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 toxaphene. 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.

Toxaphene is a manufactured pesticide composed of over 670 different constituents; the relative proportions of the major components of the pesticide are essentially the same in different formulations. The production and use of toxaphene have been banned in the United States and all of its territories since 1990 (EPA 1990b). Nevertheless, because of its earlier widespread use, persistence in the environment, and storage in waste sites, exposure to toxaphene and its persistent congeners is still possible.

Following its release to the environment, technical toxaphene undergoes biotic and abiotic "weathering" processes, resulting in congener mixtures that differ from those of technical toxaphene (EPA 2010a; Ruppe et al. 2003, 2004; Simon and Manning 2006). Because toxaphene has not been used as a pesticide in the United States since 1990, exposure to persistent toxaphene congeners from weathered toxaphene is of primary health concern. Major congeners of toxaphene that have been found to persist in fish, marine mammals, and human serum and breast milk include Parlars p-26, p-40/41, p-44, p-50, and p-62 (Simon and Manning 2006). Pooled results of studies that assessed levels of these congeners in human serum and/or breast milk (Gill et al. 1996; Polder et al. 2003; Sandanger et al. 2003; Skopp et al. 2002b; Walker et al. 2003) indicate that p-26, p-50, and p-62 comprise approximately 33, 55, and 6%, respectively, of the total toxaphene body burden (Simon and Manning 2006).

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

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of toxaphene are indicated in Table 3-2 and Figure 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 also shows a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

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

3.2.1 Inhalation Exposure

Very little information is available regarding the health effects of toxaphene following inhalation exposure in humans. Most of the existing data come from case reports and long-term studies of pesticide workers and are of limited value. In such studies, precise levels of exposure are usually not provided, and concurrent exposure to several pesticides confounds the interpretation of the results.

Limited information is available regarding health effects in animals following inhalation exposure to toxaphene. Secondary sources have cited unpublished results of studies for Hercules Incorporated, a major U.S. producer while toxaphene was registered for use as a pesticide in the United States. Although the primary study reports have not been made available to ATSDR, the results are presented in Section 3.2.1 as summarized in the Drinking Water Criteria Document for Toxaphene (EPA 1985).

3.2.1.1 Death

No studies were located regarding death in humans following inhalation exposure to toxaphene.

A 40% toxaphene dust (3,000–4,000 mg/m^3) caused death in about one-half of an exposed group of rats after 1 hour of exposure (EPA 1985). Unpublished results of repeated inhalation exposure studies cited by EPA (1985) include the death of all rats (number unspecified) exposed to toxaphene dust at 250 mg/m^3 for up to 1 week, unspecified numbers of deaths among rats, dogs, and guinea pigs exposed at 12 mg/m^3 for up to 3 months (but no deaths at 4 mg/m^3), and no mortality in rats and rabbits exposed at 500 mg/m^3 for 3 weeks.

3.2.1.2 Systemic Effects

No studies were available regarding cardiovascular, gastrointestinal, musculoskeletal, endocrine, or ocular effects in humans or animals following inhalation exposure to toxaphene.

One controlled human study investigated the general effects of inhaled toxaphene. Keplinger (1963) reported that no toxic effects were seen in 25 human subjects (15 males, 10 females) exposed to an aerosol containing a maximum of 500 mg toxaphene/m^3 for 30 minutes/day for 10 days. The author estimated the absorbed dose to be as much as 60 mg/person/day. After a 3-week period, these same subjects were exposed for three more 30-minute periods. Examinations of these subjects by a dermatologist and an internist (some of them using blood tests and urinalysis) indicated no effects. Due

to the limited information reported in this study and the unusual exposure conditions, it is difficult to assess the adequacy of these data. Nevertheless, the study is referenced below for the appropriate systemic end points.

The highest NOAEL values for humans for each effect are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Pulmonary hypersensitivity reactions to toxaphene were suspected in two Egyptian agricultural pesticide workers in 1958. In these cases, men involved in the spraying of toxaphene (formulated as 60% toxaphene, 35% kerosene, 3% xylol, and 2% emulsifier) for approximately 2 months suffered from acute pulmonary insufficiency (Warraki 1963). Chest x-rays revealed extensive miliary shadows, and one man exhibited marked bilateral hilar lymphadenopathy. The diagnosis in both cases was extensive bilateral allergic bronchopneumonia as a result of insecticide exposure. Both patients recovered quickly and completely with cortisone, streptomycin, and isoniazid treatment. Although the clinical sequelae observed in these two patients could be associated with toxaphene exposure, the effects could have been caused by other components of the spray. In one of the cases, pulmonary tuberculosis was ruled out because sputum testing for the acid-fast bacilli and tuberculin tests were negative (Warraki 1963). No similar cases have been reported since 1958.

No studies were located regarding respiratory effects in animals following inhalation exposure to toxaphene.

Hematological Effects. No blood abnormalities were observed in a group of volunteers exposed to toxaphene spray 30 minutes/day for 10 days at a maximum nominal concentration of 500 mg/m^3 (Keplinger 1963). Clinical findings in two male Egyptian agricultural pesticide workers involved in the spraying of toxaphene (formulated as 60% toxaphene, 35% kerosene, 3% xylol, and 2% emulsifier) for approximately 2 months included elevated sedimentation rates, the presence of blood eosinophilia, and high serum globulin (Warraki 1963).

Hepatic Effects. No studies were available regarding hepatic effects in humans following inhalation exposure to toxaphene.

Slight hepatocellular necrosis was reported in some female rats that survived inhalation exposure to toxaphene dust (4 or 12 mg/m^3) for 3 months (EPA 1985).

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

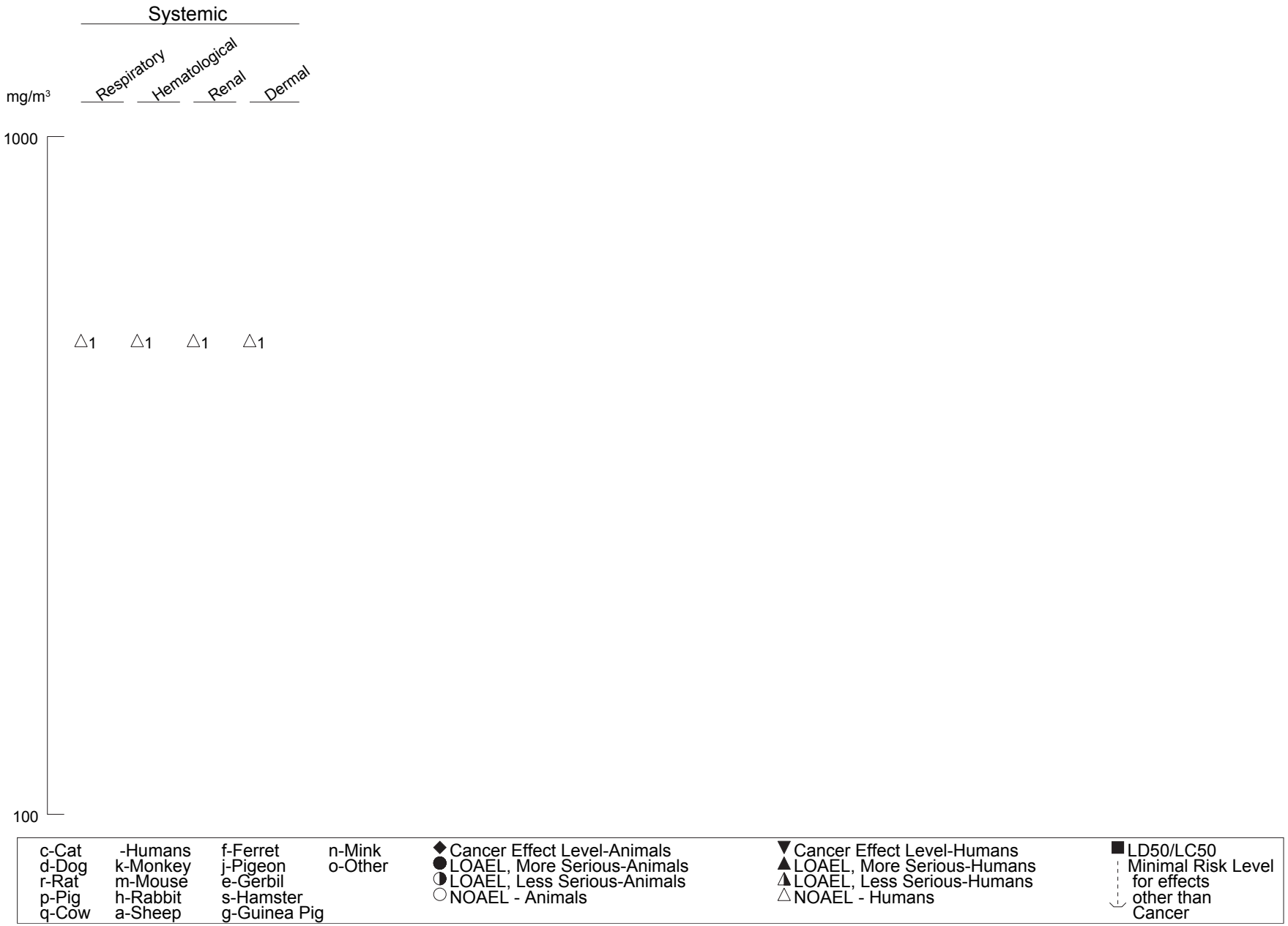
Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m^3)	LOAEL		Reference Chemical Form	Comments
					Less Serious (mg/m^3)	Serious (mg/m^3)		
ACUTE EXPOSURE								
Systemic								
1	Human	10 d 30 min/d	Resp	500			Keplinger 1963	
			Hemato	500				
			Renal	500				
			Dermal	500				

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

d = day(s); Hemato = hematological; LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory

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

Acute (≤14 days)



Renal Effects. Urinalyses were normal for a group of volunteers exposed to toxaphene spray 30 minutes/day for 10 days at a maximum nominal concentration of 500 mg/m^3 (Keplinger 1963).

No studies were located regarding renal toxicity in animals following inhalation exposure to toxaphene.

Dermal Effects. There were no signs of exposure-related dermal effects in a group of 25 volunteers exposed to an aerosol containing a maximum nominal concentration of 500 mg/m^3 toxaphene for 30 minutes/day for 10 days (Keplinger 1963).

No studies were located regarding dermal effects in animals following inhalation exposure to toxaphene.

Body Weight Effects. No studies were available regarding body weight effects in humans or animals following inhalation exposure to toxaphene.

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

3.2.1.3 Immunological and Lymphoreticular Effects

3.2.1.4 Neurological Effects

3.2.1.5 Reproductive Effects

3.2.1.6 Developmental Effects

3.2.1.7 Cancer

Limited human data do not provide convincing evidence that toxaphene causes cancer in humans. In a prospective cohort study of more than 50,000 licensed pesticide applicators enrolled in the Agricultural Health Study and assessed by interview and/or questionnaire for total lifetime exposure days to various pesticides, a statistically significant increased risk for rectal cancer (relative risk [RR] 2.0, 95% confidence interval [CI] 1.1–3.5) was noted among those with self-reported exposure to toxaphene (Purdue et al. 2006). However, the results were based on small numbers of rectal cancer cases. In the same study, a statistically significant increased risk for melanoma (RR 2.9; 95% CI 1.1–8.1) was noted among those subjects reporting more than 25 lifetime days of exposure to toxaphene (based on small numbers of cases). There was no statistically significant association between toxaphene exposure and

risk of leukemia or non-Hodgkin's lymphoma (NHL), or cancer of the prostate, lung, colon, or bladder within this study group.

Lee et al. (2007) used pesticide applicators from the same Agricultural Health Study to assess the risk of colon and/or rectal cancer among those with self-reported exposure to toxaphene; a statistically significantly increased risk was noted for rectal cancer (odds ratio [OR] 2.1; 95 CI 1.2–3.6) among ever-exposed subjects (based on 25 cases among exposed and 50 cases among nonexposed) and among those with reported toxaphene lifetime exposure days ≥56 days (OR 4.3; 95% CI 1.2–15.8), based on three cases in 93 toxaphene-exposed applicators. This study found no statistically significantly increased risk for colon cancer or combined colon and rectal cancer and no significant trend for increased risk of colon, rectal, or colorectal cancer with increasing toxaphene exposure.

Kamel et al. (2012) evaluated the risk of amyotrophic lateral sclerosis (ALS) among private pesticide applicators and their spouses from the Agricultural Health Study. Although an elevated OR was reported for ever use of toxaphene, the association was not statistically significant (OR 2.0; 95% CI 0.8–4.9). The study was based on a small number of toxaphene-exposed ALS cases (n=7).

Mills et al. (2005) reported a significant association (OR 2.20; 95% CI 1.04–4.65) between risk of leukemia and exposure to toxaphene in a nested case-control study of 131 lymphohematopoietic cancer cases (leukemia, multiple myeloma, NHL) diagnosed between 1988 and 2001 among members of the United Farm Workers (UFW) labor union in California (cohort of 139,000 workers). For each case, five gender- and age-matched members of the UFW without any cancer diagnoses were selected as controls. Crop and pesticide exposures were estimated by linking job history information from union records with California Department of Pesticide Regulation pesticide use reports during the 20-year period prior to cancer diagnosis. There was no significant association between exposure to toxaphene and risk of multiple myeloma or NHL.

Schroeder et al. (2001) reported a significant association (OR 3.7, 95% CI 1.9–7.0) between t(14;18)-positive NHL cases (n=5) and toxaphene exposure. The FARM (Factors Affecting Rural Men) case-control study included 182 NHL cases assayed for the t(14;18) translocation. This translocation is a common somatic mutation associated with B cell CLL/lymphoma-2 gene expression. Controls consisted of 30 participants who did not report use of toxaphene on farms where they worked. The study authors mentioned that chromosomal damage has been reported to be higher in peripheral blood lymphocytes

during the peak spraying season (Schroeder et al. 2001). However, this study is limited by the small numbers of cases and controls.

Studies of other groups of pesticide applicators found no significant association between toxaphene and the occurrence of NHL (Cantor et al. 1992; De Roos et al. 2003; Hoar et al. 1986; Zahm et al. 1993).

No studies were located regarding cancer effects in animals following inhalation exposure to toxaphene.

3.2.2 Oral Exposure

Toxaphene is toxic following short-term, high-dose oral exposure. Several cases of fatal and nonfatal poisoning have been reported in humans following the accidental or intentional ingestion of toxaphene or food contaminated with large amounts (gram quantities) of toxaphene. In such instances of acute poisoning, toxaphene stimulates the central nervous system like other chlorinated hydrocarbon pesticides. Long-term animal studies indicate that toxaphene causes central nervous system toxicosis and hepatic hypertrophy accompanied by increased microsomal enzyme activity and histological changes in liver cells. The kidneys, spleen, immunological system, and adrenal gland have also been identified as targets of toxaphene toxicity.

3.2.2.1 Death

Ingestion of large doses of toxaphene by humans can be fatal. Six case studies of acute poisoning were reported, three of which (all children) were fatal (McGee et al. 1952). In all cases, an unknown quantity of toxaphene was ingested, either alone or as a residue of spray on food. Symptoms were usually abruptly manifested by 7 hours post-ingestion and consisted of intermittent convulsions, generally without abdominal pain, vomiting, or diarrhea. Death was attributed to respiratory failure resulting from the seizures. An approximate minimum lethal dose in humans was estimated to be 2–7 g (CDC 1963); however, the available report did not provide a basis for the estimate.

In animals, single-dose gavage administration of toxaphene resulted in estimated oral LD_{50} values of 80–293 mg/kg for rats (Boyd and Taylor 1971; Gaines 1969; Jones et al. 1968) and 25 mg/kg for dogs (Lackey 1949). A 300 mg/kg dose was reported to be lethal to male guinea pigs within 72 hours postdosing; the study authors indicated that the 300 mg/kg dose represented an LD_{50} dose level, but did not provide more detailed dosing information (Chandra and Durairaj 1995). Gavage administration of

toxaphene to heifers (136–232 kg) at 50, 100, or 150 mg/kg resulted in 2/8, 6/7, and 5/6 deaths, respectively (Steele et al. 1980).

Mortality was also reported in animals following repeated gavage dosing. Epstein et al. (1972) observed the death of 2/12 and 9/12 male mice administered toxaphene by daily gavage on 5 consecutive days at 40 and 80 mg/kg, respectively. Chernoff and Carver (1976) administered toxaphene to rat and mouse dams on gestation days 7–16 at gavage doses of 0 (vehicle controls), 15, 25, or 35 mg/kg/day. Mortality was noted in 0/33, 2/39, 3/39, and 5/16 of the rat dams (0, 15, 25, and 35 mg/kg/day dose levels, respectively) and 1/75, 0/26, 0/45, and 07/90 of the mouse dams. In a separate study, Chernoff et al. (1990) observed mortality in 50% of the rat dams (n~25) administered toxaphene at 32 mg/kg/day during gestation days 6–15. In a 28-day oral toxicity study, Waritz et al. (1996) administered toxaphene (in corn oil) by daily gavage to a group of 40 male rats. An initial dose level of 100 mg/kg/day was reduced to 75 mg/kg/day after 2/40 of the treated rats died after three doses; no additional unscheduled deaths were observed. No treatment-related deaths were observed in pregnant rats administered 6 mg/kg/day by gavage from gestational day 7 to parturition (Crowder et al. 1980).

The vehicle used to deliver toxaphene may influence its toxicity (Lackey 1949). Among groups of dogs administered toxaphene once via gavage in corn oil at 15, 20, 25, 30, 40, or 50 mg/kg, mortalities were noted in 2/8, 1/5, 6/7, 4/7, 3/7, and 5/5 animals, respectively. However, when toxaphene was administered in kerosene (a poorly absorbed solvent) at 25–250 mg/kg, mortalities were observed only at doses ≥200 mg/kg.

The nutritional status of an animal influences its susceptibility to the lethal effects of ingested toxaphene. Boyd and Taylor (1971) found that the oral LD_{50} for rats fed a protein-deficient diet was 80 mg/kg/day, whereas the oral LD_{50} for rats fed a control diet was 220 mg/kg/day. This has important implications for the possible increased susceptibility of humans who ingest a protein-deficient diet and live in areas of potential exposure to toxaphene.

No treatment-related deaths were observed in a one-generational two-litter study of rats administered toxaphene in the diet for 48 weeks at estimated doses up to 46 mg/kg/day (Chu et al. 1988). The lack of lethality at a dietary dose within the range of LD_{50} doses noted previously is likely a reflection of differences in dose rate (i.e., bolus gavage dosing versus a slower dose rate from feeding). Treatment-related mortality was not observed in rats following gavage administration of 6 mg/kg/day for 21 days (Crowder et al. 1980). In a 6-week range-finding study that employed groups of male and female

B6C3F1 mice (5/sex/group), estimated toxaphene doses of 58 and 115 mg/kg/day to males resulted in 1/5 and 2/5 deaths, respectively; estimated toxaphene doses of 62 and 125 mg/kg/day to females resulted in 1/5 and 4/5 mortalities, respectively (NCI 1979). In similarly-treated Osborne-Mendel rats, estimated doses of 112 and 224 mg/kg/day to males resulted in 0/5 and 1/5 deaths, respectively; estimated doses of 121 and 242 mg/kg/day to females caused 0/5 and 2/5 deaths, respectively. In a subsequent cancer bioassay in which toxaphene was administered in the diet to Osborne-Mendel rats and B6C3F1 mice for 80 weeks, estimated TWA doses as high as 83 and 34 mg/kg/day, respectively, did not appear to significantly affect survival (NCI 1979). No treatment-related mortality was observed in parental male or female Sprague-Dawley rats administered toxaphene in the diet for up to 42 weeks at estimated doses up to 8.6 mg/kg/day (males) or 9.8 mg/kg/day (females) (Kennedy et al. 1973).

The LD_{50} values and doses associated with death in each species following acute and intermediate oral exposure are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located regarding musculoskeletal effects following oral exposure of humans or animals to toxaphene. The systemic effects of oral toxaphene exposure are described below.

The highest NOAEL values and all reliable LOAEL values for each species and duration of exposure for each systemic effect are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. Available information regarding respiratory effects in humans following oral exposure to toxaphene is limited to an account of congestion and edema of the lungs at autopsy of a 2-year-old boy who ingested an unspecified but lethal amount of toxaphene (McGee et al. 1952).

In rats, the acute oral administration of toxaphene has been shown to cause congestion and parenchymal hemorrhage, indicative of a generalized inflammatory response (Boyd and Taylor 1971). The study was limited by the fact that the dose was not specified. The chronic administration of toxaphene in feed to rats or mice at doses of 27 and 12.9 mg/kg/day, respectively, has been shown to cause dyspnea (NCI 1979). Bryce et al. (2001) noted no remarkable histopathology following examination of tissues (including lung tissue) from cynomolgus monkeys administered toxaphene by oral capsule at 1 mg/kg/day for 52 weeks.

Table 3-2 Levels of Significant Exposure to Toxaphene - 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 (Wistar)	once (GO)				220 M (LD50 for standard laboratory chow diet)	Boyd and Taylor 1971	
2	Rat (Wistar)	once (GO)				80 M (LD50 low protein diet)	Boyd and Taylor 1971	
3	Rat (Wistar)	once (GO)				293 M (LD50 for normal-protein diet)	Boyd and Taylor 1971	
4	Rat (CD)	Gd 7-16 1x/d (GO)				35 F (5/16 maternal deaths)	Chernoff and Carver 1976	
5	Rat (Sprague-Dawley)	Gd 6-15 1x/d (GO)				32 F (50% maternal lethality)	Chernoff et al. 1990	
6	Rat (Sherman)	once (GO)				90 M (LD50) 80 F (LD50)	Gaines 1969	
7	Rat (NS)	once (G)				283 (LD50)	Jones et al. 1968	
8	Mouse (CD-1)	Gd 7-16 1x/d (GO)				35 F (7/90 maternal deaths)	Chernoff and Carver 1976	
9	Mouse (ICR/Ha Swiss)	5 d 1x/d (G)				40 M (death; 2/12)	Epstein et al. 1972	

Table 3-2 Levels of Significant Exposure to Toxaphene - 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
10	Gn Pig (NS)	once (GO)				300 M (death)	Chandra and Durairaj 1995	
11	Dog (NS)	once (GO)				15 (death of 2/8 dogs)	Lackey 1949	
12	Dog (NS)	once (GO)				200 (death in 1/5 dogs)	Lackey 1949	
Systemic								
13	Rat (CD)	Gd 7-16 1x/d (GO)	Hepatic	35 F			Chernoff and Carver 1976	
			Bd Wt			15 F (22% reduced maternal weight gain)		
14	Rat (Sprague-Dawley)	Gd 6-15 1x/d (GO)	Bd Wt			32 F (up to 50% depressed maternal weight gain)	Chernoff et al. 1990	
15	Rat (Sprague-Dawley)	8 d ad lib (F)	Hepatic		10 M (23% decline in biliary excretion of imipramine metabolites)		Mehendale 1978	
16	Rat (Osborne-Mendel)	80 wk ad lib (F)	Bd Wt	147 F	130 M (14% lower mean body weight)		NCI 1979	Body weight results during the first 2 weeks of an 80-week dietary exposure period

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
17	Rat (NS)	once (C)	Hepatic		120 M (9% increased relative liver weight)		Peakall 1976	
18	Rat (Sprague-Dawley)	14 d ad lib (F)	Hepatic	13.5 M	18 M (20% increased relative liver weight)		Trottman and Desaiah 1980	No effects on body, heart, or kidney weights
			Bd Wt	18 M				
19	Rat (Sprague-Dawley)	14 d 1 x/d (GO)	Endocr		75 M (increased TSH; thyroid histopathology)		Waritz et al. 1996	
20	Mouse (CD-1)	Gd 7-16 1x/d (GO)	Hepatic		15 F (23% increased relative liver weight)		Chernoff and Carver 1976	
			Bd Wt	15 F		25 F (22% depressed body weight gain)		
21	Mouse (CD-1)	7 d 1 x/d (GO)	Hepatic	25 M	50 M (48% increased relative liver weight)		Hedli et al. 1998 toxaphene	
			Bd Wt	100 M				
22	Gn Pig (NS)	once (GO)	Hepatic	300 M			Chandra and Durairaj 1992	
			Renal	300 M				

Table 3-2 Levels of Significant Exposure to Toxaphene - 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
Immuno/ Lymphoret								
23	Rat (Sprague-Dawley)	14 d ad lib (F)		9 M	13.5 M (36% decreased relative thymus weight)		Trottman and Desaiah 1980	
Neurological								
24	Rat (Sprague-Dawley)	3 d 1x/d (GO)			25 M (mild tremors, nervousness)		Rao et al. 1986	
25	Gn Pig (NS)	once (GO)				300 M (sedation, convulsions)	Chandra and Durairaj 1995	
26	Dog (Beagle)	13 wk (C)		5[b]		10 (convulsions, salivation, and vomiting in 1/6 males and 2/6 females)	Chu et al. 1986	Neurological effects observed during the first 2 treatment days at 10 mg/kg/day, but not 5 mg/kg/day from treatment day 3 onward
27	Dog (NS)	once (GO)		5		10 (convulsions)	Lackey 1949	
28	Bovine (Mixed-breed)	once (GW)				50 (convulsions)	Steele et al. 1980	
Developmental								
29	Rat (Sprague-Dawley)	Gd 6-15 1x/d (GO)			32 (significantly increased incidence of fetal supernumerary ribs)		Chernoff et al. 1990	

Table 3-2 Levels of Significant Exposure to Toxaphene - 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
30	Rat (CD)	Gd 7-16 1x/d (GO)			12.5 F (decreased fetal renal protein)		Kavlock et al. 1982	Decreased fetal renal protein may indicate retardation in fetal growth
31	Mouse (CD-1)	Gd 7-16 1x/d (GO)		35 F			Chernoff and Carver 1976	
INTERMEDIATE EXPOSURE								
Death								
32	Rat (Osborne-Mendel)	6 wk ad lib (F)				224 M (death in 1/5 males) 242 F (death of 2/5 females)	NCI 1979	
33	Mouse (B6C3F1)	6 wk ad lib (F)				57.7 M (death of 1/5 males) 31.2 F (death of 1/5 females)	NCI 1979	
Systemic								
34	Monkey (Cynomolgus)	52 wk 1 x/d (C)	Ocular		1 (inflammation and/or enlargement of tarsal glands during treatment weeks 8-13; impacted diverticulae of eyelids during treatment weeks 10-41)		Bryce et al. 2001	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
35	Rat (Sprague-Dawley)	13 wk ad lib (F)	Hemato	45.9 M 63 F			Chu et al. 1986	
			Hepatic	0.35 M 0.5 F	1.8 M (nuclear necrosis, anisokaryosis) 2.6 F (nuclear necrosis, anisokaryosis)			
			Renal	0.35 M	1.8 M (tubular injury) 0.5 F (tubular necrosis, anisokaryosis)	8.6 M (focally severe tubular injury) 45.9 M (multiple and relatively severe kidney lesions) 12.6 F (focally severe tubular injury)		
			Endocr	0.35 M 12.6 F	1.8 M (moderate morphological changes in thyroid) 63 F (moderate morphological changes in thyroid)			
			Bd Wt	45.9 M 63 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
36	Rat (Sprague-Dawley)	26 wk ad lib (F)	Hemato	45 M 46 F			Chu et al. 1988	
			Hepatic	45 M 46 F				
			Renal	9.2 M 8.5 F	45 M (18% increased kidney weight, increased incidence of tubular injury) 46 F (increased incidence of renal tubular injury)			
			Endocr	9.2 M 8.5 F	45 M (cytoplasmic vacuolation in thyroid) 46 F (cytoplasmic vacuolation in thyroid)			
			Bd Wt	45 M 46 F				
37	Rat (Sprague-Dawley)	21 d 1x/d (GO)	Bd Wt	6			Crowder et al. 1980	

Table 3-2 Levels of Significant Exposure to Toxaphene - 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
38	Rat (Sprague-Dawley)	39-42 wk ad lib (F)	Cardio	8.6 M 9.8 F			Kennedy et al. 1973	
			Hepatic	2.2 M 2.5 F	8.6 M (cytoplasmic vacuolation) 9.8 F (cytoplasmic vacuolation)			
			Renal	8.6 M 9.8 F				
			Endocr	8.6 M 9.8 F				
			Bd Wt	8.6 M 9.8 F				
39	Rat (Sprague-Dawley)	6-9 wk ad lib (F)	Hepatic	2.6 M	26 M (24% liver weight increase and hepatic degeneration)		Koller et al. 1983	
			Endocr	26 M				
			Bd Wt	26 M				
40	Rat (Osborne-Mendel)	6 wk ad lib (F)	Bd Wt	224 M 242 F			NCI 1979	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
41	Rat (Sherman)	up to 9 mo ad lib (F)	Renal	20 M 22.6 F			Ortega et al. 1957	
			Bd Wt	20 M 22.6 F				
42	Rat (Sprague-Dawley)	28 d 1 x/d (GO)	Endocr		75 M (increased TSH; thyroid histopathology)		Waritz et al. 1996	
43	Mouse (Swiss Webster)	8 wk ad lib (F)	Resp	39 F			Allen et al. 1983	
			Cardio	39 F				
			Gastro	39 F				
			Hepatic	2 F	19.5 F (increased relative liver weight, variation in cell size with some fatty infiltration)			
			Renal	39 F				
			Endocr	39 F				
			Bd Wt	39 F				
44	Mouse (B6C3F1)	6 wk ad lib (F)	Bd Wt	57.7 M			NCI 1979	

Table 3-2 Levels of Significant Exposure to Toxaphene - 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
45	Dog (Beagle)	13 wk 7 d/wk 1x/d (C)	Hemato	5			Chu et al. 1986	
			Hepatic	2	5 (increased liver weight, increased serum alkaline phosphatase, hepatomegaly)			
			Renal	5				
			Bd Wt	5				
Immuno/ Lymphoret								
46	Monkey (Cynomolgus)	52 wk 1 x/d (C)		1			Tryphonas et al. 2000	Antibody response testing was performed during treatment weeks 36-51
47	Monkey (Cynomolgus)	Up to 75 wk 1 x/d (C)		0.1 F	0.4 F[c] (depressed humoral immunity)		Tryphonas et al. 2001	27-35% depressed humoral immunity expressed as reduced anti-SRBC IgM response during treatment weeks 45-49
48	Rat (Sprague-Dawley)	39-42 wk ad lib (F)		8.6 M 9.8 F			Kennedy et al. 1973	

Table 3-2 Levels of Significant Exposure to Toxaphene - 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
49	Rat (Sprague-Dawley)	9 wk ad lib (F)				2.6 M (46% decreased IgG primary antibody response at day 15 postimmunization)	Koller et al. 1983	
50	Mouse (Swiss Webster)	8 wk ad lib (F)		2 F	19.5 F (depressed humoral immunity)		Allen et al. 1983	
Neurological								
51	Rat (Sprague-Dawley)	21 d 1x/d (GO)		6			Crowder et al. 1980	
52	Dog (NS)	44 or 106 d 1x/d (C)				4 (convulsions)	Lackey 1949	
Reproductive								
53	Rat (Sprague-Dawley)	48 wk ad lib (F)		46			Chu et al. 1988	
54	Rat (Sprague-Dawley)	39-42 wk ad lib (F)		8.6 M 9.8 F			Kennedy et al. 1973	
55	Mouse (Swiss)	multigen (F)		4.9 F			Keplinger et al. 1970	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Developmental								
56	Rat (Sprague-Dawley)	Gd 7-21 1x/d (GO)			6 (transiently delay in development of righting reflex)		Crowder et al. 1980	
57	Mouse (Swiss Webster)	9.5 wk ad lib (F)		2	19.5 (suppression of macrophage phagocytic function)		Allen et al. 1983	Immune function was tested in 8-week-old mice from dams that had been administered toxaphene in the diet during gestation and lactation
CHRONIC EXPOSURE								
Systemic								
58	Monkey (Cynomolgus)	75 wk 1 x/d (C)	Hemato	0.8 F			Arnold et al. 2001	
			Bd Wt	0.8 F				
59	Monkey (Cynomolgus)	52 wk 1 x/d (C)	Resp	1			Bryce et al. 2001	
			Cardio	1				
			Gastro	1				
			Hemato	1				
			Hepatic	1				
			Renal	1				
			Endocr	1				
			Bd Wt	1				

Table 3-2 Levels of Significant Exposure to Toxaphene - 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
60	Rat (Osborne-Mendel)	80 wk ad lib (F)	Resp		39 M (dyspnea, epistaxis)		NCI 1979	
			Gastro		39 M (abdominal distension, diarrhea)			
			Hepatic	77.9 M 83.3 F				
			Renal		39 M (hematuria)			
			Dermal		39 M (alopecia, dermatitis, rough hair coats)			
			Bd Wt	77.9 M	41.6 F (up to 15% lower mean body weight)			
61	Mouse (B6C3F1)	80 wk ad lib (F)	Resp		17 (dyspnea)		NCI 1979	
			Gastro		17 (abdominal distension, diarrhea)			
			Dermal		17 (alopecia, rough hair coat)			
			Bd Wt	34				

Table 3-2 Levels of Significant Exposure to Toxaphene - 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
Immuno/ Lymphoret								
62	Monkey (Cynomolgus)	Up to 75 wk 1 x/d (C)		0.4 F	0.8 F (depressed humoral immunity)		Tryphonas et al. 2001	Depressed humoral immunity expressed as reduced anti-TT titers during treatment weeks 53-63
Neurological								
63	Monkey (Cynomolgus)	52 wk 1 x/d (C)		1			Bryce et al. 2001	
64	Rat (Osborne-Mendel)	80 wk ad lib (F)				39 M (leg paralysis, ataxia, epistaxis)	NCI 1979	
						41.6 F (leg paralysis, ataxia, epistaxis)		
65	Mouse (B6C3F1)	80 wk ad lib (F)		34 F	17 M (hyperexcitability)		NCI 1979	
Reproductive								
66	Monkey (Cynomolgus)	75 wk 1 x/d (C)		0.8 F			Arnold et al. 2001	NOAEL is for menstrual status
67	Monkey (Cynomolgus)	52 wk 1 x/d (C)		1			Bryce et al. 2001	

Table 3-2 Levels of Significant Exposure to Toxaphene - 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
68	Rat (Osborne-Mendel)	80 wk ad lib (F)			41.6 F (vaginal bleeding)		NCI 1979	
Cancer								
69	Rat (Osborne-Mendel)	80 wk ad lib (F)				77.9 M (CEL: follicular -cell carcinomas, thyroid adenomas)	NCI 1979	
						83.3 F (CEL: thyroid adenomas)		
70	Mouse (B6C3F1)	80 wk ad lib (F)				17 M (CEL: hepatocellular carcinoma)	NCI 1979	
						17 F (CEL: hepatocellular carcinoma or neoplastic nodule)		

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

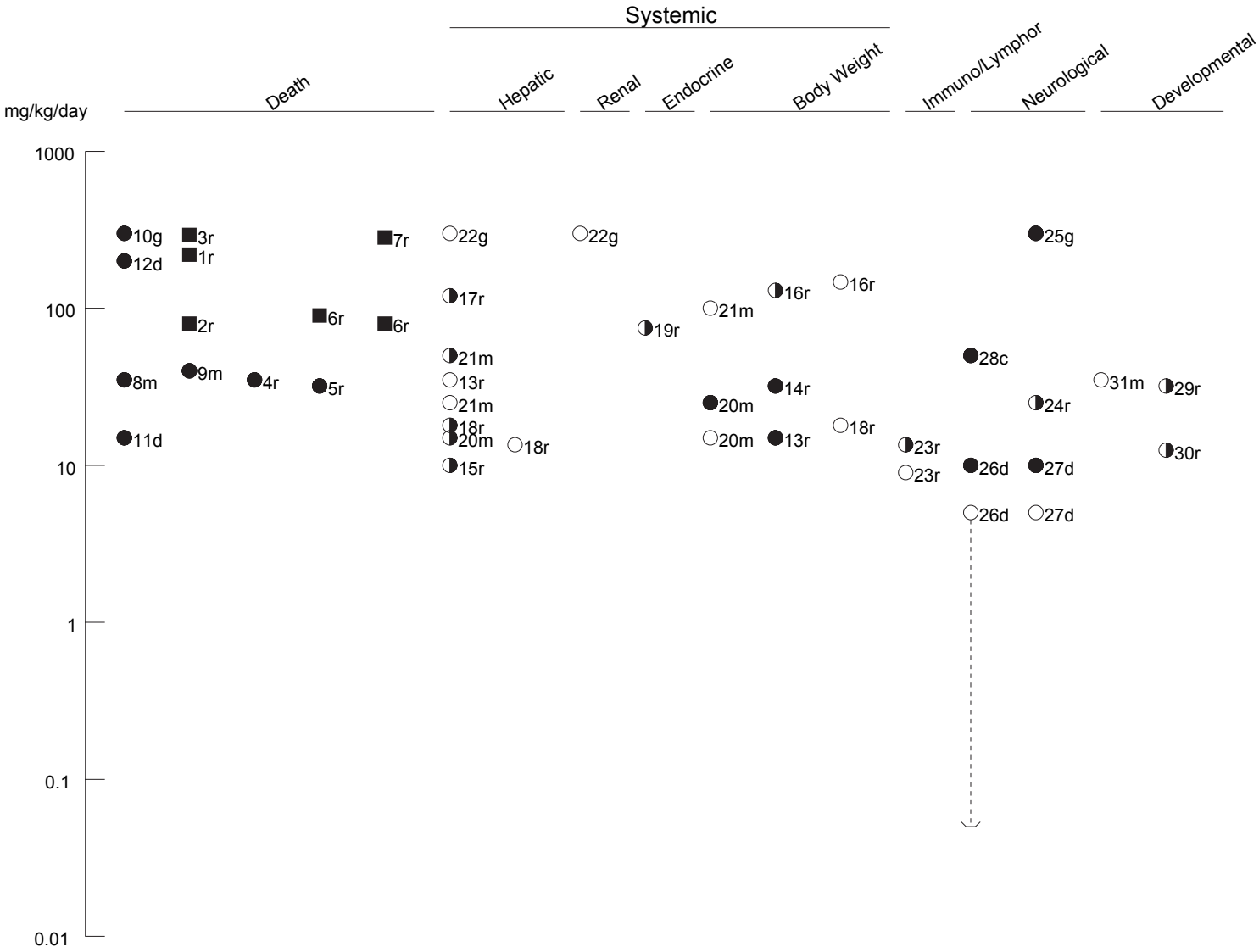
b Used to derive an acute-duration oral minimal risk level (MRL) of 0.05 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

c Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.002 mg/kg/day; the BMDL1SD of 0.22 mg/kg/day, based on benchmark dose analysis of anti-SRBC (IgM) titers as an indicator of humoral immunity, was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ad lib = ad libitum; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F)= feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; x = time(s); wk = week(s)

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

Acute (≤14 days)



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

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

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

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

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

Intermediate (15-364 days)

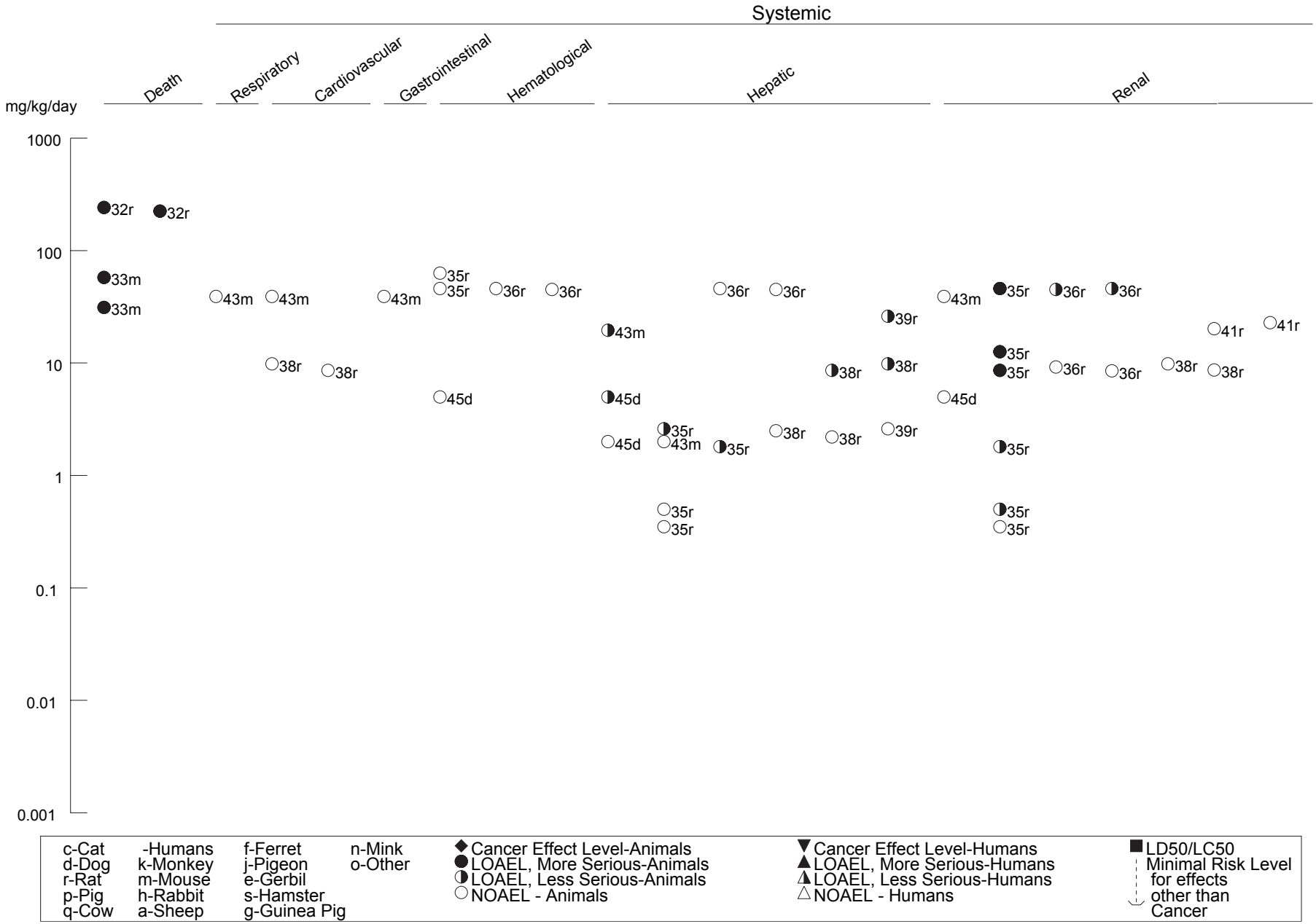


Figure 3-2 Levels of Significant Exposure to Toxaphene - Oral *(Continued)*

Intermediate (15-364 days)

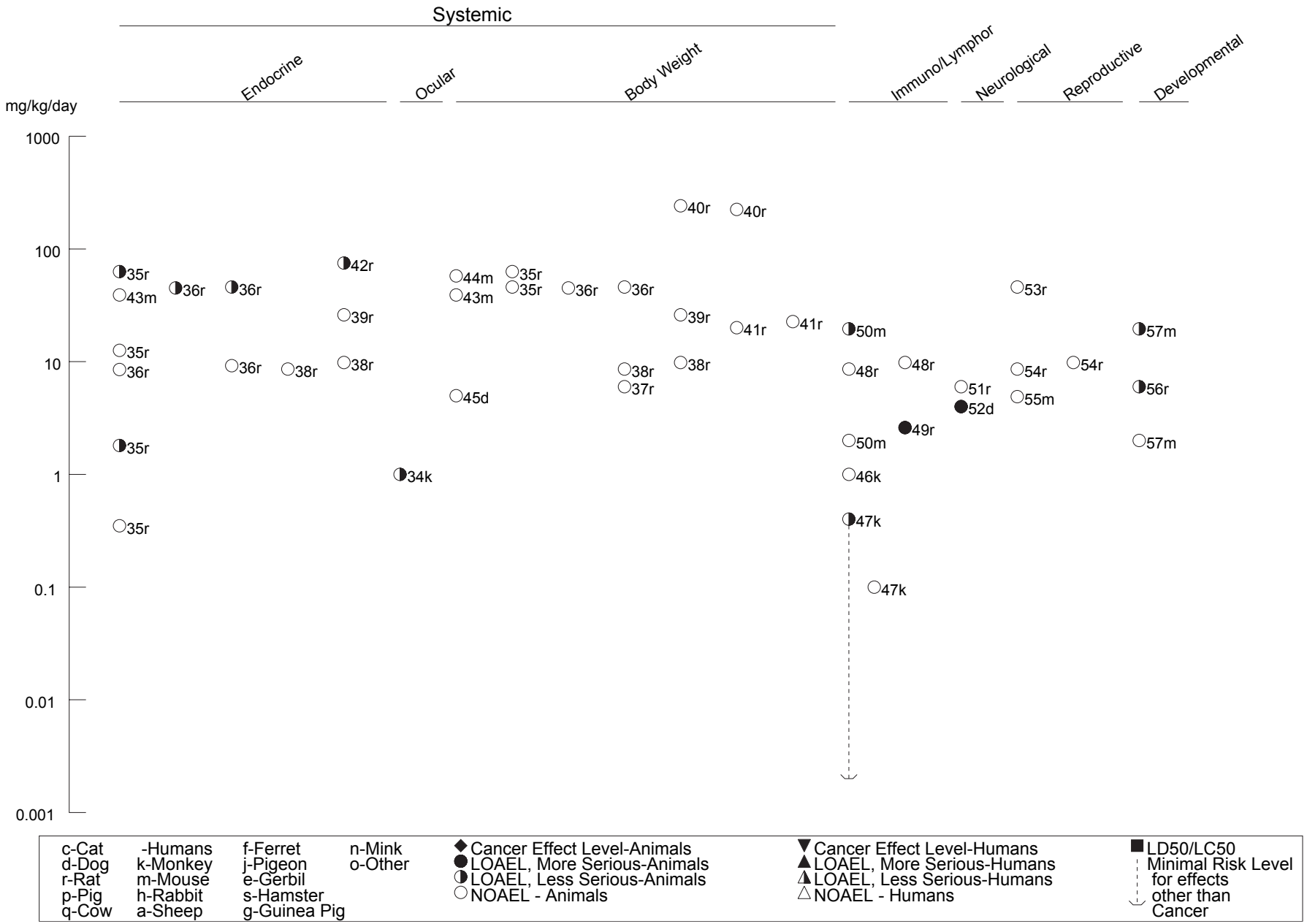
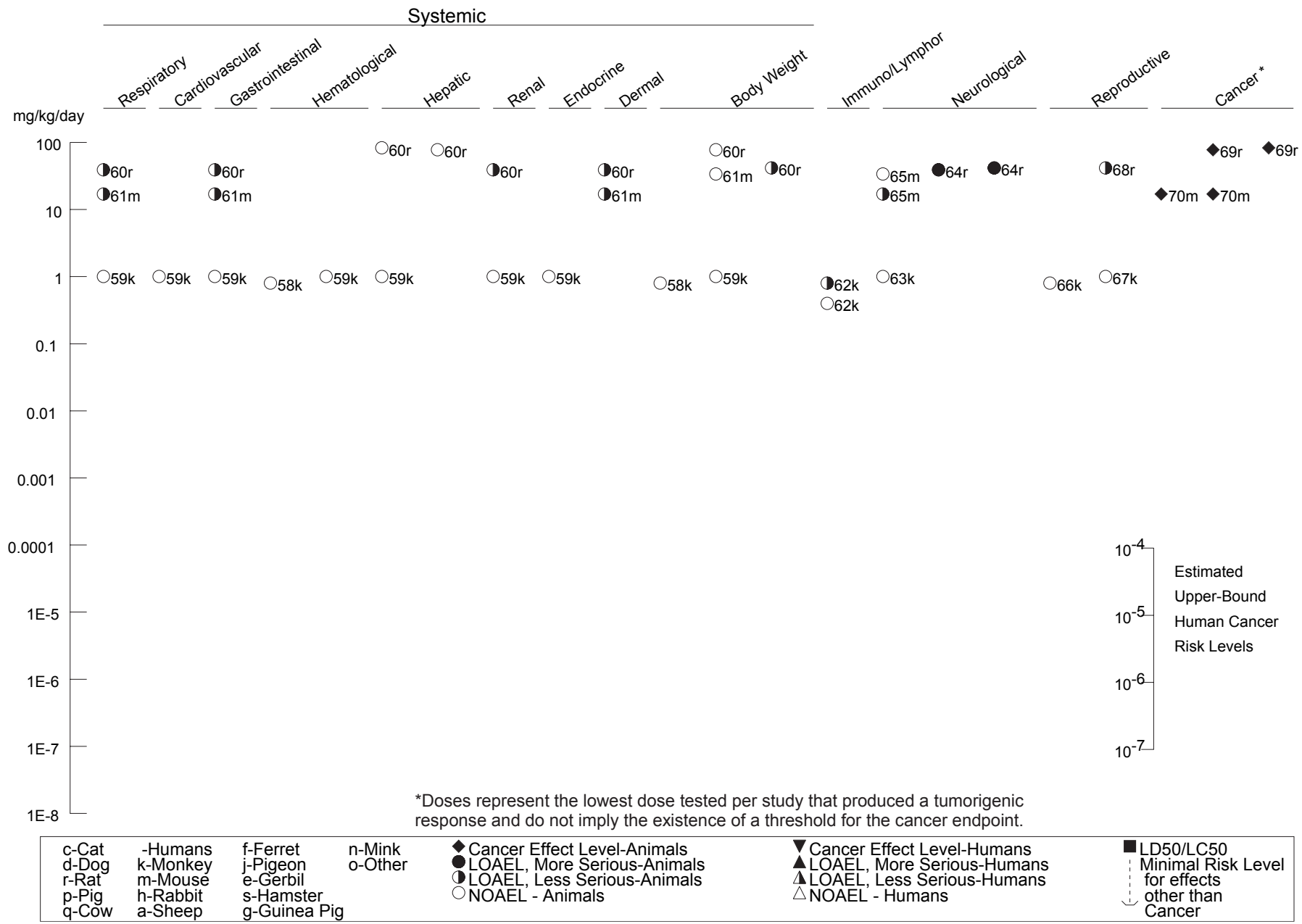


Figure 3-2 Levels of Significant Exposure to Toxaphene - Oral *(Continued)*

Chronic (≥365 days)



Cardiovascular Effects. Available information regarding cardiovascular effects in humans following oral exposure to toxaphene is limited to a report of dilatation of the heart at autopsy of a 2-year-old boy who ingested an unspecified but lethal amount of toxaphene (McGee et al. 1952).

Available information in animals is limited. Congestion and hemorrhage of cardiac capillaries were observed in rats that died following single gavage administration of an unspecified dose of toxaphene (Boyd and Taylor 1971). These effects are indicative of a generalized inflammatory response. Increased heart rate, in the absence of apparent effects on the vascular system, was noted in dogs following administration of a 10 mg/kg dose of toxaphene (Lackey 1949). Progressive neural degeneration was noted in the hearts of pregnant rats following daily gavage administration of toxaphene at 12 mg/kg/day during pregnancy (Badaeva 1976). However, the methods used to identify the lesions in this study are not well described and the effects were not quantitatively evaluated.

No treatment-related effects on heart weight were observed in rats fed toxaphene in the diet for 14 days at 10 mg/kg/day (Trottman and Desaiah 1980) or up to 42 days at 8.6 mg/kg/day (males) or 9.8 mg/kg/day (females) (Kennedy et al. 1973). Bryce et al. (2001) noted no remarkable histopathology following examination of tissues (including heart tissue) from cynomolgus monkeys administered toxaphene by oral capsule at 1 mg/kg/day for 52 weeks.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans following oral exposure to toxaphene.

Gastric ulcers and local gastroenteritis (an inflammatory reaction) were observed in rats administered a single unspecified oral dose of toxaphene (Boyd and Taylor 1971). In this study, animals fed a low protein (3.5%) diet had a greater incidence of toxaphene-induced gastritis than rats fed normal chow or a test diet with normal protein content, in keeping with the apparent "diet-dependency" of toxaphene toxicity. Abdominal distension and diarrhea were observed in rats and mice receiving toxaphene from the diet during 80 weeks at doses ≥39 mg/kg/day (rats) or ≥17 mg/kg/day (mice) (NCI 1979). These effects were most prominent in the high-dose (78 mg/kg/day) male rats. Bryce et al. (2001) noted no remarkable histopathology following examination of tissues (including esophagus, stomach, and small and large intestine) from cynomolgus monkeys administered toxaphene by oral capsule at 1 mg/kg/day for 52 weeks.

Hematological Effects. No studies were located regarding hematologic effects in humans following oral exposure to toxaphene.

No adverse effects on standard hematological parameters were noted in dogs dosed with up to 5 mg/kg/day by capsule for 13 weeks (Chu et al. 1986); dogs dosed with 4 mg/kg/day by capsule for 44 or 106 days (Lackey 1949); male and female rats receiving toxaphene from the diet for 13 weeks at estimated doses of 45.9 or 63 mg/kg/day, respectively (Chu et al. 1986); or male rats receiving 45 mg/kg/day from the diet for 26 weeks (Chu et al. 1988). There was no evidence of treatment-related hematological effects in cynomolgus monkeys administered toxaphene in oral capsules at 1 mg/kg/day for 52 weeks (Bryce et al. 2001) or 0.1–0.8 mg/kg/day for up to 75 weeks (Arnold et al. 2001). Abnormalities in the blood-forming elements were observed in the spleens of rats that died following the oral administration of a single unspecified dose of toxaphene; the study authors attributed this to a generalized stress reaction (Boyd and Taylor 1971).

Hepatic Effects. Little information was located regarding hepatic effects in humans following oral exposure to toxaphene. Transiently-elevated liver lactate dehydrogenase and serum glutamic oxaloacetic transaminase indicative of reversible liver injury were observed in a 26-year-old man who attempted suicide by ingesting the insecticide Tox-Sol, which contains toxaphene as the active ingredient (Wells and Milhorn 1983).

Oral administration of toxaphene has been shown to result in increased liver weight in some studies of rats and mice (Allen et al. 1983; Chernoff and Carver 1976; Chu et al. 1986, 1988; Hedli et al. 1998; Koller et al. 1983; Peakall 1976).

Chandra and Durairaj (1992) reported 13% increased absolute (but not relative) liver weight in guinea pigs administered a single gavage dose of toxaphene at 300 mg/kg (in groundnut oil) in the absence of indications of treatment-related histopathologic liver lesions. In a subsequent study by the same investigators (Chandra and Durairaj 1995), a similar exposure scenario resulted in significantly decreased hepatic phospholipid content and significantly increased hepatic neutral lipid content. Similar effects were noted in other guinea pigs receiving toxaphene by gavage at 5 mg/kg/day for 60 days (Chandra and Durairaj 1995).

Inhibition of hepatobiliary function was reported in perfused livers from male rats exposed to 5 mg/kg/day toxaphene in feed for 8 days (Mehendale 1978). Induction of hepatic microsomal enzymes

and increased liver weights were noted in male rats given 120 mg/kg/day by capsule or 10 mg/kg/day in feed for 14 days (Peakall 1976; Trottman and Desaiah 1980). Increased gamma-glutamyl transpeptidase (GGTP) activity was observed in male rat liver plasma membranes and blood serum after a single gavage exposure to 110 mg/kg toxaphene (Garcia and Mourelle 1984). Increased hepatic microsomal activity (aminopyrene, ethoxyresorufin, and methoxyresorufin) was noted in male and female cynomolgus monkeys (two per sex) administered toxaphene in glycerol/corn oil via gelatin capsule at 1 mg/kg/day for 52 weeks compared to vehicle controls (Bryce et al. 2001). However, the majority of these studies did not report any other evidence of hepatic toxicity. Therefore, enzyme induction in the absence of other signs of liver toxicity is not generally considered adverse, but enzyme induction may precede the onset of more serious hepatic effects.

Morphological and degenerative changes were observed in the livers of dogs (Chu et al. 1986; Lackey 1949), rats (Chu et al. 1988; Kennedy et al. 1973; Koller et al. 1983; Ortega et al. 1957), and mice (Allen et al. 1983) following intermediate-duration exposure to 4, 5–45, and 13 mg/kg toxaphene, respectively. These changes included generalized hydropic degenerative changes, cytoplasmic vacuolization, centrilobular cell hypertrophy, peripheral migration of basophilic cytoplasmic granules, and the presence of lipospheres. Hepatic enzyme induction was also observed in rats following intermediate exposure to toxaphene at 2.4 mg/kg/day (Peakall 1976) or 16.5 mg/kg/day (Garcia and Mourelle 1984). The study of Peakall (1976) did not include a concurrent control group. Toxaphene may also induce hypoxia and alter hepatic energy metabolism because it has been shown to decrease lactate dehydrogenase activity (Gertig and Nowaczyk 1975; Kuz'minskaya and Alekhina 1976). The intermediate-duration oral administration of 2 mg/kg/day toxaphene to dogs caused increased relative liver weight, hepatomegaly, and hepatocellular cytoplasmic vacuolation (Chu et al. 1986). This study is limited by the fact that the high-dose dogs were inadvertently fed the wrong dose for part of the study period. In rats, biochemical and histological evidence of toxaphene-induced liver toxicosis was observed in F_0 male and female rats fed toxaphene at 45 mg/kg/day for at least 26 weeks (Chu et al. 1988).

Liver necrosis was observed in dogs chronically administered 5 mg/kg/day toxaphene in the feed (EPA 1985; summary of an unpublished report for Boots Hercules Agrochemicals). The unpublished report was not available to ATSDR. Histopathologic examinations of livers from male and female rats and mice receiving toxaphene from the diet for up to 80 weeks at estimated time-weighted average (TWA) doses as high as 78–83 mg/kg/day (rats) and 34 mg/kg/day (mice) revealed no evidence of treatment-related nonneoplastic lesions (NCI 1979). See Section 3.2.2.7 for discussion of cancer results from the NCI (1979) study. Bryce et al. (2001) noted no remarkable histopathology following examination of tissues

(including liver) from cynomolgus monkeys administered 1 mg/kg/day of toxaphene by oral capsule for 52 weeks.

Renal Effects. Little information was available regarding renal effects in humans following oral exposure to toxaphene. Renal function was temporarily compromised in a 26-year-old man who attempted suicide by ingesting an unknown quantity of a toxaphene-containing pesticide (Wells and Milhorn 1983). Swelling of the kidney was observed in a 2-year-old boy following acute exposure to a lethal amount of toxaphene (McGee et al. 1952).

Toxaphene has been shown to be nephrotoxic in laboratory animals. A single unspecified, but lethal, oral dose of toxaphene induced cloudy swelling of the proximal and distal convoluted tubules and congestion of the loop of Henle in rats (Boyd and Taylor 1971). However, no renal effects were seen in male rats exposed to up to 10 mg/kg/day of toxaphene in feed for 14 days (Trottman and Desaiah 1980). Renal injury has also been reported to occur following intermediate exposure to toxaphene. Guinea pigs given toxaphene orally at 2 or 5 mg/kg/day for 60 days exhibited histopathologic lesions that included intense vacuolation in the kidney's collection cells and glomerulus, cortical tubule cellular degeneration, and tubular epithelial cell vacuolation (Chandra and Durairaj 1992). Ultrastructural evaluation revealed an increase in the number of mitochondria in the tubular epithelial cells. However, NOAEL and LOAEL values for this study were not established because the study report did not include incidence data for the kidney lesions. Dose-dependent injuries of the proximal convoluted tubules that were focally severe were observed in rats fed 8.6 (males) and 12.6 (females) mg/kg/day toxaphene for 13 weeks (Chu et al. 1986). Chu et al. (1988) reported 18% increased kidney weight and renal tubular injury in male rats receiving toxaphene from the diet for 26 weeks at 45 mg/kg/day and renal tubular injury in similarly-treated female rats. However, Ortega et al. (1957) reported that a dose level of 10 mg/kg/day of toxaphene was not nephrotoxic to rats. Marked degenerative fatty changes of the kidney tubular epithelium were observed in dogs following intermediate-duration exposure to 4 mg/kg/day toxaphene (Lackey 1949). Eosinophilic inclusions that were occasionally accompanied by focal necrosis have also been observed in dogs after intermediate exposure to 2 mg/kg/day toxaphene (Chu et al. 1986).

Hematuria was reported in rats receiving toxaphene from the diet for up to 80 weeks at doses in the range of 39–83 mg/kg/day (NCI 1979). Bryce et al. (2001) noted no remarkable histopathology following examination of tissues (including kidney and urinary bladder) from cynomolgus monkeys administered toxaphene by oral capsule at 1 mg/kg/day for 52 weeks.

Endocrine Effects. No information was located regarding endocrine effects in humans following oral exposure to toxaphene.

Histopathological evidence of toxaphene-related effects on the thyroid gland (angular collapse of follicles, increased epithelial height with multifocal papillary proliferation, and reduced colloid density) of male rats was observed following intermediate-duration oral administration at 1.8 mg/kg/day (Chu et al. 1986). The morphological changes were dose-dependent, considered mild to moderate in severity, and adaptive in nature. The LOAEL for histopathologic thyroid lesions in female rats was 63 mg/kg/day; NOAELs were 0.35 mg/kg/day for males and 12.6 mg/kg/day for females. A similarly-designed study by Chu et al. (1988) found no evidence of treatment-related histopathological thyroid lesions at 1.8 mg/kg/day (male rats) and 1.9 mg/kg/day (female rats).

In a 28-day gavage study designed to assess thyroid function in toxaphene-treated male rats, Waritz et al. (1996) reported significant ($p<0.05$) time-related increases in serum TSH and histopathological evidence of treatment-related effects that included increased incidences of thyroid follicular cell hypertrophy, diffuse intrafollicular hyperplasia, and decreased follicular size (indicative of depletion of colloid stores). This study employed a single dose level of 75 mg toxaphene/kg (100 mg/kg/day for the first 3 treatment days); the thyroid effects were considered to be associated with increased excretion of T_3 and/or T_4 resulting from the induction of hepatic CYPs.

In a study of male rats receiving toxaphene from the diet for 14 days, respective mean relative thymus weights at estimated doses of 13.5 and 18 mg/kg/day were 36 and 27% lower than that of controls; these effects were not seen at doses of 4.5 or 9 mg/kg/day (Trottman and Desaiah 1980). In another rat study, dietary exposure at estimated doses up to and including 8.6 mg/kg/day (males) and 9.8 mg/kg/day (females) for 39–42 weeks did not affect spleen or thymus weights (Kennedy et al. 1973).

Bryce et al. (2001) noted no remarkable histopathology following examination of tissues (including thyroid, pituitary, and adrenal glands) from cynomolgus monkeys administered toxaphene by oral capsule at 1 mg/kg/day for 52 weeks.

Dermal Effects. No information was located regarding dermal effects in humans following oral exposure to toxaphene.

Alopecia and rough hair coats were reported in rats and mice receiving toxaphene from the diet for up to 80 weeks at estimated TWA doses ≥39 and ≥17 mg/kg/day, respectively (NCI 1979).

Ocular Effects. No information was located regarding ocular effects in humans following oral exposure to toxaphene.

Available information in animals is limited to a report of inflammation and/or enlargement of tarsal glands of the eye in three of four cynomolgus monkeys and impacted diverticulae of the eyelid of all four monkeys during oral administration of toxaphene at 1 mg/kg/day for 52 weeks (Bryce et al. 2001).

Body Weight Effects. No information was located regarding body weight effects in humans following oral exposure to toxaphene.

The influence of oral toxaphene on body weight has been widely studied in laboratory animals. Some rat studies employed relatively low single- or multiple-dose levels (<20 mg/kg/day) and found no evidence of toxaphene-induced body weight effects following acute- or intermediate-duration oral exposure (Crowder et al. 1980; Kennedy et al. 1973; Ortega et al. 1957; Trottman and Desaiah 1980). There was no evidence of toxaphene-related body weight effects in intermediate-duration multiple-dose studies of rats or mice where the highest toxaphene doses ranged from 26 to 39 mg/kg/day (Allen et al. 1983; Chu et al. 1986, 1988; Koller et al. 1983).

NCI (1979) found no evidence of a treatment-related effect on body weight in a range-finding study of male and female rats and mice administered toxaphene in the diet for 6 weeks at doses as high as 242 mg/kg/day (rats) and 250 mg/kg/day (mice). However, in the subsequent chronic study that included 80 weeks of dietary exposure to toxaphene, groups of male rats receiving toxaphene from the diet at 131 and 270 mg/kg/day for the first 2 treatment weeks exhibited approximately 14 and 26% lower mean body weights, respectively, than their matched controls (NCI 1979). Throughout the remaining 78 weeks of treatment, which included two 50% reductions in toxaphene concentrations (after treatment weeks 2 and 53 due to clinical signs of neurotoxicity), body weights of the toxaphene-treated male rats appeared similar to those of matched controls. Estimated TWA doses of approximately 42 and 83 mg/kg/day to the low- and high-dose female rats resulted in lower mean body weights throughout most of the study, as much as 10 and 16% lower than those of matched controls (NCI 1979). In the mouse portion of the study (estimated toxaphene doses of 17 and 34 mg/kg/day for the low- and high-dose groups, respectively), mean body weights of the high-dose males were slightly lower than those of matched controls; there

appeared to be no treatment-related effects on body weight in low-dose males or low- or high-dose females.

Lackey (1949) reported weight loss during the initial portion of a study in which four dogs were administered toxaphene by oral capsule at 4 mg/kg/day for 44 or 106 days; convulsions were seen on occasion. Another dog study found no indication of treatment-related effects on body weight during oral dosing of toxaphene at 0.2–5 mg/kg/day for 13 weeks (Chu et al. 1986). There were no signs of treatment-related effects on body weight in cynomolgus monkeys administered toxaphene by oral capsule for 52–75 weeks, but doses were ≤1 mg/kg/day (Arnold et al. 2001; Bryce et al. 2001).

Available animal data indicate that pregnant rats may be particularly sensitive to toxaphene-induced effects on body weight. Rat dams administered toxaphene by gavage at 32 mg/kg/day (the only dose level tested) gained only 50% of the weight gained by control rats (Chernoff et al. 1990). In another developmental toxicity study, rat dams administered toxaphene by gavage at 15 mg/kg/day (the lowest dose tested) on gestation days 7–16 exhibited 22% decreased body weight gain relative to controls (Chernoff and Carver 1976). Crowder et al. (1980) found no evidence of treatment-related effects on body weight in pregnant rats administered toxaphene at 6 mg/kg/day (the only dose tested) from gestation day 7 to parturition.

Metabolic Effects. Available information in humans is limited to a single case report of lactic acidosis in a 26-year-old male who had ingested a substance containing toxaphene as the major component in an apparent suicide attempt; the lactic acidosis was considered secondary to seizures (Wells and Milhorn 1983). No information was located regarding metabolic effects in animals following oral exposure to toxaphene.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects of toxaphene in humans following oral exposure.

Toxaphene has been reported to induce immunosuppressive effects (primarily humoral) in laboratory animals. Toxaphene impaired antibody (IgG) production at some, but not all, stages of the IgG response in male rats receiving toxaphene from the diet for 9 weeks at an estimated dose of 2.6 mg/kg/day (Koller et al. 1983). Similar results were obtained in female mice receiving toxaphene from the diet for 8 weeks

at an estimated dose of 19.5 mg/kg/day, but not at 2 mg/kg/day (Allen et al. 1983). The study of Allen et al. (1983) found no evidence of a delayed hypersensitivity response.

Immunological end points have also been assessed in cynomolgus monkeys administered toxaphene by oral capsule for periods up to 75 weeks (Tryphonas et al. 2001). Groups of 10 female cynomolgus monkeys were administered toxaphene in capsules at doses of 0 (vehicle controls), 0.1, 0.4, or 0.8 mg/kg/day for 75 weeks. Groups of male cynomolgus monkeys (5/group) were dosed at 0 (vehicle controls) or 0.8 mg/kg/day. Flow cytometry, lymphocyte transformation, natural killer cell activity, and serum cortisol levels were evaluated during treatment weeks 33–46. Immunization with SRBC was performed on treatment week 44 for a primary response and week 48 for a secondary response (observations made through treatment week 52). Immunizations with tetanus toxoid (TT) and pneumococcus antigens were performed on treatment week 53 (observations made through treatment week 63). Delayed type hypersensitivity testing was initiated on treatment week 66 and completed on treatment week 70. No treatment-related effects were observed in the 0.1 mg/kg/day group of treated female monkeys. Treatment with toxaphene at 0.4 mg/kg/day resulted in significant ($p<0.05$) reductions in mean primary anti-SRBC IgM responses (indicative of depressed humoral immunity) at post-immunization weeks 1 and 4 (27 and 35% lower than that of controls) and secondary anti-SRBC IgM responses at post-immunization week 5 (10% lower than that of controls). The dose level of 0.8 mg/kg/day resulted in significantly reduced mean primary anti-SRBC IgM responses at post-immunization weeks 1–4, significantly reduced mean secondary anti SRBC IgM response at post-immunization weeks 5 and 8, and significantly reduced primary anti-SRBC IgG responses at post-immunization weeks 2 and 3 (51 and 43% lower than that of controls). In males, 0.8 mg/kg/day toxaphene induced a significant reduction in mean primary anti-SRBC IgM response at post-immunization weeks 1–3. The mean anti-TT titers were significantly reduced in 0.8 mg/kg/day females at post-immunization weeks 2–4. Flow cytometry tests showed that the only effect on leukocyte and lymphocyte subsets was a reduction in absolute B lymphocytes (CD20) in 0.8 mg/kg/day females (62% lower than controls). There were no detectable treatment-related effects on natural killer cell activity, delayed type hypersensitivity, lymphoproliferative response to mitogens, or serum cortisol levels. The toxaphene-induced reduction in mean primary anti-SRBC IgM response served as the critical effect for deriving an intermediate-duration oral MRL for toxaphene.

All reliable LOAEL values for immunological and lymphoreticular effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

Signs of central nervous system stimulation are the hallmark of acute toxaphene intoxication in both humans and animals. Case reports of accidental or intentional toxaphene ingestion indicate that toxaphene poisoning is usually accompanied by convulsive seizures that can be controlled with barbiturates or diazepam (McGee et al. 1952; Wells and Milhorn 1983). In a case of a 9-month-old infant that died after presenting with symptoms including intermittent muscle fasciculations, pupillary constriction, and seizures after playing with a bag containing DDT and toxaphene dust, autopsy revealed toxaphene in brain tissue at approximately 14 ppm; residue was found on skin and in the mouth (Haun and Cueto 1967). The dose necessary to induce nonfatal convulsions in humans has been estimated to be approximately 10 mg/kg (CDC 1963). Contaminated collard greens coated with toxaphene, eaten on empty stomachs, caused convulsive seizures followed by periods of memory loss in three females between the ages of 12 and 20, as well as nausea in a 49-year-old woman (McGee et al. 1952).

Convulsions and other clinical signs of toxaphene-induced neurotoxicity have been observed in laboratory animals. Chandra and Durairaj (1995) reported clinical signs that included convulsions and sedation in guinea pigs receiving a single oral dose of toxaphene at 300 mg/kg. Lackey (1949) administered single gavage doses of toxaphene to dogs at doses ranging from 5 to 50 mg/kg and noted convulsions at dose levels ≥10 mg/kg. In the same study report, occasional convulsions were noted in dogs dosed at 4 mg/kg/day during 44- and 106-day treatment periods. Single oral administration of toxaphene to heifer calves at 50–150 mg/kg elicited numerous clinical signs that included hyperexcitability, nystagmus, convulsions, and seizures (Steele et al. 1980). In a 13-week oral toxicity dog study, clinical signs of neurotoxicity (convulsions, salivation, and vomiting) were elicited during the first 2 days of oral dosing at 10 mg/kg; these clinical signs were no longer elicited after the dose was reduced to 5 mg/kg/day on treatment day 3 (Chu et al. 1986). Tremors and nervousness were reported in rats administered toxaphene by gavage for 3 days at doses ≥25 mg/kg/day; a NOAEL was not identified (Rao et al. 1986). Hyperreflexia was observed in rats at an unspecified dose (Boyd and Taylor 1971).

In a chronic toxicity and carcinogenicity study of male and female rats and mice (NCI 1979), hyper-excitability was reported in high-dose male rats during the first 2 weeks of exposure to toxaphene in the diet when the initial concentration (2,560 ppm) delivered an estimated dose of 270 mg/kg/day; this effect was not observed in male rats receiving 130 mg/kg/day or in groups of similarly-treated females receiving toxaphene at 70 or 147 mg/kg/day during the same 2-week period. Based on the hyperexcitability in the high-dose male rats, dietary concentrations were reduced by 50% at treatment week 3 and another 50% at

treatment week 55 when generalized body tremors were noted in most high-dose male and female rats. The study authors reported clinical signs that included tremors, leg paralysis, and ataxia from treatment weeks 52 through 80, predominantly in toxaphene-treated rats. In the mouse study, hyperexcitability was reported during treatment weeks 60–76 in the low-dose male mice (estimated dose of 17 mg/kg/day), but not in the high-dose males (34 mg/kg/day); there were no signs of hyperexcitability in either dose group (17 and 34 mg/kg/day) of female mice.

There were no clinical signs or histopathological evidence of toxaphene-induced neurological effects in male or female cynomolgus monkeys administered toxaphene in a daily capsule for 52 weeks at 1 mg/kg/day (Bryce et al. 2001).

The electroencephalographic (EEG) pattern of squirrel monkeys was altered by exposure to 1 mg/kg toxaphene (Santolucito 1975). In addition to affecting behavior, an oral dose of 120 mg/kg toxaphene was reported to alter brain catecholamine metabolism in rats (Kuz'minskaya and Ivanitskiĭ 1979). Badaeva (1976) reported brain cell death in pregnant rats gavaged with toxaphene at 12 mg/kg/day during gestation. However, the methods used to identify the lesions are not well described in this study and the effects were not quantitatively evaluated.

Dietary administration of toxaphene to rats for 14 days at estimated doses as high as 18 mg/kg/day did not affect whole brain weight in rats (Trottman and Desaiah 1980), but this is a gross measure and effects on specific neuronal populations would not be detected by this measure. Toxaphene-related decreases in brain weight were reported in guinea pigs following a single oral dose of 300 mg/kg toxaphene (Chandra and Durairaj 1992). The same exposure scenario resulted in a significant decrease of brain phospholipid content and a significant increase in brain neutral lipid and cholesterol content (Chandra and Durairaj 1995). Similar effects were observed in other guinea pigs receiving toxaphene by gavage at 5 mg/kg/day for 60 days (Chandra and Durairaj 1995). However, the toxicological significance of these finding is uncertain.

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

Oral toxicity animal studies that assessed neurodevelopmental end points are summarized in Section 3.2.6 (Developmental Effects).

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following oral exposure to toxaphene.

Testicular weight was not affected in rats receiving toxaphene from the diet for 14 days at estimated doses as high as 18 mg/kg/day (Trottman and Desaiah 1980). Vaginal bleeding was reported in rats receiving toxaphene from the diet for 80 weeks at estimated TWA doses of 41.6 and 83.3 mg/kg/day; incidence data were not provided in the study report (NCI 1979). There were no effects on litter sizes, pup survival, or weanling body weights and no evidence of treatment-related teratogenic effects in a three-generation study of male and female rats that received toxaphene from the diet for up to 42 weeks at estimated doses as high as 8.6 and 9.8 mg/kg/day, respectively (Kennedy et al. 1973). In another reproductive toxicity study (Chu et al. 1988), fertility and offspring growth and viability were not affected by dietary exposure of male and female rats to toxaphene at estimated doses as high as 45–46 mg/kg/day; the treatment period included 13 weeks prior to mating and continued through the production of F_{1a} and F_{1b} litters. Plasma testosterone levels were not affected in male rats administered a single gavage dose of 120 mg toxaphene/kg/day or in other rats dosed at 2.6 mg/kg/day for up to 6 months (Peakall 1976).

Keplinger et al. (1970) performed a multi-generation reproductive toxicity study in which male and female Swiss mice received toxaphene from the diet at estimated dose of 4.5 and 4.9 mg/kg/day, respectively, through the production of five generations of offspring. There were no indications of toxaphene-related adverse effects on lactation, reproduction, average litter size, or offspring growth or on viability through five generations.

Bryce et al. (2001) noted no remarkable histopathology following examination of tissues (including reproductive tissues) from cynomolgus monkeys administered toxaphene by oral capsule at 1 mg/kg/day for 52 weeks. Arnold et al. (2001) found no evidence of toxaphene-related effects on menstrual cycle in cynomolgus monkeys administered the chemical in capsules at daily doses ranging from 0.1 to 0.8 mg/kg/day for up to 75 weeks.

The highest NOAEL values 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 studies were located regarding developmental effects in humans following oral exposure to toxaphene.

Available developmental toxicity studies in animals indicate that toxaphene is not teratogenic. No major anatomical defects were seen in rat or mouse fetuses following gestational oral exposure of pregnant dams at doses in the range 0.05 to 75 mg/kg/day (Allen et al. 1983; Chernoff and Carver 1976; Chernoff and Kavlock 1982; Chernoff et al. 1990; Crowder et al. 1980; Kavlock et al. 1982; Kennedy et al. 1973; Olson et al. 1980).

Toxaphene (15, 25, or 35 mg/kg/day) administered to mice by gavage from gestational days 7–16 produced no adverse effects on fetal growth, viability, or gross morphology even though the toxaphene-treated dams displayed dose-dependent reductions in weight gain (Chernoff and Carver 1976). Keplinger et al. (1970) found no evidence of treatment-related effects on litter size or offspring growth and viability in mice receiving toxaphene from the diet at approximate doses of 4.5–5 mg/kg/day throughout the production of five generations of offspring.

Some of the available developmental toxicity animal studies reported treatment-related effects on development. Chernoff and Kavlock (1982) noted transient decreases in offspring body weight on postnatal day 1 following gavage administration of toxaphene to rat dams at 75 mg/kg/day on gestation days 8–12; however, the dose was maternal toxic, as evidenced by 2/25 maternal deaths and >45% depressed maternal weight gain. Chernoff et al. (1990) reported significantly increased incidences of supernumerary ribs in fetuses from rat dams gavaged at 32 mg/kg/day during gestation days 6–15; however, 50% of the treated dams died. Chernoff and Carver (1976) reported significantly decreased numbers of sternal ossification centers in 21-day-old fetuses from rat dams administered toxaphene by gavage at 15 or 25 mg/kg/day during gestation days 7–16, but not at a dose level of 35 mg/kg/day.

Kavlock et al. (1982) reported significantly decreased renal protein in the kidneys of 21-day-old rat fetuses whose mothers had been administered toxaphene by gavage at 12.5 or 25 mg/kg/day during gestation days 7–16 and significantly decreased alkaline phosphatase activity in fetal kidneys of the 25 mg/kg/day dose group.

Allen et al. (1983) assessed immunological end points in 8-week-old offspring of mouse dams that had received toxaphene from the diet at estimated doses of 2, 19.5, or 39 mg/kg/day for 3 weeks premating and throughout mating, gestation, and lactation. Assessment included a delayed-type hypersensitivity assay for cell-mediated immune response, an enzyme-linked immunosorbent assay for humoral immune response, and a phagocytosis assay to assess the ability of peritoneal macrophages to engulf SRBCs.

Toxaphene treatment resulted in significant suppression of macrophage phagocytic function in the offspring at all dose levels (32, 79, and 63% suppression in the 2, 19.5, and 39 mg/kg/day dose groups, respectively) compared to controls. The humoral antibody response was significantly suppressed at 19.5 mg/kg/day, but was significantly enhanced at 39 mg/kg/day. The cell-mediated immune response was suppressed at 19.5 mg/kg/day, but was not significantly different from controls at the low- and high-dose levels. These results indicate that the perinatal immunological system may be at risk for toxaphene toxicity.

Crowder et al. (1980) assessed the effects of toxaphene on results of selected behavioral tests (grasp-hold, righting, startle, and placing reflexes; open field and maze performance) in pups from rat dams administered toxaphene by gavage at 6 mg/kg/day during mating and throughout gestation (Crowder et al. 1980). Reflex testing, initiated at 7 days postpartum, revealed no significant treatment-related effects on performance; however, the study authors stated that 3 more days were required for 90% of the pups from the toxaphene-treated group to correctly respond in the righting reflex test compared to control pups ($p \leq 0.05$). There were no significant differences between toxaphene-treated groups and control in maze performance assessed beginning at 55 days postpartum.

Olson et al. (1980) examined the effects of toxaphene on selected behavioral parameters in pups of rat dams ingesting the chemical at 0.05 mg/kg/day from gestation day 5 until postpartum day 30. Tests of swimming ability and righting reflex were performed daily on postpartum days 7–16. Significantly retarded swimming ability was noted on postpartum days 10 ($p<0.001$), 11 ($p<0.0001$), and 12 ($p<0.05$); however, the effect was transient and pups exhibited normal swimming ability at testing on postpartum day 16. The study authors stated that pups of the toxaphene treatment group also exhibited significantly retarded overall righting reflex ($p<0.005$), but quantitative data were not provided in the study report. Maze testing, initiated on postpartum day 70, revealed no apparent treatment-related effect.

The highest NOAEL values and all reliable LOAEL values 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 in humans following oral exposure to toxaphene.

NCI (1979) assessed the toxicity and carcinogenicity of toxaphene in groups of male and female Osborne-Mendel rats and male and female B6C3F1 mice exposed to the chemical for 80 weeks via the diet followed by 28–30 weeks of recovery prior to terminal sacrifice. Among the mice, respective incidences of neoplastic liver nodules or hepatic carcinomas (combined) in concurrent controls, pooled controls, and low- and high-dose animals were 2/10, 7/48, 40/49, and 45/46 for males and 0/9, 0/48, 18/49, and 40/49 for females. Incidences at the low- and high-dose levels (estimated TWA doses of 77.9 and 83.3 mg/kg/day, respectively) were significantly higher than those of respective concurrent controls. Among the rats, incidences of thyroid follicular cell adenomas or carcinomas (combined) in concurrent controls, pooled controls, and low- and high-dose animals were 1/7, 2/44, 7/41, and 9/35 for males and 0/6, 1/46, 1/43, and 7/42 for females. Incidences in high-dose male and female rats (estimated TWA doses of 77.9 and 83.3 mg/kg/day, respectively) were significantly higher than those of respective controls. Significantly increased incidences of hepatocellular carcinomas were observed in low- and high-dose (17 and 34 mg/kg/day, respectively) male mice and high-dose (34 mg/kg/day) female mice. In light of more contemporary diagnostic criteria for classification of histopathological liver tumors, an expert pathology working group (PWG) was convened to review the original liver slides from the male and female mice. A primary report of the PWG findings was not available to ATSDR. However, Goodman et al. (2000) provided a summary of the results which indicates that, although many of the tumors originally classified as carcinomas were reclassified as adenomas, the incidences of reclassified combined adenomas or carcinomas were similar to the incidences of combined neoplastic nodules or carcinomas presented in the original study report.

Litton Bionetics, Inc. produced an unpublished report of a cancer bioassay in mice administered toxaphene in the diet. The study results were summarized by EPA in an Ambient Water Quality Criteria document (EPA 1980). EPA's Integrated Risk Information System includes a summary for toxaphene in which the results of the unpublished study were used by EPA to derive an oral slope factor of 1.1 per mg/kg/day (IRIS 2002). The following summary of the unpublished study was extracted from EPA (1980) because the study was not available to ATSDR: Groups of male and female B6C3F1 mice (54/sex/group) were administered toxaphene in the diet at 0, 7, 20, or 50 ppm for 18 months followed by a 6-month observation period. Based on EPA (1988) chronic reference values for body weight and food consumption in male and female B6C3F1 mice, respective estimated doses were 0, 1.2, 3.4, and 8.6 mg/kg/day for the males and 0, 1.2, 3.5, and 8.6 mg/kg/day for the females. At unscheduled or terminal sacrifice, histopathological evaluation of major organs was initiated. A statistically significant ($p=0.048$) excess of hepatocellular tumors (adenomas plus carcinomas) was noted in the high-dose male mice (18/51 versus 10/53 in controls). The Cochran Armitage trend test was significant ($p=0.020$) for

dose-related increased incidence of hepatocellular tumors in the male mice. There were no significant treatment-related effects on the incidence of hepatocellular tumors in any group of treated female mice.

In a study designed to assess the effect of insecticides (including toxaphene) on the induction of lung tumors by benzo[a]pyrene, toxaphene was given to female A/J mice (11–12/group) for 12 weeks at 0, 100, or 200 ppm (Triolo et al. 1982). The study assessed a limited number of end points and found no evidence for toxaphene-related lung or stomach tumors.

The oral doses associated with individual, lifetime upper-bound cancer risk of 10^{-4}–10^{-7} are $9x10^{-5}$–$9x10^{-8}$ mg/kg/day, assuming that a 70-kg human ingests 2 L water/day. The 10^{-4}–10^{-7} risk levels are indicated in Figure 3-2.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding lethal effects in humans following dermal exposure to toxaphene.

Acute dermal LD_{50} values obtained in laboratory animals range from 780 to 4,556 mg/kg (Gaines 1969; Industrial Biotest 1973; Johnston and Eden 1953; Jones et al. 1968). All of these studies except Gaines (1969) reported LD_{50} values of 1,075 and 780 mg/kg/day for male and female Sherman rats, respectively; these values are plotted in Table 3-3. The other studies are limited in design and/or reporting which preclude their inclusion in Table 3-3.

3.2.3.2 Systemic Effects

No studies were located regarding cardiovascular, musculoskeletal, endocrine, or body weight effects in humans or animals following dermal exposure to toxaphene. The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 3-3.

Respiratory Effects. In humans, fluoroscopic examination of the lungs following acute dermal exposure to 500 mg/m^3 toxaphene did not reveal abnormalities (Keplinger 1963).

Toxicosis was observed in a herd of pigs that had been treated with a 61% toxaphene solution (equivalent to 13.5 g/kg). The symptoms generally subsided when the animals were sprayed with warm water

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

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL				Reference Chemical Form	Comments
				Less Serious		Serious			
ACUTE EXPOSURE									
Death									
Rat (Sherman)	once					1075 M mg/kg	(LD50)	Gaines 1969	
						780 F mg/kg	(LD50)		
Systemic									
Rabbit (New Zealand)	4 hr	Dermal		130 B mg/kg	(erythema and edema)			International Research and Development Corporation 1973	
Pig (NS)	once	Resp		13500 mg/kg	(lung congestion and presence of peribronchia lymphoid follicles)			Dipietro and Haliburton 1979	
		Renal		13500 mg/kg	(cystic kidney cortex)				
Neurological									
Pig (NS)	once					13500 mg/kg	(convulsions)	Dipietro and Haliburton 1979	

B = both; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory

(DiPietro and Haliburton 1979). Various lung lesions were observed in three affected pigs that were not treated for toxicosis by spraying with warm water. These lesions differed in the three affected pigs examined and included congested cranial lung lobes, numerous peribronchial lymphoid follicles, and moderate congestion of the lungs. Hyperemic lungs also were observed in rabbits that died following a 24-hour dermal application of 3,038 mg/kg toxaphene (Industrial Biotest 1973). It should be noted that some studies performed by Industrial Biotest have been found to be less than reliable; thus, the accuracy of the above data cannot be assured.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans following dermal exposure to toxaphene.

Dilation of veins and intestinal hemorrhage were observed in rabbits dipped in an unspecified dose suspension of a wettable powder of toxaphene for 2 minutes (Johnston and Eden 1953).

Hematological Effects. In humans, blood tests conducted after acute dermal exposure to 500 mg/m^3 toxaphene did not reveal any abnormalities (Keplinger 1963).

No studies were located regarding hematological effects in animals following dermal exposure to toxaphene.

Hepatic Effects. No studies were located regarding hepatic effects in humans following dermal exposure to toxaphene.

Rabbits dipped in an unspecified dose suspension of a wettable powder of toxaphene for 2 minutes had pale and mottled livers (Johnston and Eden 1953). Toxaphene applied to intact or burned skin of rabbits for 24 hours caused enlarged gall bladders in both the intact and burned groups at doses ≥3,038 mg/kg (Industrial Biotest 1973). DiPietro and Haliburton (1979) reported extensive interlobular fibrosis of the liver in one or more pigs following dermal application of toxaphene (to control sarcoptic mange) at 10 times the recommended dosage.

Renal Effects. In humans, urinalysis conducted after acute dermal exposure to 500 mg/m^3 toxaphene did not reveal any abnormalities (Keplinger 1963).

Pigs exhibited renal cortical cysts and enlarged renal pelvis and ureters following acute dermal exposure to 13.5 mg/kg/day toxaphene (DiPietro and Haliburton 1979).

Dermal Effects. In humans, acute dermal exposure to 500 mg/m^3 toxaphene did not produce dermal irritation (Keplinger 1963).

Dermal application of 3,038 mg/kg toxaphene (90% weight to volume [w/v] ratio in xylene) to the skin of rabbits caused moderate to severe edema and erythema followed by severe desquamation following a 24-hour exposure (Industrial Biotest 1973). The skin irritation may have been caused by xylene, which has been reported to cause dermal irritation in guinea pigs (Anderson et al. 1986). Exposure to toxaphene (500 mg) for 4 hours caused rabbit skin to be only mildly irritated (International Research and Development Corporation 1973).

Ocular Effects. No studies were located regarding ocular effects in humans following dermal exposure to toxaphene.

Mild irritation to the eyelids and loss of eyelid hair were observed after 14 applications of a 20% toxaphene solution in kerosene to the eyes of guinea pigs. The eyes were not affected, and the lids cleared completely in 10 days (EPA 1985; summary of an unpublished report for Boots Hercules Agrochemicals). The unpublished report was not available to ATSDR.

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans or animals following dermal exposure to toxaphene.

3.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans following dermal exposure to toxaphene.

Signs of central nervous system toxicity were observed in 40 of 150 pigs 36 hours after being sprayed with 300 mL of a 61% toxaphene solution in water (equivalent to 13.5 g/kg). This dose is about 10 times the recommended dose for treatment of sarcoptic mange (DiPietro and Haliburton 1979). Inhalation and/or oral exposure may have also occurred. Clinical signs included head-pressing, ataxia, depression,

lethargy, diarrhea, and convulsive seizures. Within a day after spraying with warm water, the animals were much improved, and complete recovery was seen within 5 days. Muscular weakness was reported in rabbits exposed by 24-hour dermal application of a 90% w/v solution of toxaphene in xylene at doses ≥6,834 mg/kg, but not at doses ≤4,556 mg/kg (Industrial Biotest 1973); however, this study was limited in that the solvent, xylene, was not tested alone. Similar dermal application to burned skin resulted in muscular weakness at doses ≥3,038 mg/kg; there was no indication of muscular weakness at a dose of 2,025 mg/kg.

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

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

Available *in vitro* assays provide equivocal evidence for toxaphene-induced genotoxicity. Information regarding the potential genotoxicity of toxaphene *in vivo* is extremely limited; available results have not suggested a toxaphene-induced genotoxic effect.

Table 3-4 summarizes available *in vivo* genotoxicity information for toxaphene. A higher incidence of chromosomal aberrations was observed in cultured lymphocytes taken from the blood of eight women exposed to toxaphene than in lymphocytes taken from unexposed women (Samosh 1974). The exposed women had entered a field that had recently been sprayed with an analog of toxaphene and were described as presenting "mild to moderate" clinical symptoms. The nature of the symptoms was not reported. The women were likely to have been exposed by both inhalation and dermal routes. The small sample size precludes drawing conclusions regarding the potential for toxaphene to induce chromosomal aberrations.

In a dominant lethality test, toxaphene did not cause increased fetal death or decreased numbers of implants in mouse dams mated to males that had been administered toxaphene orally at doses of 40 or 80 mg/kg/day for 5 days or by single intraperitoneal injection at 36 or 180 mg/kg (Epstein et al. 1972).

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

Species (test system)	End point	Results	Reference
Mammalian systems			
Human lymphocytes/occupational exposure	Chromosomal aberrations	–	Samosh 1974
Mouse dominant lethal test	Gene mutation	–	Epstein et al. 1972
Mouse liver cells	DNA adducts	–	Hedli et al. 1998

– = negative

Mortality was noted in 9/12 and 2/9 of the high-dose orally- and intraperitoneally-exposed males, respectively, indicating that sufficiently high doses were tested.

Toxaphene did not cause liver deoxyribonucleic acid (DNA) damage in 90-day-old female Sprague-Dawley rats administered the chemical twice (21 and 4 hours prior to sacrifice) by gavage at up to 36 mg/kg/dose (Kitchin and Brown 1994). Hedli et al. (1998) found no evidence of DNA adduct formation in livers of male CD-1 mice administered toxaphene by gavage for 7 days at doses up to and including 100 mg/kg/day.

Table 3-5 summarizes available *in vitro* genotoxicity information for toxaphene. Toxaphene was mutagenic in reverse mutation assays using *Salmonella typhimurium* strains TA98 and/or TA100 (containing the pKm101 plasmid) in the absence of metabolic activation systems (Hooper et al. 1979; Mortelmans et al. 1986; Schrader et al. 1998; Steinberg et al. 1998; Young et al. 2009). However, mutagenic responses were diminished or abolished in some assays upon the addition of mammalian hepatic activation systems that play a role in xenobiotic metabolism (Hooper et al. 1979; Schrader et al. 1998). Negative or only weakly positive results were obtained in reverse mutation assays using *S. typhimurium* strains TA 1535 and TA1537 (non-plasmid containing strains).

Hooper et al. (1979) determined that certain components of the mixture of chemicals making up technical toxaphene were much less mutagenic than the mixture as a whole. Specifically, the components that were considered to possess the highest insecticidal or acute mammalian toxicity activity (e.g., heptachlorobornane, *gem*-dichloro components, and nonpolar fractions) were less mutagenic to *S. typhimurium* strain TA100 than was the complete toxaphene mixture (or the polar fraction). These findings may have relevance to public health in that the components of complex mixtures such as toxaphene may distribute unevenly in the environment (see Chapter 6). Steinberg et al. (1998) found no evidence of a mutagenic effect for four congeners of toxaphene (Parlars 26, 32, 50, and 62), indicating that selected congeners of weathered toxaphene may be less mutagenic than technical toxaphene. Young et al. (2009) reported a mutagenic response to technical toxaphene in *S. typhimurium* strain TA100 both with and without exogenous metabolic activation. The mutagenic response of two specific toxaphene congeners (hexa- and heptachlorobornane) found to accumulate over time in both soil and fish extracts was less than or equivalent to that of technical toxaphene.

Positive results were obtained in an assay for the induction of λ prophage in *Escherichia coli* (Houk and DeMarini 1987). Significantly increased frequency of sister chromatid exchanges in the presence and

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

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms				
Salmonella typhimurium strain TA100	Gene mutation	–	+	Hooper et al. 1979
S. typhimurium strain TA98	Gene mutation	ND	+	Hooper et al. 1979
S. typhimurium strain TA98	Gene mutation	–	+	Mortelmans et al. 1986
S. typhimurium strain TA100	Gene mutation	+	+	Mortelmans et al. 1986
S. typhimurium strain TA1535	Gene mutation	–	–	Mortelmans et al. 1986
S. typhimurium strain TA1537	Gene mutation	–	(+)	Mortelmans et al. 1986
S. typhimurium strain TA98	Gene mutation	ND	+	Steinberg et al. 1998
S. typhimurium strain TA100	Gene mutation	ND	+	Steinberg et al. 1998
S. typhimurium strain TA100	Gene mutation	+	+	Young et al. 2009
S. typhimurium strain TA97	Gene mutation	(+)	+	Schrader et al. 1998
S. typhimurium strain TA98	Gene mutation	(+)	+	Schrader et al. 1998
S. typhimurium strain TA100	Gene mutation	+	+	Schrader et al. 1998
S. typhimurium strain TA102	Gene mutation	–	–	Schrader et al. 1998
S. typhimurium strain TA104	Gene mutation	(+)	(+)	Schrader et al. 1998
S. typhimurium strain TA1535	DNA damage (*umu*C test)	NT	–	Bartoš et al. 2005
Escherichia coli K-12	Λ prophage induction	+	+	Houk and DeMarini 1987
E. coli PQ37	DNA damage (SOS chromotest)	NT	+	Bartoš et al. 2005
Plasmid DNA isolated from *E. coli*	DNA damage	ND	–	Griffin and Hill 1978
Mammalian cells				
Human lymphoid cells LAZ-007	Sister chromatid exchange	–	–	Sobti et al. 1983
Chinese hamster V79 fibroblasts	Gene mutation	–	–	Schrader et al. 1998
Chinese hamster V79 fibroblasts	Sister chromatid exchange	–	NT	Schrader et al. 1998
Chinese hamster lung (Don) cells	Sister chromatid exchange	NT	±	Steinel et al. 1990

ND = no data; NT = not tested; – = negative; + = positive; (+) = weakly positive; (±) = equivocal

absence of metabolic activation (S9) were reported in a cultured cell line derived from human lymphoid cells; however, the increases were <2-fold greater than solvent controls (Sobti et al. 1983). Significantly increased frequency of sister chromatid exchanges were observed in toxaphene-treated Chinese hamster lung cells; the increase was slightly <2-fold higher than that of controls (Steinel et al. 1990). Toxaphene did not significantly increase the frequency of sister chromatid exchanges in Chinese hamster V79 cells with or without metabolic activation and did not significantly alter the frequency of HGPRT mutations at concentrations up to and including those resulting in cytotoxicity (Schrader et al. 1998). Bartoš et al. (2005) reported positive results for toxaphene-induced DNA damage in an SOS Chromotest using *E. coli* PQ37 in the absence of metabolic activation, but reported negative results in a *umu*C test for DNA damage in *S. typhimurium* strain TA1535/pSK1002 in the absence of metabolic activation. Both assays test induction of the SOS repair system. Toxaphene did not induce DNA damage in plasmid DNA isolated from *E. coli* in the absence of metabolic activation (Griffin and Hill 1978).

3.4 TOXICOKINETICS

Studies in laboratory animals indicate that toxaphene is well absorbed by the intestinal tract and probably well absorbed by the lungs. Dermal absorption has also been demonstrated. Once absorbed, toxaphene distributes throughout the body. Studies using radiolabeled toxaphene indicate that distribution to fat predominates over distribution to other organs, and levels are detectable in fat tissue for several months following exposure. Toxaphene is rapidly and extensively degraded in mammals following oral administration. *In vivo* and *in vitro* studies indicate that the principal metabolic pathways involve dechlorination, dehydrodechlorination, and oxidation. Conjugation is also likely, but it is not a major route of metabolism. The primary route of excretion is via the feces (70% of an administered dose), but toxaphene is also excreted in the urine.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Limited human data indicate that inhaled toxaphene is absorbed (Keplinger 1963; Warraki 1963); however, no quantitative data are available. Limited unpublished animal data also indicate that inhaled toxaphene is absorbed; hepatic effects were reported in rats that survived inhalation exposure to toxaphene dust (4 or 12 mg/m^3) for 3 months (cited in EPA 1985 as an unpublished report for Hercules Incorporated; the primary report was not available to ATSDR).

3.4.1.2 Oral Exposure

No data were located regarding the extent of oral absorption of toxaphene in humans. However, accounts of death and systemic effects from accidental ingestion of toxaphene-contaminated food provide evidence that gastrointestinal absorption occurs (McGee et al. 1952). A 9-month-old infant died within a few hours following accidental exposure to a product containing 7.04% DDT and 13.8% toxaphene; autopsy revealed toxaphene levels of 14.03 ppm, 7.85 ppm and 6.75 ppm in the brain, liver and kidney, respectively (Haun and Cueto 1967).

The presence of toxaphene residues in the fat of rats (Mohammed et al. 1985; Pollock and Kilgore 1980b; Saleh and Casida 1978; Saleh et al. 1979), mice (Crowder and Whitson 1980; Saleh et al. 1979), guinea pigs, hamsters, rabbits, monkeys, and chickens (Saleh et al. 1979) following ingestion demonstrates that ingested toxaphene is absorbed. The identification of toxaphene in the milk of cows following ingestion is also evidence of its absorption (Claborn et al. 1963; Zweig et al. 1963).

Although there are no direct studies regarding the extent of toxaphene absorption, 56.5% of an orally administered dose was present in the feces and 9% of the dose was present in the urine of rats, mostly as metabolites. Very little was present as the parent compound, indicating that considerable metabolism had occurred (Chadurkar and Matsumura 1979). Less than 10% of the administered dose was detected in tissues 1 day after oral administration of radiolabeled toxaphene to rats, suggesting that absorption and redistribution may have occurred over the 24 hours following administration (Crowder and Dindal 1974). The proportion of the administered dose that was not redistributed may have been metabolized and eliminated.

The data presented above suggest that toxaphene would be absorbed by humans following the consumption of drinking water or food contaminated with the chemical. Its absorption appears to be extensive and is enhanced when it is dissolved in a vehicle that is readily absorbed. The bioavailability of toxaphene is increased when it is administered in or with vegetable oils like corn oil or peanut oil, and the toxicity of toxaphene is potentiated (EPA 1980). Thus, toxaphene may be more toxic when ingested in oily foods than when ingested in contaminated water.

3.4.1.3 Dermal Exposure

No studies were located in humans regarding the dermal absorption of toxaphene.

The detection of high toxaphene levels in cow's milk (21–45 ppm) after dipping the cattle in a toxaphene solution (0.25% w/w toxaphene plus 0.03% w/v dioxathion) indicates that toxaphene is absorbed following dermal exposure (Keating 1979). Toxaphene toxicosis was reported in swine 36 hours after the dermal application of toxaphene in a 61% solution (equivalent to 13.5 g/kg); necropsy revealed toxaphene residues in brain and body fat (DiPietro and Haliburton 1979).

Under conditions of high dosage, dermal absorption of toxaphene may be efficient enough to cause toxicosis or to produce detectable residues in cow's milk. Toxaphene appears to be well absorbed following dermal exposure in animals, but the extent of absorption has not been quantified. Other evidence suggests that absorption in humans may also be substantial following dermal exposure (Keplinger 1963).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were available in humans or animals regarding the distribution of toxaphene following inhalation exposure. Although cases of inhalation exposure have been reported, there were no data that detailed distribution of toxaphene residues in various tissues.

3.4.2.2 Oral Exposure

Limited information is available regarding distribution in humans following oral exposure to toxaphene. Toxaphene residues have been detected in samples of adipose tissue taken from children (Witt and Niessen 2000) and in maternal and cord blood (Butler Walker et al. 2003). Ingestion of contaminated food, particularly fish and marine mammals, was the assumed exposure route.

Results of tissue analysis following the oral administration of radiolabeled toxaphene to rats indicate that fat is the principal storage tissue (Ohsawa et al. 1975; Pollock and Kilgore 1980b). Other evidence in animals indicates that muscle may also be a storage site for toxaphene as suggested by the observation of a high distribution of toxaphene in muscle following an oral dose in rats, and by evidence that toxaphene residues persist in muscle for up to 20 days post-administration (Crowder and Dindal 1974). The oral administration of ^{14}C-toxaphene in olive oil to rats at a dose of 10 mg/kg resulted in toxaphene residue levels of 6.4 mg/kg in fat 7 days following administration. Residue levels in all other tissues were <0.2 mg/kg (Pollock and Kilgore 1980b). The oral administration of ^{14}C-toxaphene in corn oil to rats at

doses of 19 and 8.5 mg/kg resulted in residue levels of 0.78 and 0.52 mg/kg, respectively, in fat 14 days after administration. Residue levels in all other tissues were <0.3 mg/kg (Ohsawa et al. 1975). Although the levels detected in fat by Pollock and Kilgore (1980b) are higher than those detected by Ohsawa et al. (1975), a direct comparison cannot be made because the two studies used different sized rats, analyzed their tissues at different times after administration, and used different vehicles.

The highest level of activity, except for the gastrointestinal tract, was in the brown fat following administration of 16 mg/kg ^{14}C-toxaphene in peanut oil to rats (Mohammed et al. 1985). High concentrations of toxaphene residues were also detected in the adrenal cortex, bone marrow, liver, and kidney. Levels of radioactive residues peaked at 3 hours. At 24 hours after administration, most radioactivity was found in the white fat. Lesser amounts of the radiolabel were detected in liver and kidney.

Mice that received an oral dose of 25 mg/kg ^{36}Cl-toxaphene in corn oil retained ^{36}Cl activity in fat, brain, kidney, liver, muscle, and testes. Levels were highest in fat (10.6 ppm) when tissues were analyzed 8 days after administration (Crowder and Whitson 1980).

Toxaphene and its metabolites were detected in the liver, kidney, bone, brain, heart, lung, muscle, spleen, and testes of rats 14 days after the oral administration of 8.5 and 19 mg/kg ^{14}C-toxaphene (Ohsawa et al. 1975). After the oral administration of a single dose of 20 mg/kg ^{36}Cl-toxaphene to rats, the greatest levels of radioactivity were seen at 12 hours in almost all tissues. Levels in blood cells peaked after 3 days. The total fat content after 12 hours was only 0.86% of the total dose, but this exceeded the fraction of the dose found in the kidney (0.43%), testes (0.28%), and brain (0.23%) (Crowder and Dindal 1974). Approximately 77% of the dose was detected in the stomach at 12 hours, and <10% of the dose remained in the body after 1 day. At 12 hours after administration, 5.3% of the dose was present in the muscle. Although this was significantly more than the amount seen in fat and other tissues, the concentration of activity in muscle is low due to the large amount of muscle in the body. Crowder and Dindal (1974) only determined the fraction of the dose based on proportions of radioactivity found in each tissue that may have been derived from a component of the original mixture or a metabolite.

Cynomolgus monkeys were administered toxaphene in glycerol/corn oil via gelatin capsule at 1 mg/kg/day for 1 year (Andrews et al. 1996). At 10 weeks, the blood levels appeared to peak out at approximately 40 ppb; levels in adipose tissue leveled out at 4,000 ppb between weeks 15 and 20.

Heifer calves receiving toxaphene at oral bolus doses of 50, 100, or 150 mg/kg ^{14}C-toxaphene had measurable toxaphene residues in the liver, kidney, and brain 7 days after administration. These tissues were the only ones sampled, so it is not possible to assess the amount of toxaphene that distributed to fat (Steele et al. 1980). This study found that liver residues varied exponentially with dosage, as shown in Table 3-6.

Furthermore, liver residue levels correlated with predicted fatality with an accuracy of about 80%. Based upon these tissue distribution results, the authors concluded that liver residue values could serve as a biomarker of toxaphene poisoning. Kidney and brain levels of toxaphene could not be used as biomarkers, because residue levels of the pesticide in these organs did not correlate with observed mortality. Additionally, brain levels are not as consistent as liver values. Oral administration of 16 mg/kg ^{14}C-toxaphene to rats resulted in distribution of radioactivity to the adrenal cortex, primarily localized in the *zona fasciculata*. Only low levels of radioactivity were detected in the *zona glomerulosa* and the *zona reticularis*, and no radioactivity was found in the medulla (Mohammed et al. 1985). The *zona fasciculata* is responsible for glucocorticoid synthesis. A toxaphene-induced 50% inhibition of ACTH-stimulated adrenal corticosterone synthesis *in vitro* is supported by this pattern of toxaphene distribution *in vivo*. Pretreatment of rats with toxaphene in their diet for 5 weeks also resulted in a significant inhibition of corticosteroid synthesis when compared to controls. Hence, the distribution of toxaphene to the *zona fasciculata* was correlated with an adverse physiological effect.

Administration of ^{14}C-toxaphene in olive oil at a dose of 2.6 mg/kg to pregnant rats resulted in its distribution to the fat. Fetuses contained the lowest levels of radioactivity relative to other tissues analyzed (Pollock and Hillstrand 1982). After 1 day, the residue level in the fetus was 84 ppb; the residue level after 3 days averaged 28 ppb. Residue levels in the fat of the mothers exceeded 7,000 ppb. The authors reported that the overall amount of placental transfer was similar to that of polychlorinated biphenyls (PCBs), of which <1% of the dose was transferred.

All studies reviewed consistently demonstrate that toxaphene is distributed throughout the body and preferentially stored in fat. Although toxaphene was identified in the fat up to 30 days after administration, the overall tissue activity level was very low. Apparently, toxaphene is rapidly metabolized, and its metabolites and components are not persistent. However, it is not known whether the toxaphene metabolites or the original components that persist in fat are toxic. Therefore, these persistent residues could theoretically reenter the circulation from the fat stores and cause additional delayed toxicity. Toxaphene has been shown to cross the placenta and become localized in the fetal

Table 3-6. Mean Toxaphene Residues in Cows Following Oral Exposure to Toxaphene

	Toxaphene residue		
Dose (mg/kg)	Liver (ppm)	Kidney (ppm)	Brain (ppm)
50[a]	2.88	3.45	2.67
100[b]	7.66	2.75	4.02
150[a]	22.26	5.50	3.88

[a]Values represent mean of six animals.
[b]Values represent mean of seven animals.

Source: Steele et al. 1980

adrenal. Based on the findings in all animals (Saleh et al. 1979), it would seem likely that fat would also be a principal storage site for toxaphene in humans following its ingestion. Toxaphene localizes in the liver after initial exposure but then redistributes to fat over a longer period of time. Tissue samples obtained from a chronic dog study demonstrated that after 2 years exposure, toxaphene (as estimated from tissue chlorine levels) was measurable only in fat (Hercules Research Center 1966). The levels in liver, kidney, and brain were negligible. Fat samples obtained at the interim periods of 6 and 12 months had toxaphene levels comparable to those seen at 24 months, indicating that accumulation of toxaphene in adipose tissue may reach a saturation point, resulting in steady-state levels, with uptake being equal to excretion.

3.4.2.3 Dermal Exposure

No human or animal data were located regarding distribution of toxaphene following dermal exposure.

3.4.2.4 Other Routes of Exposure

Intravenous administration of ^{14}C-toxaphene to mice at a dose of 16 mg/kg resulted in the appearance of radioactivity in the liver, fat, bile, adrenal glands, kidneys, and ovaries within 20 minutes of administration. The distribution significantly changed after 4 hours, with an increase in radioactivity in the abdominal fat and the intestinal contents. There were decreases in other tissues after 4 hours. Highest levels of radioactivity were still localized in the fat 16 days after administration (Mohammed et al. 1983). In autoradiographic studies of pregnant albino mice intravenously injected with ^{14}C-toxaphene (16 mg/kg), Mohammed et al. (1983) found low levels of activity in fetal tissues. This activity was highly concentrated in the fetal liver and adrenal gland. These results, as after oral administration, suggest that the transplacental transfer of toxaphene after intravenous administration is relatively low. The tissue accumulation of intravenously administered ^{14}C-toxaphene was also examined in normolipidemic and hypolipidemic female NMRI mice (Mohammed et al. 1990b). In normolipidemic mice, the radiolabel first distributed to the liver and adrenal glands 20 minutes after administration of the labeled toxaphene. After 4 hours, the label was primarily found in the abdominal fat. The distribution of the radiolabel in the hypolipidemic mice was different from the controls. After 20 minutes, the labeled toxaphene was found in the liver, adrenal gland, heart, and kidneys. After 4 hours, nearly all of the label was found in the liver. The results of the study indicate that lipid metabolism may play an important role in the tissue distribution of toxaphene and thus its toxicity.

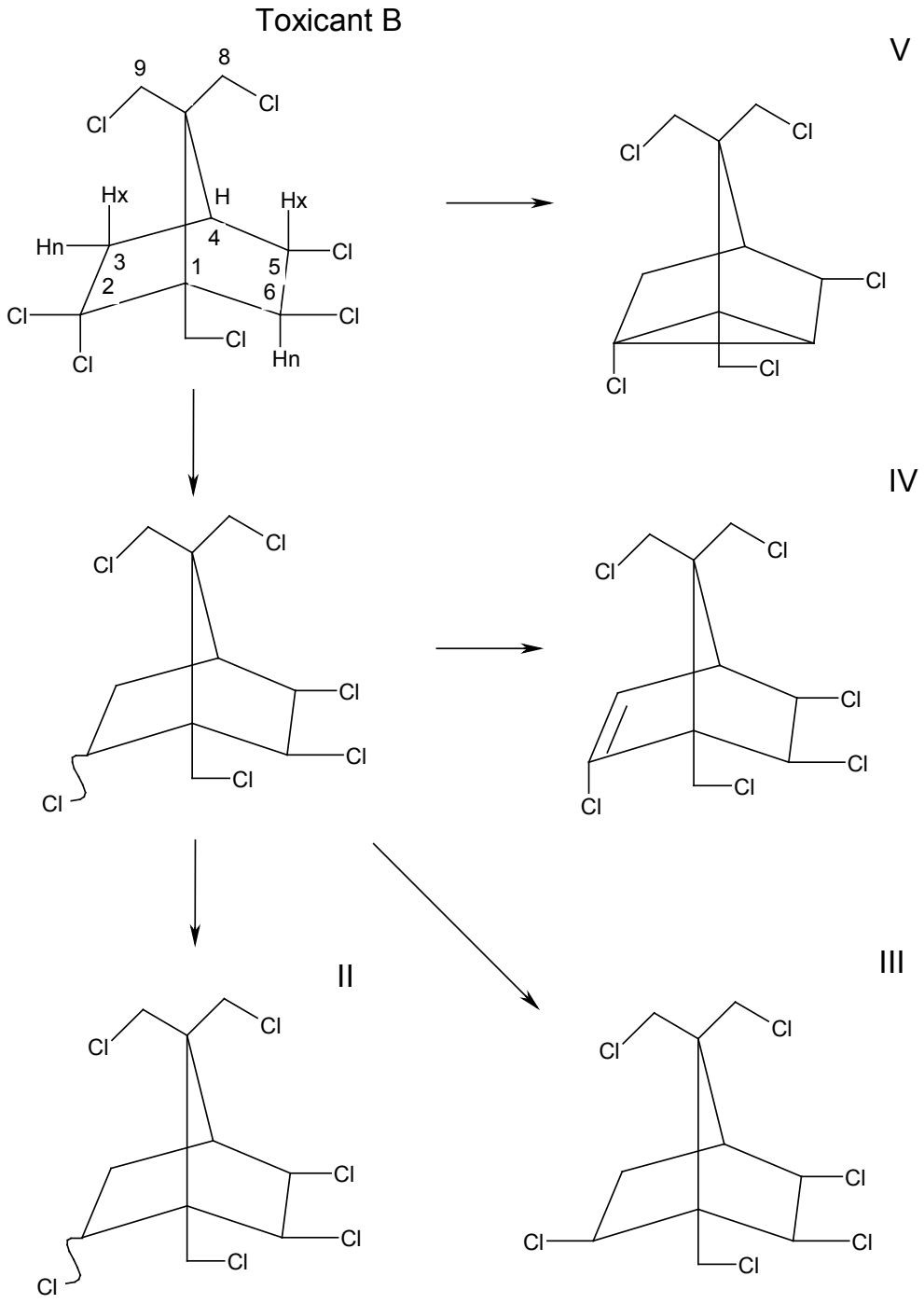
3.4.3 Metabolism

Toxaphene is rapidly and extensively degraded in mammals following oral administration (Figure 3-3). *In vivo* and *in vitro* studies indicate that the principal metabolic pathways involve dechlorination, dehydrodechlorination, and oxidation. Conjugation is also likely, but it is not a major route of metabolism. Administration of ^{36}Cl-toxaphene to rats at a dose of 13 mg/kg resulted in the excretion of ^{36}Cl-chloride ion in the urine. This was the only metabolite identified in the urine by Ohsawa et al. (1975), and it accounted for 50% of the administered radioactivity. Results obtained with ^{36}Cl- and ^{14}C-toxaphene differed. With either label, the hexane extracts of urine and feces contained some unmetabolized material. The percentage of administered activity was negligible in urine and approximately 8–12% in feces. Hence, most excreted material consisted of metabolites from toxaphene components. The combined chloroform extracts of urine and feces contained a much higher proportion of the administered ^{14}C-activity (27%) than of the ^{36}Cl-activity (11.2%). These results indicate that the chloroform fraction consists of partially dechlorinated metabolites, and a predominance of these products were found in the urine. The aqueous fraction contained 11.4% of the ^{14}C-dose and 0.5% of the ^{36}Cl dose. The low amount of ^{36}Cl activity in the aqueous extracts indicated that this fraction contained metabolites (5–10%) that had been completely dechlorinated (Ohsawa et al. 1975). About 2% of the ^{14}C-activity appeared as expired products, probably ^{14}C-carbon dioxide. Thus, these results indicate that toxaphene is metabolized mostly to partially dechlorinated products, with a small proportion being completely dechlorinated and a small proportion unmetabolized.

Pollock and Kilgore (1980b) confirmed the observations of Ohsawa et al. (1975). Less than 5% of the total activity from an orally-administered dose of 10 mg/kg ^{14}C-toxaphene was extractable from urine into hexane. Thin-layer chromatography (TLC) of the urine extract indicated that the components in the urine were more polar than toxaphene. No parent compound was found in the urine. These results provide additional evidence that most of the toxaphene absorbed is metabolized.

The complexity of toxaphene makes it difficult to understand its metabolism fully. It appears that all of its components undergo rapid metabolism, yet each component has its own rate of biotransformation. A small fraction of fecal radioactivity that was extractable into hexane indicated that some toxaphene components could be excreted unchanged. However, it is possible that some metabolite residues may share chromatographic properties similar to the original component of toxaphene.

Figure 3-3. Proposed Metabolic Scheme for a Toxicant Isolated from Toxaphene



Note: Toxicant B = 2,2,5-endo-6-exo-8,9,10-heptachlorobornane
Metabolite II = 2,5-endo-6-exo-8,9,10-hexachlorobornane
Metabolite III = 2,-exo-5-endo-6-exo-8,9,10-hexachlorogornane
Metabolite IV = 2,5-endo-6-exo-8,9,10-heptachlorogornene
Metabolite V = 2,5-endo-8,9,10-pentachlorotricyclene

Source: Saleh and Casida 1978

Pollock and Kilgore (1980b) also extracted the lipid tissue of rats treated with either ^{14}C-labeled toxaphene, Fraction 2, or Fraction 7. Fractions 2 and 7 are nonpolar and polar components, respectively, of toxaphene obtained from chromatographic separation of the toxaphene mixture. When compared to the chromatograms of extracts from fat fortified with ^{14}C-toxaphene, the fat of treated rats had 12% more activity in its polar region. Chromatograms of fat extracts from rats treated with each fraction indicated that two additional compounds were generated that accounted for 11% of the administered activity. With Fraction 2, the additional compounds were of greater polarity. In contrast, the additional compounds generated from Fraction 7 were less polar. The decreased polarity of these metabolites may result in their persistence in the fat and decrease the excretion of Fraction 7. The study does not indicate whether these new compounds were identical.

Metabolism of toxicant B (2,2,5-endo-6-exo-8,9,10-heptachlorobornane), a toxic component of toxaphene, yielded several fecal metabolites when administered orally to mice, rats, hamsters, guinea pigs, rabbits, monkeys, and chickens (Saleh et al. 1979). The greatest amount of fecal metabolites was seen in monkeys and rabbits (20%), with 3–9% in other species, indicating that species differ with respect to metabolic rate and/or pathway (Saleh et al. 1979). The extensive metabolism seen in monkeys suggests that similar findings may result in humans; however, urinary metabolites were not monitored.

The chromatographic pattern of these fecal metabolites was characterized by short retention times, which suggested that dechlorination occurred (Ohsawa et al. 1975; Saleh and Casida 1978; Saleh et al. 1979). In several *in vitro* systems, especially in rat microsomes under anaerobic conditions with NADPH, and in rats under *in vivo* conditions, toxicant B is dechlorinated at the germinal dichloro group to yield 3,5-endo-6-exo-8,9,10-hexachlorobornane (II) and 2-exo-5-endo-6-exo-8,9,10-hexachlorobornane (III) (Figure 3-3). Toxicant B is also dehydrodechlorinated to 2,5-endo-6-exo-8,9,10-hexachloroborn-2,3-ene (IV) and 2,5-endo-8,9,10-pentachlorotricyclene (V) in rats *in vivo* and in other *in vitro* systems (Saleh and Casida 1978). There is no evidence that humans either do or do not metabolize toxaphene via this pathway.

Rat liver microsomes did not transform metabolite I unless they were fortified with NADPH, indicating that cytochrome P-450 was required. Furthermore, the direction of metabolism was dependent upon the oxidative conditions. Only under anaerobic conditions did dechlorination of toxicant B occur, yielding metabolites II and III. Since most gastrointestinal reactions are anaerobic, it follows that metabolites II and III would also be present in the feces (Saleh and Casida 1978). The hexachlorobornane ratio (III/II) was relatively equivalent in the feces, fat, and liver of rats treated with toxicant B, in addition to the

microsomal system. The consistency of this ratio suggested that the mechanism involved in this reaction was similar among tissues (Saleh and Casida 1978). An alternative (and perhaps more likely) explanation is that most of the metabolism occurs in the anaerobic conditions of the intestine. Then compounds II and III are absorbed and distributed to the various tissues, thus keeping the original ratio found in the intestines.

Dechlorination of toxicant B resulted under aerobic conditions in the generation of five nonhydroxyl compounds in rat microsomes fortified with NADPH (Chadurkar and Matsumura 1979). As reported by Saleh and Casida (1978), toxicant B was metabolized to a greater extent under anaerobic conditions than under aerobic conditions. It is possible that this dechlorination reaction was representative of reductive reactions that would be more favorably executed under anaerobic conditions.

Metabolites II and III were not produced under aerobic conditions. However, other unidentified products were generated. The requirement of NADPH and anaerobic conditions for production of metabolites II and III suggests the involvement of the mixed function oxidase systems (Chadurkar and Matsumura 1979; Saleh and Casida 1978).

Acetonitrile extracts of feces and urine from rats receiving a single oral dose of ^{14}C-toxaphene at 15 mg/kg confirmed previously discussed findings that most of the toxaphene was metabolized. Gas-liquid chromatography/electron capture (GLC/EC) analysis of TLC fractions from urine and feces revealed the presence of methylation products. This showed that fecal and urinary metabolites included acidic and other hydroxyl compounds (Chadurkar and Matsumura 1979). Further analysis indicated that approximately 9 and 1% of the urinary and fecal metabolites, respectively, were sulfate conjugates. Glucuronide conjugates comprised 9.5 and 7.5% of the urinary and fecal metabolites, respectively. The presence of sulfate and glucuronide conjugates supported the conclusion that oxidative metabolism occurred.

Drenth et al. (1998) noted that toxaphene induced hepatic CYP450 activity in the rat at a single oral dose level of 40 mg/kg, but not at lower dose levels.

Toxaphene has been shown to induce selected CYP450 isozymes both *in vitro* and *in vivo*. Hedli et al. (1998) reported dose-related increased levels of total CYP450 and cytochrome b_5 in hepatic microsomal fractions from male CD-1 mice administered toxaphene by daily gavage for 7 days at doses of 0, 10, 25, 50, or 100 mg/kg/day. Significant, toxaphene-induced increases in immunodetectable levels of CYP2B,

but not CYP4A1, were detected. Dehn et al. (2005) observed significantly increased CYP1A and CYP2B activities in human HepG2 cells exposed to toxaphene for 24 hours at high concentrations (1, 5, or 10 mM) or 48 hours at lower concentrations (0.01–1 mM). The increases in CYP2B were of greater magnitude than those of CYP1A. Dehn et al. (2005) noted that glutathione levels declined when CYP2B activity was significantly elevated, but increased significantly in the absence of significant CYP450 activation, suggesting that activities of glutathione and CYP450 isozymes may influence one another.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No human or animal data were located regarding excretion following known inhalation exposure to toxaphene.

3.4.4.2 Oral Exposure

It is evident from distribution studies that toxaphene and its metabolites are not persistent in tissues; ^{36}Cl-labeled metabolites remained for 9 days and ^{14}C-labeled metabolites remained for 16 days in the fat of animals. Metabolism studies indicate that toxaphene is rapidly and extensively biodegraded. Consequently, the rate of toxaphene elimination is very high. Table 3-7 summarizes excretion results from studies in which rats were orally administered radiolabeled toxaphene and its components.

An average of 52.6% of an orally administered 20 mg/kg ^{36}Cl-toxaphene dose was excreted over 9 days (approximate half-life of excretion). Approximately 30% of this amount was excreted in the urine and 70% was excreted in the feces. Fecal excretion reached a plateau 2–3 days after administration. The cumulative urinary excretion steadily increased over the 9 days. Much of the activity in the urine and feces was attributable to the ^{36}Cl-chloride ion. Therefore, dechlorination is a principal metabolic route of toxaphene that facilitates its elimination (Crowder and Dindal 1974). In an excretion study conducted by Ohsawa et al. (1975) in rats with ^{36}Cl-toxaphene, a 13 mg/kg dose resulted in the excretion of 76% of the radioactivity after 14 days. Approximately 50% of the activity was detected in the urine. The amount of activity excreted in the urine apparently followed the pattern established by Crowder and Dindal (1974) where the cumulative urinary excretion of the dose steadily increased and eventually equaled the fecal elimination. Ohsawa et al. (1975) also found that the ^{36}Cl-chloride ion appeared almost entirely in the urine. The half-time for the elimination of ^{36}Cl was 2–3 days, a rate equivalent to the excretion of ^{36}Cl-sodium chloride.

Table 3-7. Summary of Excretion Data: Percentage of Dose Excreted in Urine and Feces Following Oral Administration to Rats of Radiolabeled Toxaphene and its Components

Chemical	Dose (mg/kg)	Vehicle	Days after administration	Percent dose		Reference
				Urine	Feces	
^{36}Cl-Toxaphene	20	Peanut oil/gum acacia	1	1.5	23.4	Crowder and Dindal 1974
^{36}Cl-Toxaphene	20	Peanut oil/gum acacia	9	15.3	37.3	Crowder and Dindal 1974
^{36}Cl-Toxaphene	14	Corn oil	14	49.1	26.9	Ohsawa et al. 1975
^{14}C-Toxaphene	8.5	Corn oil	14	21.3	34.7	Ohsawa et al. 1975
^{14}C-Toxaphene	19	Corn oil	14	31.8	27.8	Ohsawa et al. 1975
^{14}C-Toxaphene	2.6	Olive oil	5	22.0	28.3	Pollock and Hillstrand 1982
^{14}C-Toxaphene	10	Olive oil	7	22.5	35.7	Pollock and Kilgore 1980b
^{14}C-Fraction 2	1	Olive oil	7	30.8	38.6	Pollock and Kilgore 1980b
^{14}C-Fraction 7	0.6	Olive oil	7	23.5	32.6	Pollock and Kilgore 1980b
^{14}C-Toxicant A	0.84	Corn oil	14	28.3	38.4	Oshawa et al. 1975
^{14}C-Toxicant B	2.6	Corn oil	9	26.7	47.8	Ohsawa et al. 1975

Rats treated orally with 8.5 and 19 mg/kg of ^{14}C-toxaphene showed no dose-related differences with respect to the excretion of radioactivity (Ohsawa et al. 1975). After 14 days, >50% of the total activity was excreted in urine. Only 8–12% of the dose detected in the feces was suspected of being parent compound. The remainder of the activity in the urine and the feces was thought to be partially or completely dechlorinated products.

Radiolabeled toxicants A and B, obtained by chromatographic separation of ^{14}C-toxaphene, were orally administered to rats at doses of 0.84 and 2.6 mg/kg, respectively. Radioactivity from the ^{14}C-radiolabeled toxicants was excreted rapidly and to a slightly greater extent than toxaphene (Ohsawa et al. 1975). Parent compounds constituted only 8.6 and 2.6% of the fecal residues of toxicants A and B, respectively. However, the dosages used were lower than for toxaphene, and only one animal was tested. Rats orally administered 10 mg/kg ^{14}C-toxaphene in olive oil excreted 58% of the total activity in urine and feces within 7 days after administration (Pollock and Kilgore 1980b). This agreed closely with the excretion pattern reported by Ohsawa et al. (1975). Rats were also orally administered the ^{14}C-labeled isolated fractions of toxaphene, Fraction 2 and Fraction 7, which are nonpolar and polar, respectively. Of these three compound mixtures, the greatest percentage of excreted dose was seen with Fraction 2; the least was seen with Fraction 7. The metabolites derived from polar Fraction 7 were less polar, which resulted in greater persistence in fat and a reduced rate of excretion. In contrast, the nonpolar Fraction 2-derived polar metabolites were more rapidly excreted. Radioactivity measured in the urine of rats receiving Fraction 2 was significantly higher than from those administered Fraction 7 or toxaphene.

Another possible explanation for the unexpected order of excretion is the unexplained contribution of methanol-insoluble activity in the feces. Only the methanol-extractable activity was reported. Ohsawa et al. (1975) reported that some fecal radioactivity was methanol-insoluble and was not detected. Consequently, this may have significantly altered the measurements of total excreted activity. Less polar metabolites from Fraction 7 may be present in the methanol-insoluble extract from feces.

Excretion of radioactivity derived from ^{14}C-toxaphene in pregnant rats was found to be similar to that of virgin female rats (Pollock and Hillstrand 1982). Although there was a weight difference between the pregnant and nonpregnant rats, approximately 50% of the total activity was excreted in the urine and feces over 5 days after the oral administration of 2.6 mg/kg in olive oil. The increased amount of fatty tissue had no effect on the excretion of ^{14}C-toxaphene.

Toxaphene fed to cows in their feed at levels of 20, 60, 100, and 140 ppm for 8 weeks was excreted at all dosage levels. Residues in milk increased rapidly and reached a maximum within 4 weeks after feeding commenced. The levels of toxaphene found in milk were dose-dependent. Upon the cessation of toxaphene administration, there was a rapid decrease in toxaphene residues in the milk. The rate of decrease was the same at all dosage levels during the 1st week. Decreases in milk levels after the first week were slower for animals fed toxaphene at levels >20 ppm (Claborn et al. 1963), as shown in Table 3-8. Detectable amounts of toxaphene were found in the milk of cows 7–9 days after feeding of toxaphene at levels of 2.5–20 ppm commenced (Zweig et al. 1963). As with the higher feeding levels discussed above (Claborn et al. 1963), plateaus were achieved after the fourth week, except at the lowest dose of 2.5 ppm, where a maximum was achieved at 9 days. The animals were fed toxaphene for 1–2.5 months. Toxaphene was no longer detected in the milk within 14 days after cessation of toxaphene administration (Zweig et al. 1963).

The high concentration of radioactivity in the gall bladder from ^{14}C-toxaphene orally administered to quail confirmed the likelihood that the biliary pathway plays an important role in toxaphene excretion (Biessmann et al. 1983).

The detection of toxaphene residue in human breast milk samples is testament to its pharmacokinetics in humans. Ingestion of contaminated food and/or water is the most likely primary source of exposure. Toxaphene residues were detected at a mean concentration of 67.7 ng/g fat in human milk samples collected between July 1996 and April 1997 from 12 residents of Kewatin, an arctic region of northern Canada (Newsome and Ryan 1999). Mean toxaphene residue concentrations ranging from 6.03 to 12.1 ng/g fat were determined from human milk samples that had been collected from other regions of Canada at earlier times (Newsome and Ryan 1999). Toxaphene residues detected in breast milk samples from women living in different parts of Finland were estimated to be 10 ng/g fat (Mussalo-Rauhamaa et al. 1988). Toxaphene-like chlorinated bornanes have been measured in breast milk samples from women in areas of Russia (Polder et al. 1998, 2003), Germany (Skopp et al. 2002b), Belgium (Colles et al. 2008), and Hong Kong and south China (Hedley et al. 2010).

3.4.4.3 Dermal Exposure

No human data were located regarding excretion following dermal exposure to toxaphene.

Table 3-8. Toxaphene Levels in Milk from Cows Fed Toxaphene in Their Diet

Diet concentration (ppm)	Concentration of milk (ppm)[a]				
	Weeks of feeding			Weeks after cessation of toxaphene feeding	
	1	4	8	1	3
20	0.20	0.36	0.23	0.07	–
60	0.56	0.68	0.48	0.13	0.07
100	0.87	1.15	0.91	0.15	0.12
140	1.44	1.89	1.82	0.32	0.20

[a]Values represent means of three samples.

Source: Claborn et al. 1963

Information regarding the excretion of toxaphene in animals following dermal absorption is limited. Evidence for the excretion of toxaphene in milk is found in a study conducted with cows that were sprayed twice daily with 1 ounce of 2.0% toxaphene oil solution or sprayed twice at 3-week intervals with 0.5% sprays of toxaphene. Residues of toxaphene in milk resulting from daily oil sprays reached a maximum after the third day of spraying. When cows were sprayed twice at 3-week intervals, maximum residues in milk were detected 1 or 2 days after spraying (Claborn et al. 1963). Cows that were dipped in a solution containing 0.25% toxaphene also excreted toxaphene in the milk at levels of 21–45 ppm 1 day after dipping. Toxaphene levels fell to 5 ppm 19 days after exposure ceased (Keating 1979). The absorption, distribution, and excretion of toxaphene were evident from these studies, but insufficient information regarding the dose of toxaphene precludes any estimation of the extent and rate of excretion.

3.4.4.4 Other Routes of Exposure

Mohammed et al. (1983) reported that ^{14}C-toxaphene was rapidly distributed to most tissues and organs following intravenous administration in mice. Between 20 minutes and 4 hours after injection, there was a significant increase in the radioactivity observed in the intestinal contents. The presence of radioactivity in the intestine probably represented the biliary excretion of ^{14}C-toxaphene and its metabolites. Sixteen days after administration, the tissue showing the highest concentration of ^{14}C toxaphene was abdominal fat, which had concentrations about 10% of those found 4 hours after administration.

Based on the rapid and extensive metabolism seen in all animals, the fate of toxaphene in humans is probably similar. The negligible quantities of parent compound in the excreta and the lack of persistence of metabolites in the tissues indicate that toxaphene and its components are readily removed from the body. Low-level exposure is not expected to cause significant harm to humans. Theoretically, however, acute high-level exposure may saturate metabolic pathways and consequently allow toxaphene to accumulate in the tissues for a longer period of time (>16 days).

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

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based

pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

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

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

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

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste

sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

If PBPK models for toxaphene 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.

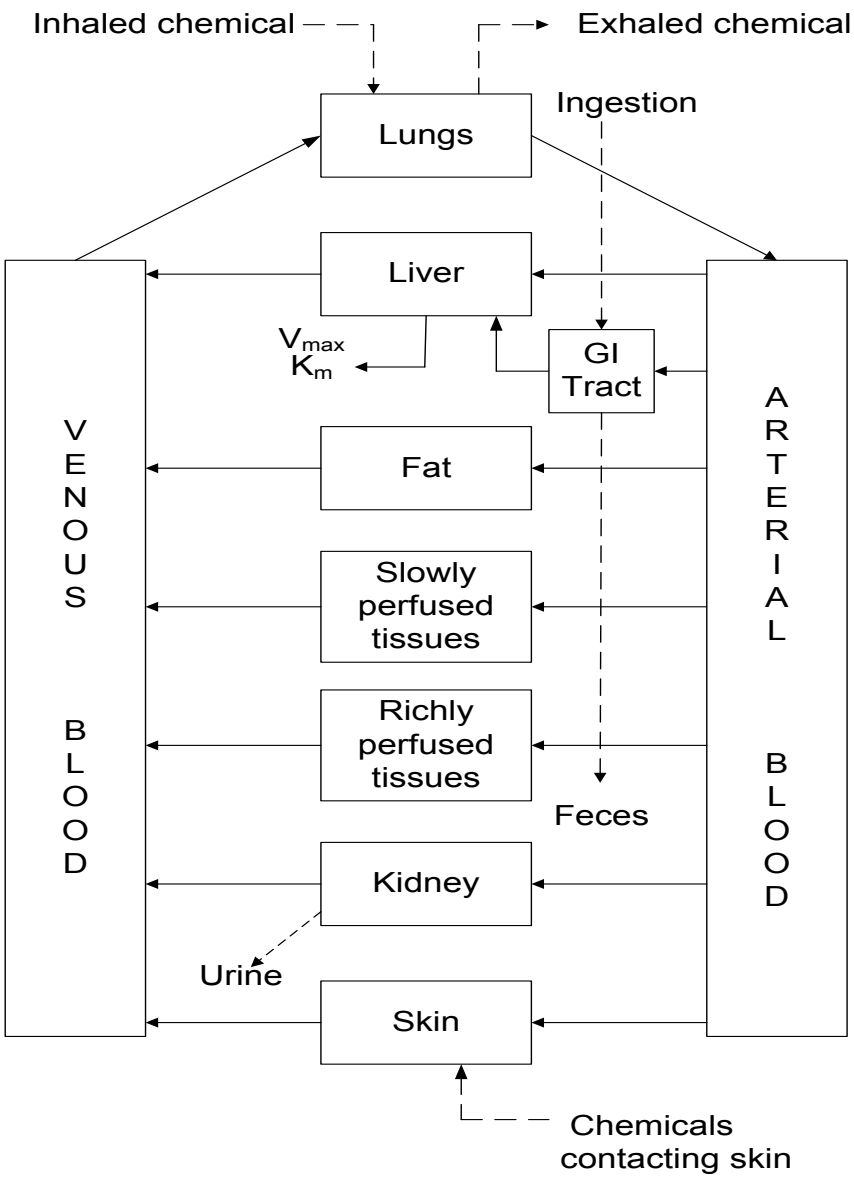
Wen and Chan (2000) developed a two-compartment pharmacokinetic model to predict absorption, elimination, and tissue burden of toxaphene in rats; it does not incorporate data regarding biotransformation or clearance of toxaphene metabolites. The model includes six tissue compartments (blood, brain, liver, muscle, fat, and carcass) and incorporates dose-dependent flux rates and first-order absorption and elimination kinetics. Time-course tissue distribution data from male albino rats administered ^{36}Cl-toxaphene served as basis for model development. The model was partially validated using time-course tissue distribution and depuration data from pregnant Sprague-Dawley rats administered ^{14}C-toxaphene orally. Pharmacokinetically based dosimetry indicated that absorption of toxaphene was fast in fat, whole body, carcass, and blood; relatively slow in liver and muscle; and slow in brain. Elimination was rapid in whole body, muscle, and blood; moderate in carcass and brain; and slow in liver and fat. Tissue burden was highest in fat, whole body, and blood; intermediate in liver; and lowest in brain. In male rats, fecal and urinary excretion represented the major and minor elimination routes, respectively. Fecal and urinary excretion were of approximately equal magnitude in pregnant female rats.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Pharmacokinetic mechanisms of action for toxaphene have not been well studied. Toxaphene is rapidly absorbed by the gastrointestinal tract and lungs. Absorption through the skin can also occur, but appears to be less efficient because dermal doses that cause overt toxicity in laboratory animals are an order of magnitude higher than those causing similar toxicity following oral exposure. Toxaphene is more rapidly absorbed if it is mixed in oily (lipophilic) solvents, probably because interactions with polar areas on the cell membrane are reduced. Once absorbed, toxaphene rapidly distributes to all organs of the body; however, the pesticide tends to concentrate in fatty tissues and muscle from which it is slowly released over a period of weeks. Circulating toxaphene is primarily metabolized by hepatic mixed-function

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



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

Source: adapted from Krishnan and Andersen 1994

oxidases. Toxaphene and its metabolites are excreted in the feces and urine, and most of it is eliminated from the body within a few days.

3.5.2 Mechanisms of Toxicity

Toxaphene-induced neurological effects may result from a general disruption of nervous system function. Toxaphene has been shown to inhibit brain ATPases (Fattah and Crowder 1980; Moorthy et al. 1987; Morrow et al. 1986; Rao et al. 1986; Trottman and Desaiah 1979; Trottman et al. 1985). Results of *in vitro* assays performed by Morrow et al. (1986) indicated that polar toxaphene fractions were more potent inhibitors of rat brain ATPase than other nonpolar or intermediate polar fractions or even toxaphene itself. Pollock and Kilgore (1980a) reported that nonpolar fractions of toxaphene were more toxic to houseflies and mice (*in vivo*) than polar fractions. Morrow et al. (1986) proposed that this discrepancy may be explained by the fact that *in vivo*, the ATPases are membrane-bound in a hydrophobic environment, whereas preparation of cell membranes in the *in vitro* assays could cause disruption within the hydrophobic environment and result in the exposure of polar groups. In any event, diminished ATPase activity in nervous tissue could have a profound effect on neural transmission because of the tissue's high metabolic rate.

Toxaphene has the potential to alter central nervous system neurotransmitter activity. Toxaphene acted as a noncompetitive γ-aminobutyric acid (GABA-A) antagonist at the chloride channel (also known as the picrotoxin binding site) in brain synaptosomes (Lawrence and Casida 1984; Matsumura and Tanaka 1984). GABA is an inhibitory neurotransmitter; antagonism of GABAergic neurons within the central nervous system leads to generalized central nervous system stimulation by inhibiting chloride influx, leading to hyperpolarization and increased neuronal activity. Moreover, the ability of toxaphene to induce convulsions is closely related to its affinity for the picrotoxin binding site. Toxaphene has also been shown to alter catecholamine metabolism in the brain (Kuz'minskaya and Ivanitskiĭ 1979).

Kuz'minskaya and Alekhina (1976) and Gertig and Nowaczyk (1975) reported that both short- and long-term oral administration of toxaphene to rats caused disturbances in energy metabolism as evidenced by changes in hepatic lactate dehydrogenase activity. However, results of Peakall (1979) indicated that these changes are not severe enough to have definite physiological consequences (measured as serum lactate and pyruvate levels) under nonstress conditions. The results of Kuz'minskaya and Alekhina (1976) and Gertig and Nowaczyk (1975) suggest that toxaphene exposure, coupled with stress, could result in detrimental effects on hepatic energy utilization and, ultimately, in hepatic injury.

Several investigators have demonstrated that toxaphene inhibits ATPases in the liver and kidney (e.g., Fattah and Crowder 1980; Mourelle et al. 1985; Trottman and Desaiah 1979; Trottman et al. 1985). These enzymes are involved in all aspects of cellular activity, and their inhibition can ultimately result in disturbances in hepatic and renal function, which could trigger injury responses. Choi et al. (2011) identified numerous genes that were upregulated or downregulated by toxaphene in human hepatocellular carcinoma (HepG2) cells using microarray and gene ontology analysis; changes in expression of these genes may be involved in toxaphene hepatotoxicity.

Mechanisms responsible for toxaphene-induced immunosuppressive effects in laboratory animals are not presently known. Gauthier and coworkers demonstrated that toxaphene induces phagocytosis, production of reactive oxygen species (ROS), and apoptosis in human neutrophils *in vitro* (Gauthier et al. 2001), and that caspases and ROS are likely involved in the degradation of cytoskeletal proteins (Lavastre et al. 2002).

Possible modes of action for toxaphene carcinogenicity have been assessed to some extent; available information is summarized in reports of de Geus et al. (1999), Goodman et al. (2000), Lamb et al. (2008), and Simon and Manning (2006).

Results of available *in vivo* genotoxicity assays have not demonstrated a genotoxic response. Although toxaphene was mutagenic in bacterial systems in the absence of metabolic activation, the mutagenic effect was reduced or abolished in the presence of metabolic activation; this finding suggests that toxaphene may be inactivated *in vivo*. There was no evidence of toxaphene-induced DNA adduct formation or peroxisomal proliferation in hepatic DNA or microsomes from mice administered toxaphene at oral doses as high as 100 mg/kg (Hedli et al. 1998).

As summarized by Waritz et al. (1996) and discussed by Lamb et al. (2008), toxaphene induces rodent liver enzymes (including cytochrome P450 and uridine diphosphate [UDP]-glucuronyl transferase) and causes liver enlargement and increased smooth endoplasmic reticulum in rats. Toxaphene stimulated the production of thyroid-stimulating hormone in rats (a consequence of its strong hepatic cytochrome P450 inducing capability), resulting in thyroid follicular epithelial hyperplasia and hypertrophy and reduction of follicular colloid stores (characteristics indicative of a hyperactive thyroid) (Waritz et al. 1996). The changes observed by Waritz et al. (1996) are consistent with a mechanism of action whereby toxaphene would cause the induction of P450 liver enzymes, increased excretion of T3 and/or T4, and eventual

thyroid tumor development. Hedli et al. (1998) observed significant increases in immunodetectable levels of hepatic CYP 2B in liver preparations from mice administered oral doses of toxaphene. The effects of toxaphene are similar to those elicited by phenobarbital, a nongenotoxic rodent tumor promoter.

Toxaphene and phenobarbital have each been shown to inhibit gap junction intercellular communication (GJIC), a mechanism associated with a nongenotoxic mode of action for tumor induction (Kang et al. 1996; Trosko et al. 1987). Inhibition of GJIC was observed in mouse primary hepatocytes exposed to technical toxaphene, simulated weathered toxaphene, or congeners associated with weathered toxaphene; these results were elicited at noncytotoxic concentrations of the test substance (Lamb et al. 2010) and suggest similarities in the tumor-promoting capability of both technical toxaphene and weathered toxaphene. Weathered toxaphene is the most likely source of potential toxaphene exposure of humans because toxaphene has been banned since the 1980s. Thus, toxaphene may act as a nongenotoxic tumor promoter via inhibition of GJIC which may eventually lead to liver tumor development in mice. Besselink et al. (2008) exposed mouse primary hepatocytes to technical toxaphene, fish-borne residues of toxaphene (cod liver extract), or UV-irradiated toxaphene (as a representative mixture for non-biological weathering) and observed dose-and time-dependent inhibition; the cod liver extract was more potent than technical toxaphene or UV-irradiated toxaphene.

Hou et al. (2013) reported a significant trend ($p=0.04$) for decreased buccal cell telomere length in in association with increased lifetime days of reported toxaphene use among 1,234 cancer-free white male pesticides applicators. However, the study authors noted that chance alone or bias due to uncontrolled confounding may have influenced the result.

3.5.3 Animal-to-Human Extrapolations

Due to a lack of information regarding potential interspecies differences in the toxicokinetics of toxaphene, it is assumed that humans and animals share similar metabolic pathways. Comparative information regarding the toxic effects of toxaphene includes findings of similar neurological effects in humans and laboratory animals following exposure to high levels of toxaphene. As discussed in Section 3.5.2, toxaphene and phenobarbital share similarities in proposed tumor-promoting mechanisms in rats and mice. Although the tumorigenicity of phenobarbital in rats and mice has been well documented, Whysner et al. (1996) reviewed available animal and human data and suggested that humans may not be at particular risk for phenobarbital-related tumors based, in part, on findings that long-term phenobarbital treatment has not been linked to liver or thyroid cancer in epilepsy patients. In the absence

of more convincing information regarding the potential carcinogenicity of toxaphene in humans, it is assumed that the effects observed in laboratory animals are of human relevance as well.

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).

Results of some assays suggest that toxaphene may be weakly estrogenic. Toxaphene was reported to induce weak estrogenic effects in most assays of human breast estrogen-sensitive MCF7 cells (Jørgensen

et al. 1997; Soto et al. 1994, 1995; Stelzer and Chan 1999) and one assay of rat uterine leiomyoma-derived cells (Hodges et al. 2000). In an assay that employed human hepatoma cells transfected with estrogen receptor and luciferase reporter gene, Kim et al (2004) reported that toxaphene exerted an agonistic effect on estrogen receptor α and an antagonistic effect on estrogen receptor β. Results of Graham et al. (2003) indicate that toxaphene may exhibit estrogen-like activity by modulating prolactin mRNA levels in GH_3 pituitary-derived cells. Toxaphene treatment increased the production of vitellogenin in adult male African clawed frogs, suggesting a possible estrogenic effect (Palmer et al. 1998). Toxaphene did not induce an estrogenic response in other assays using mouse uterus cells and MCF7 cells (Arcaro et al. 2000; Ramamoorthy et al. 1997) or yeast-based estrogen reporter genes (Ramamoorthy et al. 1997; Rehmann et al. 1999). Yang and Chen (1999) reported that toxaphene acted as an antagonist of estrogen-related receptor α-1. Drenth et al. (2000) reported that technical toxaphene exerted an antiestrogenic effect in stably transfected human T47D.Luc breast cancer cells. In an *in vivo* assay, immature ovariectomized rats were administered toxaphene via daily intraperitoneal injection for 7 days followed by intraperitoneal injection of estrone; controls received the estrone treatment without prior toxaphene treatment (Welch et al. 1971). Pretreatment with toxaphene resulted in increased metabolism of estrone and inhibition of estrone-stimulated increased uterine weight; these results suggest a potential antiestrogenic effect for toxaphene.

Overall, available *in vivo* and *in vitro* data indicate that toxaphene has the potential to exert weak estrogenic or antiestrogenic effects at relatively high exposure levels. However, limited concern for toxaphene-induced endocrine modulation is apparent at present environmentally-relevant exposure levels.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

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

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The fetus/infant has an immature (developing) blood-brain barrier that past literature has often described as being 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 blood-brain barrier, there are differences between fetuses/infants and adults which 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; as it raises a very important toxicological question as to whether these mechanisms provide protection for the developing brain or do they render it more vulnerable to toxic injury. Each case of 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 who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

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

It is not known whether children are more susceptible than adults to toxaphene toxicity. No human data are available regarding age-related susceptibility to toxaphene. Olson et al. (1980) reported significantly retarded swimming ability and righting reflex in 10–12-day-old pups of rat dams that ingested toxaphene throughout gestation and lactation. However, the effect was transient, and pups exhibited normal swimming ability at testing on postpartum day 16; thus, the toxicological significance of this effect is questionable. Immunosuppression has been demonstrated in some animals exposed to toxaphene (Allen et al. 1983; Koller et al. 1983; Tryphonas et al. 2001). Because the immune system of infants and children does not mature until 10–12 years of age (Calabrese 1978), indications of toxaphene-induced immunosuppression in animals suggest a potential concern to children.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to toxaphene 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 toxaphene 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 Toxaphene

Following acute exposure to high doses, toxaphene can be readily detected in human blood (Griffith and Blanke 1974; Taylor et al. 1979; Tewari and Sharma 1977). If exposure is via inhalation, however, absorption is probably not sufficient to yield quantifiable levels in the blood (EPA 1980). Toxaphene and/or toxaphene residues have been detected in breast milk, urine, stomach washings, umbilical cord blood, and adipose tissue samples from children (Munn et al. 1985; Tewari and Sharma 1977; Vaz and Blomkvist 1985; Butler Walker et al. 2003; Witt and Niessen 2000). Tissue samples taken from dogs sacrificed at intervals in a 2-year study demonstrated that levels of toxaphene in fat were proportional to the levels in the feed, and that tissue levels were essentially stable over the period of 2 years (Hercules Research Center 1966). Levels detected in tissues generally reflect only very recent exposures (<1 week) because toxaphene is rapidly cleared from the body. Metabolites of toxaphene are excreted predominantly in the urine and feces; however, analytic procedures for detecting toxaphene metabolites are not sensitive or reliable enough to allow for screening for metabolites in the blood or excreta.

3.8.2 Biomarkers Used to Characterize Effects Caused by Toxaphene

Toxaphene causes a number of physiological effects including central nervous system excitation, liver enzyme induction, renal tubular degeneration, and immune suppression. However, none of these effects is specific to toxaphene exposure.

For more information on biomarkers for renal and hepatic effects of chemicals see Agency for Toxic Substances and Disease Registry/CDC (1990) and for information on biomarkers for neurological effects, see OTA (1990).

3.9 INTERACTIONS WITH OTHER SUBSTANCES

Toxaphene is likely to interact with other chemicals, such as other pesticides, that also induce hepatic microsomal mixed-function oxidase systems. For example, Deichmann and Keplinger (1970) observed that the toxaphene 96-hour LD_{50} values were increased by about 2 times in rats pretreated with aldrin and dieldrin, and these values were increased by about 3 times in rats pretreated with DDT. Aldrin, dieldrin, and DDT are all known to induce microsomal enzymes. Equitoxic concentrations of toxaphene plus parathion, diazinon, or trithion yielded LD_{50} values that were higher than expected based on an

assumption of additivity, indicating that toxaphene antagonized the lethal effects of these three pesticides (Keplinger and Deichmann 1967).

Another example of microsomal enzyme induction by toxaphene resulting in altered activity of other chemicals was reported by Jeffery et al. (1976). They described the case of a farmer who was being treated with warfarin for thrombophlebitis and was observed to have a loss of warfarin effect that coincided with exposures to a toxaphene-lindane insecticide. The authors concluded that the toxaphene mixture induced the hepatic microsomal enzymes (for up to 3 months), thereby increasing the metabolism of warfarin.

Triolo et al. (1982) investigated the effects of toxaphene administered in the diet on benzo(a)pyrene (BP)-induced lung tumors in mice (BP was administered by oral intubation). There was no increase in the incidence of these tumors when toxaphene was administered alone, but toxaphene significantly reduced the incidence of BP-induced lung tumors when given in combination. This reduction correlated with a toxaphene-induced reduction in BP hydroxylase activity in the lung. The results of this study suggest that toxaphene antagonizes the tumorigenic effect of BP, possibly by inhibiting the biotransformation of BP to a reactive metabolite or by promoting degradative metabolism of BP to nonactive forms in the target tissue.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

Subsets of the human population that may be unusually susceptible to the toxic effects of toxaphene include pregnant women, their fetuses, nursing babies, young children, people with neurologic diseases (particularly convulsive disorders), and individuals with protein-deficient diets. Others at increased risk include people with hepatic, renal, or respiratory diseases, those with immune system suppression, and those ingesting alcohol or consuming therapeutic or illicit drugs.

Pregnant women, fetuses, nursing infants, and very young children may be at greater risk of adverse health effects from pesticide exposure than the general population (Calabrese 1978). Exposure to organochlorine insecticides, such as toxaphene, may adversely affect reproductive physiology (i.e., hormonal balance) in certain women (Calabrese 1978). Embryos, fetuses, and neonates up to age 2–3 months may be at increased risk of adverse effects following pesticide exposure because their enzyme detoxification systems are immature (Calabrese 1978). Animal studies suggest that detoxification of the toxaphene mixture may be less efficient in the immature human than the metabolism and detoxification of the single components such as toxicant A or B (Olson et al. 1980). Infants and children are especially susceptible to immunosuppression because their immune systems do not reach maturity until 10–12 years of age (Calabrese 1978).

Placental transfer of toxaphene has been documented in animals (Pollock and Hillstrand 1982). Toxaphene residues have also been detected in the milk of exposed cows (Claborn et al. 1963; Zweig et al. 1963). Adverse effects have been observed in the offspring of experimental animals exposed to toxaphene during gestation and nursing at doses that typically elicited maternal toxicity. Results of experimental studies indicate that maternal toxaphene exposure may induce behavioral effects in neonates and in nursing babies (Crowder et al. 1980; Olson et al. 1980). It has been suggested that toxaphene exposure during gestation and nursing might be associated with immunosuppression in offspring (Allen et al. 1983). Other effects of maternal toxaphene exposure observed in the offspring were histologic changes in fetal liver, thyroid, and kidney tissues (Chu et al. 1988).

Toxaphene exposure by inhalation, ingestion, or dermal application has induced neurotoxic effects manifested in part by seizures and other functional, biochemical, and morphological alterations (Badaeva 1976; Dille and Smith 1964; DiPietro and Haliburton 1979; Kuz'minskaya and Ivanitskiĭ 1979; Lawrence and Casida 1984; McGee et al. 1952; Wells and Milhorn 1983). Persons with latent or clinical neurologic diseases may be at an increased risk of adverse effects following toxaphene exposure.

Persons consuming diets deficient in protein may also be at increased risk of adverse effects from exposure to toxaphene. It has been estimated that 30% of women and 10% of men aged 30–60 ingest less than two-thirds of the required daily allowance (RDA) for protein (Calabrese 1978). An experimental study showed that central nervous system effects occurred sooner and at lower doses in rats ingesting toxaphene and diets deficient in protein (Boyd and Taylor 1971).

People with liver disease of a genetic origin (i.e., Gilbert's syndrome) and viral infections are at increased risk of developing toxic effects due to insecticide exposure (Calabrese 1978). Liver effects have been observed in both humans and animals following acute exposure to toxaphene. Liver enzymes were transiently elevated in a young man who attempted suicide by ingesting toxaphene (Wells and Milhorn 1983). Liver effects were observed in experimental studies with animals following acute, intermediate, or chronic exposure to toxaphene (Allen et al. 1983; Boyd and Taylor 1971; Chandra and Durairaj 1992, 1995; Chu et al. 1986, 1988; Garcia and Mourelle 1984; Gertig and Nowaczyk 1975; Hedli et al. 1998; Kennedy et al. 1973; Koller et al. 1983; Kuz'minskaya and Alekhina 1976; Lackey 1949; Mehendale 1978; Peakall 1976; Trottman and Desaiah 1980).

Persons with diseases that affect renal, adrenal gland, or respiratory function may be at increased risk of adverse effects due to toxaphene exposure. Renal function was temporarily affected in a young man who attempted suicide by ingesting toxaphene (Wells and Milhorn 1983). Respiratory function was adversely affected in two men occupationally exposed to toxaphene (Warraki 1963). The kidney (Boyd and Taylor 1971; Chu et al. 1986; Fattah and Crowder 1980; Trottman and Desaiah 1979; Trottman et al. 1985) and adrenal gland (Kuz'minskaya and Ivanitskiĭ 1979; Mohammed et al. 1985) are recognized as target organs of toxaphene toxicity in experimental animals.

People susceptible to the toxic effects of toxaphene may develop compromised immune function. People with suppressed immune systems may also be at increased risk of developing more severe effects from toxaphene exposure. Toxaphene has produced primarily humoral immunosuppressive effects in experimental animals (Allen et al. 1983; Koller et al. 1983; Tryphonas et al. 2001).

The induction of hepatic microsomal enzymes, such as mixed function oxidases, by pesticides such as toxaphene may also affect the metabolism of some drugs and alcohol (Calabrese 1978). The efficacy of prescription drugs may be reduced because of the increased rate of metabolism. For example, Jeffery et al. (1976) observed a decrease in the effectiveness of warfarin in a farmer who had been exposed to a toxaphene-lindane insecticide. Furthermore, because toxaphene is a neurotoxic agent, neurological effects associated with other agents or drugs may be exacerbated in persons exposed concomitantly to toxaphene.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

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

Dart RC, ed. 2004. Pesticides. In: Medical toxicology. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 1489-1496.

Holland MG. 2002. Insecticides: Organochlorines, pyrethrins, and DEET. In: Goldfrank LR, Flomenbaum NE, Lewin NA, et al., eds. Goldfrank's toxicologic emergencies. 7th ed. New York, NY: Mc-Graw-Hill Medical Publishing Division, 1366-1378.

Leikin JB, Paloucek FP, eds. 2002. Leikin and Paloucek's poisoning and toxicology handbook. 3rd ed. Hudson, OH: Lexi-Comp, Inc., 1199.

Rhee JW, Aks SE. 2007. Organochlorine insecticides. 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, 1231-1236.

3.11.1 Reducing Peak Absorption Following Exposure

Human exposure to toxaphene may occur by inhalation, ingestion, or by dermal contact. Toxaphene and other chlorinated hydrocarbons are efficiently absorbed from the gastrointestinal tract, particularly in the presence of dietary lipids. Although relatively nonvolatile, absorption following inhalation exposure to dusts and sprays probably occurs through mucociliary trapping and transport followed by gastrointestinal absorption. Toxaphene caused toxicosis following dermal application of the pesticide to farm animals; therefore, absorption across the skin is a health concern as well.

For oral exposure, do not induce emesis. Gastric lavage may be considered if the patient presents in a timely manner. Precaution should be taken to avoid aspiration, however. Activated charcoal may be administered to potentially decrease absorption following ingestion, although the efficacy of this treatment is uncertain.

For inhalation exposure, treatment commonly includes moving the exposed individual to fresh air, then monitoring for respiratory distress. Injuries to the airways and lungs are likely to be manifested as severe

respiratory irritation and persistent cough. Provide emergency airway support and 100% humidified supplemental oxygen with assisted ventilation if needed.

Decontamination is the first step in reducing absorption following dermal exposure. Contaminated clothing should be removed. Skin should be washed with soap and water. Decontamination includes irrigation of the eyes with copious amounts of water or saline (if available).

3.11.2 Reducing Body Burden

Once absorbed, toxaphene bioaccumulates in adipose tissue and is metabolized and excreted over several days to a few weeks following exposure. For organochlorines that undergo enterohepatic and enteroenteric recirculation, cholestyramine has been administered to potentially increase fecal elimination, although the efficacy of this treatment for toxaphene poisoning is uncertain. Exchange transfusion, peritoneal dialysis, hemodialysis, and hemoperfusion are not likely to be beneficial because of the large volume of distribution of toxaphene, resulting in a small proportion of removable toxin.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

The most serious toxicological effects of exposure to chlorinated hydrocarbon pesticides are central nervous system excitability. Organochlorine compounds are thought to interfere with the normal flux of sodium and potassium ions across the axon membrane, disrupting central nervous system activity and resulting in generalized central nervous system excitation, which may lead to convulsions and seizures in severe cases. Toxaphene-induced central nervous system stimulation is believed to result from the noncompetitive inhibition of γ-aminobutyric acid-dependent chloride ion channels that are found on the neuron. The putative role of γ-aminobutyric acid in the central nervous system is to suppress neuronal activity. Thus, if its actions are blocked, neuronal activity increases. Unchecked neuronal excitation can lead to tremors, convulsions, seizures, and death.

Although mechanisms of neurotoxicity vary among individual chlorinated hydrocarbon pesticides, toxaphene, DDT, chlordane, lindane, heptachlor, kepone, and mirex are each considered moderately toxic following acute oral administration (animal LD_{50} >50 mg/kg). Several cases of toxaphene-induced seizures in humans have been reported (McGee et al. 1952; Wells and Milhorn 1983). The acute management of seizures with anticonvulsants such as diazepam (a γ-aminobutyric acid agonist), phenobarbital, and phenytoin has been recommended (Schenker et al. 1992). These drugs tend to suppress neuronal activity, thus counteracting the stimulatory effects of toxaphene. High exposures to

organochlorines can lead to stimulation of the peripheral nervous system. Seizure control can be achieved by administration of GABA agonists such as benzodiazepines; phenobarbital or propofol may be considered if benzodiazepines are ineffective.

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 toxaphene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of toxaphene.

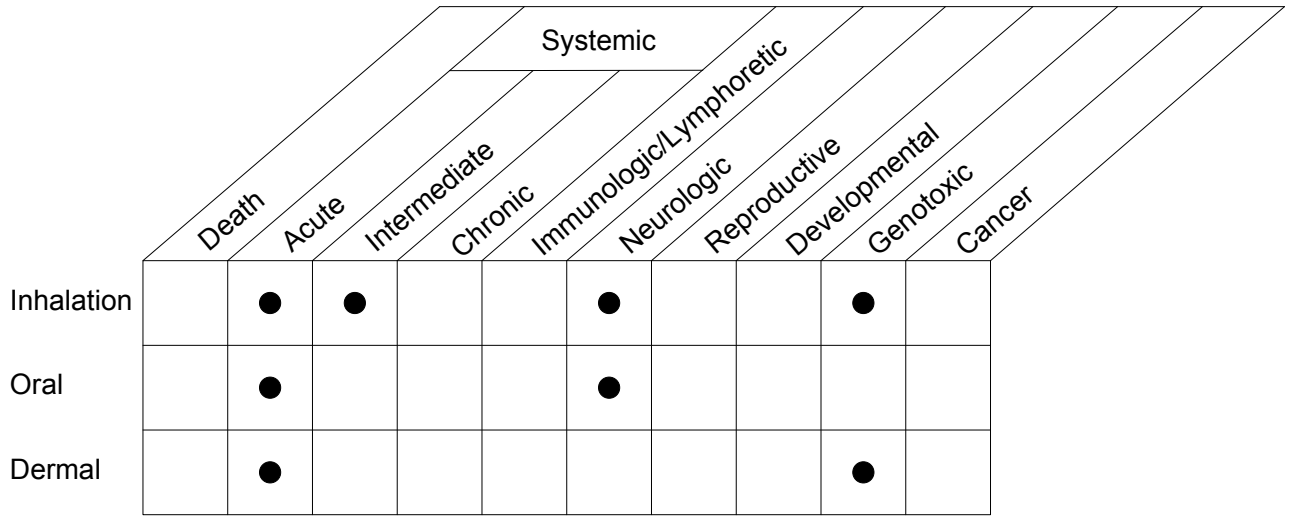
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean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

3.12.1 Existing Information on Health Effects of Toxaphene

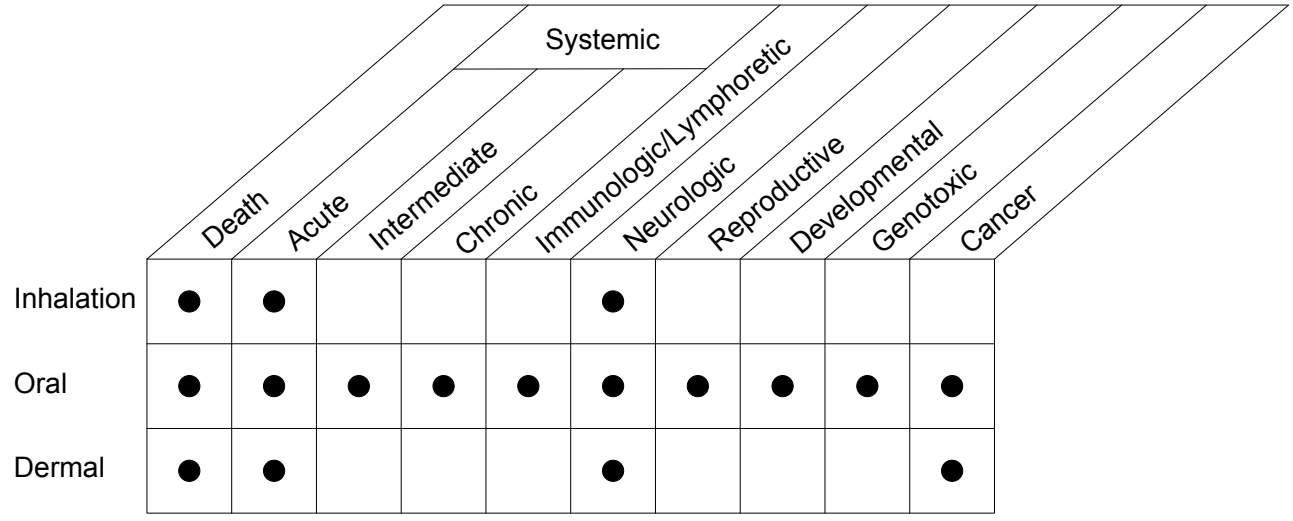
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to toxaphene are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of toxaphene. Each dot in the figure indicates that one or more 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.

The data describing the toxic effects of toxaphene in humans are generally limited to a small number of case reports of toxicity following ingestion, inhalation, or dermal contact. Some controlled studies in humans exist, but the data are incomplete or unreliable. Thus, although human toxicity information

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



Human



Animal

● Existing Studies

exists, animal data must be considered in order to adequately assess the risk of toxaphene exposure. The database for the health effects of toxaphene following ingestion in experimental animals is substantial. However, as can be seen in Figure 3-5, very little information is available on the effects of inhalation and dermal exposure to toxaphene in animals. Furthermore, the health effects associated with acute-duration exposure are more fully characterized than those associated with intermediate or chronic-duration exposure.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Limited human and animal data are available regarding effects of acute-duration inhalation exposure to toxaphene. One controlled human study reported a NOAEL of 500 mg/m^3 for repeated 30-minute exposures to toxaphene aerosols (Keplinger 1963). Animal data are restricted to secondary source accounts of death and hepatocellular necrosis in laboratory animals exposed to toxaphene by inhalation; the primary sources for this information were unpublished reports that were not available to ATSDR. Data on the acute effects of inhaled toxaphene do not appear necessary because all uses for toxaphene have been banned in the United States and its territories (EPA 1990b).

No adequate human data are available regarding the effects of acute-duration oral exposure to toxaphene. Available animal data include acute lethality studies in multiple laboratory species (Boyd and Taylor 1971; Chandra and Durairaj 1995; Chernoff and Carver 1976; Chernoff et al. 1990; Epstein et al. 1972; Gaines 1969; Jones et al. 1968; Lackey 1949). Acute-duration oral exposure to toxaphene has resulted in adverse effects on the nervous system, immunological system, body weight, and liver (Chandra and Durairaj 1995; Chernoff and Carver 1976; Chernoff et al. 1990; Chu et al. 1986; Hedli et al. 1998; Lackey 1949; Rao et al. 1986; Steele et al. 1980; Trottman and Desaiah 1980; Waritz et al. 1996). The study of Chu et al. (1986) identified the highest NOAEL (5 mg/kg/day) associated with the lowest LOAEL (10 mg/kg/day for clinical signs of neurotoxicity in dogs) and serves as the point of departure for deriving an acute-duration oral MRL for toxaphene. Additional animal studies of acute-duration oral exposure to toxaphene are not necessary.

Data from animal studies indicate that dermal exposure to toxaphene can be lethal, but at doses that are an order of magnitude higher than those for oral administration of the pesticide (Gaines 1969; Johnston and Eden 1953; Jones et al. 1968). Additional acute-duration dermal studies do not appear necessary because all uses for toxaphene have been banned in the United States and its territories (EPA 1990b).

Intermediate-Duration Exposure. Available information regarding intermediate-duration exposure to toxaphene is limited to animal studies that employed the oral exposure route and identified neurological, hepatic, renal, developmental, and immunological end points (Allen et al. 1983; Chu et al. 1986, 1988; Kennedy et al. 1973; Koller et al. 1983; Lackey 1949; NCI 1979; Olsen et al. 1980; Tryphonas et al. 2001; Waritz et al. 1996). The study of Tryphonas et al. (2001) identified the highest NOAEL (0.1 mg/kg/day) associated with the lowest reliable LOAEL (0.4 mg/kg/day for depressed humoral immunity in cynomolgus monkeys) and served as the basis for deriving the MRL. Additional animal studies do not appear necessary. All uses for toxaphene have been banned in the United States and its territories (EPA 1990b). Monitoring of workers at waste sites where toxaphene is found and people living in close proximity to such sites might provide useful information. Additional information regarding the potential for health effects in human populations consuming food sources such as fish with documented levels of toxaphene residues would be useful.

Chronic-Duration Exposure and Cancer. Available information regarding noncancer and cancer effects is limited to chronic-duration oral studies in animals. Neurological, immunological, and body weight effects were reported in rats and mice (NCI 1979) and cynomolgus monkeys (Arnold et al. 2001; Bryce et al. 2001; Tryphonas et al. 2001) administered toxaphene orally for chronic durations. A chronic-duration oral MRL was not derived for toxaphene because the study that identified the highest NOAEL (0.4 mg/kg/day) associated with the lowest LOAEL (0.8 mg/kg/day for significantly depressed humoral immune response) after 52 weeks of toxaphene treatment identified a LOAEL of 0.4 mg/kg/day for another measure of humoral immune response after <52 weeks of toxaphene treatment (Tryphonas et al. 2001). Therefore, the intermediate-duration oral MRL should be protective of immunological effects following chronic-duration oral exposure to toxaphene.

Some case-control studies of farm workers and prospective cohort studies of pesticide applicators have reported statistically significant associations between exposure to toxaphene and risk of cancers such as leukemia (Mills et al. 2005), rectal cancer (Lee et al. 2007; Purdue et al. 2006), and melanoma (Purdue et al. 2006). Conflicting results have been reported regarding exposure to toxaphene and risk of NHL. Most studies found no statistically significant association (Cantor et al. 1992; De Roos et al. 2003; Hoar et al. 1986; Mills et al. 2005; Purdue et al. 2006; Schroeder et al. 2001; Zahm et al. 1993); however, Schroeder et al. (2001) reported a significant association (OR 3.7, 95% CI 1.9–7.0) between t(14;18)-positive NHL cases (n=5) and toxaphene exposure. Lifetime oral studies found increased incidences of thyroid tumors in rats and hepatic tumors in mice exposed to high oral doses of toxaphene (NCI 1979). Additional

chronic-duration toxicity and carcinogenicity studies in animals do not appear necessary. All uses for toxaphene have been banned in the United States and its territories (EPA 1990b). Monitoring of workers at waste sites where toxaphene is found and people living in close proximity to such sites might provide useful information. Additional information regarding potential for health effects in human populations consuming food sources such as fish with documented levels of toxaphene residues would be useful.

Genotoxicity. Limited information is available regarding the genotoxicity of toxaphene in humans. A higher incidence of chromosomal aberrations was observed in cultured lymphocytes taken from the blood of eight women exposed to toxaphene compared to lymphocytes taken from unexposed women (Samosh 1974). The small sample size precludes drawing conclusions regarding the potential for toxaphene to induce chromosomal aberrations. Additional assessment of the genotoxic potential of toxaphene would be helpful in the unlikely event that populations with significant exposure to toxaphene can be identified.

Available *in vivo* genotoxicity data from animals are limited to a dominant lethality test in which toxaphene did not cause increased fetal death or decreased numbers of implants in mouse dams mated to males that had been administered toxaphene orally (Epstein et al. 1972); a study that reported the lack of DNA damage in rats administered toxaphene once by gavage at up to 36 mg/kg/day (Kitchin and Brown 1994); and a study that found no evidence of DNA adduct formation in livers of mice administered toxaphene by gavage for 7 days at doses up to and including 100 mg/kg/day (Hedli et al. 1998).

Available *in vitro* assays provide equivocal results (Bartoš et al. 2005; Griffin and Hill 1978; Hooper et al. 1979; Houk and DeMarini 1987; Mortelmans et al. 1986; Schrader et al. 1998; Sobti et al. 1983; Steinberg et al. 1998; Steinel et al. 1990). Bacterial reverse mutation assays provide some evidence of a mutagenic effect in the absence of metabolic activation systems. However, mutagenic responses were typically diminished or abolished with the addition of mammalian hepatic activation systems that play a role in xenobiotic metabolism. Additional *in vitro* data would be useful to more rigorously assess the potential genotoxicity of toxaphene.

Reproductive Toxicity. No information was located regarding toxaphene-induced reproductive effects in humans. The available information from multigeneration studies in rats indicates that toxaphene does not adversely affect reproductive end points (Kennedy et al. 1973; Keplinger et al. 1970). Additional studies do not appear necessary.

Developmental Toxicity. No information was located regarding toxaphene-induced developmental effects in humans. Toxaphene was reported to cause inferior swimming and righting ability in young mouse pups (9–12 days postpartum, but not at 16 days postpartum) of dams administered toxaphene by gavage at 0.05 mg/kg/day (the only dose tested) throughout gestation and lactation (Olson et al. 1980). Allen et al. (1983) reported suppression of phagocytic function in peritoneal macrophages from offspring of rat dams receiving toxaphene in the diet at 19.5 mg/kg/day prior to mating and during gestation and lactation; however, phagocytic function was enhanced at a higher dose level (39 mg/kg/day). Crowder et al. (1980) found no evidence of developmental toxicity following oral exposure of rat dams to toxaphene at 6 mg/kg/day (the only dose tested) during gestation. A comprehensive developmental toxicity study could be designed to provide supporting or refuting evidence to the findings of Olson et al. (1980).

Immunotoxicity. No information was located regarding immunologic effects of toxaphene in humans. Toxaphene-related depressed IgG production was reported in adult rats (Koller et al. 1983). Depressed humoral responses were noted in rat neonates exposed via their mothers (Allen et al. 1983) and in cynomolgus monkeys administered toxaphene orally for up to 75 weeks (Tryphonas et al. 2001). Additional studies of toxaphene-induced immunotoxicity in laboratory animals do not appear necessary at this time.

Neurotoxicity. Neurological effects have been reported in several cases of inadvertent or intentional ingestion of unknown “large amounts” of toxaphene (McGee et al. 1952; Wells and Milhorn 1983). Convulsions were induced in dogs (Chu et al. 1986; Lackey 1949) and heifers (Steele et al. 1980) following acute oral exposure to toxaphene. NCI (1979) reported clinical signs of neurotoxicity in rats and mice administered toxaphene orally for chronic durations. The animal data indicate that dogs are particularly sensitive to toxaphene neurotoxicity. Additional studies of the neurological effects in toxaphene-exposed animals do not appear necessary. All uses for toxaphene have been banned in the United States and its territories (EPA 1990b). Monitoring of workers at waste sites where toxaphene is found and people living in close proximity to such sites might provide useful information. Additional information regarding the potential for health effects in human populations consuming food sources such as fish with documented levels of toxaphene residues would be useful.

Epidemiological and Human Dosimetry Studies. Most of the available information on the effects of toxaphene in humans comes from cases of acute poisoning following accidental or intentional ingestion of toxaphene and from occupational exposures in agricultural industries. Limitations inherent in these studies include unquantified exposure concentrations and durations, and concomitant exposure to

other pesticides. Despite their inadequacies, those studies suggest that toxaphene can adversely affect the liver, kidneys, lungs, and central nervous system (McGee et al. 1952; Warraki 1963; Wells and Milhorn 1983). All uses for toxaphene have been banned in the United States and its territories (EPA 1990b). Monitoring of workers at waste sites where toxaphene is found and people living in close proximity to such sites might provide useful information. Additional information regarding the potential for health effects in human populations consuming food sources such as fish with documented levels of toxaphene residues would be useful.

Biomarkers of Exposure and Effect.

Exposure. Toxaphene levels have been measured in blood, fat, urine, and feces (Ohsawa et al. 1975; Pollock and Kilgore 1980b). No studies demonstrate a reliable correlation between blood levels and levels of exposure. Fat samples contain toxaphene levels proportional to treatment levels (Pollock and Kilgore 1980b), but fat samples are difficult to obtain from humans. Levels of toxaphene in milk fat may provide a more accurate estimate of exposure than body fat or blood (Keating 1979), but these samples can only be obtained from a small portion of the population. Because toxaphene is rapidly eliminated from the body, tissue levels are a useful measure only shortly following exposure to toxaphene. Persistent toxaphene congeners in fat might serve as useful biomarkers of exposure and would be most likely associated with exposure to weathered toxaphene.

Effect. No specific biomarkers of effects have been identified for toxaphene. Toxaphene has been demonstrated to cause a number of adverse health effects including central nervous system excitation, liver and kidney damage, and developmental and immunosuppressive effects. These effects are not specific for toxaphene and no studies exist that demonstrate good correlation of toxaphene levels with human health effects. Neurological tests such as electroencephalographic monitoring can record levels of central nervous system activity. Liver and kidney function tests exist that detect hepatic and renal impairment. Microsomal enzyme activity may indicate early effects in the liver. Effects on the immune system can be measured by measuring immunoglobulin levels. Although each of these tests can indicate the presence of disease in the systems affected by toxaphene, the effects can be caused by a number of other disease states.

Absorption, Distribution, Metabolism, and Excretion. Quantitative evidence on the absorption of toxaphene in humans and animals following all routes of exposure is very limited. Female animals dipped in toxaphene excrete the substance in milk and also sometimes experience toxicosis (Claborn et al.

1963). Humans and animals have become seriously ill following accidental or intentional ingestion of toxaphene. The evidence clearly indicates that toxaphene is absorbed. Reports that specifically evaluate its rate or extent of absorption as a result of inhalation, oral, and dermal exposure would be useful.

No studies were located regarding the distribution of toxaphene in humans or animals following inhalation or dermal exposures. No evidence is available regarding the distribution of toxaphene in humans following ingestion. However, animal studies conducted in several species indicate that distribution following oral absorption is similar across species (Mohammed et al. 1983; Ohsawa et al. 1975; Pollock and Kilgore 1980b), and it is assumed that distribution of the pesticide in humans would be similar. Once absorbed, toxaphene and its components are distributed initially throughout the blood compartment and then to fat. Studies that investigate the distribution of toxaphene following inhalation or dermal exposure would be helpful in order to evaluate whether toxaphene behaves similarly across all routes of exposure.

Information was not available regarding the metabolism of toxaphene following dermal or inhalation exposure in animals or humans. This information would be useful for estimating health effects by these routes. Moreover, no information was available regarding the metabolites formed by humans following ingestion. Evidence from animals receiving toxaphene orally indicates that dechlorination, dehydrodechlorination, and oxidation are principal metabolic pathways (Crowder and Dindal 1974; Ohsawa et al. 1975). Although several metabolites have been isolated and identified (Ohsawa et al. 1975), several others remain unknown. Their identification will help elucidate the toxaphene metabolic pathway(s).

Quantitative information regarding the metabolites produced would suggest which biodegradation pathways are favored and provide insight into the enzyme kinetics. Information regarding the overall rate of metabolism and the rates of specific reactions would be useful. In addition, such studies might also provide information to help facilitate the metabolism of the toxaphene mixture in accidentally exposed humans.

No studies in humans were found regarding the excretion of toxaphene. Excretion data from animal studies are available for oral and dermal exposure routes. Mice that received toxaphene intravenously were found to have toxaphene present in the intestinal content, suggesting biliary excretion (Mohammed et al. 1983). The presence of several metabolites in the urine and feces suggests that toxaphene degradation is extensive and complex (Ohsawa et al. 1975; Pollock and Kilgore 1980b). Though

metabolism of toxaphene facilitates its excretion, and the kinetics of toxaphene metabolism are related to the kinetics of excretion, they are not the same. Since metabolites may also contribute to the toxic effects attributed to toxaphene, it would be beneficial to conduct studies that would establish elimination rates for each toxaphene metabolite or for similar metabolic products. Such studies may also provide information to facilitate the rapid removal of toxaphene and its metabolites in exposed people.

Virtually all toxicokinetic properties reported in this profile were based on results from acute-duration exposure studies. Very limited information was available regarding intermediate- or chronic-duration exposure to toxaphene. Since toxaphene is known to induce hepatic enzymes, the kinetics of metabolism during chronic exposure probably differ from those seen during acute exposure. Thus, additional studies on the metabolism of toxaphene during intermediate- or chronic-duration exposure would be useful.

Comparative Toxicokinetics. The absorption, distribution, metabolism, and excretion of toxaphene have been studied in animals, but only information on absorption is available in humans. In several mammalian species, it is evident that toxaphene is absorbed, metabolized in the liver (with some elimination probably occurring via the hepatobiliary system), and then possibly some parent compound and metabolites are distributed to fat (Ohsawa et al. 1975; Pollock and Kilgore 1980b). Very little is excreted unchanged. In studies of mammals, the extent of metabolism increased with the physiological complexity of the species. Based on this trend, humans would be expected to metabolize toxaphene extensively in a manner qualitatively similar to animals.

Methods for Reducing Toxic Effects. The medical procedures used to reduce the toxic effects of toxaphene are well established and are those used to treat organochlorine poisoning or poisoning due to other chemicals with central nervous system stimulatory properties. However, data on how to best reduce body burden and also on how to prevent the inhibition of γ-aminobutyric acid-dependent chloride ion channels would be useful.

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

No human data are available regarding age-related susceptibility to toxaphene. Results of one animal study (Olsen et al. 1980) suggest that critical stages of neurological development could represent periods of increased vulnerability. However, the results do not provide convincing evidence that toxaphene is a

developmental toxicant. As identified in the Developmental Toxicity subsection, a well-designed, developmental toxicity animal study could provide additional insight into age-related susceptibility to toxaphene. Immunosuppression has been demonstrated in some animals exposed to toxaphene (Allen et al. 1983; Koller et al. 1983; Tryphonas et al. 2001), and it is known that infants and children are especially susceptible to immunosuppression because their immune systems do not reach maturity until 10–12 years of age (Calabrese 1978). However, additional studies of toxaphene-induced immunotoxicity in laboratory animals do not appear necessary at this time.

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

Researchers in the Klaunig Lab (Klaunig 2013) are conducting studies on the mechanisms of action by which toxaphene and other chlorinated insecticides induce liver tumors in rodents. According to RePORTER (2013), Dr. Nancy Denslow at the University of Florida is studying molecular mechanisms of endocrine disruption in largemouth bass exposed to organochlorine pesticides, including toxaphene. Aims of the research include development of biomarkers of exposure *in vivo* via use of microarrays and proteomics methodologies, determination of effects of organochlorine pesticide exposure on steroid synthesis and metabolism, and evaluation of the effects of organochlorine pesticides on molecular mechanisms of action of the three estrogen receptors. The research is funded by the National Institute of Environmental Health Sciences.