted and were unremarkable in a 3-year-old girl who ingested an estimated 800 mg DEET. No explicit statements regarding liver function tests were provided in other cases of acute intoxication with DEET that were reviewed.

No significant changes in clinical chemistry parameters or in gross or microscopic appearance of the liver were reported in Golden Syrian hamsters dosed with up 940 mg DEET/kg/day for 90 days (EPA 1990b). In a 52-week study in male Beagle dogs treated with 400 mg DEET/kg/day, a significant increase in serum alkaline phosphatase activity (49% at 6 months) and a significant reduction in serum cholesterol (37% at 6 months and 35% at 12 months were observed) (Schoenig et al. 1999). No significant changes occurred in dogs dosed with 100 mg DEET/kg/day. Gross and microscopic examination of the dogs' liver did not show treatment-related alterations. New Zealand White rabbits treated with 528 mg DEET/kg/day for 15 days showed changes consisting of rare to minimal fatty change in hepatocytes (Army 1980b). These changes were seen primarily midzonal, but clear vacuolated hepatocytes were also seen in central and portal areas. Clinical chemistry tests showed significant increases in serum cholesterol and triglycerides (about 4-fold each). The NOAEL for liver effects was 264 mg DEET/kg/day. A chronic-duration dietary study in CD rats also reported significant increases in serum cholesterol (2–4-fold) during the study in females dosed with 400 mg DEET/kg/day, but not 100 mg DEET/kg/day (Schoenig et al. 1999). In the chronic study there were no gross or microscopic lesions in the liver attributable to treatment with DEET.

Renal Effects. Petrucci and Sardini (2000) mentioned that creatinine levels were within normal limits in a 3-year-old girl who ingested an estimated 800 mg of DEET. No further explicit information regarding renal effects in humans following oral exposure to DEET was located.

In an early dietary study in albino rats, examination of the kidneys of the animals treated with the highest doses (701 mg/kg/day in males and 863 mg/kg/day in females) for 200 days only showed increased relative weight of the organs (12%) (Ambrose 1959). There were no treatment-related gross or microscopic alterations in the kidney that were not seen in control rats. Interestingly, in a 2-generation reproductive study in Sprague-Dawley rats, hyaline nephropathy was reported in adult F1 males from all

treated groups (doses of DEET mixed in the food were 0, 25, 100, or 250 mg/kg/day) (EPA 1989). F1 males had been produced by F0 females that had been dosed with DEET for at least 80 days before mating and presumably during gestation and lactation. F1 males were therefore exposed *in utero* and then directly for at least 93 days. The kidneys from F0 males were not examined microscopically because they did not show gross alterations (EPA 1989). Other intermediate-duration studies did not observe adverse kidney effects in Golden Syrian hamsters dosed with up to 940 mg DEET/kg/day for 90 days (EPA 1990b), Beagle dogs dosed with up to 400 mg DEET/kg/day for 52 weeks (Schoenig et al. 1999), or New Zealand White rabbits dosed with up to 528 mg DEET/kg/day for 15 days (Army 1990).

Chronic-duration studies in rats and mice did not report treatment-related kidney lesions at termination, but did report relative high incidences of chronic progressive nephropathy in male and female CD rats and chronic nephritis in male and female CD-1 mice, which also occurred in control groups and were considered unrelated to the test material (Schoenig et al. 1999). In these studies, male and female rats were dosed with up to 100 and 400 mg DEET/kg/day, respectively, for 104 weeks and male and female mice were dosed with up to 1,000 mg DEET/kg/day for 78 weeks.

Endocrine Effects. Several intermediate-duration and one chronic-duration oral study conducted gross and microscopic examinations of endocrine glands of animals following oral exposure to DEET and did not find significant treatment-related alterations. Glands examined included the adrenals, pituitary, thyroid, and parathyroid. Reported NOAELs included 701 and 863 mg DEET/kg/day in male and female albino rats, respectively, treated for 200 days (Ambrose 1959); 940 mg DEET/kg/day in male and female Golden Syrian hamsters treated for 90 days (EPA 1990b); 400 mg DEET/kg/day in Beagle dogs treated for 52 weeks (Schoenig et al. 1999); and 528 mg/DEET/kg/day in New Zealand White rabbits treated for 15 days (Army 1980b).

In chronic-duration studies, the highest doses of DEET tested (100 mg/kg/day in CD male rats; 400 mg/kg/day in CD female rats; 1,000 mg/kg/day in male and female CD-1 mice) did not induce morphological alterations in adrenals, thyroid, or pituitary glands (Schoenig et al. 1999).

Dermal Effects. No treatment-related skin alterations were reported in Beagle dogs dosed with up to 400 mg DEET/kg/day for 52 weeks (Schoenig et al. 1999) or in New Zealand White rabbits dosed with up to 528 mg DEET/kg/day for 15 days (Army 1980b). Similar observations were made regarding the skin of CD-1 mice dosed with up to 1,000 mg DEET/kg/day for 78 weeks or male and female CD rats dosed with up to 100 or 400 mg DEET/kg/day, respectively, for 104 weeks (Schoenig et al. 1999).

Ocular Effects. Lacrimation and chromodacryorrhea were reported in rats administered lethal doses of DEET by gavage (Ambrose 1959). Three studies provided additional data regarding ocular effects in animals exposed orally to DEET. No significant morphological alterations were seen in the eyes of Beagle dogs dosed with up to 400 mg DEET/kg/day for 52 weeks (Schoenig et al. 1999), New Zealand White rabbits dosed with up to 528 mg DEET/kg/day for 15 days (Army 1980b), or male and female CD rats dosed with up to 100 or 400 mg DEET/kg/day, respectively, for 104 weeks (Schoenig et al. 1999).

Body Weight Effects. Several studies provide information regarding body weight effects in animals after oral exposure to DEET; not all of the studies, however, provided data on food consumption. In general, reductions in body weight gain relative to controls were associated with reductions in food consumption. In acute-duration studies, Schoenig et al. (1993) reported that a single dose of up to 500 mg DEET/kg did not affect body weight or food consumption in CD rats over a 14-day observation period.

Administration of 750 mg DEET/kg/day to pregnant CD rats on GDs 6–15 or 325 mg DEET/kg/day to pregnant New Zealand White rabbits on GDs 6–18, however, reduced maternal weight gain by 35 and 69%, respectively (Schoenig et al. 1994); the corresponding NOAELs were 250 and 100 mg/kg/day. In both cases, food consumption was reduced.

In intermediate-duration oral studies, significant reductions in terminal body weight (≥10% differences with controls) were seen at DEET doses of 701 mg/kg/day in albino rats (Ambrose 1959), 500 mg/kg/day in CD rats (Schoenig et al. 1993), 624 mg/kg/day in Golden Syrian hamsters (EPA 1990b), and 400 mg/kg/day in Beagle dogs (Schoenig et al. 1999). In these studies, DEET was administered via the food, except in dogs, which were treated with DEET in capsules. In a 15-day study in male New Zealand White rabbits in which DEET was administered by gavage, doses of 528 mg DEET/kg/day caused a 22% reduction in body weight (rabbits lost weight) (Army 1980b); body weight showed a rapid and linear decrease throughout the study with no indication of reversal. No significant effects appeared to occur at 264 mg/kg/day, while the 132 mg/kg/day animals retained a higher portion of initial body weight compared to controls, and by day 12, diverged positively from controls. No data on food consumption were provided in this study. In the 2-generation reproductive study in Sprague-Dawley rats (EPA 1989), decreased body weight was reported in adult F0 and F1 males and females at various time points during the study and occurred mainly in rats in the 100 and 250 mg/kg/day dose groups. Some of differences with the control groups were statistically significant, but only in one case (high-dose F1 females at 18 weeks of age) was the difference with controls >10% (15.4%). Significant decreases in food

consumption relative to controls were also reported at various times. Only in adult F1 high-dose females was reduced food consumption >10% (15 and 14% at 12 and 16 weeks of age, respectively).

In chronic-duration studies, doses of 400 mg DEET/kg/day reduced terminal body weight in female CD rats by 10% and a similar effect was reported in CD-1 mice dosed with 1,000 mg DEET/kg/day (Schoenig et al. 1999); the corresponding NOAELs were 100 and 500 mg/kg/day.

Metabolic Effects. Serum electrolytes within normal limits were reported in an 18-month-old child who had ingested an unknown, but probably small, amount of an insect repellent containing DEET the day prior to being admitted to the hospital (Zadikoff 1979). Petrucci and Sardini (2000) also reported electrolytes and glucose within normal limits in a 3-year-old girl who ingested an estimated 800 mg of DEET. Metabolic acidosis was reported in a 37-year-old man who ingested 6 ounces of a repellent containing 40% DEET (approximately 748 mg DEET/kg) (Wiles et al. 2014). No further relevant information was located regarding metabolic effects in humans after oral exposure to DEET.

A few alterations in serum electrolytes were reported in studies in animals. Increases in serum potassium of 10 and 16% were reported in male and female Golden Syrian hamsters, respectively, following doses of 940 mg DEET/kg/day for 90 days (EPA 1990b); no significant changes were reported at ≤624 mg DEET/kg/day. Serum potassium was also significantly increased (23%) in male Beagle dogs after receiving doses of 400 mg DEET/kg/day for 6 months; no significant alterations were reported in dogs dosed with ≤100 mg DEET/kg/day or in dogs dosed with 400 mg DEET/kg/day for 12 months (Schoenig et al. 1999). In a study in male New Zealand White rabbits, serum calcium was significantly decreased (14%) following dosing with 528 mg DEET/kg/day, but not 264 mg/kg/day, for 15 days (Army 1980b). No significant alterations in serum electrolytes or glucose were reported in a chronic-duration study in CD rats (Schoenig et al. 1999); the highest doses tested were 100 mg DEET/kg/day in males and 400 mg/kg/day in females.

3.2.2.3 Immunological and Lymphoreticular Effects

No information was located regarding immunological effects in humans following oral exposure to DEET.

No relevant information was located regarding effects in acute-duration studies in animals. Intermediate-duration studies did not find gross or microscopic lesions in lymphoreticular organs of Beagle dogs

(Schoenig et al. 1999), albino rats (Ambrose 1959), Golden Syrian hamsters (EPA 1990b), or New Zealand White rabbits (Army 1980b) exposed to doses of DEET ranging from 400 to 863 mg/kg/day. Similar results were reported in a chronic-duration study in CD rats dosed with up to 400 mg/kg/day DEET or CD-1mice dosed with up to 1,000 mg/kg/day DEET (Schoenig et al. 1999). None of these studies, however, conducted tests to examine immunocompetence.

The highest NOAEL values for effects on lymphoreticular organs in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

All of the reported cases of acute oral intoxication with insect repellents containing DEET reported adverse neurological effects in the patients described by some as toxic encephalopathy. Opisthotonic episodes followed by generalized seizures with clonic movement of the facial muscles were described in a 3-year-old girl who ingested an estimated 800 mg of DEET (Petrucci and Sardini 2000). Opisthotonus is a postural abnormality characterized by hyperextension of the back and neck muscles, with retraction of the head, and arching forward of the trunk. Edwards and Johnson (1987) described a similar case of a child who developed toxic encephalopathy after ingesting an unknown amount of a product containing 10% DEET. The five cases of oral ingestion of DEET described by Tenenbein (1987) showed neurological signs within several hours of ingestion of the chemical including a hypertonic condition with Babinski signs, tremors, seizures, opisthotonic spells, and coma. Opisthotonic posture and bizarre movements were also described in a young child who ingested a small amount of an insect repellent containing DEET (Zadikoff 1979). Wiles et al. (2014) reported that a man suffered a seizure within minutes of ingesting 6 ounces of a repellent containing 40% DEET (approximately 748 mg DEET/kg) and was unresponsive and areflexic over the next 3 days before being declared brain dead.

Oral studies in animals have examined neurobehavioral parameters as well as the gross and microscopic morphology of tissues of the nervous system following exposure to DEET. An acute-duration study that performed a functional observational battery (FOB) and a motor activity test in CD rats reported a decrease in vertical activity and delayed response to thermal stimuli following a single dose of 500 mg/kg, the highest dose tested; the NOAEL was 200 mg/kg (Schoenig et al. 1993). A similar study in Sprague-Dawley rats, however, did not report significant alterations in locomotor activity and thigmotaxis (response to touch) following a dose of 500 mg DEET/kg (Hoy et al. 2000a). In a study aimed at determining oral LD_{50} values for DEET in Wistar rats, no clinical signs were seen in rats treated

with single doses of <1,500 mg DEET/kg in arachis oil (Verschoyle et al. 1992). Doses between 2,000 and 3,000 mg/kg, however, decreased reactivity and muscle tone. Central nervous system depression was occasionally interrupted by seizures; mostly qualitative data were presented in this study. Spikes in the electroencephalogram (EEG) arising from the auditory cortex were recorded in rats with implanted electrodes. In rats given single doses of 1,000–3,000 mg DEET/kg, light microscopy showed histological changes in the brain consisting of vacuolization of myelin sheaths mainly in cerebellar roof nuclei. Axons usually appeared normal. Also seen were single or multiple, clear cytoplasmic clefts in neurons diffusely distributed throughout the brain. Rats with these lesions usually were severely prostrated or ataxic. Electron microscopy showed extensive edematous swelling of the inner loop of the myelin sheaths and splitting of the innermost myelin lamellae occurring at the intraperiod line.

In intermediate-duration studies, dietary treatment of albino rats with up to 863 mg DEET/kg/day for 200 days did not induce gross or microscopic alterations in the brain (Ambrose 1959). A similar lack of effects was reported in multiple tissues of the central and peripheral nervous tissue from CD rats following dietary doses of up to 500 mg DEET/kg/day for 9 months, but this dietary level of DEET induced transient increases in motor activity (Schoenig et al. 1993). Golden Syrian hamsters dosed with up to 940 mg DEET/kg/day for 90 days (EPA 1990b), New Zealand white rabbits dosed with up to 528 mg DEET/kg/day for 15 days (Army 1980b), or Beagle dogs dosed with up to 400 mg DEET/kg/day for 52 weeks (Schoenig et al. 1999) did not show gross or microscopic alterations in tissues of the nervous system. In the study in dogs, doses of 400 mg DEET/kg/day induced occasional tremors in some dogs as well as excessive salivation.

In chronic-duration studies, dietary doses of up 100 mg DEET/kg/day in male CD rats, 400 mg DEET/kg/day in female CD rats, or 1,000 mg DEET/kg/day in CD-1 mice did not induce morphological alterations in central or peripheral nervous tissues (Schoenig et al. 1999).

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

3.2.2.5 Reproductive Effects

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

Intermediate- and chronic-duration studies provide information regarding reproductive effects in animals after oral exposure to DEET. In a 2-generation continuous feeding study in Sprague-Dawley rats, fertility was not affected by treatment with up to approximately 250 mg DEET/kg/day (EPA 1989). In addition, gross and microscopic examination of the reproductive organs of the F0 generation and F1 weanlings did not show morphological alterations. In an earlier study, treatment of male and female albino rats with up to 701 or 863 mg DEET/kg/day, respectively, in the diet for 200 days did not induce gross or microscopic changes in the reproductive organs (Ambrose 1959). A 52-week study in male and female Beagle dogs dosed with up to 400 mg DEET/kg/day via capsules (Schoenig et al. 1999) or a 15-day study in male New Zealand White rabbits treated with up to 528 mg/DEET/kg/day (Army 1980b) also did not observe morphological alterations in the animals' reproductive organs. A 90-day dietary study in male and female Golden Syrian hamsters, however, reported an increased incidence of tubular degeneration in the testes and accumulation of cellular debris in the lumens of the epididymides from males dosed with ≥624 mg DEET/kg/day; the NOAEL was 305 mg DEET/kg/day (EPA 1990b). No significant alterations were observed in females.

Chronic-duration studies did not report gross or microscopic alteration in the reproductive organs from male CD rats dosed with up to 100 mg DEET/kg/day, female CD rats dosed with up to 400 mg DEET/kg/day, or male and female CD-1 mice dosed with up to 1,000 mg DEET/kg/day (Schoenig et al. 1999).

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

3.2.2.6 Developmental Effects

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

Limited data are available in animals. Gavage administration of 750 mg DEET/kg to pregnant CD rats on GDs 6–15 resulted in a 6% reduction in fetal weight measured at sacrifice on GD 21 (Schoenig et al. 1994). The NOAEL was 250 mg DEET/kg/day (Schoenig et al. 1994). It should be noted that the 750 mg/kg/day dose level induced neurological signs in the dams during treatment as well as a significant (35%) reduction in maternal weight gain relative to controls. Examination of the fetuses did not show treatment-related increases in external, visceral, or skeletal variations or malformations. In the same study, gavage administration of up to 325 mg DEET/kg to pregnant New Zealand White rabbits on

GDs 6–18 did not result in embryotoxic or teratogenic effects in the offspring, despite the fact that maternal weight gain was reduced by about 69% during treatment.

In a 2-generation continuous feeding study in Sprague-Dawley rats that included exposure for at least 80 days before mating, treatment of the F0 generation and later of the F1 generation with 250 mg DEET/kg/day resulted in significantly reduced (>10%) F1 and F2 pup weights on lactation days 14 and 21 (EPA 1989). No significant differences with controls were observed at 100 mg DEET/kg/day compared to controls. In addition, F1 males from all treated groups (25, 100, and 250 mg/kg/day) showed a dose-related increased incidence of gross and microscopic lesions in the kidneys. The lesions included inflammation, hyaline droplet and granular cast formation, and regeneration of tubules. The reduction in pup weights was used to derive an intermediate-duration oral MRL for DEET.

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

3.2.2.7 Cancer

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

One publication of three studies was located that examined the potential carcinogenicity of DEET in animals following oral exposure, and negative results were reported in the three species tested (dogs, rats, and mice) (Schoenig et al. 1999). Male and female Beagle dogs were dosed by capsule with up to 400 mg DEET/kg/day for 52 weeks; male CD rats were dosed with up to 100 mg/kg/day and female CD rats were dosed with up to 400 mg/kg/day via the diet for 104 weeks; and male and female CD-1 mice were dosed with up to 1,000 mg/kg/day via the diet for 78 weeks.

3.2.3 Dermal Exposure

3.2.3.1 Death

Five deaths have been associated with dermal exposure to DEET and three of them occurred in children. Zadikoff (1979) reported the case of a 5-year-old girl who had been sprayed nightly for almost 3 months with an insect repellent containing 10% DEET and subsequently developed progressively severe headaches starting 10 days prior to hospitalization. On admission, the child was extremely agitated, restless, and irritable with constant involuntary movements involving the head, trunk, and all limbs. This

was interrupted periodically by short episodes of quiet, shaking, crying, and screaming. Shortly thereafter, she developed a generalized convulsion and for the next 24 days, she was treated with various combinations and doses of drugs to control the hyperactivity, which eventually become intractable even with haloperidol treatment. An autopsy revealed generalized edema of the brain with intense congestion of the brain and meninges. The second case was a 6-year-old girl who in response to repeated black fly bites used a spray containing 15% DEET on at least 10 occasions on extensive areas of skin and developed a clinical picture similar to Reye syndrome or ornithine carbamoyl transferase deficiency (Heick et al. 1980). On the fifth day in the hospital, the child developed generalized convulsions followed by coma, and on the seventh and eighth day, the EEG became flat and supportive therapy was discontinued. Autopsy showed edematous brain. Based on tests, it was hypothesized that the child might have been a carrier of OCT deficiency, a potential lethal hyperammonemic condition, which may have contributed to her death. Pronczuk de Garbino et al. (1983) briefly described the case of a 17-month-old girl who was admitted to the hospital with a diagnosis of acute encephalopathy of unknown origin. During 3 weeks prior to admission, the child had received repeated applications of a lotion containing DEET. The child rapidly deteriorated and died before further toxicological information could be obtained, but DEET-induced toxicity was strongly suspected. In a study of insect repellent reports to the AAPCC, Bell et al. (2002) identified two deaths (a 26-year-old male and a 34-year-old female) following dermal exposure to >50% DEET. The male, who had applied a 52% DEET repellent liberally throughout the day, developed dyspnea, seized, vomited, unsuccessfully underwent cardio-pulmonary resuscitation, and was taken to the emergency department where he died within 2 hours of the initial seizure. Of the tissues tested, DEET levels were elevated only in the blood. Little information was available for the female, but exposure duration was reported as chronic with the only effect being an adverse dermal reaction. It should be noted that in all of these cases, excessive exposure appears to have occurred.

Limited information was located regarding lethal doses in animals exposed dermally to DEET. Carpenter et al. (1974) reported that the dermal LD_{50} in New Zealand White rabbits was 3,167 mg/kg (per Table 3-3). DEET was applied to a shaved area of the skin that was covered for 24 hours; the observation period was 14 days. EPA (1998b) indicated that the dermal LD_{50} in rabbits (strain not specified) was 4,280 mg/kg.

The LD_{50} from the Carpenter et al. (1974) is presented in Table 3-3.

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL				Reference Chemical Form	Comments
				Less Serious		Serious			
ACUTE EXPOSURE									
Death									
Rabbit (New Zealand)	24 hr					3167 M mg/kg	(LD50)	Carpenter et al. 1974	
Systemic									
Gn Pig (albino)	10 d 1 x/d	Dermal		1 mL	(slight erythema)			Ambrose 1959	10% DEET was used. DEET was not a skin sensitizer.
Rabbit (albino)	24 hr	Dermal	4000 mg/kg					Ambrose 1959	Reported erythema was due to mechanical irritation.
Rabbit (albino)	once	Ocular				0.05 mL	(severe eye irritation)	Ambrose 1959	
Rabbit (New Zealand)	once	Ocular		10 mg	(moderate eye irritation)			MacRae et al. 1984	

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL		Reference Chemical Form	Comments
				Less Serious	Serious		
INTERMEDIATE EXPOSURE							
Systemic							
Rat (CD)	13 wk 1 x/d	Resp	1000 B mg/kg/day			EPA 1988	NOAELs are for organs or tissue histopathology.
		Cardio	1000 B mg/kg/day				
		Gastro	1000 B mg/kg/day				
		Hemato	1000 B mg/kg/day				
		Musc/skel	1000 B mg/kg/day				
		Hepatic	1000 B mg/kg/day				
		Renal		100 M mg/kg/day (granular casts; inflammation, hyaline droplets)			
		Endocr	1000 B mg/kg/day				
		Dermal		100 B mg/kg/day (skin scaling; acanthosis/hyperkeratosis)			
		Ocular	1000 B mg/kg/day				
		Bd Wt	1000 B mg/kg/day				

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL		Reference Chemical Form	Comments
				Less Serious	Serious		
Rat (CD)	13 wk 1 x/d	Metab	1000 B mg/kg/day			EPA 1988	NOAELs are for organs or tissue histopathology.
Rat (CD)	90 d 5 d/wk	Renal		1000 M mg/kg/day (hyaline nephropathy)		EPA 1990a	
		Dermal		1000 M mg/kg/day (increased incidence of erythema)			
		Bd Wt	1000 M mg/kg/day				
Rat (Sprague-Dawley)	9 wk 5 d/wk	Hepatic	1000 M mg/kg/day			Lebowitz et al. 1983	Liver and kidney NOAELs are for organ weight.
		Renal	1000 M mg/kg/day				
		Bd Wt	1000 M mg/kg/day				
Rabbit (albino)	13 wk 5 d/wk	Dermal		1000 B mg/kg (skin irritation)		Ambrose 1959	
		Bd Wt	1000 B mg/kg				

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL		Reference Chemical Form	Comments
				Less Serious	Serious		
Pig Micropigs	90 d 5 d/wk	Resp	1000 B mg/kg/day			EPA 1992a	NOAELs are for organ and tissue histopathology.
		Cardio	1000 B mg/kg/day				
		Gastro	1000 B mg/kg/day				
		Hemato	1000 B mg/kg/day				
		Musc/skel	1000 B mg/kg/day				
		Hepatic	1000 B mg/kg/day				
		Renal	1000 B mg/kg/day				
		Endocr	1000 B mg/kg/day				
		Dermal		100 B mg/kg/day	(skin desquamation; hyperkeratosis)		
		Ocular	1000 B mg/kg/day				
		Bd Wt	1000 B mg/kg/day				

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL				Reference Chemical Form	Comments
				Less Serious		Serious			
Pig Micropigs	90 d 5 d/wk	Metab	1000 B mg/kg/day					EPA 1992a	NOAELs are for organ and tissue histopathology.
Immuno/ Lymphoret									
Rat (CD)	13 wk 1 x/d		1000 B mg/kg/day					EPA 1988	NOAEL is for histopathology of lymporeticular organs.
Pig Micropigs	90 d 5 d/wk		1000 B mg/kg/day					EPA 1992a	NOAEL is for histopathology of lymphoreticular tissues.
Neurological									
Rat (Sprague-Dawley)	60 d 1 x/d					40 M mg/kg/day	(diffuse neuronal cell death in brain regions)	Abdel-Rahman et al. 2001	
Rat (Sprague-Dawley)	30 d 1 x/d					40 M mg/kg/day	(neuronal degeneration in brain; impaired neurobehavior)	Abdel-Rahman et al. 2004	
Rat (Sprague-Dawley)	60 d 7 d/wk			4 M mg/kg/day	(impaired sensorimotor performance)			Abou-Donia et al. 2001a	
Rat (Sprague-Dawley)	45 d 1 x/d			40 M mg/kg/day	(impaired sensorimotor function)			Abou-Donia et al. 2001b	

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Rat (CD)	13 wk 1 x/d		1000 B mg/kg/day			EPA 1988	NOAEL is for histopathology of the brain and spinal cord.
Rat (Sprague-Dawley)	30 d 1 x/d		40 B mg/kg/day			Fediuk et al. 2010	NOAEL is for neurobehavioral function.
Pig Micropigs	90 d 5 d/wk		1000 B mg/kg/day			EPA 1992a	NOAEL is for histopathology of brain, spinal cord, and sciatic nerve.
Reproductive							
Rat (CD)	13 wk 1 x/d		1000 B mg/kg/day			EPA 1988	NOAEL is for histopathology of the reproductive organs.
Rat (Sprague-Dawley)	9 wk 5 d/wk		1000 M mg/kg/day			Lebowitz et al. 1983	NOAEL is for testes histopathology and sperm count and viability.
Pig Micropigs	90 d 5 d/wk		1000 B mg/kg/day			EPA 1992a	NOAEL is for histopathology of reproductive organs.

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL		Reference Chemical Form	Comments
				Less Serious	Serious		
CHRONIC EXPOSURE							
Systemic							
Mouse Swiss	140 wk 2 x/wk	Dermal	20 F mg			Stenback 1977	
		Bd Wt	20 F mg				
Rabbit (New Zealand)	90 wk 2 x/wk	Dermal	20 B mg			Stenback 1977	

B = both; Bd Wt = body weight; d = day(s); F = Female; Gn pig = guinea pig; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; wk = weeks(s); x = time(s)

3.2.3.2 Systemic Effects

No studies were located regarding body weight effects in humans following dermal exposure to DEET.

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

Respiratory Effects. The only information regarding respiratory effects in humans exposed to DEET is that provided by a survey of 143 workers of the Everglades National Park, Florida (NIOSH 1986). Based on the reported use of insect repellent sprays or lotions, the workers were classified as low exposure (n=44, non-users), medium exposure (n=55; <4.25 g DEET/week), or high exposure (n=44, >4.25 g DEET/week). Concentrations of DEET in the repellents used varied from 15 to 75% in the sprays and from 30 to 100% in the lotions. The survey found that complaints of chest pain or wheezing were significantly elevated ($p<0.05$) in the high-exposure group (30%) compared to the medium- (9%) or low-exposure (11%) groups. It should be noted that exposure was inferred from only survey responses, a notable weakness. Because no actual quantification was possible, the findings from this report should be interpreted with caution.

Application of up to 1,000 mg DEET/kg/day for 13 weeks onto the shaved back of micropigs or CD rats did not induce gross or microscopic alterations in the respiratory tract, although details were not reported (EPA 1988, 1992a).

Cardiovascular Effects. Hypotension and orthostatic change in blood pressure were described in a case of an adult woman after spraying herself with a DEET-containing insect repellent (Clem et al. 1993). For years she had used a product containing 14.25% DEET without adverse effects, but, that day she used a product with higher (but unspecified) percent of DEET and completely wetted most of her body with it. An ECG performed on admission showed marked sinus bradycardia (44 beats/min), but a repeat ECG performed 1 hour later showed no abnormalities. In a case report of an 18-month-old boy who was having seizures after being applied an unknown amount of an insect repellent containing 17.6% DEET, an ECG performed on arrival to a medical center was within normal limits (Briassoulis et al. 2001).

Blood pressure appeared unaffected in two case reports of death from DEET overexposure, with respective values of 110/65 mm Hg for a 5-year-old girl (Zadikoff 1979) and 108/68 mm Hg for a 6-year-old girl (Heick et al. 1980), but was slightly elevated at 140/86 mm Hg in a 27-year-old man who

survived after significant medical treatment (Hampers et al. 1999). Heart rate was elevated in the first case (135/min), normal in the second (66/min), and elevated in the third (104/min). A study conducted in nine volunteers to determine the impact of 33% DEET lotion on various physiological measures, including heart rate, during exercise-heat stress reported no significant differences in heart rate between controls and those who applied DEET (Kenefick et al. 2011). The investigators had hypothesized that DEET lotion would impair measures of sweating and evaporation and thus increase strain and discomfort, which it did not.

Application of up to 1,000 mg DEET/kg/day, 5 days/week for 13 weeks onto the shaved back of micropigs or CD rats did not induce gross or microscopic alterations in the heart and aorta, although details were not reported (EPA 1988, 1992a).

Gastrointestinal Effects. The only relevant information with regard to gastrointestinal effects is from a report by Clem et al. (1993), which states that a 61-old-woman developed nausea, vomiting, and explosive diarrhea after spraying herself liberally with a high percentage DEET insect repellent. The possibility that this was coincidental, however, cannot be ruled out.

Application of up to 1,000 mg DEET/kg/day, 5 days/week for 13 weeks onto the shaved back of micropigs or CD rats did not induce gross or microscopic alterations in the gastrointestinal tract, although details were not reported (EPA 1988, 1992a).

Hematological Effects. Several studies provided information regarding hematological effects following dermal exposure to insect repellents that contained DEET. The majority involve single case reports of children 6-years-old or younger that found no significant deviations from normal limits for red and white blood cell counts (Briassoulis et al. 2001; Heick et al. 1980; Lipscomb et al. 1992; Roland et al. 1985; Zadikoff 1979). The one case in which leukocytosis (16,900/mm^3 or $1.69x10^{10}$/L) was observed involved an 18-months-old girl who had been sprayed daily with an insect repellent containing 20% DEET for approximately 3 months prior to admission (Edwards and Johnson 1987). Clem et al. (1993) and Hampers et al. (1999) reported single cases of intoxication in adults. Both cases had signs and symptoms severe enough to warrant a visit to the emergency department. Hematological parameters were measured and were within normal limits.

Application of up to 1,000 mg DEET/kg/day, 5 days/week for 13 weeks days onto the shaved back of micropigs or CD rats did not induce significant alterations in hematological parameters, although details were not reported (EPA 1988, 1992a).

Musculoskeletal Effects. In the survey of 143 employees of the Everglades National Park, Florida, symptoms of muscle cramping were significantly ($p<0.05$) increased in the medium- (24%) and high- (25%) exposure groups compared to the low-exposure (7%) group (NIOSH 1986).

Application of up to 1,000 mg DEET/kg/day, 5 days/weeks for 13 weeks onto the shaved back of micropigs or CD rats did not induce gross or microscopic alterations in skeletal muscle or bone (femur), although details were not reported (EPA 1988, 1992a).

Hepatic Effects. Four case reports of intoxication of children following dermal exposure to insect repellents containing DEET stated that liver function tests performed shortly after admission to an emergency center were within normal limits (Edwards and Johnson 1987; Lipscomb et al. 1992; Roland et al. 1985; Zadikoff 1979). In the case reported by Zadikoff (1979), the 5-year-old girl died 24 days after admission. In an additional case of dermal intoxication of a 6-year-old girl who eventually died 8 days after admission to the hospital, Heick et al. (1980) reported significantly elevated serum enzymes measured on the fifth day in the hospital. Necropsy showed an enlarged liver with no other abnormalities in appearance. Histological and ultrastructural examination of the liver suggested a nonspecific hepatic injury.

Liver weight was increased, but not significantly, in male Sprague-Dawley after receiving applications of up to 1,000 mg undiluted DEET/kg/day onto the shaved dorsal skin 5 days/week for 9 weeks (Lebowitz et al. 1983). Application of 1,000 mg DEET/kg/day (the highest dose tested) onto the shaved back of male and female CD rats for 13 weeks induced significant increases in absolute and relative liver weight; the same was observed in females that received doses of 300 mg DEET/kg/day (EPA 1988). In addition, males from all treated groups (100, 300, and 1,000 mg DEET/kg/day) had vacuolar change in the liver (significant only in the mid-dose group), which were considered an adaptive response; no vacuolar changes were seen in females. Application of up to 1,000 mg DEET/kg/day, 5 days/week for 13 weeks onto the shaved back of micropigs did not induce gross or microscopic alterations in the liver, nor did it affect serum levels of transaminases, although details were not provided (EPA 1992a).

Renal Effects. Few studies reported the results of renal tests following dermal intoxication with insect repellents containing DEET. Two case reports of children (Briassoulis et al. 2001; Roland et al. 1985) and one case of an adult exposed to DEET (Clem et al. 1993) all reported levels of blood urea nitrogen and creatinine within normal limits in the patients upon arrival at the emergency department.

Application of up to 1,000 mg undiluted DEET/kg/day onto the shaved back of male Sprague-Dawley rats 5 days/week for 9 weeks increased kidney weight at 36, 65, and 95 days, and the increase was significant at 65 days (Lebowitz et al. 1983). A study on male CD rats that received applications of 1,000 mg undiluted DEET/kg/day, 5 days/week for 90 days with microscopic examination of the kidneys reported a significant increased incidence of granular casts and hyaline droplets, as well as inflammation and regeneration of renal tubular epithelium in treated rats compared to controls in both castrated and non-castrated rats (EPA 1990a). The test was performed to assess whether testosterone caused greater effects of DEET on males, but both relative kidney weights and histopathology results indicated that the renal effects of DEET were not enhanced by this hormone. A similar study in CD rats reported slight, but statistically significant, increases in blood urea nitrogen in males exposed to ≥300 mg DEET/kg/day (EPA 1988). In addition, gross necropsy showed enlarged kidneys in males from all exposed groups (100, 300, and 1,000 mg DEET/kg/day), with pale and granular appearance in males exposed to ≥300 mg/kg/day. Microscopic examination showed an increased incidence of renal lesions in all males consisting of granular casts, inflammation, regeneration, and hyaline droplets. High-dose females had a small increase in hyaline casts and inflammation, but the hyaline cast in females was, reportedly, different from that of males (no further details were provided). As mentioned in Chapter 2, the hydrocarbon-induced nephropathy has only been demonstrated in adult male rats and has been linked to a specific protein, $\alpha_{2\mu}$-globulin, which is produced under hormonal control by the liver (Alden 1986; Swenberg 1993). However, the $\alpha_{2\mu}$-globulin is unique to male rats and is not present in human kidneys. Hence, this particular nephropathy has no significance for humans and would therefore be inappropriate to use for evaluation of human health effects or risk assessment. Application of up to 1,000 mg DEET/kg/day, 5 days/week for 13 weeks onto the shaved back of micropigs did not induce gross or microscopic alterations in the kidneys, nor did it affect serum creatinine levels, although details were not reported (EPA 1992a).

Endocrine Effects. In the case report of a 5-year-old girl admitted to the emergency department after being sprayed repeatedly with an insect repellent containing 10% DEET, Zadikoff (1979) stated that thyroid function studies were within normal limits. No other study provided information regarding endocrine effects in humans following dermal exposure to DEET.

Application of up to 1,000 mg DEET/kg/day, 5 days/week for 13 weeks onto the shaved back of micropigs or CD rats did not induce gross or microscopic alterations in the adrenals, thyroid, parathyroid, or pituitary glands, although details were not provided (EPA 1988, 1992a).

Dermal Effects. A low incidence of adverse dermal effects has been reported in humans following application of insect repellents containing DEET in the form of lotions or sprays. It should be noted, however, that the paucity of consumer adverse effect reports, considering the billions of product applications that have occurred in the 60-year history of DEET usage as an active ingredient in insect and acarid repellent, suggests that DEET is generally safe for consumer use if instructions for application are followed.

In an early study in five volunteers (for which further information on informed consent was not provided), application of 1mL of a 50% solution of DEET in isopropanol to the face and 2 mL to the arms once per day for 5 consecutive days did not cause irritation on the arms, but caused a feeling of dryness and astringency in both face and arms (Ambrose 1959). DEET applied to the face also caused desquamation around the nose and some feeling of dryness and astringency. One subject who applied undiluted DEET to the face for 6 weeks showed desquamation around the nose after the third day; each time desquamation appeared, applications were stopped and desquamation disappeared usually within 2 days, and then treatment was resumed. No other signs or symptoms were noted.

MMWR (1989) and Wantke et al. (1996) reported two cases of children who developed non-immunological urticaria following applications of insect repellents containing DEET. In the case described by Wantke et al. (1996), a 4-year-old boy with no history of prior insect repellent application developed urticarial and a generalized itch within minutes of applying a 25% DEET product. Patch testing revealed that the boy's skin was highly sensitive, and that his cutaneous hyperreactivity was not specific to DEET. Wantke et al. (1996) concluded that the boy appeared to have developed non-immunologic, chemical-induced generalized urticaria from the insect repellent. Roland et al. (1985) reported the case of an 8-year-old girl with reportedly sensitive skin who developed a raised, erythematous pruritic rash on her face and extremities 2 days after applying copious amounts of Off!® to those skin areas for that period. On the third day that she applied Muskol®, she experienced convulsions and seizures by the next morning, and was hospitalized. After a 2-day stay at a hospital to treat neurological effects, the rash faded. Results indicated this was a hypersensitivity reaction to DEET.

A group of soldiers developed acute dermatitis 18–24 hours after applying an insect repellent containing 50% DEET before sleep to the uncovered skin of the face, neck, upper part of the trunk, and legs (Reuveni and Yagupsky 1982). All of the subjects complained of a burning sensation and showed erythema of the antecubital fossa of one or both arms where applications could pool or be confined and macerated with sweat in the flexures. Subsequent examination showed progression of the erythema to hemorrhagic blister formation, and in some cases deep ulcerations in 1–2 days. Amichai et al. (1994) reported another single case of acute dermatitis of the antecubital fossa in a soldier who developed a burning sensation and skin eruption about 8 hours after applying an insect repellent containing 33% DEET the previous night. The symptoms did not recur following re-exposure. A survey of 143 employees of the Everglades National Park, Florida, who used DEET regularly in their work, showed that more highly exposed workers had a significantly higher ($p<0.05$) prevalence (27%) of skin rash and blisters than those with medium exposure (14%) or those with low (7%) exposure (NIOSH 1986).

An acute study with occluded application of 2 or 4 mL undiluted DEET (approximately 2,000 or 4,000 mg) to the depilated torsos of albino rabbits (of which the skin was slightly abraded on half of the animals) for 24 hours resulted in mild to moderate erythema on all animals (Ambrose 1959). Slightly more erythema was present on the abraded areas of skin and on the ventral compared with flanks and dorsal surfaces. An evaluation concluded that the greater degree of erythema observed on ventral surfaces was due to mechanical action rather than to increased heat in that area.

Uncovered repeated application of 1 mL DEET/kg (approximately 1,000 mg/kg/day) to albino rabbits 5 days/week for 13 weeks resulting in cutaneous irritation starting at about the third application of DEET Ambrose 1959). The effect was characterized by slight to moderate erythema, desquamation, and dryness of the skin. The erythema disappeared over the weekend but not the desquamation or skin dryness. The skin became leathery, hard, and dry and fissures developed after the third or fourth week of treatment in some rabbits. Desquamation persisted and remained throughout the study. Although scarring was present in some rabbits, most skin alterations had disappeared three weeks after the last dose. Application of doses of 1,000 mg neat technical DEET/kg/day to the back of male CD rats 5 days/week for 90 days resulted in increased incidence of erythema (EPA 1990a). A similar study in which CD rats received applications of 100, 300, or 1,000 mg DEET/kg/day (in volumes of 0.1, 0.3, and 1.0 mL/kg) for 13 weeks reported increased incidence of red and scabbed areas at the application site for both male and female rats (EPA 1988). Microscopic examination of the skin showed increased incidence of acanthosis and/or hyperkeratosis. Application of ≥100 mg DEET/kg/day (as a mixture consisting of equal parts of technical-grade DEET from four manufacturers) to the back of micropigs 5 days/week for 13 weeks

resulted in skin desquamation (EPA 1992a). Microscopic examination of two unspecified application sites on dorsal and lateral surfaces at termination showed dose-related increased incidence of hyperkeratosis at ≥100 mg DEET/kg/day in males and ≥300 mg DEET/kg/day in females. Acanthosis at application site A was observed only in males and showed a threshold response at 1,000 mg DEET/kg/day, while at site B/C, the effect was dose-related at ≥300 mg DEET/kg/day (EPA 1992a).

No skin lesions were reported in a chronic-duration study in male Swiss mice and in male and female New Zealand White rabbits that received applications of 0.02 mL of a 10, 50, or 100% solution of DEET (2, 10, or 20 mg DEET) for 2 times/week (140 weeks in mice, 90 weeks in rabbits) (Stenback 1977).

DEET was not a skin sensitizer in guinea pigs or in rabbits (Ambrose 1959).

Ocular Effects. In the study of 20,764 human exposures involving insect repellents containing DEET that were reported to poison control centers from 1993 to 1997, 4,422 were identified as being exposed predominantly by accidental contact of an insect repellent spray with the eyes (Bell et al. 2002). Six of those (three children and three adults) experienced major ocular symptoms. Common signs and symptoms reported in these subjects included ocular irritation/pain and lacrimation.

A study in CD rats applied up to 1,000 mg DEET/kg/day onto the shaved back daily for 13 weeks reported that ophthalmological examinations conducted at week 13 showed no compound-related effects (EPA 1988). A study in albino rabbits examined the ocular effects of three different DEET preparations: 100% undiluted (1 drop, 0.04 mg), 30% DEET in cottonseed oil (3 drops, ~0.04 mg), or a 40% emulsion in vegetable lecithin with ethanol and water (3 drop, ~0.05 mg) (Ambrose 1959). Two hours after application to the conjunctival sac, there did not seem to be significant differences in the degree of eye injury induced by the three preparations, but it appeared that the emulsion was slightly more irritating. DEET induced moderate to marked edema of the nictitating membrane, lacrimation, conjunctivitis, and pus, which were still present 48 hours after application. Three rabbits also showed some cloudiness. All treated eyes showed varying degrees of injury as revealed by fluorescein staining. Some effects seen at 48 hours were still seen 72 hours after application, but were not as severe. After 5 days, fluorescein staining was negative and all eyes were considered to have a normal appearance by the investigator, suggesting that the eye injuries were probably not permanent. Another study in rabbits reported that 0.01 mL of undiluted DEET (approximately 10 mg) caused moderate eye irritation, as indicated by increased corneal thickness and fluorescein staining, swelling of the conjunctiva, corneal cloudiness, and iris reaction (MacRae et al. 1984). The eye returned to a normal appearance by 168 hours. Application of up

to 1,000 mg DEET/kg/day 5 days/week for 13 weeks onto the shaved back of micropigs did not induce gross or microscopic alterations in the eyes, although details were not provided (EPA 1992a).

Body Weight Effects. Repeated applications of approximately 1,000 mg DEET/kg/day to a shaved area of the skin of Sprague-Dawley rats or albino rabbits did not significantly affect body weight (Ambrose 1959; Lebowitz et al. 1983). Similar findings were reported in male Swiss mice applied 20 mg DEET (approximately 666–1,000 mg/kg/day assuming a body weight of 0.02–0.03 kg for the mice) for 140 weeks (Stenback 1977). Body weight was not significantly affected (<10% difference with controls) in CD rats or micropigs that received applications of up to 1,000 mg DEET/kg/day onto the shaved back for 13 weeks (EPA 1992a).

Metabolic Effects. Several studies of children intoxicated after skin application of insect repellents containing DEET reported levels of glucose and serum electrolytes within normal limits upon admission to emergency centers (Briassoulis et al. 2001; Edwards and Johnson 1987; Gryboski et al. 1961; Heick et al. 1980; Roland et al. 1985; Zadikoff 1979). Similar findings were reported by Hampers et al. (1999) in their description of an adult case of poisoning.

Application of up to 1,000 mg DEET/kg/day, 5 days/week for 13 weeks onto the shaved back of micropigs did not induce alterations in serum electrolytes or glucose levels, although details were not reported (EPA 1992a). Tests conducted in male and female CD rats that received applications of 1,000 mg DEET/kg/day, 5 days/week for 13 weeks showed a significant decrease in serum glucose in males, which was considered not biologically significant by the investigators (EPA 1988). Serum electrolyte levels were within normal ranges in that study.

3.2.3.3 Immunological and Lymphoreticular Effects

A few cases of contact urticaria by immunological mechanisms have been reported in humans after using products containing DEET. Immunological contact urticaria is a type I hypersensitivity reaction that is mediated by antigen-specific IgE in individuals who previously have been sensitized (Shutty et al. 2013). Maibach and Johnson (1975) reported the case of an elderly woman who discovered that she had allergic contact dermatitis to DEET containing products after self-experimentation with insect repellents over four summers (applying them, observing a rash form immediately, and then noting that the rash disappeared upon washing off the 0.1, 1, and 100% substance). Patch test application of three active ingredients of repellents (dimethylphthalate, DEET, and butopyronoxyl), along with their inactive components, to intact

skin showed that DEET was the substance causing the immediate urticaria. Application of pure samples of DEET gave similar responses. Testing with 18 structurally-related analogs of DEET and similar substances showed that the response depended on the nature and positions of those substances that were substituted on the benzene ring. It was determined that active structures required *ortho*, *meta*, or both positions to be fluoromethylated, inactivation occurred if the *para* position was so filled (as if full activation needed access to the *meta* site for hydroxylation), the molecule needed a benzoyl structure, and the response may be mediated by histamine. Serum from the patient was injected into two volunteers who had an injection site response to DEET, indicating the patient's response could be passively transferred. Results suggested that the mechanism of action was immunologic, and its passive transferability indicated a deficiency in the patient's immune system. A similar case was reported by Vozmediano et al. (2000) in a 16-year-old girl who historically experienced disproportional reactions to insect bites and developed a skin reaction accompanied by increasingly evident edema and severe pruritus after regularly applying a lotion containing 20% DEET. The authors conducted an open skin test using that product and 0.1%, 1% and 100% DEET. The product and higher two concentrations resulted in a raised area, considered to be an immunologic contact urticarial. More recently, Shutty et al. (2013) described the case of a 22-year-old man who developed contact urticaria immediately after application of an insect repellent. The patient reported consistently avoiding DEET-containing products since previous contact with them had resulted in welts, and he had recently developed hives after contact with individuals who had used DEET-containing repellents. Because it was unclear what ingredient produced the urticaria, open patch testing of the patient with DEET and picaridin (another common insect repellent) was conducted. There were positive responses in tests areas receiving applications of 7% DEET and 7% DEET in ethanol, but no dermal response in areas to which 5% picaridin and 5% picaridin in ethanol were applied.

Application of up to 1,000 mg DEET/kg/day, 5 days/week for 13 weeks onto the shaved back of CD rats or micropigs did not induce gross or microscopic alterations in spleen, thymus, or lymph nodes, although details were not provided (EPA 1988, 1992a).

3.2.3.4 Neurological Effects

There have been sporadic reports over the last several decades of adverse neurological effects in adults and children following dermal application of insect repellents containing DEET. It should be noted, however, that in all cases, excessive amounts of the insect repellent may have been applied. A few representative studies are mentioned below, and additional references can be found in review articles

(Antwi et al. 2008; Bell et al. 2002; Osimitz and Murphy 1997; Qiu et al. 1998; Sudakin et al. 2003; Veltri et al. 1994). In almost all cases, exposure involved repeated applications of an insect repellent on multiple days but in at least three cases, neurological effects developed after a child received a single application (Briassoulis et al. 2001; Lipscomb et al. 1992) and after an adult received a few applications in the same day (Hampers et al. 1999). The amount applied was not known in either case, but Lipscomb et al. (1992) reported that the child received a virtual total body application of an insect repellent containing 95% DEET. Hampers et al. (1999) reported that the adult male had applied 20% DEET sunscreen lotion early in the day and a 25% DEET spray several times later that day to his arms, neck, and legs, resulting in the acute onset of parathesias of limbs and face, then progressive hallucinations and confusion, followed by combativeness. The emergency department neurological examination identified tremors in all extremities and a hypertonic state. He was unresponsive to haloperidol, diazepam, and phenytoin treatment, and required further medication, intubation, and mechanical ventilation. On day 2, he was off the ventilator, and on day 3, his mental state appeared normal, although he still had headaches. All effects had resolved after a week.

Neurological signs and symptoms reported in children and adults include seizures, ataxia, restlessness, uncontrolled limb movements, agitation, aggressive behavior, combativeness, impaired cognitive functioning, and opisthotonos (Briassoulis et al. 2001; Edwards and Johnson 1987; Gryboski et al. 1961; Hampers et al. 1999; Heick et al. 1980; NIOSH 1986; Pronczuk de Garbino et al. 1983; Roland et al. 1985; Snyder et al. 1986; Zadikoff 1979), or headaches that progressively worsen or are long lasting (Hampers et al. 1999; Zadikoff 1979). Some milder symptoms included insomnia, muscle cramping, mood disturbances, and difficulty with starting or stopping urination (NIOSH 1986). The combination of some of these signs and symptoms has been described as toxic encephalopathy, and Zadikoff (1979) considered that this spectrum of symptoms could result in misdiagnosis as viral encephalitis. Five deaths occurred among these cases (Bell et al. 2002; Heick et al. 1980; Pronczuk de Garbino et al. 1983; Zadikoff 1979). Osimitz and Murphy (1997) examined 14 cases that reported neurological effects following dermal exposure to DEET and concluded that causality is difficult to establish because of limitations in clinical details provided in the reports. The investigators noted that 8 of the 14 patients may have had idiopathic seizures, 1 may have had an exanthematous illness and a convulsion, 3 may have had an inflammatory process affecting the central nervous system, and 1 was heterozygous for ornithine carbamoyl transferase deficiency, but synergisms with DEET were not excluded. There was insufficient information on an additional patient to determine if there were alternate explanations for the patient’s encephalopathy.

In a study of 20,764 human exposures involving insect repellents containing DEET that were reported to poison control centers from 1993 to 1997, 2,179 were identified as having been exposed predominately by skin contact (Bell et al. 2002). Of these, 118 exhibited minor neurologic symptoms that included dizziness/vertigo, headache, and drowsiness/lethargy. The severe symptoms among these cases included tremors (15), single seizures (8), muscle weakness (10), muscle rigidity (5), peripheral neuropathy (5), slurred speech (3), and paralysis (1).

In an early study in five volunteers, application of approximately 1 mL of a 50% solution of DEET in isopropanol to the face (i.e., enough to completely wet each area) once per day for 5 consecutive days induced a slight tingling sensation in all the subjects (Ambrose 1959). No other neurological signs or symptoms were noted.

Haley and Kurt (1997) conducted a cross-sectional survey of 249 Gulf War veterans in order to identify risk factors of war-related syndromes. The extent of exposure to DEET was assessed by self-estimation of the number of times per day repellent was typically applied. Independent risk factors were identified by performing a series of adjusted, stepwise logistic regression analyses that required a level of significance of $p<0.005$ for a variable to enter and remain in a logistic regression model. The results of the analyses showed that the prevalence of a syndrome termed arthro-myo-neuropathy increased with the amount of insect repellent used ($p<0.005$ for a univariate association and $p<0.001$ for trend). This association held true for those who used government-issued repellent (75% DEET in ethanol) (odds ratio [OR] of 1.54; 95% confidence interval [CI] of 1.17–2.03), but not for those who reported using a formulation containing ≤31% DEET or one containing no DEET. While the latter gives biological plausibility to the results, assessing exposure by self-recollection limits the validity of the study conclusions.

A series of animal studies have been conducted to examine the neurological effects of DEET applied to the skin of animals alone and in combination with other chemicals used by military personnel in the Persian Gulf War. This section summarizes the effects of DEET alone; information regarding interactions of DEET with other chemicals is presented in Section 3.9, Interactions with Other Chemicals. It should be mentioned, however, that some of these studies seem to have some deficiencies in reliability, as explained below (Jortner 2006; Schoenig 2002).

In an intermediate-duration study, relatively low doses of 4 mg/kg/day DEET (as 10 mg/mL in 70% alcohol) applied daily to 1 in^2 of the back of the neck skin of male Sprague-Dawley rats for 60 days did

not affect simple sensorimotor reflexes tested 30–60 days after exposure ceased, but affected some sensory parameters such as performance on a beam, grip strength, and performance on an inclined plane (Abou-Donia et al. 2001a). In addition, doses ≥4 mg DEET/kg/day decreased the permeability of the blood brain barrier mainly in the brainstem but also in the cerebellum, doses ≥40 mg DEET/kg/day decreased the permeability in the midbrain, and 400 mg DEET/kg/day decreased the permeability in the cortex. In a companion study, similar treatment with 40 mg DEET/kg/day (only dose tested) for 45 days was shown to significantly increase (by ~40%) acetylcholinesterase (AChE) activity in the brainstem but not in other brain areas and also to significantly increase (by ~20%) choline acetyltransferase (ChAT) activity in the cortex but not in the brainstem (Abou-Donia et al. 2001b). DEET also significantly increased ligand binding to m2 muscarinic acetylcholine receptors in the cortex, but did not affect ligand binding of nicotinic receptors in the cortex. Gross and microscopic examination of the brain of the treated rats showed neuronal degeneration principally in the motor cerebral cortex, dentate gyrus, CA1 and CA3 subfields of the hippocampus, and the Purkinje cell layer of the cerebellum (Abdel-Rahman et al. 2001). In a subsequent study, the same group of investigators confirmed the findings regarding the neurobehavioral effects and histological effects in the various brain areas (Abdel-Rahman et al. 2004). Contrary to what was reported in a previous study (Abou-Donia et al. 2001b), however, in the more recent study (Abdel-Rahman et al. 2004), DEET was reported to statistically significantly increase (rather than have no effect) AChE activity in the cortex and cerebellum but not in the brainstem (which earlier was increased by 40%) and to have no significant effect on the ligand binding to m2 muscarinic acetylcholine receptors in the cortex. No explanation was provided for these apparent discrepancies. It should also be mentioned that Fediuk et al. (2010) applied doses of 40 mg DEET/kg/day (as 100 mL DEET in 70% alcohol) to 4 cm^2 of the shaved back of Sprague-Dawley rats for 30 days, as did Abdel-Rahman et al. (2004) to 2.5 cm^2 (dose applied in 1 mL of 70% ethanol in water) for 60 days, and reported no significant alterations in various neurobehavioral tests that assessed arousal, locomotion, habituation, and motor coordination. The reason for the difference in the result between these two studies (other than the area of skin exposed, duration of exposure, and concentration of DEET in the alcohol vehicle) is not apparent. Regarding the histological findings in the Abdel-Rahman et al. (2001, 2004) reports, it was noted that there may have been misinterpretation of the findings (Jortner 2006). The main concern is that the report of “degenerating” or “dying” neurons in this article is actually the result of poor handling and inadequate fixation of the brain tissue and is a “dark” neuron artifact. The presence of this artifact suggests that both the neuron counting and the immunostaining procedures may have been compromised.

The studies by Abou-Donia and Abou-Rahman state that the doses of DEET, pyridostigmine bromide (PB), and permethrin used in their studies of rats were considered comparable to exposures received by

service members during the Persian Gulf War. The National Academy of Sciences (NAS) stated that "the primary studies of Veterans deployed to the Gulf War compared to Veterans not deployed do not demonstrate differences in cognitive and motor measures as determined through neurobehavioral testing." The NAS update committee concluded that "there is inadequate or insufficient evidence to determine if an association exists between deployment to the Gulf War and neurocognitive and neurobehavioral performance" (DVA 2011).

In studies in CD rats and micropigs, application of up to 1,000 mg DEET/kg/day to the shaved back 5 days/week for 13 weeks did not induce gross or microscopic alterations in the brain, sciatic nerve, or spinal cord, although details were not reported (EPA 1988, 1992a).

The NOAELs and LOAELs for neurological effects from the animal studies summarized above are presented in Table 3-3.

3.2.3.5 Reproductive Effects

No information was located regarding reproductive effects in humans following dermal exposure to DEET.

Three studies were located with information regarding reproductive effects in animals following dermal exposure to DEET. Application of up to 1,000 mg undiluted DEET/kg/day onto the shaved dorsal skin of male Sprague-Dawley rats for 5 days/week over a total for 9 weeks did not significantly affect sperm count or viability, nor did it induce sperm head abnormalities (Lebowitz et al. 1983). Application of 4–400 mg DEET/kg/day for 60 days to 1 cm^2 of skin on the necks of male Sprague-Dawley rats decreased blood-testis barrier permeability (but not in a dose-related manner) to approximately 75% of the control value (Abou-Donia et al. 2001). In addition, treatment with DEET did not affect testes weight, nor did it induce compound-related lesions in the testes. Application of up to 1,000 mg DEET/kg/day to the shaved back of CD rats or micropigs 5 days/week for 13 weeks did not induced gross or microscopic alterations in the reproductive organs, although details were not reported (EPA 1988, 1992a).

The doses of 1,000 mg DEET/kg/day (the highest dose tested) in rats and pigs is listed as a NOAEL for reproductive effects in Table 3-3.

3.2.3.6 Developmental Effects

Limited information is available regarding developmental effects in humans following dermal exposure to DEET from two single cases and two cohort studies. Schaefer and Peters (1992) reported the case of a 34-year-old woman who had been working in Africa where she continuously applied a lotion containing 25% DEET in addition to taking prophylactic chloroquine against malaria. Pregnancy was without complications and she delivered a boy of normal weight at the estimated date of birth. However, the boy was born with antimongoloid slant of the palpebral fissures, hypertelorism, thin lips, poorly developed philtrum, and a broad nasal bridge. During the first months of life, the boy developed statomotor retardation, muscular hypotonia, central hearing loss, and strabismus. Genetic testing did not show inborn errors of metabolism and there was no family history of genetic disorders. A possible role for chloroquine was ruled out given the safety of its prophylactic use. A causal relationship with DEET was not established. Hall et al. (1975) described two cases of children born with cardiac anomalies leading to congestive heart failure and diagnosis of coarctation of the aorta. The mothers, who were sisters, had used large amounts of insecticides (containing N-octyl bicycloheptene dicarboximide, piperonyl butoxide, allethrin, pyrethrins, and 2,2-dichlorovinyl dimethyl phosphate) and DEET in the insect repellent Off!® during a camping trip at about 8 weeks into their pregnancies. It is worth noting that the sisters were only together during the camping trip, that defects in the aortic arch segment occur between gestational weeks 6 and 10, and that there was a family history of heart problems on the father's side of one of the boys. In this study, the role of DEET, if any, cannot be determined. Although Hall et al. (1975) indicated that that multiple cases of familial coarctation are rare, others (e.g., Perera et al. 2014; Atalay and Kichilas 2011) have reported congenital aortic coarctation phenotypic malformations, and the latter reported three such individuals in two generations of the same family.

McGready et al. (2001) studied the effects of application of 1.7 g DEET/day in the second and third trimesters of 449 pregnant women as part of a double-blind trial of insect repellents in the prevention on malaria in Thailand. Controls consisted of 449 women who did not apply DEET. Women were followed for the duration of their pregnancy. Newborns were assessed for head and arm circumference and length; gestational age was assessed within 5 days of birth and neurological tests were conducted that assessed tone, movement, behavior, and visual and auditory alertness. Infants were followed up until 12 months of age for growth and basic developmental milestones. DEET was not detected in 30 urine samples from DEET-exposed women, but was detected in 4 of 50 samples of cord blood from women exposed to DEET. The results of the analyses did not reveal significant differences in the outcomes measured between offspring from exposed and non-exposed women. More recently, Barr et al. (2010) studied the

association between exposure to various pesticides (DEET among them) in a cohort of 150 New Jersey women and birth outcomes (birth weight, head circumference, abdominal circumference, and birth length). Exposure was assessed by measuring pesticides in maternal serum prior to birth and in cord blood after delivery. DEET was one of the pesticides most frequently detected in maternal and cord serum; the corresponding mean concentrations were 3.21 ng/g (range 1.82–18.84 ng/g) and 3.12 ng/ng (range 2.06–13.07 ng/g). The results of multivariable regression analyses carried out to minimize biases due to confounding factors ascertained that there was no significant association between DEET and the birth outcomes measured. The investigators noted that since blood was collected at birth, the exposure measured did not necessarily precede the birth outcomes measured. Also, there was no documentation of exposure by environmental measurements. Finally, many of the concentrations measured were near the limit of detection (LOD) of the analytical method.

No studies were located regarding developmental effects of DEET in animals following dermal exposure.

3.2.3.7 Cancer

Limited information exists regarding exposure to DEET and cancer in humans. A case-control study of testicular cancer and occupational exposures was conducted in Sweden (Hardell et al. 1998). Exposure to multiple occupations and chemical agents was assessed by self-administered questionnaires. The final analysis comprised 148 cases and 363 controls. The risk for testicular cancer among workers who used insect repellents (most containing DEET) for <115 days was not elevated based on 15 cases (OR 1.2, 95% CI 0.6–2.5). The OR for those using repellents for >115 days, however, was 2.3 (95% CI 1.2–4.4), based on 24 cases. Little information was presented in this study regarding how potential confounders were controlled; however, multivariate analysis found a significant interaction for those who were co-exposed to insect repellents and video display units (OR 2.5, CI 1.1–5.4). The investigators also noted that a previous study of this cohort had found an increased risk for testicular cancer associated with exposure to polyvinyl chloride (PVC) and that additives in PVC may also be used in the manufacture of insect repellents. Finally, assessment of exposure by self-recollection is known to be unreliable.

Another case-control study involved 513 men with non-Hodgkin's lymphoma (NHL) and 1,506 controls (McDuffie et al. 2005). The study found that simultaneous use of the pesticide mecoprop and DEET by farmers who wore rubber gloves resulted in higher odds ratios for NHL (OR 3.86, 95% CI 1.57–9.49) than farmers who either did not use DEET or did not use rubber gloves. The results were explained by DEET presumably increasing the permeability of the gloves to the phenoxyherbicide. Co-exposure to

DEET and the herbicide dicamba also resulted in increased risk with rubber gloves (OR 2.04, CI 1.02–4.06) or without them (OR 1.84, CI 1.23–2.75).

Only one study was located regarding cancer in animals after dermal exposure to DEET. In that study, 0.02 mL of a 10, 50, or 100% solution of DEET (2, 10, or 20 mg DEET) was applied over 1 in^2 of the skin of Swiss mice (50/exposure group, 100 unexposed controls) 2 times/week for 140 weeks. The incidence of tumors on the application site or at any remote site compared to controls did not increase (Stenback 1977). Additionally, fewer DEET-exposed mice developed tumors (36–50 vs. 58% for controls), and they had higher long-term survival rates (e.g., 12–26 vs. 6% for controls at 100 weeks). Dosing New Zealand White rabbits (5/group) in the same manner for 90 weeks also yielded negative cancer results (Stenback 1977).

3.3 GENOTOXICITY

No studies were located regarding genotoxic effects in humans exposed to DEET.

The only information regarding genotoxicity following *in vivo* exposure is that from a study in which Sprague-Dawley rats were applied a single dermal dose of 400 mg DEET/kg in 70% ethanol and the urine was collected and analyzed for the biomarker of DNA damage 8-hydroxy-2'-deoxyguanosine (Abu-Qare and Abou-Donia 2000). The results showed a significant increase ($p<0.05$) in the levels of the biomarker in urine over a 72-hour period after dosing. Maximum excretion was reached 24 hours after dosing, after which time excretion of 8-hydroxy-2'-deoxyguanosine leveled off.

Few studies have examined the genotoxicity of DEET in *in vitro* assays. Exposure of primary human nasal mucosal cells from the inferior and the middle turbinate to concentrations of DEET ranging from 0.5 to 1.0 mM (~0.1–0.2 µL/mL) for 60 minutes induced significant DNA damage, as quantified by the comet assay (Tisch et al. 2002). In another study with mammalian cells, DEET assayed to a cytotoxic level of ≥1.0 µL/mL for 18–20 hours did not induce unscheduled DNA synthesis in primary rat hepatocytes (EPA 1990c). In yet another study in mammalian cells, incubation of Chinese hamster ovary cells with up to 1.0 µL DEET/mL without activation (16 hours) or up to 0.5 µL DEET/mL with activation (2 hours) did not induce chromosomal aberrations (EPA 1990c). Mutagenicity studies conducted in prokaryotic organisms with or without metabolic activation yielded negative results (EPA 1990c; Zeiger et al. 1992). The results of the *in vitro* genotoxicity studies with DEET are summarized in Table 3-4.

Table 3-4. Genotoxicity of DEET *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	EPA 1990c
S. typhimurium TA98, TA100, TA1535, TA1538	Reverse mutation	–	–	Zeiger et al. 1992
Mammalian cells:				
Cultured primary human nasal mucosal cells	DNA damage	+	No data	Tisch et al.1992
CHO cells	Chromosomal aberrations	–	–	EPA 1990c
Rat hepatocytes	Unscheduled DNA synthesis	No data	–	EPA 1990c

+ = positive results; – = negative results; DNA = deoxyribonucleic acid; CHO = Chinese hamster ovary

3.4 TOXICOKINETICS

No data on the toxicokinetics of DEET in humans exposed via inhalation or oral routes were located in the literature reviewed. Similarly, there are no animal data on toxicokinetics of inhaled DEET. Data on absorption of orally-administered DEET are limited to a single rat study (Schoenig et al. 1996) that showed rapid and nearly complete >90% absorption of DEET. Dermal absorption of DEET has been extensively studied in humans, laboratory animals and in *in vitro* test systems. The rate and extent of dermal uptake are affected by species, sex, vehicle and/or formulation in which DEET is applied, dose, and evaporation rate; thus, estimates are highly variable. Based on urinary excretion of radioactivity, the available estimates of the extent of dermal ^{14}C-DEET absorption in humans have ranged between 3.8 and 17% of the applied radioactivity (Blomquist and Thorsell 1977; Feldman and Maibach 1970; Selim et al. 1995).

No specific deposition site has been identified for DEET. After oral or dermal exposure, DEET is widely distributed. It has been detected in the brain, liver, lung, spleen, kidney, fat, lacrimal glands, and nasal mucosa of exposed animals. The one study examining the potential for transplacental transfer of ^{14}C-DEET in rabbits did not detect radioactivity in the fetuses at the end of 29 days of daily dermal applications to the does. After intravenous exposure of pregnant rabbits on 1 day, low levels of radioactivity, however, were detected in the fetuses. DEET did not bind to human serum albumin (HSA) in *in vitro* tests, but did bind to bovine serum albumin (BSA).

The primary metabolites of DEET in humans and laboratory mammals exposed via oral, dermal, or intraperitoneal injection routes are *m*-(diethylaminocarbonyl) benzoic acid (DCBA) and *m*-(ethylaminocarbonyl) benzoic acid (EACB) (Sandstrom et al. 2005; Schoenig et al. 1996; Selim et al. 1995; Taylor and Spooner 1990). It should be noted that some older studies referred to DCBA as *m*-(diethylamino carbonyl)benzoic acid and used the acronym DACB. Metabolism has not been examined in other species or after inhalation exposure. In humans, DCBA was produced by ring methyl oxidation via the intermediate N,N-diethyl-3-hydroxymethyl-benzamide (DHMB), primarily by cytochrome (CYP) 1A2 and 2B6. EACB resulted from N-dealkylation via the intermediate, N-ethyl-*m*-toluamide (ET), by CYP2C19 and CYP3A4. At low substrate concentrations, the ring methyl oxidation pathway was expected to predominate due to higher substrate affinities of the relevant cytochrome P-450 isozymes. There was evidence that DEET induced CYP3A, thereby inducing its own metabolism (Abu-Qare and Abou-Donia 2001a; Usmani et al. 2002). Other metabolites identified in human urine include N-ethyl-N-(1-hydroxyethyl)-3-methylbenzamide and 3-((carboxymethyl)

(hydroxymethyl)carbamoyl)benzoic acid (Wu et al. 1979), while *m*-(aminocarbonyl)benzoic acid (ACB) and *m*-toluic acid were also identified in rat urine (Taylor and Spooner 1990).

Available information from animal studies suggested that metabolism occurred rapidly and mainly in the liver. Limited information suggested that there might be gender differences in the metabolism of DEET, such that males might metabolize DEET faster than females. Females may produce more of the intermediate ET than DHMB at higher doses (Schoenig et al. 1996; Yeung and Taylor 1988).

DEET was rapidly cleared from the plasma after dermal exposure, with plasma elimination half-lives ranging from 2.5 to 9 hours in animals (Fediuk et al. 2011; Kasichayanula et al. 2007; Qiu et al. 1997a, 1997b). The primary route of elimination after oral, dermal, or intravenous exposure was via urinary excretion of metabolites, although some unchanged DEET was excreted in the urine after a high-dose or long-term exposures. Biliary excretion of DEET or its metabolites was observed in animals. It is not known whether this is a significant excretory pathway in humans.

3.4.1 Absorption

The available literature did not include any studies of the absorption of DEET after inhalation exposure. Data on absorption of orally-administered DEET are limited to two rat studies (Hoy et al. 2000a; Schoenig et al. 1996). Schoenig et al. (1996) showed rapid and nearly complete absorption (>90%) in CD rats, while Hoy et al. (2000a) reported that oral administration of 200 mg DEET/kg to Sprague-Dawley rats resulted in blood serum concentrations 30 minutes after administration that were approximately 3.5, 8.5, and 13 ng/mL in males, pre-estrus females, and met-estrus females, respectively. Dermal absorption of DEET has been extensively studied in humans, laboratory animals, and *in vitro* test systems. Estimates of the rate and extent of dermal uptake vary widely and these parameters may be affected by species (Moody and Nadeau 1993), sex (Snodgrass et al. 1982), vehicle and/or the formulation in which DEET is applied (Fediuk et al. 2011; Iscan et al. 2006; Karr et al. 2012; Kasting et al. 2008; Qiu et al. 1997a, 1997b), dose (Moody et al. 1995; Santhanam et al. 2005), and evaporation rate (Reifenrath et al. 1991; Santhanam et al. 2005). The sunscreen, oxybenzone, if applied after DEET application, has been shown to enhance the penetration of DEET across animal skin *in vivo* and *in vitro* (Chen et al. 2010; Gu et al. 2005; Kasichayanula et al. 2007; Ross et al. 2004; Wang and Gu 2007) as has mechanical action (Ambrose 1959).

3.4.1.1 Inhalation Exposure

No quantitative information on the absorption of DEET in humans or animals exposed via inhalation was located. Many exposures reported to poison control centers from 1993 to 1997, however, were identified as involving predominantly inhalation exposure and the adverse signs and symptoms exhibited by these subjects suggest that absorption by this route may have occurred (Bell et al. 2002). Toxic effects seen in rats and mice after acute inhalation exposure to high concentrations of DEET also provide indirect evidence of absorption of DEET through the lungs (Ambrose 1959; Army 1979; Deb et al. 2010; EPA 1998c).

3.4.1.2 Oral Exposure

The many case reports of adverse health effects in humans following accidental or intentional ingestion of insect repellents containing DEET mentioned in Section 3.2.2, Oral Exposure, provide evidence of gastrointestinal absorption of this substance.

One study in CD rats examined absorption, distribution, metabolism, and elimination of radioactivity after administration of ring-labeled ^{14}C-DEET by gavage (Schoenig et al. 1996). The time of peak radioactivity in plasma was 30 minutes postdosing in males and 2 hours postdosing in females (Schoenig et al. 1996), indicating rapid uptake. Data from this study suggest that up to 91% of an oral dose of 100–500 mg DEET/kg was absorbed based on urinary recovery of radioactivity.

Hoy et al. (2000a) demonstrated oral absorption of DEET in Sprague-Dawley rats by measuring DEET in blood serum 30 minutes after oral administration of 200 mg/kg via gavage. The measured blood serum concentrations were approximately 3.5, 8.7, and 13 μg/mL in male, pre-estrus females, and met-estrus female rats, respectively.

3.4.1.3 Dermal Exposure

Small quantities of DEET are rapidly absorbed across human skin, based on appearance in the plasma. Selim et al. (1995) evaluated the rate of absorption of DEET applied to the arms of volunteers. ^{14}C-DEET (~0.5 mg/cm^2) was applied either neat or in a 15% solution in ethanol to 24 cm^2 areas on the arms of six male volunteers and left for 8 hours. Doses of DEET in both applications were similar (~15 mg and 37 μCi in the neat application and about 12 mg and 36 μCi in ethanol). The peak radioactivity in plasma occurred 6 hours after application of neat DEET and 4 hours after application of DEET in ethanol,

indicating that the ethanol solvent slightly enhanced absorption. Smallwood et al. (1992) detected DEET in serum (by high performance liquid chromatography [HPLC] analysis) within 1 hour after dermal application of an insect repellent at estimated doses of 0.14–1.86 g. The peak serum concentration typically occurred within 1–2 hours after application of the repellent (Smallwood et al. 1992). The authors estimated dermally applied doses ranging from 0.31 to 5.99 $\mu g/cm^2x10^3$ and observed a correlation ($r=0.80$, $p=0.01$) between applied dose ($\mu g/cm^2x10^3$) and area under the serum concentration vs. time curve (through the 6-hour measurement); in units of hour · μg/g). Feldman and Maibach (1970) observed the maximum rate of absorption during the first 12 hours after administration of 4 μg DEET/cm^2 to the skin of volunteers, when the absorption rate was estimated as 0.773% per hour.

Estimates of the extent of DEET absorbed across the skin of humans have been made based on urinary excretion of radioactivity; these estimates range between 3.8 to 17% of the applied radioactivity, a primary factor for the difference may be the time that DEET was left on the skin. Based on the cumulative amount of radioactivity excreted in urine collected during the 5 days after dosing in the human study by Selim et al. (1995), 5.6– 8.3% of the applied radioactivity was absorbed on average. In two experiments with the same female volunteer exposed for 8 hours to 0.12 mg/kg ^{14}C-DEET via topical application, absorption was at least 3.8–5.5% of the applied radioactivity based on cumulative urinary excretion of radioactivity during the 48 hours following commencement of exposure (Blomquist and Thorsell 1977). Feldman and Maibach (1970) reported total absorption of ~17% of a topically applied dose of 4 μg/cm^2 (total area of 13 cm^2) ^{14}C-DEET (left untouched for 24 hours) to the forearm of volunteers (ages and genders not reported); absorption was based on cumulative urinary excretion of radioactivity over 5 days.

Dermal absorption of DEET in CD rats occurred rapidly, with peak blood levels occurring within 2–3 hours after the commencement of exposure. A single dermal application of 100 mg/kg ring-labeled ^{14}C-DEET (the vehicle was not reported) to the shaved backs (12.5 cm^2) of fasted rats was studied. Radioactivity levels in blood peaked 2 hours after application in males (at 333 dpm/0.1 mL) and 3 hours after application in females (255 dpm/0.1 mL), and persisted at a high level for the duration of the exposure, indicating ongoing absorption from the application site (Schoenig et al. 1996). The application site was covered with a glass rectangular enclosure to minimize evaporative losses. In a study in which evaporative losses were not prevented, a peak plasma concentration of 0.3 μg DEET/mL was reached 90 minutes after the end of the 24-hour exposure (Fediuk et al. 2011). Dermal absorption was rapid in Beagle dogs; plasma concentrations of DEET in dogs exposed to two different formulations (a novel formulation with 7.5% DEET and Off!: Skintastic II® containing 7.125% DEET) showed a similar time

profile, with the peak concentrations of 154.3 and 196.5 ng/mL (for the two formulations) occurring at 1.25 hours postdosing (Qiu et al. 1997a, 1997b). DEET was detected in plasma at 15 minutes postexposure for both formulations, but the rate of absorption from the formulation with 7.5% DEET was slower, based on the lower plasma concentration observed at that time (17.7 vs. 101 ng/mL) (Qiu et al. 1997a).

The extent of DEET absorbed across the skin of rats has been reported to be as low as 15% and as high as 78% due a variety of factors, including dose, vehicle and/or formulation in which DEET is applied, and evaporation rate. Schoenig et al. (1996) measured radioactivity in urine collected for 7 days after a single dermal application of 100 mg/kg ring-labeled ^{14}C-DEET (a vehicle was not reported) to the shaved backs (12.5 cm2) of fasted CD rats. Based on the urinary radioactivity levels, approximately74–78% of the applied dose was absorbed across rat skin (Schoenig et al. 1996). When male and female Wistar rats were treated topically with 50 mg/kg ^{14}C-DEET on the shaved upper back (1.5–2 cm^2 area), 52–54% of the applied radioactivity was excreted in the urine within the first 48 hours, indicating dermal absorption of at least 52% of the administered dose (Taylor and Spooner 1990). Moody et al. (1995) estimated dermal absorption of DEET by Sprague-Dawley rats to be 15.1, 26.8, and 20.3% of doses of 4.7, 6.7, and 31.8 mg DEET/cm^2 (respectively) in various formulations, based on urinary, fecal, and tissue recovery of radioactivity, indicating a J-shaped dose-response.

Available *in vivo* information on species differences in dermal uptake are limited but suggest that the differences among laboratory animals may be small. When dermal absorption was estimated based on cumulative urinary and fecal excretion of radioactivity over 7 days after a single dermal application of 4 μg ^{14}C-DEET/cm^2, estimates of 44, 33, 38, and 31% absorption were reported for male Sprague-Dawley rats, female Sprague-Dawley rats, female New Zealand White rabbits, and male Beagle dogs, respectively (Snodgrass et al. 1982). While the species differences were small in this study, the data did suggest gender differences, with male rats absorbing a greater percentage than females.

Fediuk et al. (2011) reported the relative bioavailability in rats of dermally-applied DEET in ethanol (100 mg/kg) to Sprague-Dawley rats as 1.5% based on the ratio of the dermal and intravenous plasma area under the curve values (24 hours after the end of exposure). The study authors attributed the low bioavailability in this study to the use of the ethanol vehicle which may have enhanced evaporation. However, it should be noted that others have shown that 30–45% ethanolic solutions of DEET increased permeation of DEET into human's skin *in vitro* compared to DEET alone or to 60–90% ethanolic solutions (Stinecipher and Shah 1997). These investigators suggested that at low ethanol concentrations,

extraction of skin lipids associated with alteration of polar pathways results in increased permeation of DEET. However, higher concentrations of ethanol may extract lipids from the skin and alter the barrier function and decrease uptake by the skin resulting in decreased permeation of DEET.

In addition to evaporation, a variety of other factors can affect the dermal uptake of DEET. These factors are most notably the vehicle and/or formulation of the product containing DEET, but articles often provide little or no information regarding inert ingredients in the product being evaluated or any vehicle that was used. Qiu et al. (1997a, 1997b) evaluated the dermal bioavailability of two different DEET formulations in Beagle dogs. One was a novel formulation (7.5% DEET) and the second was Off! Skintastic® containing 7.125% DEET. Using the ratio of area under the plasma concentration-time curve to dose after dermal (15 mg/kg) and intravenous (2.5 mg/kg) exposures, the study authors estimated the absolute dermal bioavailability of the two formulations to be 14% (Off! Skintastic®) and 18% (novel formulation). More recently, Brand et al. (2006) showed that administration of a single gavage dose of ≥4.3 g ethanol/kg to rats significantly increased skin absorption of DEET when a piece of the rat's skin was tested 2 hours later in an *in vitro* flow-through diffusion cell system. The ethanol-induced enhancement of DEET absorption was dose-related. A mechanism for these findings was not explored in the study. Their conclusion was that acute and chronic consumption of alcoholic beverages compromises the skin barrier and increases the dermal absorption of DEET; this enhancement remains for at least 24 hours after blood alcohol levels subside since ethanol clears from the skin more slowly than from blood.

The sunscreen, oxybenzone, has been demonstrated to enhance the dermal penetration of DEET in both *in vivo* (Kasichayanula et al. 2007; Wang and Gu 2007) and *in vitro* studies (Chen et al. 2010; Gu et al. 2005; Ross et al. 2004). Kasichayanula et al. (2007) evaluated the absorption of DEET across the shaved skin of 3-week-old piglets. The test materials were a commercial insect repellent containing 9% DEET and a combined sunscreen/repellent that contained 9% DEET. One gram of the product was applied to a surface area of 150 cm^2. Plasma samples were collected at regular intervals between 0 and 48 hours after application for HPLC analysis. When the repellent was applied alone, the concentration of DEET in the plasma peaked at ~28 μg/mL 2 hours after dosing, declined rapidly over the next 10 hours, and then declined very gradually for the subsequent 36 hours. A similar profile was seen with the combination product. The area under the curve of the plasma concentration:time plot was higher after application of the combined repellent/sunscreen product (446.21 μg hour/mL) compared with repellent alone (286.59 μg hour/mL), indicating enhanced absorption of DEET from the combination product. Wang and Gu (2007) used the same experimental design as Gu et al. (2005) and had similar methodological issues. For example: (1) the human skin samples were prepared very aggressively with freezing, thawing, scraping,

and cutting, but the integrity of the skin samples used for the experiments was only visual; a more rigorous and standardized approach to evaluate the integrity of skin samples, such as $^{3}H_2O$ penetration (Santhanam et al. 2005), would have been more appropriate, and (2) it was stated that the amount of test sample in direct contact with the skin surface (0.64 cm^2) in the diffusion cells was measured to be 0.1 g (equivalent to approximately 100 µL/cell and 156,250 µg/cm^2 skin); this appears to be an enormous dose in comparison to human use of DEET and sunscreen products and to the *in vitro* study of Santhanam et al. (2005) in which Franz diffusion cells with DEET applied to human skin samples used doses of 5 µL/cell in most experiments and a maximum dose of 20 µL/cell.

Moody and Nadeau (1993) compared the *in vitro* dermal permeability of ^{14}C-DEET across skin samples from several animal species and humans. Doses varied approximately 2-fold across the experiments from 12.5 to 44.7 µg/cm^2; the skin thickness was the same (0.5±0.01 mm) for all samples except human foreskin, which was 0.3 mm. Table 3-5 shows the pharmacokinetic parameters calculated from the experiments. The maximum rate of permeability was greatest across mouse skin (2.9 µg/cm^2/hour) and lowest across guinea pig skin (0.4 µg/cm^2/hour). The maximum rate of permeability of DEET across human skin *in vitro* was between 1 and 2 µg/cm^2/hour (Moody and Nadeau 1993).

In vitro estimates of DEET skin permeability vary significantly depending on the vehicle in which it is applied (Qiu et al. 1998; Moody et al. 1995; Stinecipher and Shah 1997). Qiu et al. (1998) reported 10–23% reductions in the flux of DEET across the skin with the use of 20% (w/w) PEG 400, 1% (w/w) Tween 80, or 75% (v/v) ethanol in Carbopol 940 NF and Pemulun TR-2 formulations when compared with a commercially-available preparation containing an equivalent concentration of DEET. *In vitro* measurements of flux and permeability across human skin were higher for DEET in 30–45% ethanol solutions compared with 75–90% ethanol solutions (Stinecipher and Shah 1997). Similarly, in a study by Iscan et al. (2006), *in vitro* skin permeation rates were shown to vary depending on the concentration of ethanol; flux rates were 0.41, 0.14, and 0.09 mg/cm^2-second at 45, 70, and 95% ethanol, respectively (Iscan et al. 2006).

The percent of applied dose that is absorbed across human skin appears to depend on dose and integrity of the skin samples. Santhanam et al. (2005) evaluated *in vitro* permeability of DEET across human cadaver skin at doses ranging from 0.02 to 11,000 µg/cm^2. The percent penetration increased with dose up to 680 µg/cm^2 and then declined at higher doses. Moody et al. (1995) observed lower absorption of ^{14}C-DEET from higher doses of DEET. Based on a methodology of recovery from receiver solution, skin extraction with methanol and skin digest, the authors estimated 48, 36, and 17% absorption across human

Table 3-5. Species Differences in *In Vitro* Estimates of DEET Dermal Permeability

Species (site of skin sample)	Applied dose (µg/cm^2)	Permeability based on receiver solution (%)	Permeability based on receiver solution, skin wash and skin digest (%)	Maximum rate of permeability (µg/cm^2/hour)	Lag time[a] (hours)
Mouse (back)	33.3	36.2±27.5	39.0±29.0	2.9±1.81	1.4±0.45
Rat (back)	38.7	21.4±2.17	64.8±2.70	1.3±0.07	1.9±0.08
Guinea pig (back)	12.5	10.9±1.40	38.4±4.46	0.4±0.14	1.3±0.20
Yorkshire pig (back)	19.4	15.3±0.82	28.9±5.17	0.7±0.12	1.4±0.20
Human (abdomen)	44.7	27.7±4.24	28.1±4.28	2.0±0.58	0.6±0.28
Human (neonatal foreskin)	27.9	13.1±9.58	13.8±9.59	0.98±0.91	1.6±1.73

[a]Time to appearance in receiver fluid.

Source: Moody and Nadeau (1993)

skin at applied doses of 16.7, 25.3, and 97.3 mg DEET/cm^2, respectively, in three different commercial preparations. Similar experiments conducted with rat skin showed little or no difference in percent absorption across a similar dose range and the same preparations (Moody et al. 1995).

A number of studies have examined alternative formulations of DEET intended to minimize skin permeation and maximize the duration of insect repellent effectiveness by prolonging evaporation time.

Karr et al. (2012) showed that microencapsulation formulations resulted in lower penetration across split-thickness human cadaver skin tested in Franz cells modified to allow controlled airflow trapping. Kasting et al. (2008) reported a similar observation; microencapsulation using walled polysaccharide microcapsules diminished the dermal uptake of DEET by 25–35% (compared with an ethanol vehicle) in *in vitro* tests using human cadaver skin. Similarly, Iscan et al. (2006) incorporated DEET into solid lipid particles as a colloidal solution and observed decreased permeation across human donor skin from plastic surgery patients when compared to free DEET in the same preparation. Wang et al. (2014) showed that oil-in-water emulsions significantly lowered percutaneous permeation of DEET through isolated human skin compared to water-in-oil emulsions. Experiments also showed that the addition of xanthan gum to the oil-in-water emulsion reduced the size of oil droplets containing DEET and increased penetration of DEET through human skin.

Exposure to other compounds prior to dermal exposure to DEET may also alter skin permeability. Kaushik et al. (2010) observed that pretreatment of human skin *in vitro* with different compounds (laurocapram, iminosulfuram, and others) could enhance or retard the rate of skin penetration depending on the vehicle in which the pretreatment was applied.

Airflow across exposed skin affects dermal penetration of DEET by its effect on volatilization. Santhanam et al. (2005) observed lower penetration across human skin tested *in vitro* under a fume hood with higher airflow compared with tests conducted on a laboratory workbench with lower airflow. Reifenrath et al. (1991) observed reduced (one-third as high) dermal penetration of ^{14}C-DEET across excised pig skin *in vitro* when air flow was increased by 10-fold.

3.4.2 Distribution

Limited data regarding distribution of DEET in humans indicate that DEET can distribute to cord blood following dermal exposure of pregnant women (Barr et al. 2010; McGready et al. 2001). No data were located regarding distribution following inhalation or oral exposure.

Distribution data are available in animals exposed orally or dermally. A single study of CD rats exposed via gavage (Schoenig et al. 1996) indicated distribution to a number of organs (liver, lung, spleen, kidney, and fat) without identifying specific deposition sites for DEET. Similar findings were reported after dermal exposure to DEET in rats, rabbits, and dogs (Fediuk et al. 2010; Schoenig et al. 1996; Snodgrass et al. 1982). An older study that used whole-body autoradiography to assess distribution after dermal exposure to ^{14}C-DEET reported high levels of radioactivity in the lacrimal glands and nasal mucosa of albino mice (Blomquist and Thorsell 1977); these tissues were not assessed in other studies. When pregnant New Zealand White rabbits were exposed topically to ^{14}C-DEET (50, 100, or 500 mg/kg/day) for 29 days, radioactivity was not detected in the fetuses at the end of treatment (Snodgrass et al. 1982).

After intravenous exposure, DEET undergoes extensive extravascular distribution; estimates of steady-state volume of distribution in beagle dogs (Qiu et al. 1997b) and Sprague-Dawley rats (Fediuk et al. 2011) exceeded the total body water of these species. Some evidence for transplacental transfer of intravenously-administered DEET was provided in studies of pregnant mice and rabbits exposed intravenously to ^{14}C-DEET; radioactivity was detected at low levels in the fetuses of both species (Snodgrass et al. 1982; Blomquist et al. 1975).

DEET did not bind to plasma proteins in *in vitro* tests conducted with HSA; the fraction of unbound DEET after a 60-minute incubation of DEET in saline with up to 10 μg/mL HSA was 95% (Abu-Qare and Abou-Donia 2002). Kasting et al. (2008) measured the binding of DEET to BSA in saline using equilibrium dialysis in side-by-side diffusion cells (one side containing BSA and one side saline only). Equilibrium was reached in 2–3 days, and the fraction unbound was calculated to be 0.189±0.008 (Kasting et al. 2008).

3.4.2.1 Inhalation Exposure

No information on the tissue distribution of DEET in humans or animals exposed via inhalation was located.

3.4.2.2 Oral Exposure

When CD rats were given single doses of 100 or 500 mg/kg ring-labeled ^{14}C-DEET in corn oil via gavage and sacrificed 7 days later for tissue analysis, total tissue residues of ^{14}C activity ranged from 0.15 to 0.67% of administered radioactivity and the distribution of radioactivity showed highest concentrations in the liver, lung, spleen, kidney, and fat. The percent of administered radioactivity reaching systemic circulation and the tissues was much higher for animals administered ^{14}C-DEET orally than for animals administered ^{14}C-DEET dermally (Schoenig et al. 1996).

3.4.2.3 Dermal Exposure

McGready et al. (2001) studied the distribution of 1.7 g DEET/day in the second and third trimesters of 449 pregnant women as part of a double-blind trial of insect repellents in the prevention of malaria in Thailand. DEET was not detected in 30 urine samples from DEET-exposed women, but was detected in 4 of 50 samples of cord blood from women exposed to DEET. Barr et al. (2010) assessed the distribution of DEET by measuring pesticides in maternal serum prior to birth and in cord blood after delivery. DEET was one of the pesticides most frequently detected in maternal and cord serum; the corresponding mean concentrations were 3.21 ng/g (range 1.82–18.84 ng/g) and 3.12 ng/ng (range 2.06–13.07 ng/g).

Schoenig et al. (1996) measured tissue concentrations of radioactivity in CD rats 7 days after a single dermal application of 100 mg/kg ring-labeled ^{14}C- DEET. Radioactivity levels were low (<0.4 ppm) in all tissues; apart from the carcass, the highest concentrations were in the liver (0.21 and 0.22 ppm in males and females, respectively), kidneys (0.08 and 0.02 ppm in males and females, respectively), and blood (0.04 and 0.05 ppm in males and females, respectively).

Fediuk et al. (2010) detected DEET in the liver and brain of male and female Sprague-Dawley rats that had received daily topical applications of 40 mg DEET/kg (2,500 μg/cm^2 skin), either alone or in combination with the sunscreen, oxybenzone, for 30 days. The median concentration in liver was significantly higher when DEET was applied with oxybenzone (350.8 ng/g) than when applied alone (95.9 ng/g). In contrast, the concentrations in the brain (7.6–8.5 ng/g) were similar with both treatments (Fediuk et al. 2010). In a related study also conducted in rats, a single, 24-hour dermal exposure to 100 mg DEET/kg yielded liver and kidney concentrations of 14.6 and 12.2 ng/g, respectively (Fediuk et al. 2011). The study authors noted that the use of an ethanol vehicle for the latter study likely facilitated evaporation of DEET, decreasing the quantities that reached the liver and kidney.

Snodgrass et al. (1982) measured the tissue distribution of ^{14}C-DEET in small groups of Sprague-Dawley rats (n=6/sex), New Zealand White rabbits (n=6 females), and Beagle dogs (n=3 males) 7 days after dermal application of 4 μg/cm^2, and observed the highest levels in the lung and spleen of dogs, lung of rabbits, and liver and kidney of female rats. The levels, however, varied among individual animals; radioactivity was not detected in these organs in some animals. Using whole-body autoradiography, Blomquist and Thorsell (1977) measured the highest levels of radioactivity in the lacrimal gland, liver, kidney, and nasal mucosa of albino mice 2 hours after a topical application of 15 mg ^{14}C-DEET/kg for 2 hours.

Dermal application of ^{14}C-DEET at doses of 50, 100, or 500 mg/kg/day to pregnant New Zealand White rabbits on GDs 1–29 did not result in detectable radioactivity in the fetuses at sacrifice at the end of exposure (Snodgrass et al. 1982).

3.4.2.4 Other Routes of Exposure

Intravenously administered DEET undergoes extensive extravascular distribution, as shown by the volume of distribution in dogs and rats. Qiu et al. (1997b) calculated a mean steady-state volume of distribution of 6.21 L/kg in Beagle dogs exposed to 2.5 or 6.0 mg DEET/kg via intravenous injection; this volume is 10 times higher than the total body water of a lean dog (~0.6 L/kg; Davies and Morris 1993) and demonstrates extravascular distribution. Similarly, Fediuk et al. (2011) calculated a steady-state volume of distribution of 5.6 L/kg in Sprague-Dawley rats receiving intravenous injections of DEET (2 mg/kg); this compares with a total body water of ~0.7 L/kg in rats (Davies and Morris 1993).

In albino mice exposed to ^{14}C-DEET (0.05 μCi/g body weight) via intravenous injection and evaluated by whole-body autoradiography, the highest concentrations of radioactivity were detected (in descending order) in the lacrimal gland, liver, kidney, and nasal mucosa (Blomquist et al. 1975). Radioactivity persisted for 24 hours postdosing in the lacrimal gland, liver, nasal mucosa, urinary bladder, and intestinal contents, but none was detected on the autoradiograms 3 days later (four days post-dosing). When male albino mice were fasted for 18–24 hours prior to treatment, lower concentrations of radioactivity were detected in the liver and kidney compared with levels in mice allowed to feed prior to exposure (Blomquist et al. 1975).

A similar pattern of radioactivity was seen in a pregnant albino mouse examined 20 minutes after injection of DEET; the concentration of radioactivity in the fetus was low. The highest concentrations in

the fetus were in the kidney, urinary bladder, gastric mucosa, lens, and liver. Very little radioactivity was detected in the fetus 4 hours after exposure of the pregnant dam (Blomquist et al. 1975). Snodgrass et al. (1982) observed low levels of radioactivity (about one-sixth of the corresponding maternal blood levels) in the fetuses of New Zealand White rabbits treated with a single intravenous injection of 140.6 μg ^{14}C-DEET on GD 15.

3.4.3 Metabolism

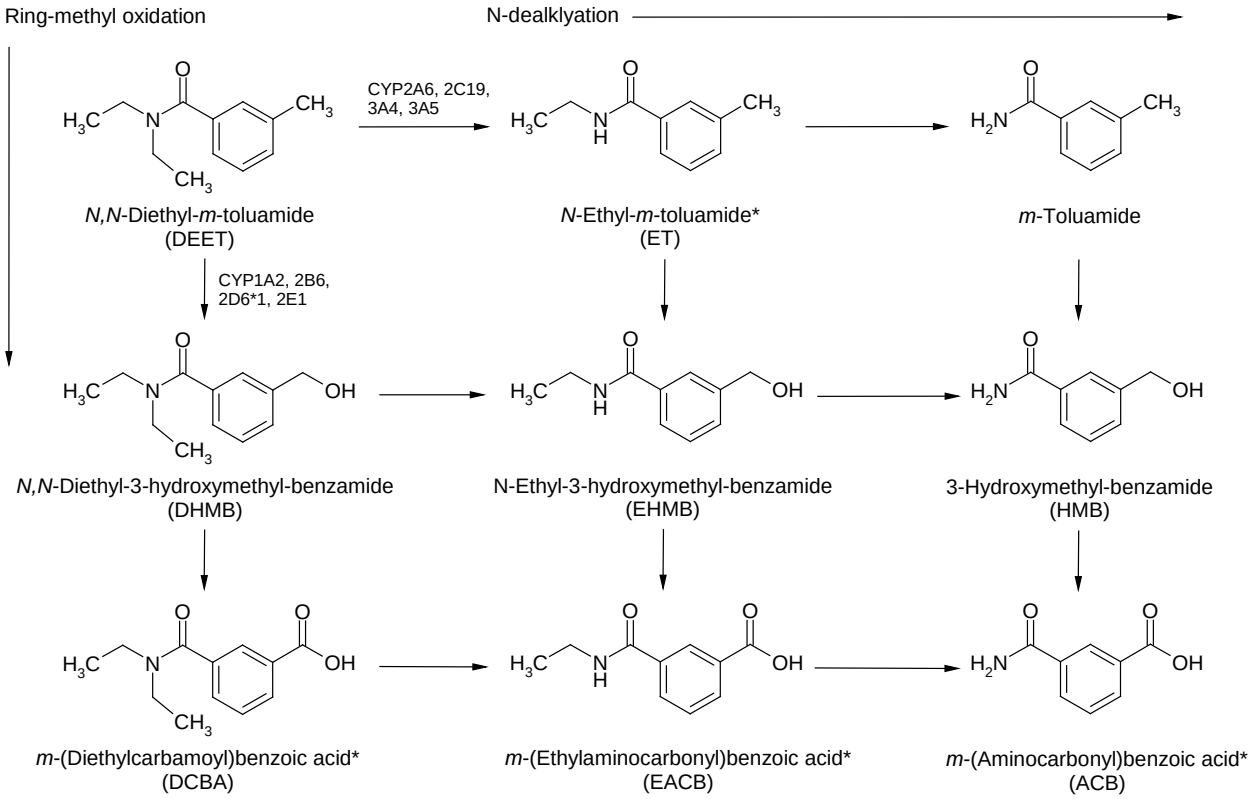
The major metabolic pathways for DEET are shown in Figure 3-3. The primary metabolites of DEET in humans exposed dermally (Selim et al. 1995) and in rats exposed via oral, dermal, or intraperitoneal injection routes (Schoenig et al. 1996; Taylor and Spooner 1990) are DCBA and EACB; metabolism has not been examined after inhalation exposure. ET occurs in urine as the glucuronide conjugate and has been identified in acid-hydrolyzed human (Tian and Yiin 2014; Wu et al. 1979) and rat (Taylor and Spooner 1990) urine. Other metabolites identified in human urine include N-ethyl-N-(1-hydroxyethyl)-3-methylbenzamide and 3-((carboxymethyl)(hydroxymethyl)carbamoyl)benzoic acid (Wu et al. 1979), while ACB and *m*-toluic acid have also been identified in rat urine (Taylor and Spooner 1990).

DHMB is produced by oxidation of the methyl group on the benzene ring to carboxylic acid, a reaction mediated primarily by CYPs 1A2 and 2B6 in humans (Usmani et al. 2002). ET results from dealkylation of the amide group; the primary cytochrome P-450 isozymes that catalyze this reaction are CYP2C19 and CYP3A4 (with small contributions from CYPs 2A6 and 3A5) in humans (Usmani et al. 2002). Other minor metabolites have been observed after incubation of DEET with liver microsomes, including N,N-diethyl-*m*-formylbenzamide and N-ethyl-*m*-hydroxymethylbenzamide (EHMB) (Taylor et al. 1986).

Metabolism via one or the other of these pathways will be favored in humans with higher levels of the corresponding CYPs (Usmani et al. 2002). At low substrate concentrations, the ring methyl oxidation pathway is expected to predominate due to higher substrate affinities of the relevant cytochrome P-450 isozymes (Usmani et al. 2002). There is evidence that DEET can induce CYPs 3A4, 2B6, 2A6, 1A1, and 1A2 translation and transcription, thereby inducing its own metabolism (Abu-Qare and Abou-Donia 2001a; Usmani et al. 2002).

Available information suggests that metabolism occurs rapidly; metabolites were detected in the plasma of rats as soon as 30 minutes after a 24-hour dermal exposure (Fediuk et al. 2012). The liver is the primary site of metabolism (Abu-Qare and Abou-Donia 2008). Limited *in vivo* and *in vitro* data suggest

Figure 3-3. Primary Metabolic Pathways of DEET in Rodents and Humans



*Detected in human urine as free or conjugated metabolite

Sources: Constantino and Iley 1999; Schoenig et al. 1996; Selim et al. 1995; Taylor 1986; Taylor and Spooner 1990; Usmani et al. 2002; Wu et al. 1979

the possibility of gender differences in metabolism of DEET (Schoenig et al. 1996; Yeung and Taylor 1988); males may metabolize DEET faster than females, and females may produce more EACB than DCBA at higher doses.

3.4.3.1 Inhalation Exposure

No information on the metabolism of DEET in humans or animals exposed via inhalation was located.

3.4.3.2 Oral Exposure

A single rat study provides information on metabolism of DEET after oral exposure. Schoenig et al. (1996) administered ^{14}C-DEET in corn oil via gavage to male and female CD rats and collected urine samples over the next 36–72 hours. Analysis of urine samples by HPLC showed complete metabolism of DEET at both low (100 mg/kg) and high (500 mg/kg) doses (no DEET was detected in urine). The majority of the excreted radioactivity (~50–60% of administered radioactivity depending on dose and regimen) was associated with the metabolite DCBA, which resulted from oxidation of the methyl group to carboxylic acid. A second metabolite, EACB, resulting from dealkylation of the amide group, accounted for between 3 and 17% of the administered radioactivity (Schoenig et al. 1996). No effort was made to identify the minor metabolites.

3.4.3.3 Dermal Exposure

Selim et al. (1995) analyzed the urine of human volunteers exposed to undiluted DEET or 15% DEET in ethanol via dermal application (~0.5 mg/cm^2). Unchanged DEET was not detected in the urine. Six peaks were separated by HPLC; these six accounted for the majority (>60%) of urinary radioactivity after either form of DEET was applied. By comparing the HPLC profile from human urine with the profile from the urine of rats treated with DEET, the authors identified two of the metabolites; 24–42% of the urinary radioactivity consisted of DCBA, while between 7.6 and 26% consisted of EACB (a coeluting peak prevented more precise quantification of this metabolite).

Wu et al. (1979) identified (but did not quantify) DEET metabolites in the urine of a 30-year old, 78 kg male subject who applied 10.4 g of DEET in a repellent to ~75% of his body; the duration of treatment was not specified. Four possible metabolic pathways were identified, and five metabolites resulting from three of the pathways were identified by electron impact ionization and chemical ionization mass spectrometry. The metabolites were identified as: DCBA; the glucuronide conjugate of ET; N-ethyl-

N-(1-hydroxyethyl)-3-methylbenzamide; and 3-((carboxymethyl)(hydroxymethyl)carbamoyl)benzoic acid and a small amount of DHMB was tentatively identified.

Tian and Yiin (2014) reported that application of 10 mL of a repellent containing 12% DEET to the arms or legs of children (5–7 years old) and adults (23–25 years old) resulted in urinary excretion mainly of DCBA and ET and a small amount of unchanged DEET over an 8-hour period after the application. DCBA constituted 78.2% of the total metabolites in children and 46.1% in adults. No significant differences regarding metabolic profile were observed between male and female subjects. Expressed as DEET equivalents, a greater amount of metabolites were recovered from children (1,116 µg) than from adults (446 µg). The reason for this difference was not totally clear, but no details were provided regarding the exposure conditions.

The urinary metabolite profile observed in Wistar rats exposed to 100 mg DEET/kg via dermal application was similar to that seen after oral exposure (Schoenig et al. 1996). The major urinary metabolites were DCBA, which represented 47–48% of the administered radioactivity, and EACB, which represented 3–13% of administered radioactivity. Female rats excreted higher amounts of EACB (13%) than males (3%). Taylor and Spooner (1990) administered 50 mg ^{14}C-DEET/kg by topical application to the backs of male and female Wistar rats. DCBA and EACB were the primary urinary metabolites, accounting for ~36–37% and ~11–12% of the administered radioactivity, respectively, in the first 48 hours (metabolites were quantified in two 24-hour urine samples). Minor metabolites identified in the rat urine were ACB and *m*-toluic acid; these were not quantified. Analysis of acid-hydrolyzed urine revealed the presence of ET; the authors suggested that it was conjugated with glucuronide in urine. In contrast to the results reported by Schoenig et al. (1996), Taylor and Spooner (1990) detected unchanged DEET in the urine, accounting for 4.7–5.5% of the applied dose in the first 48 hours after dosing.

Fediuk et al. (2012) measured the levels of DHMB and ET in the plasma of Sprague-Dawley rats as soon as 30 minutes after the end of a 24-hour dermal treatment with 100 mg/kg (4 mg/cm^2) DEET. Plasma was not analyzed for any other metabolites or for DEET. The plasma concentrations of both DHMB and ET continued to increase for 24 hours postexposure. At the end of the 24-hour observation period, concentrations of DHMB and ET were ~140 and 120 ng/mL, respectively. In a repeated exposure experiment conducted by the same authors, the concentrations of DHMB and ET were ~150 and 350 ng/mL, respectively, after a 30-day repeated application of 40 mg/kg (10 mg/cm^2) DEET.

In liver samples taken at sacrifice at the end of the 24-hour exposure to 100 mg DEET/kg, the concentrations of DHMB and ET were similar at 29±2.9 and 36±4.2 ng/g, respectively (Fediuk et al. 2012). In contrast, after 30 days of repeated dermal dosing with 40 mg DEET/kg, the concentration of DHMB in the liver was ~3 times higher (384.3±87.3 ng/g) than that of ET (139.6±57.1 ng/g).

DHMB and ET were measured in urine samples taken from piglets 48 hours after dermal application of a repellent lotion containing 9% DEET (1 g quantity) to a shaved area of 150 cm^2 (Kasichayanula et al. 2005). Urine was not analyzed for other metabolites. The concentration of DHMB in urine was about twice that of ET (0.99 vs. 0.55 μg/mL, respectively); no parent compound was detected.

3.4.3.4 Other Routes of Exposure

After intraperitoneal administration of 50 mg ^{14}C-DEET/kg, male Wistar rats excreted 55–66% of the administered dose as DCBA in the first 24 hours after dosing; an additional 16–22% was excreted as EACB in the same time period (Taylor and Spooner 1990). As with dermal exposure, two additional urinary metabolites were identified (*m*-aminocarbonyl) benzoic acid and *m*-toluic acid; these metabolites were not quantified (Taylor and Spooner 1990). Acid hydrolysis of the urine revealed ET, which was likely present in urine as the glucuronide conjugate (Taylor and Spooner 1990).

In vitro experiments with liver microsomes show metabolism of DEET to DHMB and ET. Based on studies in DEET-exposed humans and rats (Schoenig et al. 1996; Selim et al. 1995; Taylor and Spooner 1990), these compounds appear to be intermediates and/or minor *in vivo* metabolites of DEET. Incubation of 1,000 nmol DEET with phenobarbital-induced male rat liver microsomes for 45 minutes resulted in metabolism of at least 65% of the DEET (of the total 71% recovered; Taylor 1986). The major metabolite was DHMB (422.2 nmol), followed by ET (222.5 nmol); only 62.8 nmol of DEET was recovered at the end of the experiments (Taylor 1986). Metabolism varied with pH. At pH 8.6, two minor metabolites, N,N-diethyl-*m*-formylbenzamide and EHMB were detected at low levels. At pH (7.4), metabolism of DEET was virtually abolished (<7% was metabolized).

Using baculovirus-infected insect cells expressing specific human cytochrome P-450 isozymes, Usmani et al. (2002) demonstrated that the two primary metabolites of DEET are each produced by specific isozymes, with no cross-reactivity. Specifically, DHMB is formed by CYPs 1A2, 2B6, 2D6*1, and 2E1, while ET is formed by CYPs 2A6, 2C19, 3A4, and 3A5. Table 3-6 shows the activity of each isozyme on the DEET substrate; as the table shows, CYP2E1 has relatively low activity compared with the other

Table 3-6. CYP-Specific Metabolism of DEET by Human CYPs Expressed in Baculovirus-Infected Insect Cells

		Kinetic parameters		
CYP isoform	Activity at 1,000 or 3,000 µM DEET (nmol/nmol isoform/minute)	V_{max} (nmol/mg protein/minute)	K_m (µM)	CL_{int} (10^{-6}/nmol isoform/minute)
DHMB formation (ring methyl oxidation)				
CYP1A2	68.94±2.64	24.5±1.2	41.0±2.0	598.7±39.6
CYP2B6	69.51±1.83	22.3±2.1	40.2±1.2	552.0±40.4
CYP2D6*1	56.56±2.52			
CYP2E1	3.34±0.17			
ET formation (N-deethylation)				
CYP2A6	4.55±0.30			
CYP2C19	8.96±0.82			
CYP3A4	5.05±0.20			
CYP3A5	5.81±0.24			

Source: Usmani et al. (2002)

DHMB-forming isozymes. Usmani et al. (2002) also evaluated species differences in metabolism of DEET by human, Long-Evans rat, and CD-1 mouse microsomes *in vitro*; the calculated intrinsic clearances of human and mouse microsomes were similar, while much higher clearance (>2-fold) was calculated from data in rat microsomes (Table 3-7).

Abu-Qare and Abou-Donia (2008) showed that DEET is primarily metabolized in the liver rather than in plasma. The authors incubated human plasma with DEET for 60 minutes and measured the disappearance of DEET over time; the half-life for DEET in plasma was calculated to be 665 minutes. In contrast, when DEET was incubated with human liver microsomes, the disappearance half-life was 60 minutes. When the experiments were conducted in the presence of pyridostigmine bromide and/or permethrin, the plasma disappearance half-life decreased by more than half (indicating accelerated metabolism), while the liver microsome half-life increased by 2.6–5.3-fold (indicating slowed metabolism) (Abu-Qare and Abou-Donia 2008). The study authors identified *m*-toluamide as a metabolite of DEET in the human liver microsomes, and reported K_m and V_{max} values of 62 μM and 112 pmol/minute/mg protein, respectively, for the formation of this metabolite.

Gender differences in the rate of DEET metabolism were demonstrated in a study by Yeung and Taylor (1988). When liver microsomes from male and female Wistar rats were incubated with DEET for 2 hours, microsomes from male rats metabolized DEET much faster than those from female rats, as measured by the disappearance of DEET from incubation solution and appearance of DHMB and ET metabolites. Table 3-8 compares the microsomal metabolism data for males and females.

3.4.4 Elimination and Excretion

There are no studies of the excretion of DEET in humans or animals exposed via inhalation in the available scientific literature.

DEET is rapidly cleared from the plasma after dermal or intravenous exposure. Wu et al. (1979) evaluated the metabolism of DEET in a 30-year-old, 78-kg subject who applied 10.4 g of DEET in a repellent to ~75% of his body. Urine was collected for 36 hours, and the rate of excretion of unchanged DEET via urine was estimated as 10–14% in the first hour and was reduced to 2% by the fourth hour. After dermal exposure, the plasma elimination half-life has been reported to be ~6–9 hours in rats (Fediuk et al. 2011), 2.5–2.7 hours in Beagle dogs (Qiu et al. 1997a, 1997b), and 7.3 hours in piglets

Table 3-7. *In vitro* Liver Microsomal Metabolism Parameters of DEET

Species	V_{max} (nmol/mg protein/minute)	K_m (μM)	CL_{int} (10^{-6}/mg protein/minute)
DHMB formation (ring methyl oxidation)			
Human	12.9±1.6	67.6±4.2	191.5±15.4
Rat	17.6±1.2	38.3±0.2	461.3±23.5
Mouse	6.8±1.4	43.4± 0.6	156.8±23.8
Mouse treated *in vivo* (200 mg/kg/day)	16.4±3.4	42.6±13.6	385.1±52.6
ET formation (N-deethylation)			
Human	20.5±3.4	842.5±49.9	24.4±5.1
Rat	19.2±2.8	214.3±26.1	89.5±10.9
Mouse	14.5±2.9	660.6±59.5	21.7±3.9
Mouse treated *in vivo* (200 mg/kg/day)	22.9±2.9	630.9±128.0	38.3±5.1

Source: Usmani et al. (2002)

Table 3-8. Gender Differences in *In Vitro* Rat Liver Microsomal Metabolism of DEET

Parameter	Male	Female
Percent metabolized at 2 hours	58±4.8[a]	17±1.6
Rate of DEET disappearance (minute^{-1})	0.0667±0.007[b]	0.0467±0.002
Half-life for DEET disappearance (minutes)	10±1.5[b]	15±1.1
Rate of DHMB appearance (minute^{-1})	0.0777±0.009[a]	0.0273±0.001
Rate of ET appearance (minute^{-1})	0.970±0.011[a]	0.346±0.002

[a]Significantly different from female, $p<0.001$.
[b]$p<0.05$.

DHMB = N,N-diethyl-*m*-hydroxymethylbenzamide; ET = N-ethyl-*m*-toluamide

Source: Yeung and Taylor (1988)

(Kasichayanula et al. 2007). After intravenous exposure, plasma elimination half-lives of 1.7 hours in rats (Fediuk et al. 2011) and 2.56 hours in Beagle dogs (Qiu et al. 1997a, 1997b) have been reported.

After oral, dermal, or intravenous exposure, the primary route of elimination is via urinary excretion of metabolites (Blomquist and Thorsell 1977; Schoenig et al. 1996; Selim et al. 1995; Snodgrass et al. 1982). At high dermal doses and/or after long-term repeated dermal exposure, some unchanged DEET is excreted in the urine (Smallwood et al. 1992; Taylor and Spooner 1990). A small amount of DEET is eliminated via the bile (Blomquist and Thorsell 1977; Moody et al. 1995; Qiu et al. 1997b; Schoenig et al. 1996; Selim et al. 1995; Snodgrass et al. 1982).

3.4.4.1 Inhalation Exposure

No information on the elimination of DEET in humans or animals exposed via inhalation was located.

3.4.4.2 Oral Exposure

Available data on elimination of DEET after oral exposure is limited to a single study using gavage administration in CD rats (Schoenig et al. 1996). In this study, up to 91% of an administered dose of 100–500 mg DEET/kg was recovered in the urine, with 3–6% recovered in feces, within 7 days of exposure (Schoenig et al. 1996). The rate of urinary excretion was high in the first 12 hours, during which ~75% of the low dose (100 mg/kg) and ~35–50% of the high dose (500 mg/kg) was excreted. Urinary excretion was minimal after 24 hours at both doses (Schoenig et al. 1996).

3.4.4.3 Dermal Exposure

Urinary excretion was the primary route of elimination in volunteers exposed to ^{14}C-DEET via dermal application (Selim et al. 1995). After application of ~0.5 mg DEET/cm^2 (neat or as a 15% solution in ethanol) to the arms of male volunteers, 5.6–8.3% of the administered radioactivity was excreted in the urine, and 0.02–0.08% was excreted in feces over the first 5 days post-application. The highest rates of urinary excretion occurred during the first 12 hours, and cumulative excretion increased very little after 24 hours (Selim et al. 1995). When a female volunteer was exposed for 8 hours to 0.12 mg^{14}C-DEET/kg via topical application, 3.8–5.5% of the applied radioactivity was excreted via the urine during the 48 hours following commencement of exposure (Blomquist and Thorsell 1977). The maximum rate of excretion (~0.23% per hour) occurred ~4–6 hours after the end of exposure.

Smallwood et al. (1992) developed a technique to analyze DEET in urine and tested the method on eight National Park employees who regularly used a DEET-containing insect repellent and on nine naïve volunteers exposed in a laboratory. The National Park employees were observed to apply sufficient quantities of the repellent, which contained 71% DEET, to yield a daily application of approximately 1 g of DEET. Concentrations of DEET in 24-hour urine samples collected from eight employees after the third day of the work week ranged from below the quantification limit of 0.18 µg/mL up to 5.69 µg/mL. Urinary concentrations were positively correlated ($p<0.05$) with estimated exposure (details not provided) among the eight employees. When nine volunteers without prior exposure were exposed in a laboratory to a DEET-containing repellent at doses between 0.14 and 1.86 g DEET, however, only two of the nine 24-hour urine samples showed DEET concentrations above the quantification limit; concentrations reported for these two volunteers ranged between 0.31 and 2.02 µg/mL; in the remaining seven volunteers, the concentrations of DEET in urine were <0.09 µg/mL, the LOD.

Wu et al. (1979) measured DEET in the urine of a 30-year-old, 78-kg male subject who applied 10.4 g of DEET in a repellent to ~75% of his body. The duration of treatment was not specified. The rate of excretion of unchanged DEET via urine was reported to be 10–14%/hour in the first hour and 2%/hour in the fourth hour. Unchanged DEET was detected in the urine up to 18 hours after application. Because 10.4 g is a very high dose, this may have caused saturation of metabolism pathways, which may have contributed to the detection of unchanged DEET in the urine.

Fediuk et al. (2011) calculated a plasma elimination half-life of ~6 hours in Sprague-Dawley rats exposed for 24 hours to 100 mg DEET/kg in ethanol via topical treatment. Co-treatment with oxybenzone yielded a 44% decrease in plasma elimination half-life (Fediuk et al. 2011). After 30 days of topical exposure (40 mg DEET/kg/day or 2,500 µg/cm^2/day), the plasma concentration of DEET declined with an apparent elimination half-life of 9.1 hours (Fediuk et al. 2010). DEET was still detected in the plasma 24 hours after the last treatment. Qiu et al. (1997a, 1997b) reported a plasma elimination half-life of 2.5–2.7 hours in Beagle dogs exposed to two different formulations of DEET via dermal application; these values were similar to the plasma elimination half-life after intravenous exposure (2.56 hours). Kasichayanula et al. (2007) reported a plasma elimination half-life of 7.3 hours for DEET in piglets exposed via the skin to either a commercial insect repellent containing 9% DEET or a sunscreen/repellent that also contained 9% DEET (for each product, 1 g was applied to 150 cm^2).

When CD rats received a 100 mg/kg dermal application of ^{14}C- DEET under occlusion, 74–78% of the administered radioactivity was recovered in the urine and 4–7% was recovered in the feces (Schoenig et

al. 1996). The rate of urinary elimination was slower than after oral exposure; only ~22–28% of the administered radioactivity was recovered in urine samples over the first 24 hours after dermal application, compared with up to 75% after oral dosing (Schoenig et al. 1996). Snodgrass et al. (1982) compared the 7-day excretion profiles of male and female Sprague-Dawley rats, female New Zealand rabbits, and male Beagle dogs following topical application of ^{14}C-DEET. The cumulative excretion was essentially complete in all species by 3–4 days postdosing. Species differences in cumulative excretion were not apparent.

Although the group sizes were small in this study (three animals each), the excretion profiles suggested a sex difference in excretion by rats; males exhibited a higher percent total excretion (~44% including urinary and fecal excretion) compared with females (~33% including urinary and fecal excretion; Snodgrass et al. 1982).

The detection of radioactivity in feces after dermal exposure suggests that rats, rabbits, and dogs eliminate a small amount of DEET via enterohepatic circulation (Moody et al. 1995; Schoenig et al. 1996; Snodgrass et al. 1982). An older study in albino mice provided direct evidence for biliary excretion of DEET. Blomquist and Thorsell (1977) reported high levels of radioactivity in the bile and intestinal tract (as well as the urine) when whole-body autoradiography of mice was performed after 2-hour dermal exposure to 15 mg ^{14}C-DEET/g.

3.4.4.4 Other Routes of Exposure

Clearance of DEET from plasma is rapid after intravenous administration. Fediuk et al. (2011) reported a clearance rate of 67 L/hour/kg and elimination half-life of 103 minutes in Sprague-Dawley rats after intravenous injection of 2.5 mg DEET/kg. Qiu et al. (1997a, 1997b) reported a plasma elimination half-life of 2.56 hours in Beagle dogs exposed intravenously to 2.5 mg DEET/kg. Qiu et al. (1997b) calculated the clearance of DEET in this study to be 2.66 L/hour/kg, and noted that this value exceeded the renal blood flow rate (1.38 L/hour/kg) and thus provided evidence for intrahepatic clearance of DEET in beagle dogs.

Snodgrass et al. (1982) observed species differences in excretion of intravenously-administered DEET, with lower cumulative excretion (about half as much, based on visual inspection of data shown graphically) in male beagle dogs than male or female rats or female rabbits.

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. However, if the uptake and disposition of the chemical substance(s) are adequately described, this simplification is desirable because data are often unavailable for many

biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

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

If PBPK models for DEET 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.

No PBPK modeling studies were located for DEET.

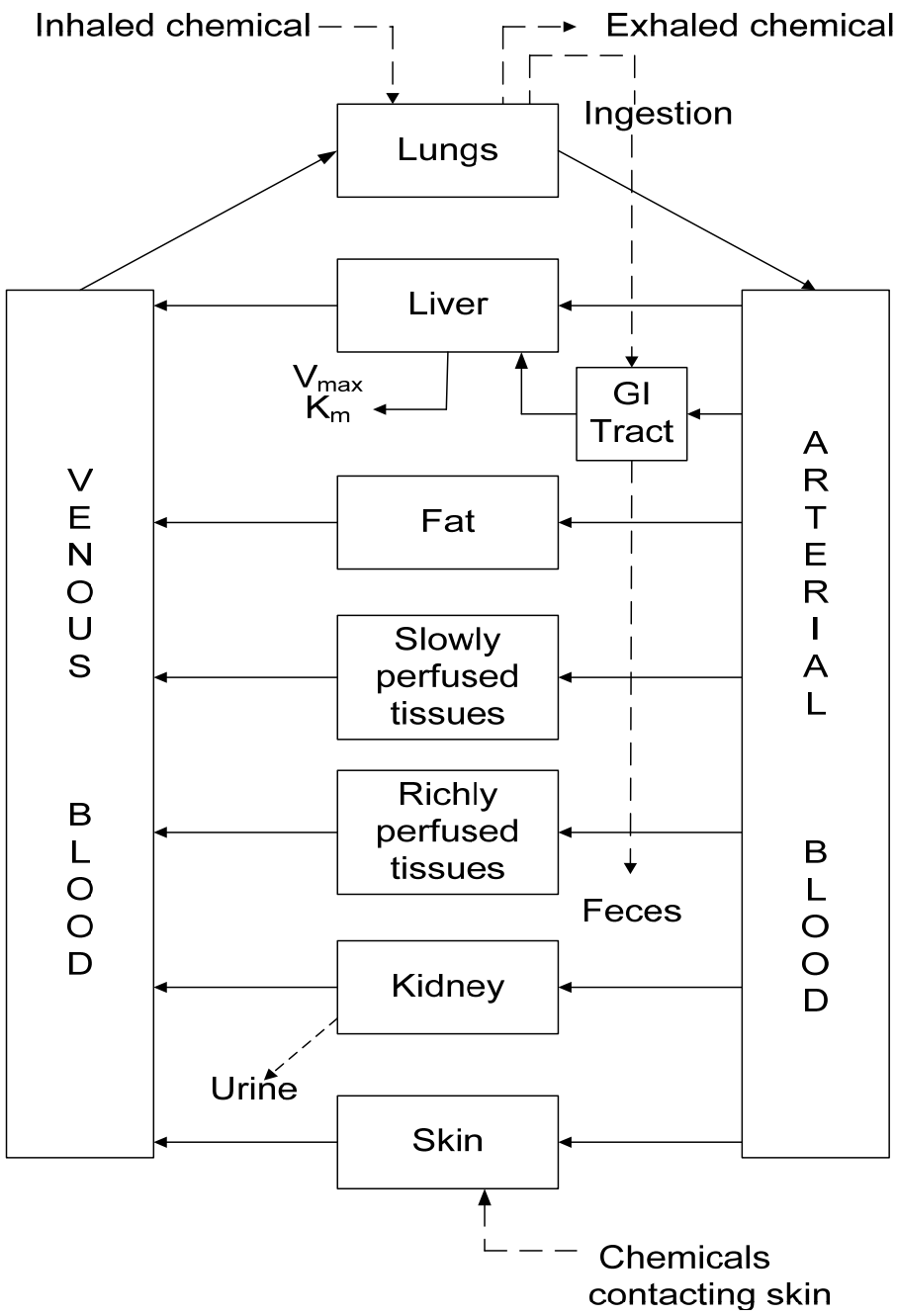
3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

As discussed in detail in Section 3.4 (Toxicokinetics), DEET is absorbed following oral or dermal exposure. No studies examining the mechanisms of DEET absorption were located in the available literature. The dermal absorption of DEET may be affected by species (Moody and Nadeau 1993), sex (Snodgrass et al. 1982), vehicle and/or formulation in which DEET is applied (Fediuk et al. 2011; Iscan et al. 2006; Karr et al. 2012; Kasting et al. 2008; Qiu et al. 1997a, 1997b), dose (Moody et al. 1995; Santhanam et al. 2005), evaporation rate (Reifenrath et al. 1991; Santhanam et al. 2005), and coexposure to other compounds. In particular, the sunscreen, oxybenzone, has been shown to increase the dermal absorption of DEET (Chen et al. 2010; Gu et al. 2005; Kasichayanula et al. 2007; Ross et al. 2004; Wang and Gu 2007).

Available studies provide somewhat disparate findings on the plasma protein binding of DEET; Abu-Qare and Abou-Donia (2002) observed little or no binding to HSA, but the incubation time was short (60 minutes). In contrast, Kasting et al. (2008) used an equilibrium dialysis method to estimate that ~81% of DEET is bound to BSA; equilibrium was reached at about 2–3 days of incubation, suggesting that the incubation time may have been too short in the earlier study. The significance of equilibrium being

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: Krishnan and Andersen 1994

reached in days is unclear since dermally applied DEET in humans is eliminated within hours (Selim et al. 1995).

The role of metabolism on the toxicity of DEET is not known. The two major pathways of DEET metabolism (ring methyl oxidation and N-deethylation) depend on specific CYP isozymes (oxidation via CYPs 1A2, 2B6, 2D6*1, and 2E1 and N-deethylation via CYPs 2A6, 2C19, 3A4, and 3A5); thus, the rate of metabolism and the nature of the metabolites produced may vary among individuals due to variations in these isozymes and their activities. Specifically, metabolism via one or the other of these pathways will be favored in humans with higher levels of the corresponding CYPs (Usmani et al. 2002). At low substrate concentrations, the ring methyl oxidation pathway is expected to predominate due to higher substrate affinities of the relevant cytochrome P-450 isozymes (Usmani et al. 2002). There is evidence that DEET can induce CYPs 3A4, 2B6, 2A6, 1A1, and 1A2 translation and transcription, thereby inducing its own metabolism (Abu-Qare and Abou-Donia 2001a; Usmani et al. 2002).

No information was located regarding mechanisms of elimination and excretion of parent compound or metabolites of DEET.

3.5.2 Mechanisms of Toxicity

In rare instances, DEET has been shown to induce adverse neurological effects in humans including seizure, ataxia, restlessness, uncontrolled limb movements, agitation, aggressive behavior, combativeness, impaired cognitive function, and opisthotonos. Studies in animals have reproduced some of these effects following high oral bolus exposure to DEET. In a repeated dose dermal study in rats, doses of ≥40 mg DEET/kg/day decreased the permeability of the blood-brain barrier (BBB) in various brain areas, but significantly only in the brainstem (Abou-Donia et al. 2001b). Investigators in this study provided several speculative hypotheses regarding DEET's effects on decreasing the permeability of the BBB, including regulation of expression of the cerebral endothelial multidrug transporter, *p*-glycoprotein (*p*-gp), which serves to protect the central nervous system by inducing efflux of drugs and chemicals; modulating levels of cyclic adenosine monophosphate (cAMP) after prolonged exposure, as high levels of cAMP have been shown to reduce BBB permeability; causing a hypothermic response, which may be responsible for the BBB permeability changes; and regulating the expression of proteins at tight junctions in the BBB resulting in reduced blood flow, and thus, reduced entry of chemicals into the brain. It should be noted that in this study, impaired sensory performance was reported at 4 mg DEET/kg/day, 1/10 the dose level that affected BBB permeability, and that doses up to 400 mg DEET/kg/day did not induce observable

clinical signs such as seizures, ataxia, or other signs. This lack of clinical signs suggests that the altered sensory performance may not be caused by alterations in BBB permeability and that changes of greater magnitude in BBB permeability or recruitment of additional brain areas are necessary for overt signs such as tremors or seizures to occur. The results of another dermal exposure study in rats from the same group of investigators suggested that DEET might affect cholinergic and noradrenergic pathways innervating brain areas involved in specific behaviors such as limb placing or beam-walking performance (Abou-Donia et al. 2001b). In a subsequent study of repeated-dosing dermal exposure of rats, DEET was shown to induce neuronal degeneration in the dentate gyrus, CA1 and CA3 subfields of the hippocampus, midbrain, brainstem, and Purkinje cell layer of the cerebellum (Abdel-Rahman et al. 2004). Possible mechanisms discussed by the investigators that could explain these morphological alterations included DEET-induced oxidative stress leading to the generation of free radicals and alterations in antioxidants, and induction of acetylcholinesterase in various brain areas leading to neuronal damage and subsequent apoptosis. As noted earlier, Jortner (2006) published his concerns about the misinterpretation of the histopathological findings reported in Abdel-Rahman et al. (2001) and other related publications. The main concern is that the report of "degenerating" or "dying" neurons in this article is the result of poor handling and inadequate fixation of the brain tissue and is a "dark" neuron artifact. The presence of this artifact suggests that both the neuron counting and the immunostaining procedures may have been compromised in the Abdel-Rahman et al. (2001) study and in subsequent studies at this laboratory.

Studies by Chaney et al. (1999) also provide information regarding possible mechanisms of DEET toxicity. Treatment of mice with DEET by intraperitoneal injection resulted in seizures that could not be prevented by pretreatment with standard anticonvulsive drugs or anticholinergic agents. These results suggested that DEET-induced seizure activity is mediated by a non-cholinergic pathway.

3.5.3 Animal-to-Human Extrapolations

Exposure of animals to DEET has resulted in effects similar to those reported in cases of intoxication in humans. There does not seem to be an animal species that can be used as a preferred animal model for studies of DEET.

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).

No studies were located regarding endocrine disruption in humans after exposure to DEET. Intermediate- (Ambrose 1959; Army 1980b; Schoenig et al. 1999) and chronic-duration (Schoenig et al. 1999) oral studies in animals that conducted gross and microscopic examination of endocrine glands found no evidence that DEET is an endocrine disruptor.

No *in vitro* studies were located regarding endocrine disruption of DEET.

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 most 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 adverse health effects from exposure to hazardous chemicals, but whether there is a difference depends on the chemical(s) (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to exposure-related 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 that are most sensitive to disruption from exposure to hazardous substances. Damage from exposure in one stage 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). Past literature has often described the fetus/infant as having an immature (developing) blood-brain barrier that is leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the brain, there are differences between fetuses/infants and adults that are toxicologically important. These differences mainly involve variations in physiological transport

systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; whether these mechanisms provide protection for the developing brain or render it more vulnerable to toxic injury is an important toxicological question. Chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

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 given their low glomerular filtration rate and not having 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).

As indicated throughout Section 3.2, Discussion of Health Effects by Route of Exposure, there are reports that provide information regarding health effects in children exposed to DEET. Most of these cases involved oral or dermal exposure and some cases resulted in death (Heick et al. 1980; Pronczuk de Garbino et al. 1983; Zadikoff 1979). The most common manifestation of intoxication were neurological effects including agitation, hypertonia, seizures, ataxia, restlessness, and uncontrolled limb movements (Edwards and Johnson 1987; Gryboski et al. 1961; Heick et al. 1980; Lipscomb et al. 1992; MMWR 1989; Petrucci and Sardini 2000; Roland et al. 1985; Tenenbein 1987; Zadikoff 1979). The combination of some of these signs and symptoms has been described as toxic encephalopathy. The putative association between exposure to DEET and seizures needs to be interpreted with caution since, as noted by Koren et al. (2003), a relatively high percentage (23–29%) of children are exposed to DEET in the United States and seizure disorders occur in approximately 3–5% of children from any cause, making it possible, just by chance alone, to erroneously find an association. This had been discussed earlier by MMWR (1989), which pointed out that "since the exact circumstances under which DEET-related neurotoxicity may occur are unclear, DEET should not be accepted as the cause of a seizure until appropriate evaluation has reliably excluded other possible etiologies." Also, in its Registration Eligibility Document for DEET, EPA (EPA 1998b) stated the following: "One possible explanation for the seizures [reported for children] is coincidence. Seizure coinciding with DEET is not unexpected, given an estimated 15,000–20,000 afebrile seizures in children (ages zero-19 years) estimated annually and an estimated 17 million children using DEET 10 times a year."

In the study of 9,086 human exposures involving insect repellents containing DEET reported to Poison Control Centers from 1985 to 1989 the majority of exposures were accidental and occurred in children (Veltri et al. 1994). These investigators also did not find a relationship between age and the severity of the reaction or with gender. They noted that "children less than six years of age were not more likely to develop adverse effects from DEET-containing products than older children or adults and the effects that did occur in children were not more serious" and that "exposed females were not more likely to develop adverse effects nor were the effects more severe than in exposed males."

An epidemiological study in which women applied DEET themselves in the second and third trimester of pregnancy did not find significant differences between exposed and controls regarding head and arm circumference or length or in a series of neurological tests in newborn infants (McGready et al. 2001). Another epidemiological study did not find significant associations between DEET concentration in maternal blood or cord serum and birth weight, head circumference, abdominal circumference, or birth length in newborn infants (Barr et al. 2010).

Studies in rats and rabbits exposed orally to DEET during gestation did not find fetotoxicity or teratogenicity (Schoenig et al. 1994). In both species, exposure to the highest doses (750 mg DEET/kg/day in rats and 325 mg DEET/kg/day in rabbits) resulted in significant reductions in maternal weight gain during the dosing period. In addition, rats treated with 750 mg DEET/kg/day showed a series of neurological signs during the dosing period including hypoactivity, ataxia, decreased muscle tone, and foot splay. A 2-generation reproductive study in rats reported significantly reduced body weight in F1 and F2 male and female pups on days 14 and 21 of lactation at maternal doses of 250 mg DEET/kg/day (EPA 1989).

Only one study was located that showed greater susceptibility to DEET in young animals compared to adults (Verschoyle et al. 1992). The study reported that the oral LD_{50} for DEET in 11-day-old Wistar rats was 4–5 times lower than in adult rats.

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).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of a generalizable sample of the exposure of the U.S. population to environmental chemicals using biomonitoring. This report is available at http://www.cdc.gov/exposurereport/. The biomonitoring data for DEET from this report is discussed in Section 6.5. 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 DEET 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 DEET 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 DEET

Measurement of DEET in urine may not be a reliable biomarker of exposure, as this compound is rapidly metabolized after oral and dermal exposure, and the parent compound has rarely been detected in urine of animals or humans (Selim et al. 1995; Schoenig et al. 1996; Taylor and Spooner 1990) other than for high doses resulting in death (Ambrose 1959). Urinary metabolites of DEET appear to be better markers of exposure than the parent compound (Calafat et al. 2016, see below).

Smallwood et al. (1992) assessed the correlation between dermal exposure to DEET and urine and serum levels of DEET in a group of chronically-exposed workers and in a group of naïve volunteers, observing that neither urine nor serum levels were related to exposure estimates at low doses, but that urine samples may be correlated with exposure at higher doses or in individuals exposed regularly. The authors attributed the lack of correlation at low doses to the complex toxicokinetic behavior of DEET applied to the skin. As part of an effort to develop techniques to analyze DEET in urine and serum, Smallwood et al. (1992) tested the method on eight National Park employees who regularly used a DEET-containing insect repellent, and nine naïve volunteers (for whom information on informed consent was not provided)

exposed in a laboratory. The National Park employees were observed to apply sufficient quantities of the repellent, which contained 71% DEET, to yield an average daily application of approximately 1 g of DEET. Concentrations of DEET in spot urine samples collected from eight employees after the third day of the work week ranged from below the quantitation limit of 0.18 μg/mL up to 5.69 μg/mL. The authors reported that urinary concentrations were positively correlated (r=0.7) with estimated exposure among the eight employees; the wide range of urinary levels (0.18–5.69 μg/mL), however, appears to have no relationship to the single 1-g dermal dose that each individual reportedly received, suggesting that there may have been a wide range of applied doses. When nine volunteers without prior exposure were exposed in a laboratory to a DEET-containing repellent at doses between 0.14 and 1.86 g DEET per volunteer, the serum of all volunteers contained detectable DEET at concentrations ranging from 0.15 to 1.17 μg/g (Smallwood et al. 1992). Spot urine samples from only two of the nine volunteers, however, showed DEET concentrations above the detection limit of 0.09 μg/mL, and these subjects did not have the highest exposures (based on flux estimates). DEET concentrations in urine reported at various times (between 4 and 22 hours after application) for these two subjects ranged between 0.31 and 2.02 μg/mL (Smallwood et al. 1992). Dermal dose and application area were not readily correlated with spot urine or serum levels at any time point up to 6 hours after application among the volunteers, but the areas under the serum DEET concentration vs. time curves for the volunteers, integrated over 6 hours, were shown to correlate with the calculated flux of DEET across the skin (flux estimates ranged between 0.31 and 5.99 μg-cm^{2}x10^9).

Urinary metabolites of DEET are useful biomarkers of exposure. Kuklenyik et al. (2013) developed an HPLC/isotope dilution tandem mass spectrometry (MS) method to measure DEET and two of its oxidative metabolites (DHMB and DCBA) in the urine of humans and tested the technique on 75 anonymously collected samples from U.S. adults without known exposure. The authors reported that DCBA was detected most frequently and at the highest concentrations, indicating that this may be a useful biomarker of DEET exposure (Kuklenyik et al. 2013). Because no volunteer had any known exposure to DEET, however, the presence of DCBA in urine may in fact be indicative of exposure to the metabolite DCBA, perhaps in drinking water. This theory is consistent with reports that DEET metabolites are not effectively removed by waste water treatment facilities and may thus be available for subsequent public exposure.

Arcury et al. (2007) analyzed the urine of children of North Carolina farmworkers for metabolites of pesticides and reported that DEET metabolites were detected in the urine of 6 of 60 children. The median concentration of DEET metabolites in urine was 0.08 μg/g creatinine. The report did not clearly report

the metabolites analyzed in the urine. Recently, Calafat et al. (2016) published the results of analyses of 5,348 urine samples from persons ≥6 years old in a representative sample of the U.S. general population in the 2007–2010 NHANES. DEET was detected in only 3% of the samples (0.08–45.1 µg/L), DCBA was detected in approximately 84% of the samples (>0.48–30,400 µg/L), and DHMB was detected in approximately 15.5% of the samples (>0.09–332 µg/L). Adjusted concentrations of DCMB were found to be dependent on season of the year (higher in May through September than in October through April), race/ethnicity highest in non-Hispanic whites, possibly reflecting different lifestyle uses), household income, and age. In general, children had higher adjusted concentrations of DCBA than adults, which the investigators attributed to a higher rate of application to children by the parents than to themselves. Urine is the normal medium for assessing human exposure to DEET, and due to the high metabolic rate of DEET, both DCBA and DHMB are more sensitive biomarkers of exposure than DEET itself.

3.8.2 Biomarkers Used to Characterize Effects Caused by DEET

There are no specific biomarkers of effect for DEET exposure. Exposure to products containing DEET has been associated with neurological effects such as seizures, ataxia, hypertonia, uncontrolled movements and agitation, as well as with skin irritation. These and other symptoms including hypotension, nausea and vomiting, and skin rashes can be the result of exposure to many other chemicals or can be caused by conditions unrelated to chemical exposures.

3.9 INTERACTIONS WITH OTHER CHEMICALS

A series of animal studies have been conducted that examined the effects of combined application of DEET and other chemicals that were implicated in the development of neurological alterations and other symptoms among veterans of the Persian Gulf War, termed Gulf War Syndromes or Gulf War illness. Some investigators (Abou-Donia et al. 1996) have proposed that the combination of chemicals, namely, DEET, permethrin, and pyridostigmine bromide (PB), possibly caused some of the range of symptoms reported. Permethrin is a type I pyrethroid insecticide that was applied to the clothing of the military personnel. Pyridostigmine bromide is a reversible inhibitor of AChE that was used orally. A summary of the findings from these studies is provided below.

A 60-day study of daily dermal applications of 4, 40, or 400 mg of 97.7% DEET/kg/day to 1 cm^2 of shaved backs of rats found decreases in permeability of the BBB [^{3}H]hexamethomium of 78, 66, and 65%, respectively, in the brainstem, which was the most sensitive area (Abou-Donia et al. 2001a). Although dermally applied permethrin at doses up to 1.3 mg/kg/day had no significant effect on BBB

permeability, DEET and permethrin in combination significantly decreased BBB permeability in the cortex. None of the treatments affected simple sensorimotor reflexes, but DEET alone at the lowest dose, 4 mg/kg, and the combination with permethrin affected some sensory parameters such as performance on a beam, grip strength, and performance on an inclined plane. The combination of the two drugs resulted in poorer performance in some tests, but only at the highest doses.

In a subsequent study of DEET (40 mg/kg/day dermally) that also included PB (1.3 mg/kg/day in drinking water) and permethrin (0.13 mg/kg/day dermally), in doses stated to be comparable to those received by service members during the Persian Gulf War, none of the single treatments affected postural reflexes, limb placing, or vibrissae touch (Abou-Donia et al. 2001b). Beam walking time (but not beam walking score) was increased over time by coexposure to DEET with PB (but not DEET with permethrin), and was affected most by the 3 substances in combination, but was unaffected by DEET or permethrin alone. Each of the 3 substances alone and in any combination reduced performance on the incline plane and especially reduced forepaw grip strength to <10% of controls. There is no indication in the literature that service members comparably exposed to these substances were unable to lift items and function on the battlefield, perhaps indicating that rats are an exquisitely sensitive species, and more sensitive than humans. DEET produced occasional diarrhea, which has not been reported in other studies conducted at higher dose levels. In general, combination with PB produced the most marked deficits. DEET alone increased AChE by about 40% in the brainstem but not in other brain areas. In combination with PB, AChE in brainstem was decreased. The three drugs together decreased AChE in brainstem and midbrain. DEET alone caused a significant increase (about 20%) in choline acetyltransferase (ChAT, the enzyme responsible for the synthesis of acetylcholine) activity in the cortex and a non-significant increase in the brainstem, the only two places measured. DEET alone and with permethrin significantly increased ligand binding density of m2 muscarinic acetylcholine receptors in the cortex. DEET alone did not affect ligand binding of nicotinic receptors in the cortex; nor did exposure to PB plus DEET only, or the three drugs together.

A study that conducted microscopic examination of the brain in rats exposed to the same service member related doses of DEET, permethrin, or the combination showed that DEET alone induced neuronal degeneration principally in the motor cerebral cortex, dentate gyrus, *cornu ammonis* (CA) subfields 1 and 3 of the hippocampus, and the Purkinje cell layer of the cerebellum (Abdel-Rahman et al. 2001). In the cerebral cortex DEET alone generally appeared to cause more damage than permethrin alone and degeneration appeared to occur earlier in rats treated with the combination than with either chemical alone, but the combination did not induce enhanced neuron loss. In the dentate gyrus, there was a greater

level of neuron loss with DEET or permethrin alone than with the combination; the authors suggested that concurrent exposure to chemicals can decrease their absorption. As mentioned earlier, concerns have been expressed about the misinterpretation of the histopathological findings reported in Abdel-Rahman et al. (2001) and other related publications. The main concern is that the report of "degenerating" or "dying" neurons in this article is actually the result of poor handling and inadequate fixation of the brain tissue and is a "dark" neuron artifact. The presence of this artifact suggests that both the neuron counting and the immunostaining procedures may have been compromised in the Abdel-Rahman et al. (2001) study and in other studies conducted at this laboratory in this timeframe.

Yet another study that included DEET, permethrin, and malathion showed that all three chemicals alone altered neurobehavioral parameters (Abdel-Rahman et al. 2004). The combination of DEET with these other chemicals altered the effects of DEET alone. DEET with permethrin significantly increased AChE activity in the cortex and cerebellum, and significantly decreased AChE activity in the midbrain. Similar, but less marked, changes were seen in the group with DEET plus malathion. DEET plus malathion and DEET plus permethrin significantly increased butyrylcholinesterase in plasma. Treatment with DEET alone or DEET combined with permethrin or malathion did not significantly affect muscarinic acetylcholine receptor binding. DEET alone induced neuronal degeneration in the dentate gyrus, CA1 and CA3 subfields of the hippocampus, midbrain, brainstem, and Purkinje cell layer of the cerebellum. These studies used doses of DEET, PB, and permethrin at doses stated to be comparable to those received by military personnel during the Persian Gulf War.

Similar studies in rats to those conducted by Abou-Donia and coworkers, but with DEET administered orally once or daily over 7 days were conducted by Hoy et al. (2000a, 2000b). In the single-dose study, up to 500 mg DEET/kg had no significant effect on locomotor activity except in met-estrus female rats for which speed was reduced at the highest dose. Administration of DEET (100 mg/kg) and permethrin (15 mg/kg) significantly reduced locomotor activity in male but not female rats to 2.06 meters/min compared to 2.24 and 2.50 meters/min achieved for each substance individually administered at twice those doses. Despite DEET having a greater effect on male mobility, uptake to blood serum was 2–3 times greater in females. Also, the administration of PB tended to decrease oral uptake of DEET in female but not in male rats. In the 7-day study, administration of DEET (200 mg/kg/day) or permethrin (60 mg/kg/day) by gavage or PB (7.5 mg/kg/day) by intraperitoneal injection did not cause significant alterations in locomotor activity. Administration of the combination PB/DEET (at half doses), however, resulted in significantly lower locomotor rates, while the combination permethrin/DEET (at half doses) significantly increased locomotor rates. Administration of the three drugs together (at one third doses)

caused no significant effect. The competition between drugs is consistent with the suggestion by Hoy et al. (2000a) that PB uptake might protect rats from the effects of permethrin.

While the results summarized above suggest that the combination of PB and insect repellents may have synergistic neurological effects, the results of a more recent acute exposure study in humans did not support these findings. Roy et al. (2006) conducted a multicenter, prospective, double-blind, placebo-controlled crossover trial, approved by human use committees at the Uniformed Services University, Bethesda, Maryland; Naval Health Research Center, San Diego, California, Office of the Surgeon General of the Army; and Navy Bureau of Medicine and Surgery. The 64 volunteers completed informed consent forms and were exposed to permethrin-impregnated uniforms continuously; a DEET-containing skin cream (33% DEET) twice daily to neck, face, and legs, and oral pyridostigmine bromide (30-mg tablets) every 8 hours for a full day before each part was conducted. The 4-part crossover design ensured exposure of all participants to all treatments and placebos under both mental plus physical stress and rest conditions. The outcomes examined included biochemical assays and parameters of physical performance, neurocognitive responses, and self-reported adverse effects. The results showed significant increases in systolic blood pressure and heart rate, and increased serum levels of adrenaline, noradrenaline, and lactate during stress sessions; however, none of these were influenced by treatment. None of the exposure combinations significantly affected diastolic blood pressure or serum dopamine or cortisol levels. In addition, neurocognitive performance, as measured by the WinSCAT battery, did not differ with exposure to treatments compared to placebos and showed a slight non-statistical improvement with stress. Finally, self-reported effects did not differ by exposure group. Roy et al. (2006) attributed the difference between their results and those of Abou-Donia and coworkers to the different dose levels used and routes of administration. Roy et al. (2006) noted that in their study, permethrin-treated uniforms did not lead to measurable permethrin in the blood stream.

In an additional study examining the interactive effects of DEET, PB, and permethrin in animals, McCain et al. (1997) reported that the simultaneous administration of the three chemicals to rats significantly increased the lethality compared to expected additive values. Concurrent administration of PB and DEET caused a significant increase in lethality compared to expected additive values. The investigators suggested that possible mechanisms could involve facilitated absorption of PB in the gut by DEET or inhibition of detoxification systems.

In studies in rats, intraperitoneal administration of 200 mg DEET/kg (at levels expected to produce 10–20% lethality) did not significantly alter the inhibition of cholinesterase activity in the heart (20%

reduction), diaphragm (30% reduction), or whole blood (15% increase), whereas intraperitoneal administration of only 1 or 3 mg PB/kg resulted in significant 80–90% reductions of cholinergic activity in those areas (Chaney et al. 2000). Co-administration of 200 mg DEET/kg with 1 or 3 mg PB/kg, however, reduced ChE activity to levels achieved solely by PB, except in whole blood for which the high PB dose resulted in levels comparable to unexposed controls. No dose of PB alone significantly altered cholinesterase activity in the brain. DEET alone slightly reduced brain ChE activity, and DEET plus the high PB dose reached statistical significance at 40% inhibition of brain cholinesterase. These results were interpreted as DEET not altering the inhibition of ChE activity induced by PB in the heart, diaphragm, or whole blood, and that DEET increased the permeability of the brain to PB rather than directly affecting PB-induced cholinesterase inhibition. The same group of investigators also reported that intraperitoneal co-administration of PB and DEET to rats caused a profound and rapid decrease in heart rate that did not occur with either chemical alone that eventually resulted in death (Chaney et al. 2002). The investigators noted that the primary cause of death appeared to be circulatory failure and proposed the following sequence of events: DEET may have depressed central cardiorespiratory centers and altered sympathetic outflow from the brain. PB aggravated DEET-induced toxicity presumably by promoting accumulation of acetylcholine at peripheral cholinergic receptor sites. This accumulation at cholinergic sites resulted in bradycardia and further reduced cardiac output, which caused the development of progressive circulatory shock. It is worth noting that the relevance of injection studies of DEET to safety assessment of DEET exposure in humans is, at best, questionable.

It should also be noted that while the studies that examined the interactive action of DEET, permethrin, and PB provide valuable information for understanding potential mechanisms that could explain some health outcomes manifested in the Gulf War Syndrome, it is difficult to see their relevance to civilian exposures to DEET and other chemicals.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to DEET than will most persons exposed to the same level of DEET in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of DEET, or compromised function of organs affected by DEET. Populations who are at greater risk due to their unusually high exposure to DEET are discussed in Section 6.7, Populations with Potentially High Exposures.

No populations that are unusually susceptible to adverse health effects from exposure to DEET were identified in the literature reviewed. A study of 9,086 human exposures involving insect repellents containing DEET reported to Poison Control Centers from 1985 to 1989 found that children younger than 6 years of age were not more likely to develop adverse effects from DEET-containing insect products than older children or adults, and the effects that occurred in children were not more serious (Veltri et al. 1994). AAPCC (2013) reported that 57%, or 2,316 case reports, of exposure to DEET were in children ≤5 years of age. This may indicate a propensity for parents to apply DEET more liberally to protect their youngest children from insect bites, rather than a differential susceptibility. Neurological effects, specifically seizures, have been reported in children and adults following oral or dermal exposure to products containing DEET. Thus, the question arose as to whether subjects with known prior seizure disorders would be more susceptible to DEET. In a study of 296 major and moderate severity cases included in the DEET Registry from 1995 to 2001, people with an underlying neurological disorder or a history of seizures prior to the first documented use of DEET were not disproportionally represented in the Registry (Osimitz et al. 2010).

Verschoyle et al. (1992) evaluated acute toxicity in rats and noted that neonates were significantly more sensitive than adult rats to DEET-induced lethality; however, no sensitivity generalizations can be made based on a single study, nor is it possible to make inferences about sensitivity at the lower doses to which humans are more likely to be exposed.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to DEET. 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 DEET. When specific exposures have occurred, poison control centers, board certified medical toxicologists, board-certified occupational medicine physicians and/or other medical specialists with expertise and experience treating patients overexposed to DEET can be consulted for medical advice.

DEET has a low order of toxicity if used properly; however, it is prudent to avoid the overuse of DEET, which could result in adverse health effects (AAP 2015; Holland 2015).

The following texts provide specific information about treatment following exposures to DEET:

Borron SW. 2007. Pyrethrins, repellents, and other pesticides. In: Shannon MW, Borron SW, Burns MJ, eds. Haddad and Winchester's clinical management of poisoning and drug overdose. 4th ed. Philadelphia, PA: Saunders Elsevier, 1185-1194.

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

Holland MG. 2015. Insecticides: Organochlorines, pyrethrins/pyrethroids, and insect repellents. In: Hoffman RS, Howland MA, Lewin NA, eds. Goldfrank's toxicologic emergencies. 10th ed. New York, NY: McGraw-Hill Education, 1435–1448.

Additional relevant information can be found in the front section of this profile under QUICK REFERENCE FOR HEALTH CARE PROVIDERS.

3.11.1 Reducing Peak Absorption Following Exposure

The following information was extracted from the books listed above; specific chapters were written by Borron (2007), Osmudsen (1998), and Holland (2015). It is recommended, however, that this information be used along with consultation with a medical specialist with expertise and experience treating/managing patients with DEET poisoning.

In cases of accidental dermal overexposure, skin decontamination with copious amounts of soap and water should be a priority to prevent further absorption. In cases of eye exposure, irrigation of the eyes with isotonic saline or copious amounts of room temperature water for at least 15 minutes is recommended. In cases of oral ingestion, one report recommended administration of a single dose of activated charcoal if clinically indicated (Holland 2015). However, only one case report of treating a DEET-overexposed individual with activate charcoal was located (Tenenbein 1987). Since the patient died, the efficacy of using activated charcoal is unclear.

3.11.2 Reducing Body Burden

No information was located regarding reducing the DEET body burden following exposure to this substance, but studies in volunteers indicate that it is rapidly cleared from the body (Selim et al. 1995; Wu et al. 1979).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Overexposure to DEET has been associated mainly with neurological effects such as seizures and hyperactivity, and skin effects, if exposure was dermal. The mechanisms by which these effects occur have not been elucidated. Management of suspected DEET-related toxicity is essentially supportive and aimed primarily to treat the neurological effects. Benzodiazepines may be used to treat seizures. Refractory seizures may be treated with phenobarbital.

3.12 ADEQUACY OF THE DATABASE

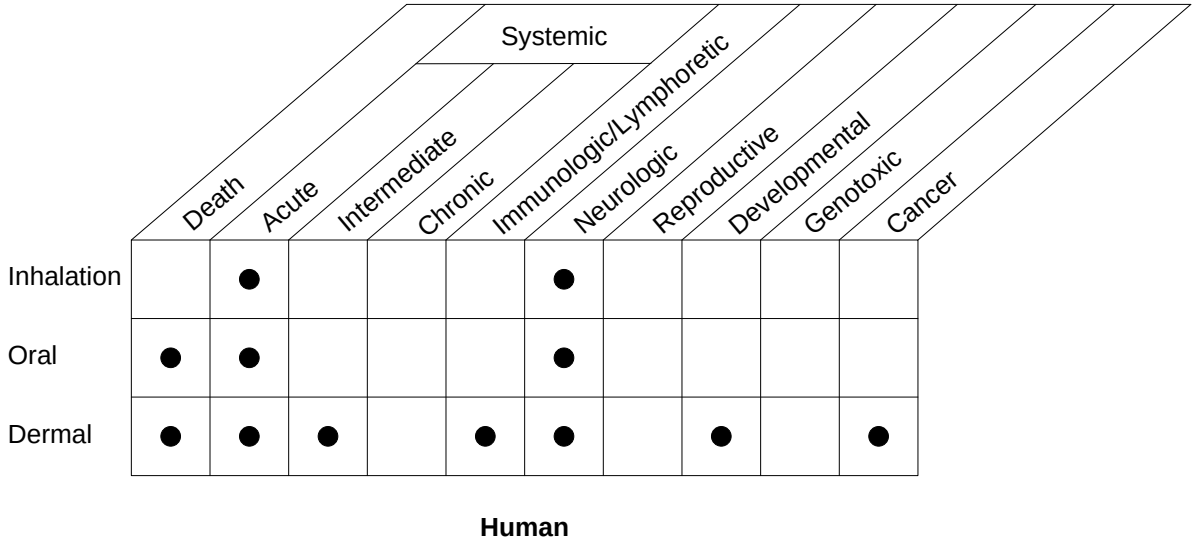
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of DEET is available. Where adequate information is not available,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

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 risk 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.

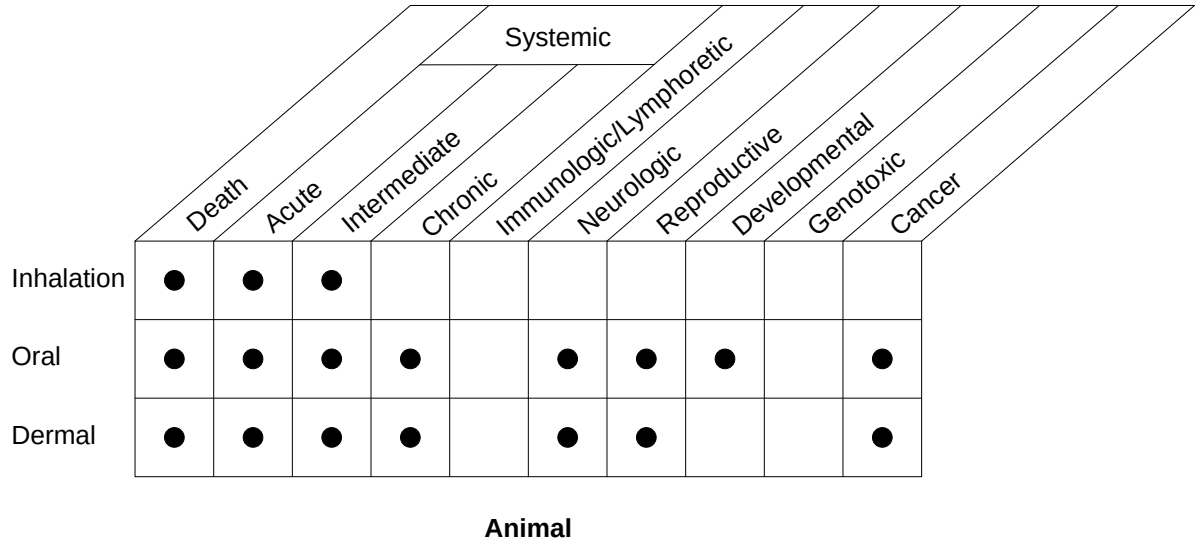
3.12.1 Existing Information on Health Effects of DEET

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

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



Human



Animal

● Existing Studies

Most of the literature reviewed concerning the health effects of DEET in humans described case reports of acute dermal exposure to DEET. There are also surveys involving great numbers of individuals from the general population compiled from records kept in public and private poison control centers. These individuals were exposed to products containing DEET by the inhalation, oral, or dermal route. Also available are a few studies of occupational exposure and controlled dermal exposure in volunteers. No reliable estimates of quantitative exposure could be obtained from case reports.

The database in animals is extensive. As can be seen in Figure 3-5, most studies in animals have been conducted by the oral and dermal routes of exposure. There is more information regarding the health effects of DEET following intermediate exposure than regarding acute or chronic exposure. There is no evidence suggesting that the toxicity of DEET is route-specific. The intake and uptake rates from oral exposure, however, are faster than those by the dermal route of exposure, so greater peak concentrations in liver and nervous system tissues are achieved.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Information is lacking concerning actual dermal exposures of individuals who use DEET-containing products, and the AAPCC database only addresses the percentage of DEET in products for which reports are made to poison control centers. Since environmental and public health agencies recommend the use of DEET to avoid insect and acarid bites, developing a standard for conducting dermal dosimetry and adding dose information to poison control center reports could help improve recommendations for safely applying DEET.

Information is available regarding the effects of acute-duration exposure in humans following inhalation (Bell et al. 2002), oral (Edwards and Johnson 1987; Fraser et al. 1995; Petrucci and Sardini 2000; Tenenbein 1987; Wiles et al. 2014; Zadikoff 1979), and dermal exposure (Ambrose 1959; Briassoulis et al. 2001; Clem et al. 1993; Edwards and Johnson 1987; Gryboski et al. 1961; Hampers et al. 1999; Heick et al. 1980; Lipscomb et al. 1992; Maibach and Johnson 1975; MMWR 1989; Reuveni and Yagupsky 1982; Roland et al. 1985; Shutty et al. 2013; Vozmediano et al. 2000; Wantke et al. 1996; Zadikoff 1979). Deaths have been reported following oral and dermal exposure to products containing DEET (Heick et al. 1980; Pronczuk de Garbino et al. 1983; Tenenbein 1987; Veltri et al. 1994; Wiles et al. 2014; Zedikoff 1979). The main target of toxicity in humans and animals following acute, high-level exposure by any route is the nervous system (Army 1979; Briassoulis et al. 2001; Gryboski et al. 1961; Lipscomb et al.

1992; Petrucci and Sardini 2000; Verschoyle et al. 1992; Zadikoff 1979). No reliable exposure concentrations were available in surveys of people reporting to emergency departments after inhalation exposure to insect repellents containing DEET; therefore, no acute-duration inhalation MRL could be derived using human data. Very limited inhalation data in animals were located and the available studies (Ambrose 1959; Army 1979; Deb et al. 2010) have significant limitations rendering them inadequate for MRL derivation. Studies that examine a comprehensive number of end points to possibly construct dose-response relationships would seem warranted; the need to fill this data gap, however, needs to be balanced with the fact that, assuming proper use, it is unlikely that significant inhalation of DEET will occur based on its estimated half-life in air of 5 hours (EPIWIN 2012), which makes persistence and long-range transport of DEET in air negligible. There is no information from humans who ingested DEET that is useful for derivation of an acute-duration oral MRL for DEET. Studies in animals provided information regarding lethal doses (Ambrose 1959; Carpenter et al. 1974; EPA 1998c; McCain et al. 1997; Verschoyle et al. 1992), developmental effects in rats and rabbits (Schoenig et al. 1994), and neurological effects in rats (Schoenig et al. 1993; Verschoyle et al. 1992). These studies did not identify a sensitive target for DEET. Studies that provide comprehensive information regarding potential gross and microscopic alterations in major organs and tissues would be valuable. Based on an incomplete database, an acute-duration oral MRL was not derived for DEET. Case reports of people who used insect repellents containing DEET in the form of aerosols or lotions provide information regarding cardiovascular, gastrointestinal, hematological, hepatic, renal, endocrine, dermal, ocular, immunological, and neurological effects (Amichai et al. 1994; Bell et al. 2002; Briassoulis et al. 2001; Clem et al. 1993; Hampers et al. 1999; Heick et al. 1980; Lipscomb et al. 1992; Maibach and Johnson 1975; MMWR 1989; Reuveni and Yagupsky 1982; Roland et al. 1985; Zadikoff 1979). A study in volunteers provided information on dermal effects of DEET (Ambrose 1959). A limited number of studies in animals provided information on lethal doses (Carpenter et al. 1974; EPA 1998c) and on dermal and ocular effects (Ambrose 1959; MacRae et al. 1984). Normal use of products containing DEET involves direct skin exposure. Therefore, animal studies that examine a wide range of end points (systemic, immunological, neurological, reproductive, and developmental) and establish dose-response relationships would be valuable.

Intermediate-Duration Exposure. There are no studies of humans exposed to DEET for intermediate durations by the inhalation or oral routes. Two inhalation studies in animals were available for review. A limited-scope study reported unspecified alterations in the lungs and trachea of rats following a 7-week exposure period (Ambrose 1959). A 13-week study in rats examined a comprehensive number of end points, including organ and tissue histopathology and hematological and

clinical chemistry parameters and found no significant effects (Army 1980a). In the absence of a LOAEL being identified, an intermediate-duration inhalation MRL was not derived for DEET. As mentioned above, it is unlikely that humans will be exposed to significant amounts of DEET in the air; therefore, it does not appear that additional inhalation studies are necessary at this time. Intermediate-duration oral studies in animals provided information on systemic (Ambrose 1959; Army 1980b; EPA 1989, 1990b; Schoenig et al. 1993, 1999), immunological (Ambrose 1959; Army 1980b; EPA 1990b; Schoenig et al. 1999), neurological (Ambrose 1959; Army 1980b; EPA 1990b; Schoenig et al. 1993, 1999), reproductive (Ambrose 1959; EPA 1989), and developmental effects (EPA 1989). Although a clear target for DEET toxicity was not apparent, a developmental study that identified the lowest LOAEL (EPA 1989) was adequate and was used to derive an intermediate-duration oral MRL for DEET. Additional intermediate oral studies do not seem necessary at this time. Limited information is available regarding intermediate-duration dermal exposure in humans. Workers at a national park who used insect repellents or lotions containing DEET repeatedly during the summer season complained more often of chest pain or wheezing, muscle cramping, skin rashes and blisters, dizziness, disorientation, and difficulty concentrating than workers who used the products less often or did not use them at all (NIOSH 1986). In addition, a case report of an 18-month-old girl who received daily applications of an insect repellent containing 20% DEET for approximately 3 months reported hematological and neurological effects, but no evidence of altered liver function (Edwards and Johnson 1987). Further studies of workers exposed seasonally to DEET would be valuable, especially if exposure can be better characterized. Intermediate-duration dermal studies in animals provide information regarding systemic effects (Ambrose 1959; EPA 1988, 1990a, 1992a; Lebowitz et al. 1983), neurological effects (Abdel-Rahman et al. 2001, 2004; Abou-Donia et al. 2001a, 2001b), and reproductive effects (Lebowitz et al. 1983). Two of these studies (EPA EPA1988, 1992a) conducted a comprehensive examination of the major organs and tissues from rats and micropigs to identify possible histological alterations; these studies also monitored hematological and clinical parameters. Therefore, additional intermediate-duration animal studies by the dermal route do not seem warranted at this time.

Chronic-Duration Exposure and Cancer. There are no studies of humans exposed chronically to DEET by any route with the exception of a study that monitored birth outcomes in women exposed to various pesticides (Barr et al. 2010) and a study of testicular cancer in Sweden (Hardell et al. 1998). The assumption is that in both studies, the subjects may have been exposed for extended periods of time (see specific sections below). There are no chronic-duration inhalation studies in animals. Based on use patterns and physical properties of DEET, however, chronic-inhalation exposure to DEET is not expected; therefore, chronic-inhalation studies may not be necessary at this time. Chronic-duration oral

studies have been conducted with DEET in rats and mice (Schoenig et al. 1999). These studies provided information on a comprehensive number of end points including clinical signs, gross and microscopic appearance of tissues and organs, hematological parameters, and clinical chemistry and ophthalmology in rats. A chronic-duration oral MRL however, was not derived because the few alterations reported were of questionable toxicological significance. In addition, the available studies did not test for end points such as subtle neurobehavioral effects, which have been reported at relative low doses in intermediate-duration dermal studies in rats (Abou-Donia et al. 2001a) and in humans following seasonal used of DEET-containing insect repellents (NIOSH 1986). It would be useful to have this information because populations living near hazardous waste sites could be exposed orally via contaminated water. Only one chronic-duration dermal study in animals was available for review. That study examined gross lesions in mice and rabbits that had DEET applied onto the skin for 140 or 90 weeks, respectively, but did not provide information regarding possible non-neoplastic changes in tissues and organs (Stenback 1977). Based on decades of experience with humans applying DEET to the skin repeatedly and because chronic dermal exposure is not expected except in unusual circumstances (e.g., long-term use in tropical areas where biting insects are active throughout the year, or in the field by military personnel), additional chronic-duration dermal studies do not seem necessary at this time.

Very limited information is available regarding exposure to DEET and cancer in humans. A study of testicular cancer and occupational exposures in Sweden found an increased risk among workers who used insect repellents for more than 115 days (Hardell et al. 1998). Studies of cancer need to be conducted among groups identified as having long-term exposure to DEET, such as those involved in the manufacture of the chemical and those who use it for extended periods during the year, such as park workers. DEET has been examined for carcinogenicity in oral studies in rats, mice, and dogs (Schoenig et al. 1999) and in dermal studies in mice and rabbits (Stenback 1977). The results were negative in all the species tested. Additional cancer studies in animals do not seem necessary.

Genotoxicity. There are no genotoxicity studies of humans exposed to DEET. Individuals involved in the manufacture and long-term use of DEET could be tested for possible genomic alterations. A single *in vivo* study reported an increase in a biomarker of DNA damage in rats that had application of a single dermal dose of DEET (Abu-Qare and Abou-Donia 2000). Additional studies *in vi*vo that examine whether DEET is a clastogenic substance would be valuable. A limited number of studies of reverse mutation in *Salmonella* gave negative results (EPA 1990c; Zeiger et al. 1992); additional studies seem unwarranted. Studies in mammalian cells gave mixed results. Evidence of DNA damage was reported in

cultured primary human nasal mucosal cells (Tisch et al. 2002). It would be useful to try to replicate the findings from Tisch et al. (2002).

Reproductive Toxicity. No studies were located regarding reproductive effects in humans exposed to DEET by any route. No data on reproductive toxicity were located in animals exposed to DEET by inhalation. Intermediate- and chronic-duration oral studies did not find significant gross or microscopic alterations of the reproductive organs of male and female animals (Ambrose 1959; Army 1980b; EPA 1989; Schoenig et al. 1999) except for tubular degeneration of the testes in hamsters (EPA 1990b). Fertility was evaluated only in a 2-generation continuous feeding study in rats and was not affected by treatment with DEET (EPA 1989). An intermediate-duration dermal exposure study in rats did not find significant histological alterations in the testes or in sperm parameters (Lebowitz et al. 1983). Two additional intermediate-duration dermal studies did not observe morphological alterations in the reproductive organs from rats or micropigs (EPA 1988, 1992a). Further reproductive studies are unlikely to provide new key information.

Developmental Toxicity. Two studies provided information regarding developmental effects in humans following exposure to DEET (Barr et al. 2010; McGready et al. 2001). In the latter study, controlled dermal exposure of pregnant women to DEET during the second and third trimesters did not affect developmental outcomes at birth and up to 1 year of age. Follow-up studies of these infants would have been useful. In the Barr et al. (2010) study of the general population, there was no significant association between levels of DEET in maternal blood and cord serum and developmental outcomes. Studies in rats and rabbits exposed orally to DEET during gestation showed only a slight decrease in fetal weight in rats and no significant fetotoxicity in rabbits at dose levels that induced maternal toxicity (Schoenig et al. 1994). No teratogenicity was reported in either study. In a 2-generation continuous feeding study in rats, the only significant effect observed was a reduction in F1 and F2 pup body weight on days 14 and 21 of lactation (EPA 1989). Since the birth weight of pups was not significantly different from controls, the possibility exists that DEET was transferred to the pups via the milk or that there was insufficient milk production by the exposed dams, or both. Experiments could be designed to test these hypotheses. Cross-fostering experiments could provide information regarding the relative importance of exposure to DEET through the placenta vs. via lactation.

Immunotoxicity. A few studies have reported that exposure to DEET can cause contact urticaria by immunological mechanisms in humans (Maibach and Johnson 1975; Shutty et al. 2013; Vozmediano et al. 2000). Intermediate- and chronic-duration oral studies in animals have mainly showed that exposure

to DEET did not induce gross or microscopic alterations in lymphoreticular organs and tissues (Ambrose 1959; Army 1980b; EPA 1990b; Schoenig et al. 1999). Because parameters of immunocompetence were affected in a study in mice administered DEET by subcutaneous injection (Keil et al. 2009), and because immunocompetence has been affected in animals by exposure to other chemicals at relatively low doses (Abadin et al. 2007), it would be useful to conduct pilot studies, especially by the dermal route of exposure, to test whether exposure to DEET can also affect immunocompetence.

Neurotoxicity. Adverse neurological effects have been reported in humans following inhalation, oral, or dermal exposure to insect repellents containing DEET. In most cases, this has occurred following exposure to what appears to have been excessive amounts. Signs and symptoms reported include seizures, ataxia, restlessness, uncontrolled limb movements, agitation, aggressive behavior, combativeness, impaired cognitive functioning, and opisthotonos (Briassoulis et al. 2001; Edwards and Johnson 1987; Gryboski et al. 1961; Hampers et al. 1999; Heick et al. 1980; NIOSH 1986; Petrucci and Sardini 2000; Pronczuk de Garbino et al. 1983; Roland et al. 1985; Snyder et al. 1986; Wiles et al. 2014; Zadikoff 1979). Continued follow-up of the individuals with the most severe effects (i.e., seizures) would provide valuable information regarding possible long-term effects (or lack thereof) due to acute exposure. In a 1-year follow-up of 35 of these cases of seizures after exposure to DEET, medical tests showed evidence of an underlying neurological disorder in 5 of these cases (Osimitz et al. 2010). Studies in animals have reported neurobehavioral alterations (Abdel-Rahman et al. 2004; Abou-Donia et al. 2001a; Army 1979; Schoenig et al. 1993), morphological alterations (Abdel-Rahman et al. 2001; Verschoyle et al. 1992), and neurochemical alterations (Abou-Donia et al. 2001b). As mentioned in Section 2.2, Summary of Health Effects, there are some unexplained inconsistencies between the results from some of these studies that need to be resolved. With regard specifically to morphological alterations, as mentioned earlier, findings reported by Abdel-Rahman et al. (2001, 2004) have been questioned as possible artifacts (Jortnet 2006), so it would be useful to try to replicate their findings. The mechanism by which DEET (or a metabolite) induces neurological alterations has not been elucidated, so further research in this area is needed.

Epidemiological and Human Dosimetry Studies. Most of the literature reviewed concerning the health effects of DEET in humans described case reports of accidental or intentional ingestion, or dermal exposure to DEET by the general population (Barr et al. 2010; Briassoulis et al. 2001; Clem et al. 1993; Edwards and Johnson 1987; Fraser et al. 1995; Gryboski et al. 1961; Hampers et al. 1999; Heick et al. 1980; Lipscomb et al. 1992; Maibach and Johnson 1975; MMWR 1989; Petrucci and Sardini 2000; Pronczuk de Garbino et al. 1983; Roland et al. 1985; Shutty et al. 2013; Tenenbein 1987; Vozmediano et

al. 2000; Wantke et al. 1996; Wiles et al. 2014; Zadikoff 1979), and exposure of workers (NIOSH 1986), volunteers (Ambrose 1959; McGready et al. 2001), and military personnel (Amichai et al. 1994; Haley et al. 1997; Reuveni and Yagupsky 1982). Only in four of these studies was there information regarding dose/exposure concentrations. Wiles et al. (2014) reported that a man who ingested 6 ounces of a repellent containing 40% DEET (748 mg/DEET/kg) suffered a seizure, became unresponsive, and was declared brain dead 3 days after poisoning. Ambrose (1959) reported that 1–2 mL of a 50% DEET solution applied on to the face of volunteers for 5 days caused some desquamation. McGready et al. (2001) reported that dermal application of 1.7 g DEET/day to women during the second and third trimester of pregnancy did not affect developmental outcomes. In NIOSH (1986), estimates of exposure to >4.25 g DEET/week (based on survey recall data) were associated with chest pain or wheezing, skin rash and blisters, and impaired cognitive functioning. Follow-up of individuals who have experienced the most severe effects (i.e., seizures) would help determine possible long-term effects of acute high exposure. Continuous evaluation of park workers who have used insect repellents during part of the year for several years could provide valuable information including reproductive data in both males and females and pregnancy outcomes in women as well as potential health effects in their offspring.

Biomarkers of Exposure and Effect.

Exposure. Further studies correlating urinary levels of DEET or its metabolites with exposure measures could provide valuable information to validate the use of these metrics as biomarkers of exposure to DEET, as available studies are limited (Calafat et al. 2016).

Effect. There are no DEET-specific effects following exposure to this substance. Neurological and dermal effects that have been associated with exposure to DEET can also be induced by exposure to other chemicals or can even be caused by conditions unrelated to chemical exposures. Any research aimed at identifying a specific biomarker of effect for DEET would be valuable.

Absorption, Distribution, Metabolism, and Excretion. No information on the toxicokinetics of DEET in humans or animals exposed via inhalation was available in the literature reviewed. Studies aimed at characterizing the behavior of DEET entering systemic circulation through the inhalation route of exposure would be valuable, even though inhalation is an unlikely route of exposure to DEET if products containing DEET are used properly. Only one study (Schoenig et al. 1996) examined the toxicokinetics of DEET after a single oral exposure in rats; studies examining other exposure regimens would provide useful information on the effects of exposure frequency or duration on toxicokinetics, and

studies in other species would help determine whether there are species differences in toxicokinetics after oral exposure. The role of metabolism on the toxicity of DEET is not known; therefore, studies designed to provide information on this issue seem warranted.

Comparative Toxicokinetics. There is suggestive evidence for species differences in the absorption, metabolism, and/or elimination of DEET. As only one study in rats examined toxicokinetics after oral exposure to DEET (Schoenig et al. 1996), the lack of information on species differences in toxicokinetics of orally-administered DEET represents an important data gap. Additional information comparing dermal absorption by rodents with absorption by humans under the same treatment conditions would be useful, as cross-study comparisons are hampered by differences in exposure conditions that can markedly affect the rate or extent of absorption. Likewise, there are suggestive, but not conclusive, data indicating gender differences in metabolism and/or excretion of DEET. Further studies comparing male and female animals or humans are needed to provide a basis for conclusions regarding gender differences in the toxicokinetics of DEET.

Methods for Reducing Toxic Effects. There are no DEET-specific effects following exposure to this chemical. Overexposure to DEET has been associated mainly with neurological effects such as seizures and hyperactivity, and dermatitis, if exposure was through skin contact. The mechanisms by which these effects occurred have not been elucidated. Management of suspected DEET-related toxicity is essentially supportive and aimed primarily to treat the neurological effects. Publishing treatments that have proved to be effective in randomized controlled trials in medical journals could improve and/or prevent secondary effects and speed recovery in the most severe cases.

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.

Information on the effects of DEET in children is derived mainly from case reports of accidental ingestion of insect repellents containing DEET or after receiving excessive dermal applications of the repellents. The most common manifestation of intoxication were neurological effects including agitation, hypertonia, seizures, ataxia, restlessness, and uncontrolled limb movements (Edwards and Johnson 1987; Gryboski et al. 1961; Heick et al. 1980; Lipscomb et al. 1992; MMWR 1989; Petrucci and Sardini 2000; Roland et al. 1985; Tenenbein 1987; Zadikoff 1979). Evaluation of poisoning cases reported to Poison Control Centers from 1983 to 1989 did not suggest that children are more sensitive to DEET than adults (Veltri et al.

1994). Only one study in animals was located that examined the acute toxicity of DEET in relation to age (Verschoyle et al. 1992). That study reported that neonatal rats were significantly more sensitive than adult rats to DEET-induced lethality. No generalization, however, can be made based on a single study, nor can inferences be made about sensitivity at the nonlethal dose levels to which humans are exposed. Additional studies would be useful.

Limited information is available regarding developmental effects of DEET in humans. A study of women who applied a known amount of DEET onto the skin during the second and third trimesters did not affect developmental outcomes at birth and up to 1 year after birth (McGready et al. 2001). Follow-up studies of these infants would have been useful. A study of the general population did not find significant associations between levels of DEET in maternal blood and cord serum and developmental outcomes (Barr et al. 2010). Conventional developmental studies in rats and rabbits did not find adverse developmental effects in the offspring at maternal sacrifice on the last day of gestation (Schoenig et al. 1994). In the 2-generation continuous feeding study in rats, male and female F1 and F2 pups, however, had significantly reduced body weight on lactation days 14 and 21 (EPA 1989). As mentioned before, because birth weight was not affected by treatment with DEET, the reduced body weight in the pups could have been due to reduced milk production or quality, or transfer of DEET and/or metabolites in the milk to the pups. Further studies regarding possible transfer of DEET and/or metabolites to the offspring via maternal milk seem appropriate.

There are no adequate data to evaluate whether pharmacokinetics of DEET in children are different from adults. To the extent that various cytochromes P450 that are involved in the metabolism of DEET in humans (Usmani et al. 2002) are developmentally regulated (Tateishi et al. 1997), the metabolism of DEET in neonates and infants, however, will likely differ from adults. Whether this would result in increased susceptibility of the young is not known because it is also not known whether metabolism of DEET represents activation or detoxification. No information was located regarding levels of DEET (or metabolites) in human milk. DEET, however, has been measured in cord blood (Barr et al. 2010). Further information on the dynamics of DEET and metabolites during pregnancy would be useful.

Biomarkers of exposure need to be further studied to better estimate human exposure at all age levels following exposure to DEET. There are no data on the interaction of DEET with other chemicals in children. The information available indicates that methods used to mitigate the effects of DEET in adults are applicable to children.

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

No relevant ongoing studies pertaining to DEET were identified in RePorter (2017).