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 trichlorobenzenes. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

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

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

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

3.2.1 Inhalation Exposure

3.2.1.1 Death

No studies were located regarding death in humans and/or animals after inhalation exposure to trichlorobenzenes.

3.2.1.2 Systemic Effects

There is very limited information regarding health effects in humans following exposure to trichlorobenzenes. A review of the literature indicates that an adult male who inhaled trichlorobenzene for several hours during the repair of a pump suffered massive hemoptysis, and that some trichlorobenzene production workers developed chloroacne (IPCS 1991). There is also a case report of aplastic anemia in a woman with prolonged exposure through the soaking of her husband’s work clothes in trichlorobenzene (Girard et al. 1969). None of these reports provided exposure details or specified the isomer involved. Citing an unpublished source, ACGIH (2001) states that minimal eye and throat irritation could occur in some people exposed to 3–5 ppm 1,2,4-trichlorobenzene.

No information was located regarding systemic effects in animals following inhalation exposure to 1,2,3-trichlorobenzene.

The highest NOAEL values and all LOAEL values of 1,2,4-trichlorobenzene and 1,3,5-trichlorobenzene from each reliable study for systemic effects in each species and duration category are recorded in Tables 3-1 and 3-2 and plotted in Figures 3-1 and 3-2.

Respiratory Effects. Continuous exposure of male cynomolgous monkeys to up to 100 ppm 1,2,4-trichlorobenzene vapors for 26 weeks had no significant effect on results of pulmonary function tests (static compliance, diffusion capacity, distribution of ventilation, and lung volumes) conducted in anesthetized monkeys at termination (Coate et al. 1977). Measurements of mechanical properties of the lungs conducted in unanesthetized monkeys also were not significantly affected by exposure to 1,2,4-trichlorobenzene. Histological examination of the lungs showed no treatment-related effects.

Continuous exposure of male rats to up to 100 ppm 1,2,4-trichlorobenzene vapors for up to 26 weeks did not induce significant histological alterations in the lungs (Coate et al. 1977). Similar results were reported in rats exposed to up to 200 ppm 1,2,4-trichlorobenzene vapors 6 hours/day for 15 exposures (Gage 1970) or in male rats exposed 7 hours/day, 5 days/week to up to 100 ppm 1,2,4-trichlorobenzene vapors for a total of 30 exposures (Kociba et al. 1981). Two intermediate-duration studies in rabbits exposed to up to 100 ppm 1,2,4-trichlorobenzene vapors continuously (Coate et al. 1977) or intermittently (Kociba et al. 1981) also reported no significant alterations in the lungs upon microscopic examination. Similar results were reported in dogs following intermittent intermediate-duration exposure to up to 100 ppm 1,2,4-trichlorobenzene (Kociba et al. 1981). The nasal mucosa was also examined in rats and rabbits in the Kociba et al. (1981) study.

The only information regarding 1,3,5-trichlorobenzene is from a study in male and female CD rats in which the animals were exposed 6 hours/day, 5 days/week for 13 weeks (Sasmore et al. 1983). Exposure to up to 130 ppm 1,3,5-trichlorobenzene vapors did not induce significant histological alterations in the lungs, trachea, or nasal passages.

Cardiovascular Effects. Continuous exposure of male monkeys, rats, or rabbits up to 100 ppm 1,2,4-trichlorobenzene vapors for 26 weeks did not induce significant gross or microscopic alterations in the heart (Coate et al. 1977). Exposure of male rats, dogs, and rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors 7 hours/day, 5 days/week for a total of 30 exposures during a 40-day period did not induce significant gross or microscopic alterations in the heart or aorta (Kociba et al. 1981).

Table 3-1 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Systemic								
1	Monkey (Cynomolgus)	26 wk 24 h/d	Resp	100 M			Coate et al. 1977 1,2,4-trichlorobenzene	NOAELs are for organ and tissues histopathology
			Cardio	100 M				
			Hemato	100 M				
			Hepatic	100 M				
			Renal	100 M				
			Dermal	100 M				
			Ocular	100 M				
			Bd Wt	100 M				
2	Rat (Sprague-Dawley)	26 wk 24 h/d	Resp	100 M			Coate et al. 1977 1,2,4-trichlorobenzene	Mild liver alterations at 4 and 13 weeks, but not 26 weeks; no quantitative data
			Cardio	100 M				
			Hemato	100 M				
			Hepatic	100 M				
			Renal	100 M				
			Dermal	100 M				
			Ocular	100 M				
			Bd Wt	100 M				

Table 3-1 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
3	Rat (Sprague-Dawley)	44 d 5 d/wk 7 h/d	Resp	100 M			Kociba et al. 1981 1,2,4-trichlorobenzene	NOAELs are for organ and tissues histopathology
			Cardio	100 M				
			Gastro	100 M				
			Hemato	100 M				
			Musc/skel	100 M				
			Hepatic	30 M	100 M (increased relative liver weight)			
			Renal	100 M				
			Endocr	100 M				
			Ocular	100 M				
			Bd Wt	100 M				

Table 3-1 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
4	Rabbit (New Zealand)	26 wk 24 h/d	Resp	100 M			Coate et al. 1977 1,2,4-trichlorobenzene	NOAELs are for organ and tissues histopathology
			Cardio	100 M				
			Hemato	100 M				
			Hepatic	100 M				
			Renal	100 M				
			Dermal	100 M				
			Ocular	100 M				
			Bd Wt	100 M				
5	Rabbit (New Zealand)	44 d 5 d/wk 7 h/d	Resp	100 M			Kociba et al. 1981 1,2,4-trichlorobenzene	NOAELs are for organ and tissues histopathology
			Cardio	100 M				
			Gastro	100 M				
			Hemato	100 M				
			Musc/skel	100 M				
			Hepatic	100 M				
			Renal	100 M				
			Endocr	100 M				
			Ocular	100 M				
			Bd Wt	100 M				

Table 3-1 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
6	Monkey (Cynomolgus)	26 wk 24 h/d		100 M			Coate et al. 1977 1,2,4-trichlorobenzene	NOAEL is for spleen histopathology
7	Rat (Sprague-Dawley)	26 wk 24 h/d		100 M			Coate et al. 1977 1,2,4-trichlorobenzene	NOAEL is for histopathology of the spleen
8	Rat (Sprague-Dawley)	44 d 5 d/wk 7 h/d		100 M			Kociba et al. 1981 1,2,4-trichlorobenzene	NOAEL is for histopathology of lymphoreticular tissues
9	Rabbit (New Zealand)	26 wk 24 h/d		100 M			Coate et al. 1977 1,2,4-trichlorobenzene	NOAEL is for histopathology of the spleen
10	Rabbit (New Zealand)	44 d 5 d/wk 7 h/d		100 M			Kociba et al. 1981 1,2,4-trichlorobenzene	NOAEL is for histopathology of lymphoreticular tissues
Neurological								
11	Monkey (Cynomolgus)	26 wk 24 h/d		100 M			Coate et al. 1977 1,2,4-trichlorobenzene	NOAEL is for operant behavior tests and brain and spinal cord histopathology

Table 3-1 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
12	Rat (Sprague-Dawley)	26 wk 24 h/d		100 M			Coate et al. 1977 1,2,4-trichlorobenzene	NOAEL is for brain and spinal cord histopathology
13	Rat (Sprague-Dawley)	44 d 5 d/wk 7 h/d		100 M			Kociba et al. 1981 1,2,4-trichlorobenzene	NOAEL is for histopathology of central and peripheral nervous tissues
14	Rabbit (New Zealand)	26 wk 24 h/d		100 M			Coate et al. 1977 1,2,4-trichlorobenzene	NOAEL is for histopathology of the brain and spinal cord
15	Rabbit (New Zealand)	44 d 5 d/wk 7 h/d		100 M			Kociba et al. 1981 1,2,4-trichlorobenzene	NOAEL is for histopathology of central and peripheral nervous tissues
Reproductive								
16	Rat (Sprague-Dawley)	44 d 5 d/wk 7 h/d		100 M			Kociba et al. 1981 1,2,4-trichlorobenzene	NOAEL is for histopathology of reproductive organs
17	Rabbit (New Zealand)	44 d 5 d/wk 7 h/d		100 M			Kociba et al. 1981 1,2,4-trichlorobenzene	No histopathological effects in reproductive organs

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

Bd Wt = body weight; (Cardio = cardiovascular; d = day(s); Endocr = endocrine; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 3-1 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Inhalation

Intermediate (15-364 days)

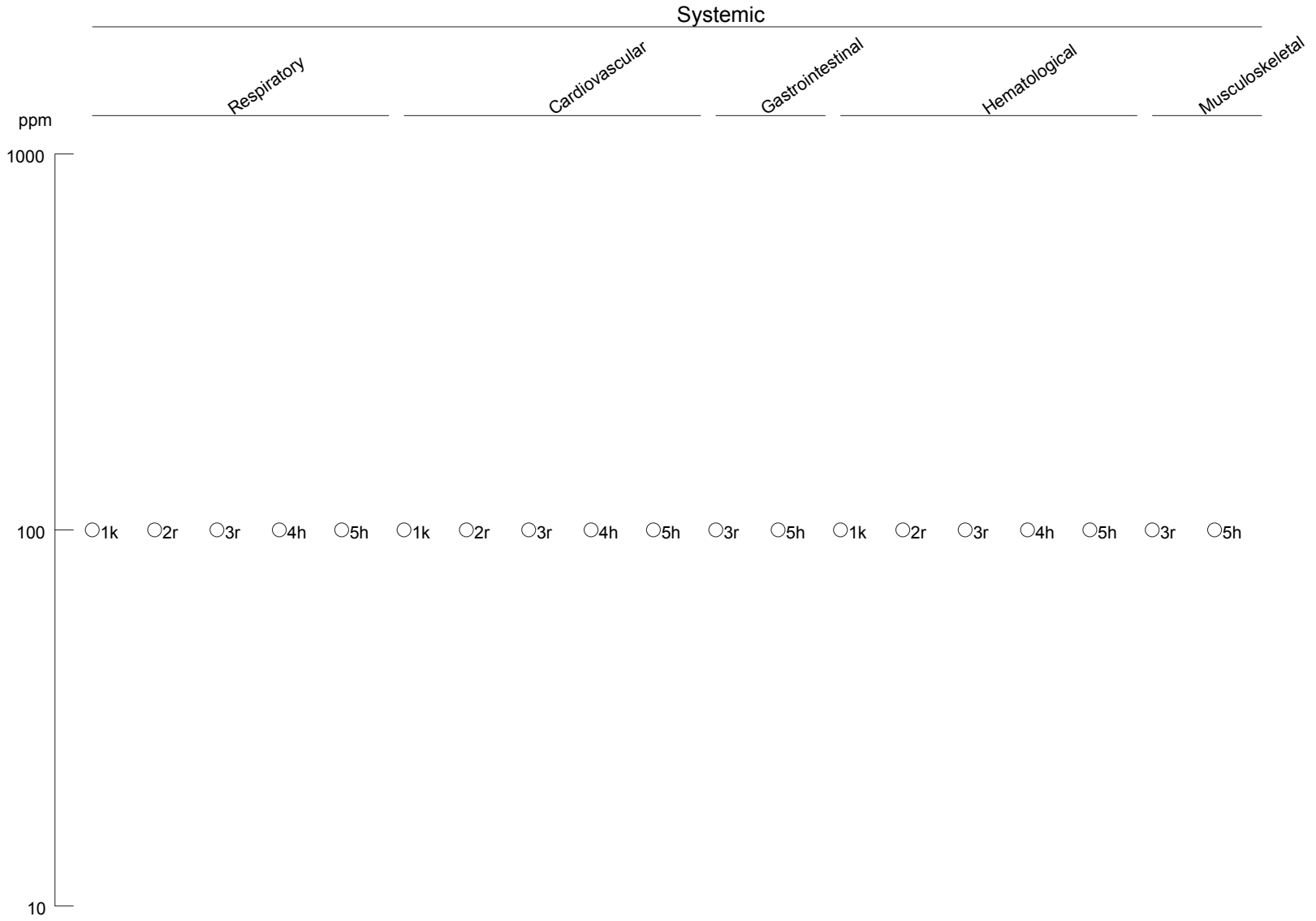


Figure 3-1 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Inhalation *(Continued)*

Intermediate (15-364 days)

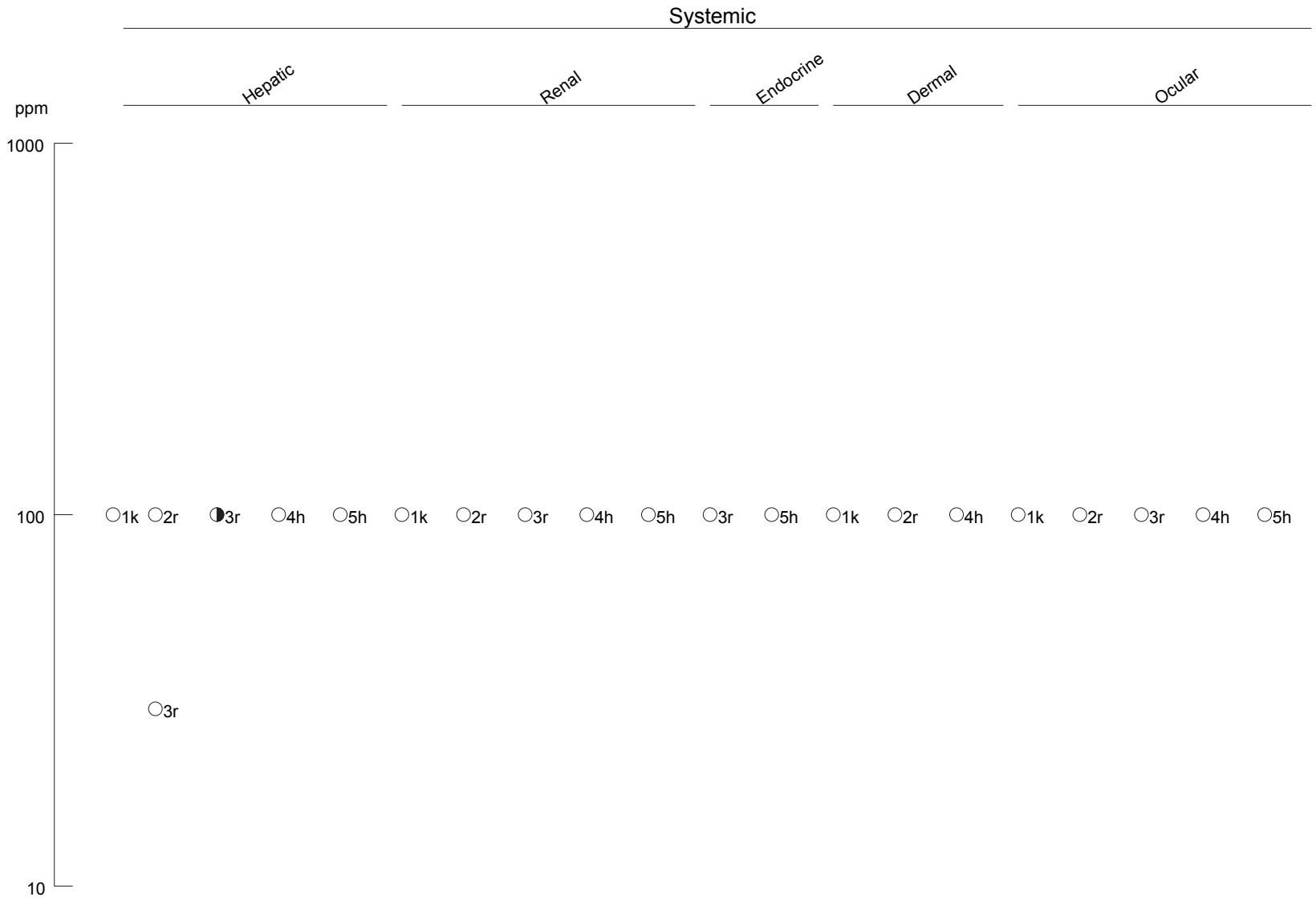


Figure 3-1 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Inhalation *(Continued)*

Intermediate (15-364 days)

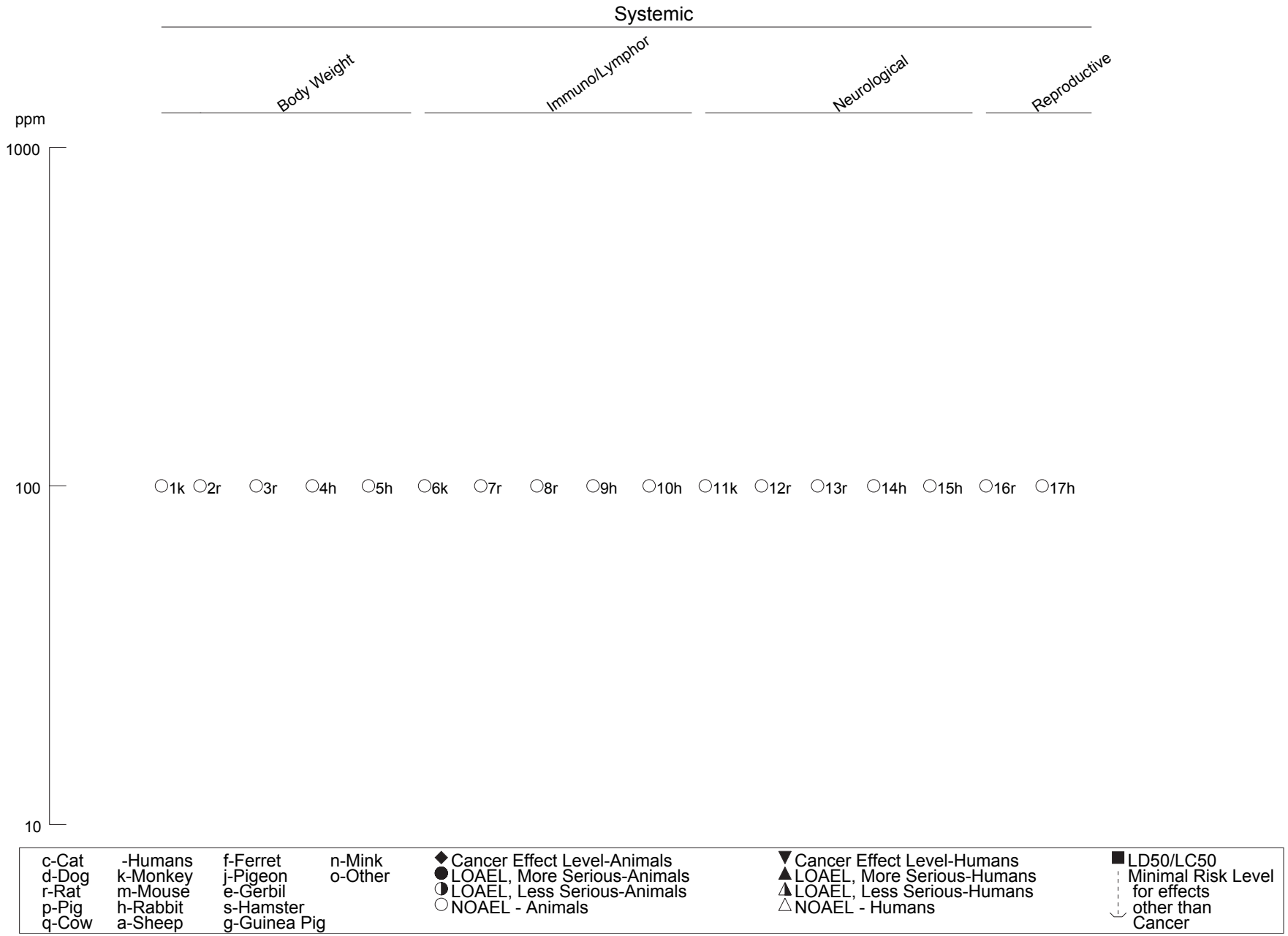


Table 3-2 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form	Comments
ACUTE EXPOSURE									
Systemic									
1	Rat (Sprague-Dawley)	1 hr	Bd Wt			1209	(44-60% less weight than controls 14 days after exposure)	Jorgenson et al. 1976 1,3,5-Trichlorobenzene	
INTERMEDIATE EXPOSURE									
Systemic									
2	Rat (CD)	13 wk 5 d/wk 6 h/d	Resp	130				Sasmore et al. 1983 1,3,5-Trichlorobenzene	NOAELs are for organ and tissue histopathology
			Cardio	130					
			Gastro	130					
			Hemato	130					
			Hepatic	130					
			Renal	130					
			Endocr	130					
			Ocular	130					
			Bd Wt	130					
Immuno/ Lymphoret									
3	Rat (CD)	13 wk 5 d/wk 6 h/d		130				Sasmore et al. 1983 1,3,5-Trichlorobenzene	NOAEL is for histopathology of lymphoreticular tissues
Neurological									
4	Rat (CD)	13 wk 5 d/wk 6 h/d		130				Sasmore et al. 1983 1,3,5-Trichlorobenzene	NOAEL is for histopathology of tissues from the nervous system

Table 3-2 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form	Comments
Reproductive								
5	Rat (CD)	13 wk 5 d/wk 6 h/d		130			Sasmore et al. 1983 1,3,5-Trichlorobenzene	NOAEL is for histopathology of reproductive organs

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

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 3-2 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Inhalation

Acute (≤14 days)

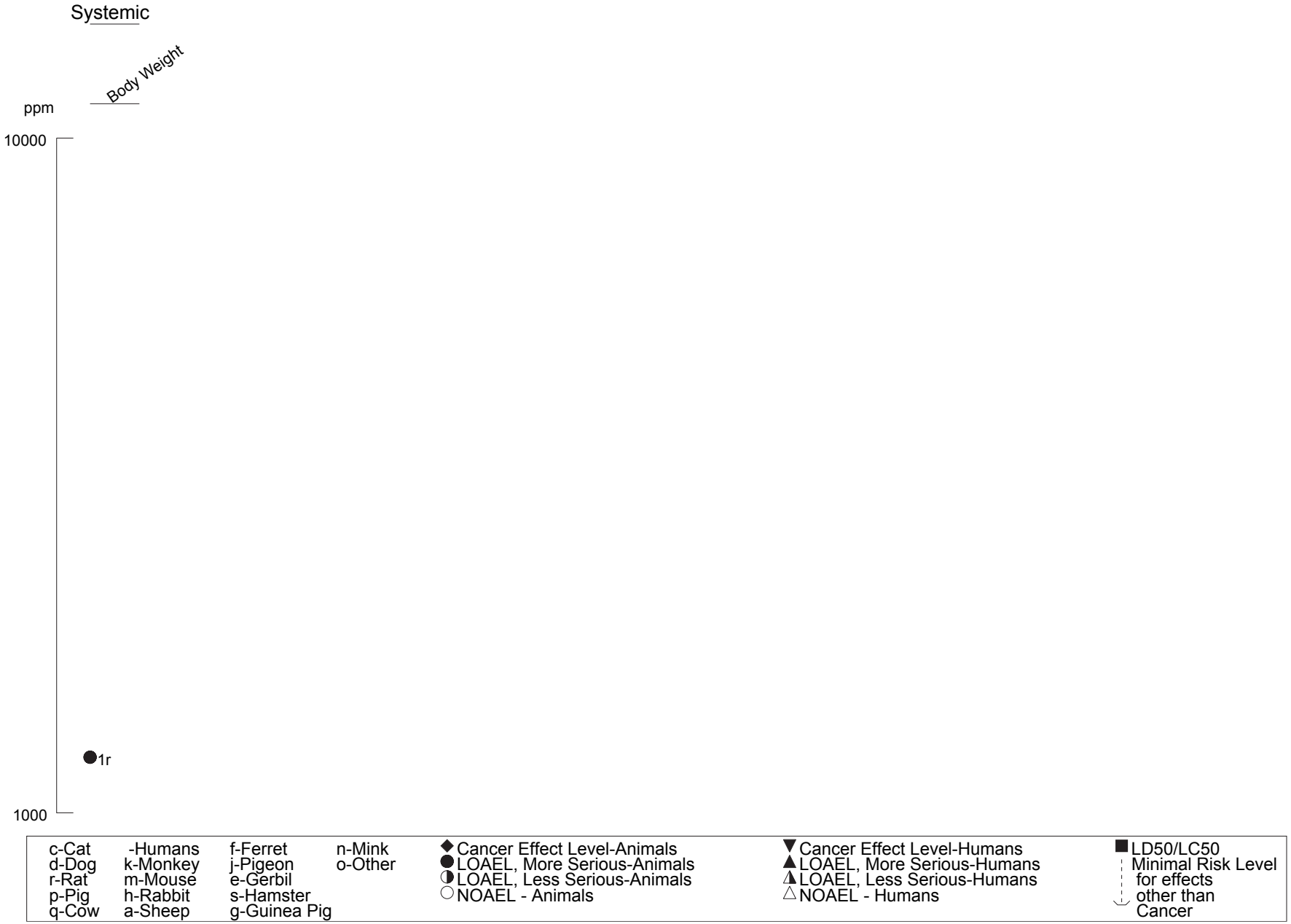
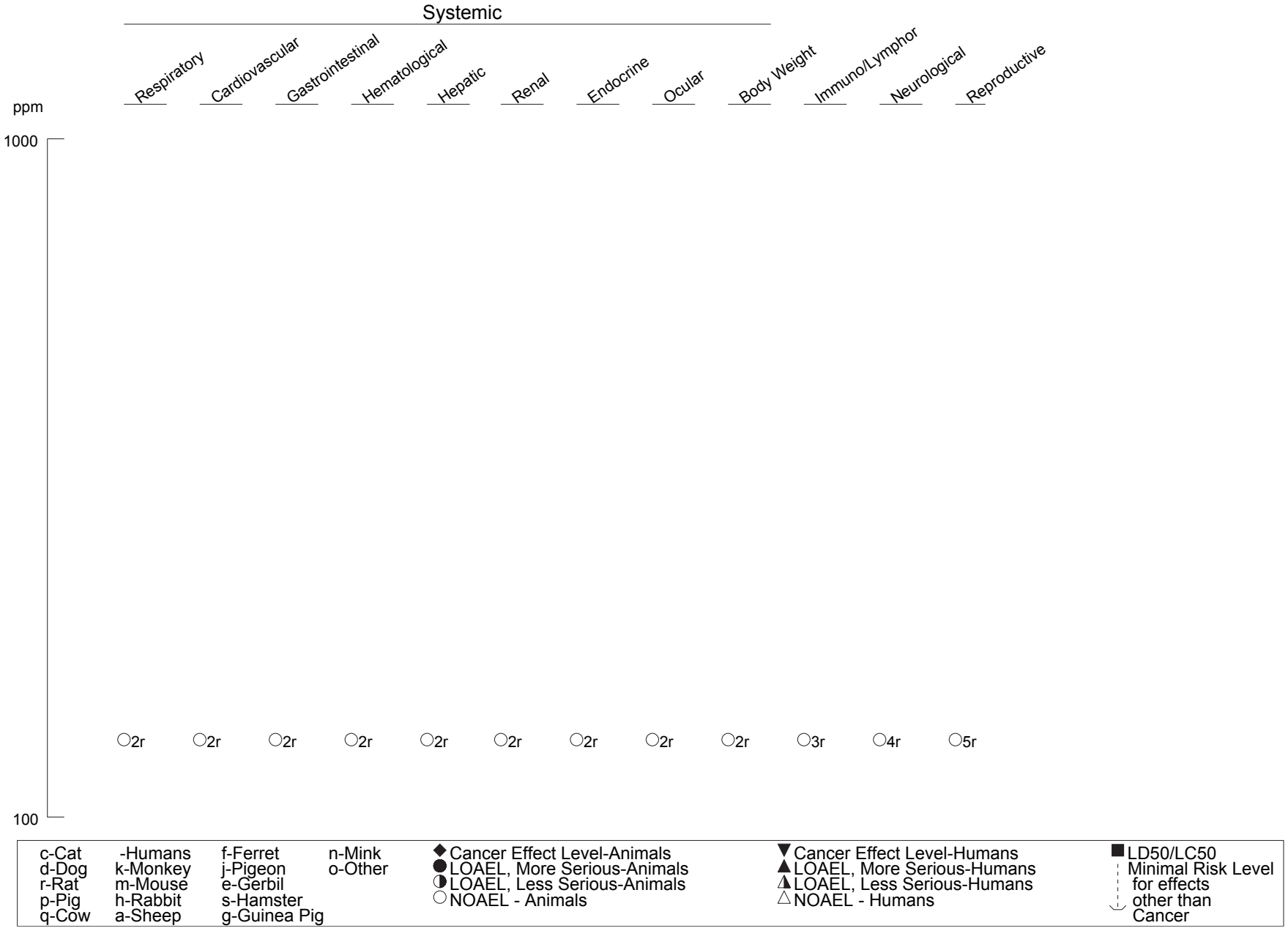


Figure 3-2 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Inhalation *(Continued)*

Intermediate (15-364 days)



Exposure of rats to up to 130 ppm 1,3,5-trichlorobenzene vapors 6 hours/day, 5 days/week for 13 weeks did not induce histological changes in the heart (Sasmore et al. 1983).

Gastrointestinal Effects. Exposure of male rats, dogs, and rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors 7 hours/day, 5 days/week for a total of 30 exposures during a 40-day period did not induce significant gross or microscopic alterations in the gastrointestinal tract (Kociba et al. 1981). Similar results were reported for the gastrointestinal tract of rats exposed to up to 130 ppm 1,3,5-trichlorobenzene vapors 6 hours/day, 5 days/week for 13 weeks (Sasmore et al. 1983).

Hematological Effects. Complete blood counts performed on male monkeys, rats, and rabbits exposed continuously to up to 100 ppm 1,2,4-trichlorobenzene vapors for up to 26 weeks did not show any significant exposure-related differences from controls (Coate et al. 1977). Kociba et al. (1981) reported no significant alterations in total red blood cells, total differential leukocytes, packed cell volume or hemoglobin concentration in blood samples from male rats, rabbits, and dogs following 30 intermittent exposures to up to 100 ppm 1,2,4-trichlorobenzene vapors.

Exposure of rats to up to 130 ppm 1,3,5-trichlorobenzene 6 hours/day, 5 days/week for 13 weeks did not significantly alter blood cell counts and mean corpuscular values, differential counts, or platelet counts (Sasmore et al. 1983). Methemoglobin was slightly higher at 13 weeks than at 4 weeks, but values did not reach significant levels (data not shown).

Musculoskeletal Effects. No significant alterations were reported in skeletal muscle from male rats, rabbits, and dogs following 30 intermittent exposures to up to 100 ppm 1,2,4-trichlorobenzene vapors (Kociba et al. 1981).

In the 13-week inhalation study with 1,3,5-trichlorobenzene (Sasmore et al. 1983), there is no explicit indication that musculoskeletal tissues were examined, although the investigators stated that 34 specific organs and tissues from the control and the high-exposure groups (130 ppm) were examined microscopically. Therefore, it is reasonable to assume that evaluations were conducted and that no alterations were observed.

Hepatic Effects. Continuous exposure of male monkeys or rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors for 26 weeks did not induce significant gross or microscopic alterations in the liver (Coate et al. 1977). However, exposure-related liver changes were described in rats (only qualitative description

provided). The changes were characterized as mild and were seen in all exposed groups (25, 50, 100 ppm) usually after 4 and 13 weeks but not at 26 weeks. Hepatocytomegaly occurred in all exposed groups but seemed more noticeable in the mid- and high-exposure groups. There was also a slight increase in the degree of vacuolization of hepatocytes in exposed rats that did not appear dose-related. Hepatocytomegaly was present in all exposed groups at 4 weeks and in the mid- and high-exposure groups at 13 weeks. Exposed rats also showed a slight increase in incidence of granuloma at 4 weeks, which did not appear to be dose-related. No histological alterations were reported in the liver from rats following 15 exposures to 200 ppm 1,2,4-trichlorobenzene each lasting 6 hours (Gage 1970).

Changes in liver weight were reported in rats, dogs, and rabbits exposed to 100 ppm 1,2,4-trichlorobenzene vapors 7 hours/day, 5 days/week for a total of 30 exposures during a 44-day period (Kociba et al. 1981). These changes were not accompanied by histological alterations or alterations in clinical chemistry tests for liver function. In rats, relative liver weight increased 11% relative to controls. Urinalyses conducted after 15 and 30 days of exposure showed statistically significant increased coproporphyrins and uroporphyrins in both the low- (30 ppm) and high-dose rats (100 ppm), which the investigators attributed to the ability of 1,2,4-trichlorobenzene to induce hepatic microsomal enzymes rather than to induce destruction of heme-containing cytochromes or inhibition of heme synthesis. In dogs, both absolute and relative liver weights were increased (27–30%). In rabbits, relative liver weight was decreased 16% relative to controls. Urinary porphyrins levels were not examined in dogs or rabbits.

Exposure of rats to up to 130 ppm 1,3,5-trichlorobenzene vapors 6 hours/day, 5 days/week for 13 weeks did not significantly affect liver weight or the gross or microscopic appearance of the liver (Sasmore et al. 1983). Urinary porphyrin levels were elevated in males at 13 weeks, but large variability rendered the differences with controls nonsignificant.

Renal Effects. Continuous exposure of male monkeys or rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors for 26 weeks did not induce significant gross or microscopic alterations in the kidneys (Coate et al. 1977). Male rats exposed in a similar manner showed kidney changes characterized as mild and present in all exposed groups usually after 4 and 13 weeks but not at 26 weeks of exposure (only a qualitative description was provided). Exposed male rats showed hyaline degeneration in the inner zone of the cortex, which appeared more severe at 4 weeks but was not dose-related; severity appeared increased at 13 weeks in high-dose rats. No histological alterations were reported in the kidneys from rats following 15 exposures to 200 ppm 1,2,4-trichlorobenzene each lasting 6 hours (Gage 1970), or in the

kidneys from rats, rabbits, and dogs exposed to up to 100 ppm 1,2,4-trichlorobenzene vapors 7 hours/day, 5 days/week for 30 exposures during a 44-day period (Kociba et al. 1981).

Exposure of rats to up to 130 ppm 1,3,5-trichlorobenzene vapors 6 hours/day, 5 days/week for 13 weeks did not result in significant gross or microscopic alterations in the kidneys (Sasmore et al. 1983).

Endocrine Effects. Fifteen 6-hour exposures to up to 200 ppm 1,2,4-trichlorobenzene vapors did not induce significant gross or histological alterations in the adrenal glands from rats (Gage 1970). Exposure of rats, dogs, and rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors 7 hours/day, 5 days/week for a total of 30 exposures did not induce significant gross or microscopic alterations in the pituitary gland, adrenal gland, thyroid, or parathyroid (Kociba et al. 1981).

In the 13-week study of rats exposed intermittently to up to 130 ppm 1,3,5-trichlorobenzene vapors, the investigators stated that 34 organs were examined in the control and high-dose groups but did not specify the organs (Sasmore et al. 1983). However, there is mention that hemosiderosis was noted in the thyroid gland, which, by the distribution and nature in the different groups, appeared unrelated to the treatment. It seems reasonable to assume that other endocrine glands were also examined and that no alterations were observed.

Dermal Effects. Continuous exposure of male monkeys, rats, and rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors for 26 weeks did not induce significant gross or microscopic alterations in a section of abdominal skin that was examined (Coate et al. 1977).

No relevant data were located regarding 1,3,5-trichlorobenzene.

Ocular Effects. Gage (1970) reported that lacrimation occurred in rats initially during the 6-hour exposures to 70 ppm 1,2,4-trichlorobenzene but not during exposures to 20 ppm 1,2,4-trichlorobenzene. Continuous exposure of male monkeys, rats, or rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors for 26 weeks did not induce significant gross or microscopic alterations in the eyes (Coate et al. 1977). The eyes from rats, dogs, and rabbits were also examined in the intermediate-duration study by Kociba et al. (1981). In that study, exposure to up to 100 ppm 1,2,4-trichlorobenzene vapors did not induce alterations in the microscopic morphology of the eye.

Body Weight Effects. Continuous exposure of male monkeys, rats, and rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors for 26 weeks (Coate et al. 1977) or intermittent exposure of rats, dogs, and rabbits to up to 100 ppm for 44 days (Kociba et al. 1981) did not significantly alter body weight. Gage (1970) reported that rats exposed to 70 ppm 1,2,4-trichlorobenzene 6 hours/day for 15 exposures showed retarded weight gain, but no data were presented.

Exposure of rats to 1,209 ppm 1,3,5-trichlorobenzene vapors for 60 minutes resulted in 44 and 60% less body weight gain in males and females, respectively, 14 days after exposure (Jorgenson et al. 1976). Body weight was not affected in rats exposed intermittently to up to 130 ppm 1,3,5-trichlorobenzene vapors for 13 weeks (Sasmore et al. 1983).

Metabolic Effects. Intermittent exposure of rats to up to 130 ppm 1,3,5-trichlorobenzene vapors for 13 weeks did not significantly affect serum electrolyte levels or electrolyte balance (Sasmore et al. 1983).

No relevant data were located regarding 1,2,4-trichlorobenzene.

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological effects in humans after inhalation exposure to trichlorobenzenes.

Continuous exposure of monkeys, rats, and rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors for 26 weeks did not induce significant gross or microscopic changes in the spleen of the animals (Coate et al. 1977). Fifteen intermittent exposures (6 hours/day) of rats to up to 200 ppm 1,2,4-trichlorobenzene vapors also did not result in significant histological alterations in the spleen (Gage 1970). Similar experiments in rats, rabbits, and dogs exposed 7 hours/day, 5 days/week to up to 100 ppm 1,2,4-trichlorobenzene vapors for a total of 30 exposures during a 44-day period did not result in significant gross or microscopic alterations in the spleen, thymus, or lymph nodes (Kociba et al. 1981).

No histological alterations were observed in lymphoreticular tissues from rats exposed to up to 130 ppm 1,3,5-trichlorobenzene vapors 6 hours/day, 5 days/week for 13 weeks (Sasmore et al. 1983).

No relevant data were located regarding 1,2,3-trichlorobenzene.

NOAELs for lymphoreticular effects of 1,2,4-trichlorobenzene and 1,3,5-trichlorobenzene are presented in Tables 3-1 and 3-2 and are plotted in Figures 3-1 and 3-2.

3.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans after inhalation exposure to trichlorobenzenes.

Lethargy was reported in rats during 6-hour exposures to 70 ppm 1,2,4-trichlorobenzene vapors (Gage 1970). No such effect was observed during exposures to 20 ppm.

Continuous exposure of monkeys, rats, or rabbits to up to 100 ppm 1,2,4-trichlorobenzene vapors for 26 weeks did not induce significant gross or microscopic changes in the brain and spinal cord (Coate et al. 1977). Operant behavior tests conducted in the monkeys throughout the study showed no exposure-related alterations. Similar lack of gross or histological alterations were reported in the brain, spinal cord, and peripheral nerves from rats, dogs, and rabbits exposed intermittently to up to 100 ppm 1,2,4-trichlorobenzene vapors during a 44-day period (Kociba et al. 1981).

No histological alterations were observed in the brain and spinal cord from rats exposed to up to 130 ppm 1,3,5-trichlorobenzene vapors 6 hours/day, 5 days/week for 13 weeks (Sasmore et al. 1983).

No relevant data were located regarding 1,2,3-trichlorobenzene.

NOAELs and LOAELs for neurological effects are presented in Tables 3-1 and 3-2 and are plotted in Figures 3-1 and 3-2.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to trichlorobenzenes.

No significant gross or histological alterations were reported in the reproductive organs from male rats, dogs, and rabbits exposed intermittently to up to 100 ppm 1,2,4-trichlorobenzene vapors for 44 days (Kociba et al. 1981). It should be noted, however, that absolute and relative testes weights were significantly increased (30 and 43%, respectively) in rabbits exposed to 100 ppm (but not 30 ppm). The

investigators considered this change unrelated to the test material since, as indicated above, microscopic examination did not reveal any significant histological changes.

No histological alterations were observed in the reproductive organs from male or female rats exposed to up to 130 ppm 1,3,5-trichlorobenzene vapors 6 hours/day, 5 days/week for 13 weeks (Sasmore et al. 1983).

No relevant data were located regarding 1,2,3-trichlorobenzene.

NOAELs for reproductive effects are presented in Table 3-1 and are plotted in Figure 3-1.

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

3.2.1.6 Developmental Effects

3.2.1.7 Cancer

3.2.2 Oral Exposure

3.2.2.1 Death

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

Information regarding acute lethality is available for the three trichlorobenzene isomers. An LD_{50} of 756 mg/kg was reported for 1,2,4-trichlorobenzene in CFE rats (Brown et al. 1969). In the same study, the investigators determined an oral LD_{50} of 766 mg/kg for 1,2,4-trichlorobenzene in C57BL/6N mice. In both species, lower doses caused depression of activity, while lethal doses induced extensor convulsions. Another study reported an LD_{50} of 880 mg/kg for 1,2,4-trichlorobenzene in Sprague-Dawley rats (Côté et al. 1988). In a developmental study, six out of six pregnant Sprague-Dawley rats died after 3 days of dosing with 1,200 mg/kg 1,2,4-trichlorobenzene (Kitchin and Ebron 1983).

Without providing further details, Côté et al. (1988) reported that the oral LD_{50} for 1,2,3-trichlorobenzene in Sprague-Dawley rats was 1,830 mg/kg. In a brief report in which groups of two rats per group were administered 1,000 or 2,000 mg/kg 1,2,3-trichlorobenzene by gavage in corn oil, one rat in the high-dose

group died within 2 days of dosing (Dow Chemical 1956). The investigators stated that slight pathology of the liver and spleen occurred, but no details were provided.

An oral LD_{50} of 2,100 mg/kg was reported for 1,3,5-trichlorobenzene in Sprague-Dawley rats (Côté et al. 1988). In another study, Jorgenson et al. (1976) reported LD_{50} values of 1,800 and 2,800 mg/kg for 1,3,5-trichlorobenzene in male and female Sprague-Dawley rats, respectively. All deaths except one occurred between 21 and 96 hours after dosing. Clinical signs observed included rough hair coat, passive tremors, depression, and inactivity leading to prostration, persistent tremor, coma, and death; necropsy revealed no gross abnormalities. The corresponding oral LD_{50} values for 1,3,5-trichlorobenzene in male and female ICR mice were 3,350 and 3,402 mg/kg, respectively (Jorgenson et al. 1976). Clinical signs observed included rough hair coat, passive tremors, depression, inactivity, prostration, persistent tremors, coma, and death. Deaths occurred between 22 and 288 hours after dosing; necropsy revealed no gross abnormalities. Increased mortality rate was reported in male rats dosed with 66.5 mg/kg/day 1,2,4-trichlorobenzene for 104 weeks (Moore 1994a). Similar results were reported in male and female mice dosed with 519.9 and 572.6 mg/kg/day 1,2,4-trichlorobenzene, respectively, for 104 weeks (Moore 1994b).

The limited data available suggest that 1,2,4-trichlorobenzene has a stronger acute toxicity than the other two isomers, and that rats may be more susceptible than mice.

Lethal doses and LD_{50} values are presented in Tables 3-3, 3-4, and 3-5 and plotted in Figures 3-3, 3-4, and 3-5.

3.2.2.2 Systemic Effects

No studies were located regarding systemic effects in humans after oral exposure to trichlorobenzenes.

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Tables 3-3, 3-4, and 3-5 and plotted in Figure 3-3, 3-4, and 3-5.

Respiratory Effects. No gross or microscopic alterations were observed in the lungs, bronchi, or trachea from rats administered up to 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3- or 1,3,5-trichlorobenzene on Gd 6–15 and sacrificed on Gd 22 (Black et al. 1988). In intermediate-duration

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - 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 (Sprague-Dawley)	once (G)				756	(LD50)	Brown et al.1969 1,2,4-trichlorobenzene	
2	Rat (Sprague-Dawley)	once (G)				880	(LD50)	Cote et al. 1988 1,2,4-trichlorobenzene	
3	Rat (NS)	once (G)				2250	(lowest lethal dose)	E.I. Dupont 1971 1,2,4-trichlorobenzene	
4	Rat (Sprague-Dawley)	Gd 9-13 1 x/d (GO)				1200 F	(6/6 died after 3 days of dosing)	Kitchin and Ebron 1983 1,2,4-trichlorobenzene	
5	Mouse (C57BL/6N)	once (G)				766	(LD50)	Brown et al.1969 1,2,4-trichlorobenzene	

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
6	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)	Resp	300 F			Black et al. 1988 1,2,4-trichlorobenzene	NOAELs are for organ and tissues histopathology
			Cardio	300 F				
			Gastro	300 F				
			Hemato	300 F				
			Musc/skel	300 F				
			Hepatic	150 F	300 F (11% increase in relative liver weight)			
			Renal	300 F				
			Dermal	300 F				
			Ocular	300 F				
			Metab	300 F				
7	Rat (albino)	14 d 1 x/d (GO)	Hemato	40 M			Carlson and Tardiff 1976 1,2,4-trichlorobenzene	
			Hepatic		10 M (15% increase in relative liver weight; increased phase I and phase II enzyme activity)			
8	Rat (NS)	3 d 1 x/d (GO)	Bd Wt		450 (4% weight loss in 3 days)		E.I. Dupont 1971 1,2,4-trichlorobenzene	

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
9	Rat (Sprague-Dawley)	Gd 9-13 1 x/d (GO)	Hepatic	36 F	120 F (increased phase I and phase II metabolic enzyme activity)		Kitchin and Ebron 1983 1,2,4-trichlorobenzene	Moderate hepatocellulalar hypertrophy at 360 mg/kg/day
			Bd Wt	120 F		360 F (17 grams lost, controls gained 37 grams)		
10	Rat (albino)	10 d 1 x/d (G)	Hepatic			500 M (intense necrosis and fatty change)	Rimington and Ziegler 1963 1,2,4-trichlorobenzene	
			Bd Wt			500 M (weight loss)		
11	Mouse (CD-1)	Gd 8-12 1 x/d (G)	Bd Wt	130 F			Chernoff and Kavlock 1983 1,2,4-trichlorobenzene	NOAEL is for maternal weight change during treatment
Immuno/ Lymphoret								
12	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		300 F			Black et al. 1988 1,2,4-trichlorobenzene	NOAEL is for histopathology of the thymus and spleen
Neurological								
13	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		300 F			Black et al. 1988 1,2,4-trichlorobenzene	NOAEL is for histopathology of the brain and peripheral nerve

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
14	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		300 F			Black et al. 1988 1,2,4-trichlorobenzene	NOAEL is for histopathology of the ovaries and uterus
Developmental								
15	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		300			Black et al. 1988 1,2,4-trichlorobenzene	Lesions in pup's lenses occurred at 75 mg/kg/day; incidences not reported
16	Rat (Sprague-Dawley)	Gd 9-13 1 x/d (GO)			360 F (retarded fetal development)		Kitchin and Ebron 1983 1,2,4-trichlorobenzene	
17	Mouse (CD-1)	Gd 8-12 1 x/d (G)		130			Chernoff and Kavlock 1983 1,2,4-trichlorobenzene	NOAEL is for neonatal weight and viability.
18	Mouse (CD-1)	Gd 8-12 1 x/d (GO)		130			Gray and Kavlock 1984 1,2,4-trichlorobenzene	NOAEL is for viability of F1 and reproductive performance of F1
19	Mouse (CD-1)	Gd 8-12 1 x/d (GO)		130			Gray et al. 1986 1,2,4-trichlorobenzene	NOAEL is for reactive locomotor activity of pups exposed in utero

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Systemic								
20	Rat (albino)	90 d 1 x/d (GO)	Hemato	40 M			Carlson and Tardiff 1976 1,2,4-trichlorobenzene	No liver histopathology
			Hepatic	20 M	40 M (14% increase in relative liver weight after 30-day recovery period; increased phase I metabolic enzymes)			
			Bd Wt	40 M				

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - 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
21	Rat (Fischer- 344)	14 wk ad lib (F)	Resp	150.6 F			CMA 1989 1,2,4-trichlorobenzene	NOAELs are for histopathology of tissues and organs.
			Cardio	150.6 F				
			Gastro	150.6 F				
			Hemato	150.6 F				
			Musc/skel	150.6 F				
			Hepatic	14.6 M[b]	45.6 M (increased liver weight, hepatocyte hypertrophy)			
			Renal	45.6 M	133.7 M (increased kidney weight; dilated tubules, granular casts; interstitial nephritis; elevated BUN)			
			Endocr	150.6 F				
			Ocular	150.6 F				
			Bd Wt	150.6 F				
			Metab	150.6 F				

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - 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
22	Rat (Sprague-Dawley)	13 wk ad lib (F)	Resp	101 F			Cote et al. 1988 1,2,4-trichlorobenzene	NOAELs are for organ histopathology
			Cardio	101 F				
			Gastro	101 F				
			Hemato	101 F				
			Musc/skel	101 F				
			Hepatic	7.8 M	82 M (13-20% increased absolute and relative liver weight)			
			Renal	7.8 M	82 M (31-36% increase absolute and relative kidney weight)			
			Dermal	101 F				
			Ocular	101 F				
			Bd Wt	101 F				
			Metab	101 F				
23	Rat (albino)	15 d 1 x/d (G)	Hepatic			730 M (intense necrosis and fatty change)	Rimington and Ziegler 1963 1,2,4-trichlorobenzene	
			Bd Wt			730 M (weight loss)		

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - 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
24	Mouse (B6C3F1)	13 wk ad lib (F)	Resp	1345 F			Hiles 1989 1,2,4-trichlorobenzene	NOAELs are for histopathology of organs and tissues
			Cardio	1345 F				
			Gastro	1345 F				
			Hemato	1345 F				
			Musc/skel	1345 F				
			Hepatic	67 M	850 M (hepatocyte hypertrophy, atrophy, vacuolar degeneration, necrosis; higher ALT and SDH activities)			
			Renal	1345 F				
			Endocr	1345 F				
			Ocular	1345 F				
			Metab	1345 F				
Immuno/ Lymphoret								
25	Rat (Fischer- 344)	14 wk ad lib (F)		150.6 F			CMA 1989 1,2,4-trichlorobenzene	NOAEL is for histopathology of lymphoreticular tissues
26	Rat (Sprague-Dawley)	13 wk ad lib (F)		101 F			Cote et al. 1988 1,2,4-trichlorobenzene	NOAEL is for histopathology of lymphoreticular organs

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - 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
27	Mouse (B6C3F1)	13 wk ad lib (F)		1345 F			Hiles 1989 1,2,4-trichlorobenzene	NOAEL is for histopathology of lymphoreticular organs
Neurological								
28	Rat (Fischer- 344)	14 wk ad lib (F)		150.6 F			CMA 1989 1,2,4-trichlorobenzene	NOAEL is for histopathology of central and peripheral nervous tissues
29	Rat (Sprague-Dawley)	13 wk ad lib (F)		101 F			Cote et al. 1988 1,2,4-trichlorobenzene	NOAEL is for histopathology of central and peripheral nervous tissue
30	Mouse (B6C3F1)	13 wk ad lib (F)		1345 F			Hiles 1989 1,2,4-trichlorobenzene	NOAEL is for histopathology of brain and spinal cord
Reproductive								
31	Rat (Fischer- 344)	14 wk ad lib (F)		133.7 M 150.6 F			CMA 1989 1,2,4-trichlorobenzene	NOAEL is for histopathology of reproductive organs
32	Rat (Sprague-Dawley)	13 wk ad lib (F)		82 M 101 F			Cote et al. 1988 1,2,4-trichlorobenzene	NOAEL is for histopathology of reproductive organs

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - 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
33	Rat (Sprague-Dawley)	90 d ad lib (W)		33 M 53.6 F			Robinson et al. 1981 1,2,4-trichlorobenzene	NOEAL is for fertility of F0 and F1 generation
34	Mouse (B6C3F1)	13 wk ad lib (F)		1222 M 1345 F			Hiles 1989 1,2,4-trichlorobenzene	NOAEL is for histopathology of reproductive organs
Developmental								
35	Rat (Sprague-Dawley)	90 d ad lib (W)		53.6 F			Robinson et al. 1981 1,2,4-trichlorobenzene	NOAEL is for standard neonatal indices, locomotor activity, clinical chemistry in offspring
CHRONIC EXPOSURE								
Death								
36	Rat (Fischer- 344)	104 wk ad lib (F)				66.5 M (decreased survival)	Moore 1994a 1,2,4-trichlorobenzene	
37	Mouse (B6C3F1)	104 wk ad lib (F)				519.9 M (decreased survival) 572.6 F (decreased survival)	Moore 1994b 1,2,4-trichlorobenzene	

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Systemic								
38	Rat (Fischer- 344)	104 wk ad lib (F)	Resp	81.4 F			Moore 1994a 1,2,4-trichlorobenzene	NOAELs are for organ and tissues histopathology
			Cardio	81.4 F				
			Gastro	81.4 F				
			Hemato	81.4 F				
			Musc/skel	81.4 F				
			Hepatic	19.4[c] M	66.5 M (increased liver weight; hepatocellular hypertrophy)			
			Renal	19.4 M	66.5 M (renal transitional cell hyperplasia)			
			Endocr	81.4 F				
			Dermal	81.4 F				
			Ocular	81.4 F				
			Bd Wt	81.4 F				

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - 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
39	Mouse (B6C3F1)	104 wk ad lib (F)	Resp	572.6 F			Moore 1994b 1,2,4-trichlorobenzene	NOAELs are for tissues and organs histopathology
			Cardio	572.6 F				
			Gastro	572.6 F				
			Hemato	572.6 F				
			Musc/skel	572.6 F				
			Hepatic	21 M	100.6 M (centrilobular hepatocytomegaly)			
					26.3 F (increased absolute and relative liver weight)			
			Renal	572.6 F				
			Endocr	572.6 F				
			Dermal	572.6 F				
			Ocular	572.6 F				
			Bd Wt	100.6 M	519.9 M (16% decreased final body weight)			
Immuno/ Lymphoret								
40	Rat (Fischer- 344)	104 wk ad lib (F)		81.4 F			Moore 1994a 1,2,4-trichlorobenzene	NOAEL is for histopathology of lymphoreticular tissues

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
41	Mouse (B6C3F1)	104 wk ad lib (F)		572.6 F			Moore 1994b 1,2,4-trichlorobenzene	NOAEL is for histopathology of lymphoreticular tissues
Neurological								
42	Rat (Fischer- 344)	104 wk ad lib (F)		81.4 F			Moore 1994a 1,2,4-trichlorobenzene	NOAEL is for nervous system histopathology
43	Mouse (B6C3F1)	104 wk ad lib (F)		572.6 F			Moore 1994b 1,2,4-trichlorobenzene	NOAEL is for histopathology of peripheral and central nervous tissues
Reproductive								
44	Rat (Fischer- 344)	104 wk ad lib (F)		66.5 M 81.4 F			Moore 1994a 1,2,4-trichlorobenzene	NOAEL is for histopathology of reproductive organs
45	Mouse (B6C3F1)	104 wk ad lib (F)		519.9 M 572.6 F			Moore 1994b 1,2,4-trichlorobenzene	NOAELs are for histopathology of reproductive organs

Table 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - 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
Cancer								
46	Mouse (B6C3F1)	104 wk ad lib (F)				100.6 M (CEL: hepatocellular carcinoma)	Moore 1994b 1,2,4-trichlorobenzene	
						127 F (CEL: hepatocellular carcinoma)		

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

b Used to derive an intermediate-duration oral MRL of 0.1 mg/kg/day for 1,2,4-trichlorobenzene; the MRL was derived by dividing the BMDL10 by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

c Used to derive a chronic-duration oral MRL of 0.1 mg/kg/day for 1,2,4-trichlorobenzene; the MRL was derived by dividing the BMDL10 by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

ad lib = ad libitum; Bd Wt = body weight; 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; Hemato = hematological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolic; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; (W) = drinking water; wk = week(s); x = time(s)

Figure 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Oral

Acute (≤14 days)

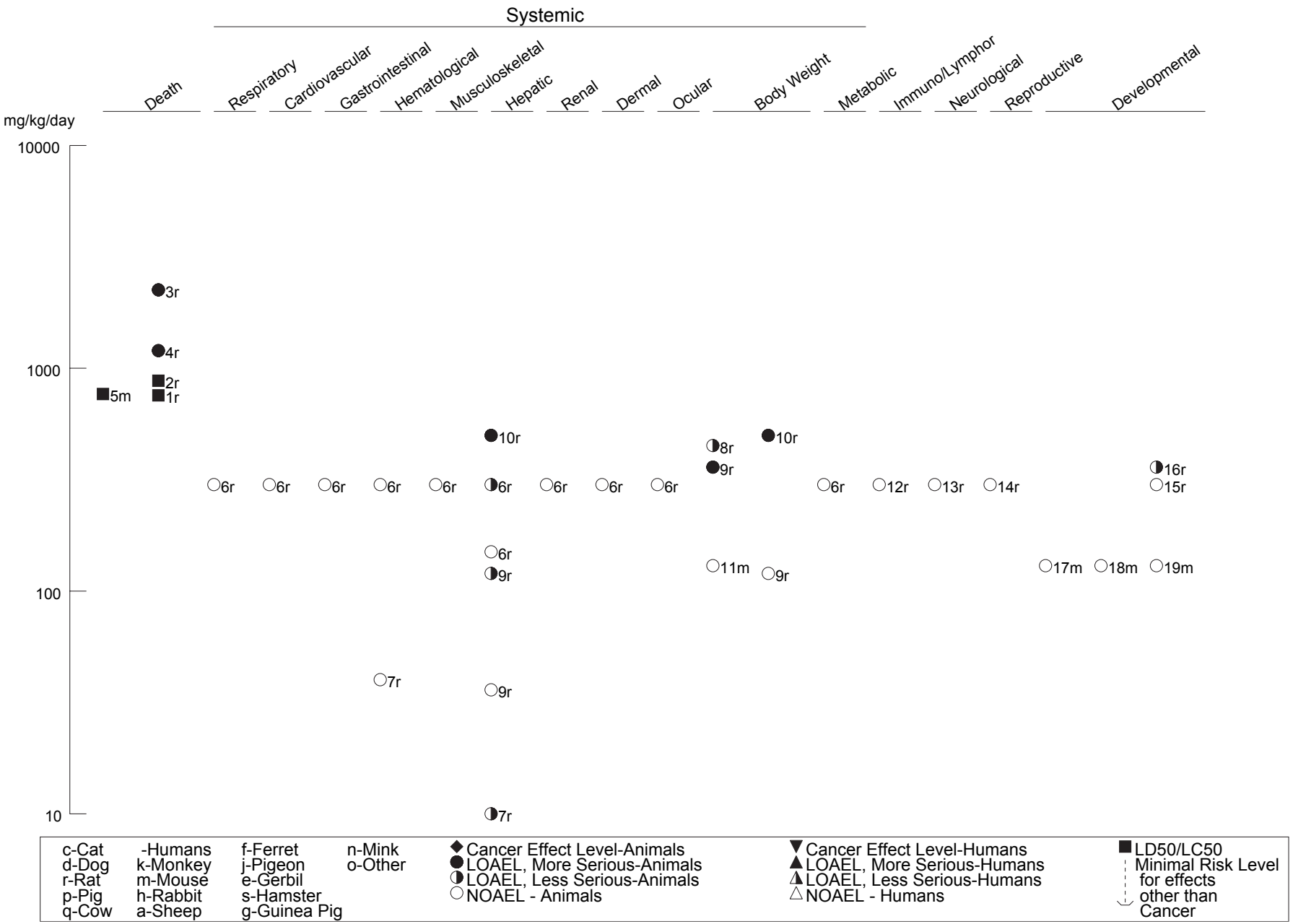


Figure 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

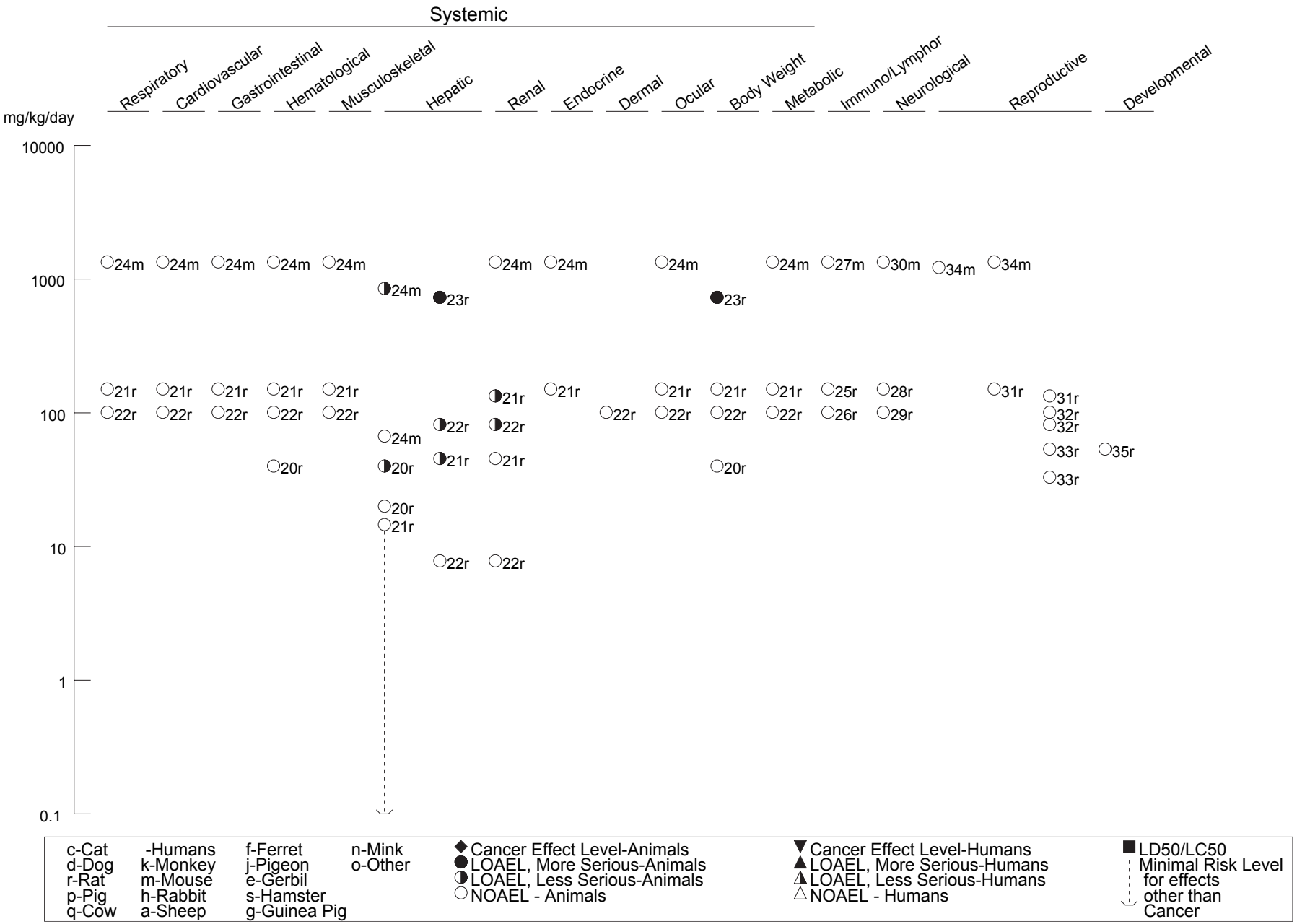
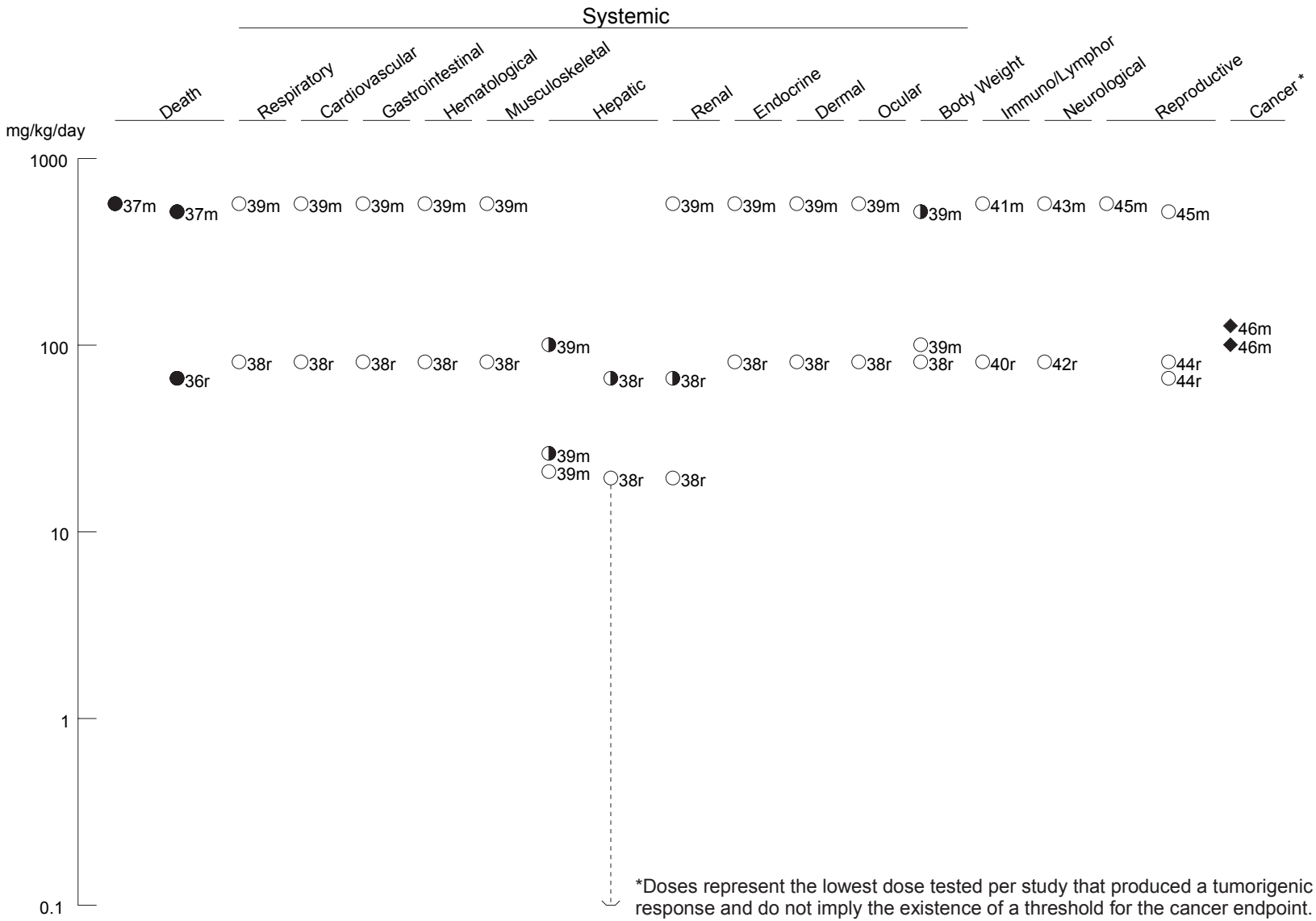


Figure 3-3 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Oral *(Continued)*

Chronic (≥365 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

Table 3-4 Levels of Significant Exposure to 1,2,3-Trichlorobenzene - 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 (Sprague-Dawley)	once (F)				1830 (LD50)	Cote et al. 1988 1,2,3-Trichlorobenzene	
2	Rat (NS)	once (GO)				2000 (1 out of 2 rats died within two days of dosing)	Dow Chemical 1956 1,2,3-Trichlorobenzene	
Systemic								
3	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)	Resp	600 F			Black et al. 1988 1,2,3-Trichlorobenzene	NOAELs are for organ and tissues histopathology
			Cardio	600 F				
			Gastro	600 F				
			Hemato	600 F				
			Musc/skel	600 F				
			Hepatic	150 F	300 F (14% increase in relative liver weight)			
			Dermal	600 F				
			Ocular	600 F				
			Metab	600 F				
4	Rat (albino)	7 d 1 x/d (G)	Bd Wt			780 M (weight loss)	Rimington and Ziegler 1963 1,2,3-Trichlorobenzene	

Table 3-4 Levels of Significant Exposure to 1,2,3-Trichlorobenzene - 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								
5	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		600 F			Black et al. 1988 1,2,3-Trichlorobenzene	NOAEL is for histopathology of spleen and thymus
Neurological								
6	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		600 F			Black et al. 1988 1,2,3-Trichlorobenzene	NOAEL is for histopathology of brain and peripheral nerve
Reproductive								
7	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		600 F			Black et al. 1988 1,2,3-Trichlorobenzene	NOAEL is for histopathology of reproductive organs
Developmental								
8	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		600			Black et al. 1988 1,2,3-Trichlorobenzene	NOAELs are for histopathology of organs and tissues from pups

Table 3-4 Levels of Significant Exposure to 1,2,3-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Systemic								
9	Rat (Sprague-Dawley)	13 wk ad lib (F)	Resp	113 F			Cote et al. 1988 1,2,3-Trichlorobenzene	NOAELs are for organ histopathology
			Cardio	113 F				
			Gastro	113 F				
			Hemato	113 F				
			Musc/skel	113 F				
			Hepatic	7.6 M	78 M	14% increase in relative liver weight)		
			Renal	7.6 M	78 M	(21% increase in relative kidney weight)		
			Dermal	113 F				
			Ocular	113 F				
			Bd Wt	7.6 M	78 M	(10.2% reduced body weight gain)		
			Metab	113 F				
Immuno/ Lymphoret								
10	Rat (Sprague-Dawley)	13 wk ad lib (F)		113 F			Cote et al. 1988 1,2,3-Trichlorobenzene	NOAEL is for histopathology of lymphoreticular tissues
Neurological								
11	Rat (Sprague-Dawley)	13 wk ad lib (F)		113 F			Cote et al. 1988 1,2,3-Trichlorobenzene	NOAEL is for histopathology of central and peripheral nerve tissues

Table 3-4 Levels of Significant Exposure to 1,2,3-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
12	Rat (Sprague-Dawley)	13 wk ad lib (F)		78 M 113 F			Cote et al. 1988 1,2,3-Trichlorobenzene	NOAEL is for histopathology of reproductive organs

a The number corresponds to entries in Figure 3-4.

ad lib = ad libitum; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); (F) = feed; F = Female; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day; (GO) = gavage in oil; Hemato = hematological; Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolic; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s)

Figure 3-4 Levels of Significant Exposure to 1,2,3-Trichlorobenzene - Oral

Acute (≤14 days)

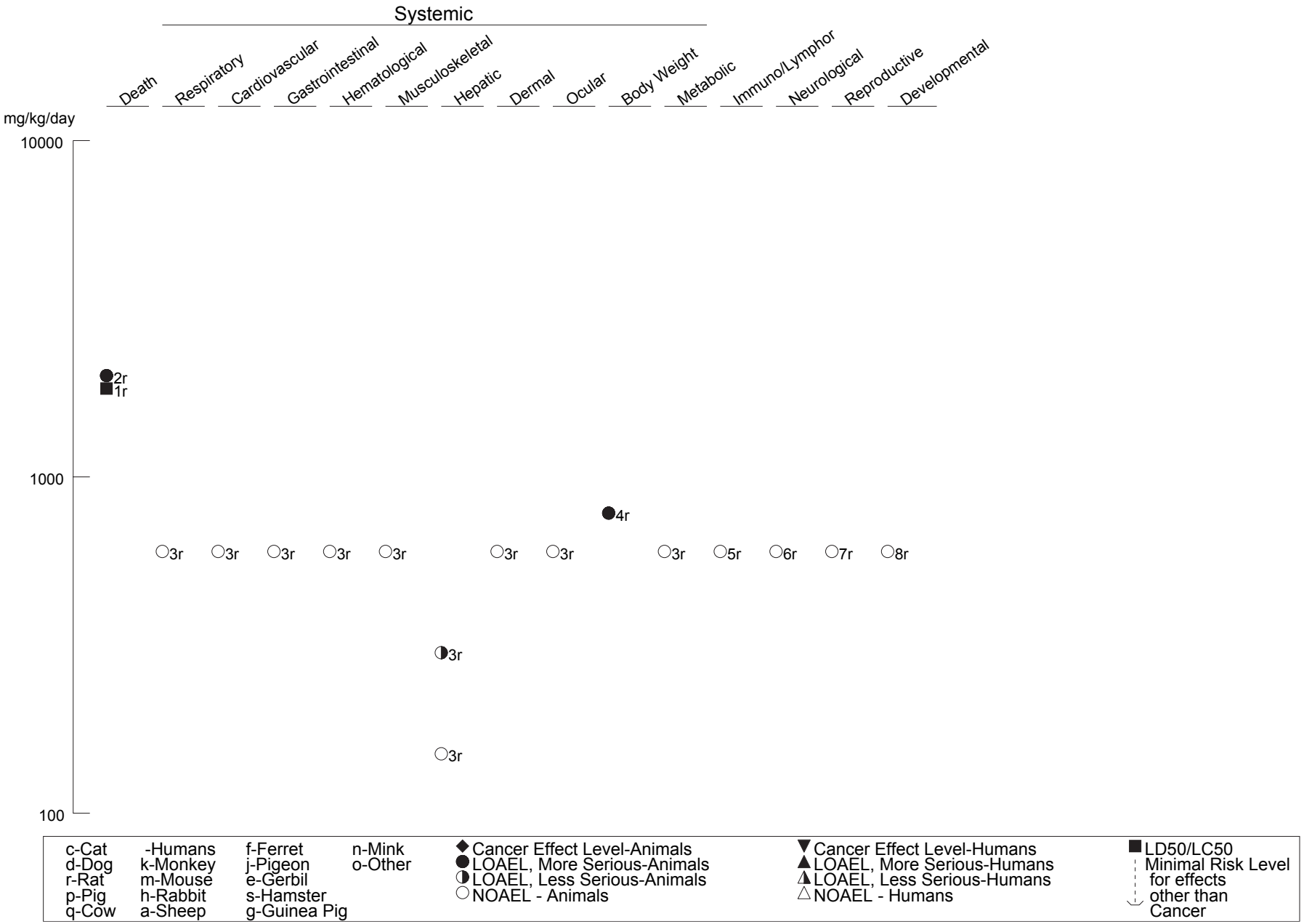


Figure 3-4 Levels of Significant Exposure to 1,2,3-Trichlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

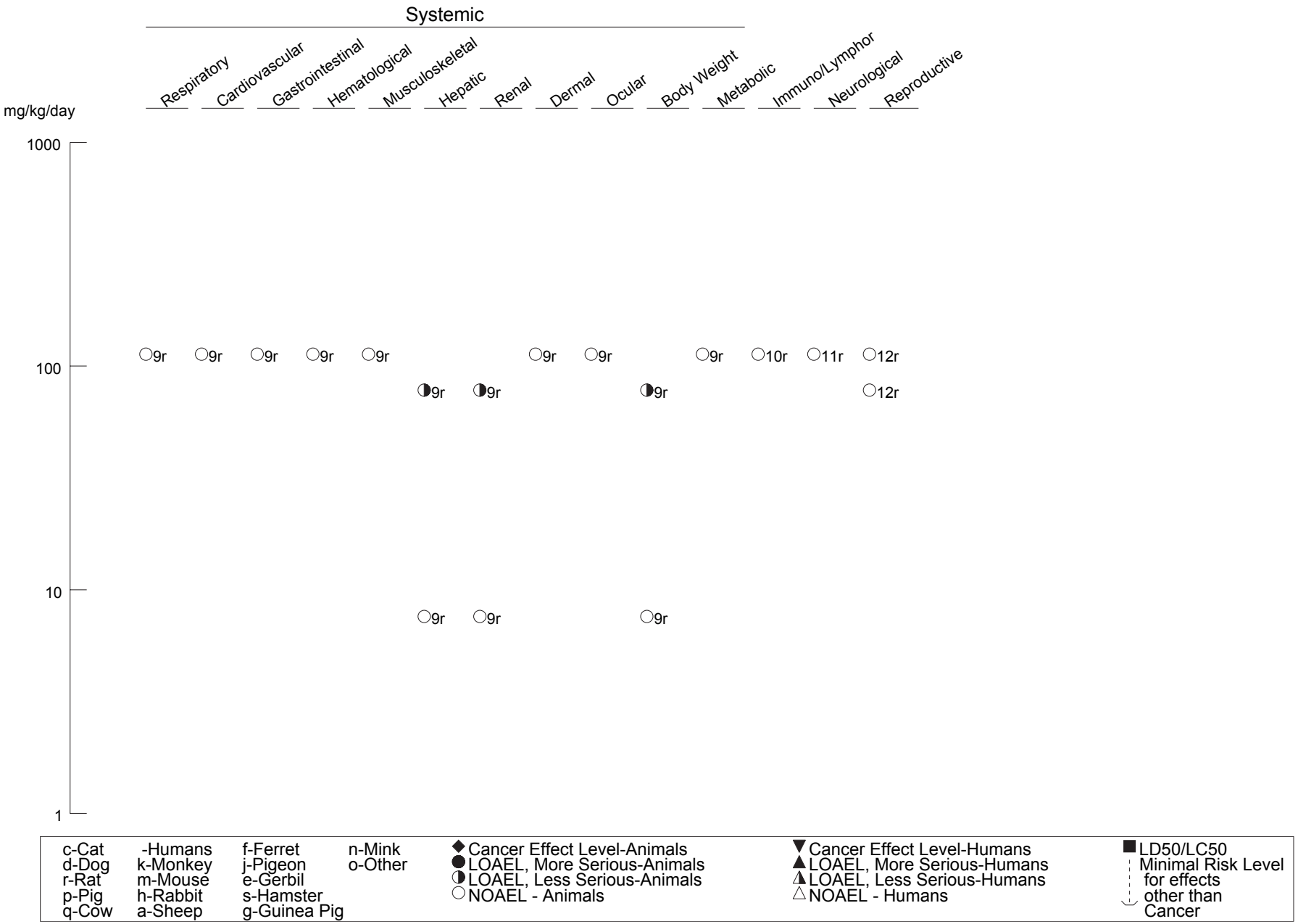


Table 3-5 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Oral

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 (Sprague-Dawley)	once (F)				2100 (LD50)	Cote et al. 1988 1,3,5-Trichlorobenzene	
2	Rat (Sprague-Dawley)	once (GO)				1800 M (LD50) 2800 F (LD50)	Jorgenson et al. 1976 1,3,5-Trichlorobenzene	
3	Mouse (ICR)	once (GO)				3350 M (LD50) 3402 F (LD50)	Jorgenson et al. 1976 1,3,5-Trichlorobenzene	
Systemic								
4	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)	Resp	600 F			Black et al. 1988 1,3,5-Trichlorobenzene	NOAELs are for organ and tissues histopathology
			Cardio	600 F				
			Gastro	600 F				
			Hemato	600 F				
			Musc/skel	600 F				
			Hepatic	300 F	600 F (25% increase in relative liver weight)			
			Dermal	600 F				
			Ocular	600 F				
			Bd Wt	300 F		600 F (34% reduced body weight gain)		
			Metab	600 F				

Table 3-5 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - 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									
5	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		600 F				Black et al. 1988 1,3,5-Trichlorobenzene	NOAEL is for spleen and thymus histopathology
Neurological									
6	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		600 F				Black et al. 1988 1,3,5-Trichlorobenzene	NOAEL is for histopathology of the brain and peripheral nerve
Reproductive									
7	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)		600 F				Black et al. 1988 1,3,5-Trichlorobenzene	NOAEL is for histopathology of reproductive organs
Developmental									
8	Rat (Sprague-Dawley)	Gd 6-15 1 x/d (GO)				150	(histological lesions in the lenses of pups)	Black et al. 1988 1,3,5-Trichlorobenzene	

Table 3-5 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
INTERMEDIATE EXPOSURE								
Systemic								
9	Rat (Sprague-Dawley)	13 wk ad lib (F)	Resp	146 F			Cote et al. 1988 1,3,5-Trichlorobenzene	NOAELs are for organ histopathology
			Cardio	146 F				
			Gastro	146 F				
			Hemato	146 F				
			Musc/skel	146 F				
			Hepatic	7.7 M	82 M 11% increase in relative liver weight)			
			Renal	0.81 M	7.7 M 25% increase in relative kidney weight)			
			Dermal	146 F				
			Ocular	146 F				
			Bd Wt	146 F				
			Metab	146 F				
Immuno/ Lymphoret								
10	Rat (Sprague-Dawley)	13 wk ad lib (F)		146 F			Cote et al. 1988 1,3,5-Trichlorobenzene	NOAEL is for histopathology of lymphoreticular organs and tissues
Neurological								
11	Rat (Sprague-Dawley)	13 wk ad lib (F)		146 F			Cote et al. 1988 1,3,5-Trichlorobenzene	NOAEL is for histopathology of brain, spinal cord and sciatic nerve

Table 3-5 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Reproductive								
12	Rat (Sprague-Dawley)	13 wk ad lib (F)		82 M 146 F			Cote et al. 1988 1,3,5-Trichlorobenzene	NOAEL is for histopathology of reproductive organs

a The number corresponds to entries in Figure 3-5.

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

Figure 3-5 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Oral

Acute (≤14 days)

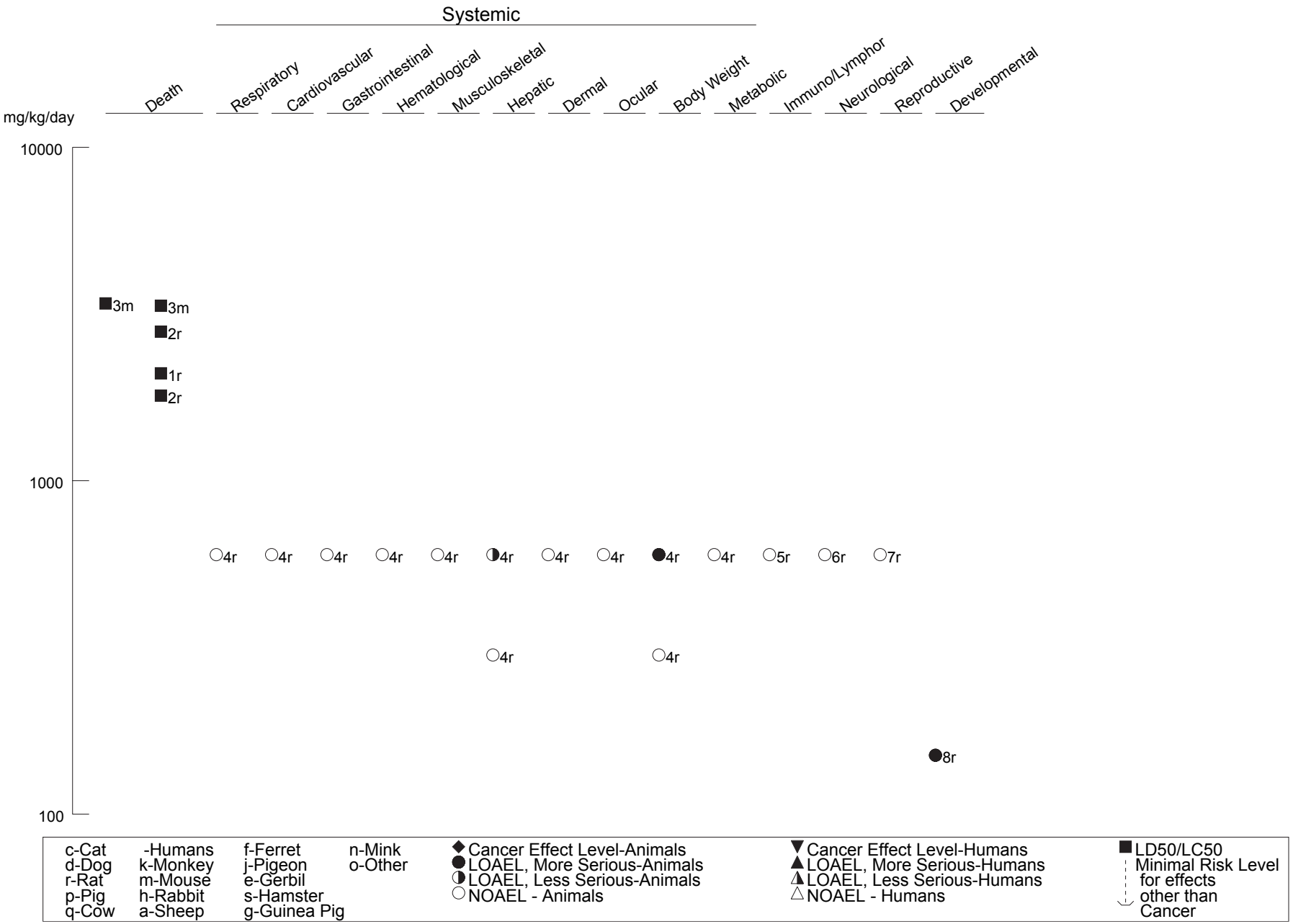
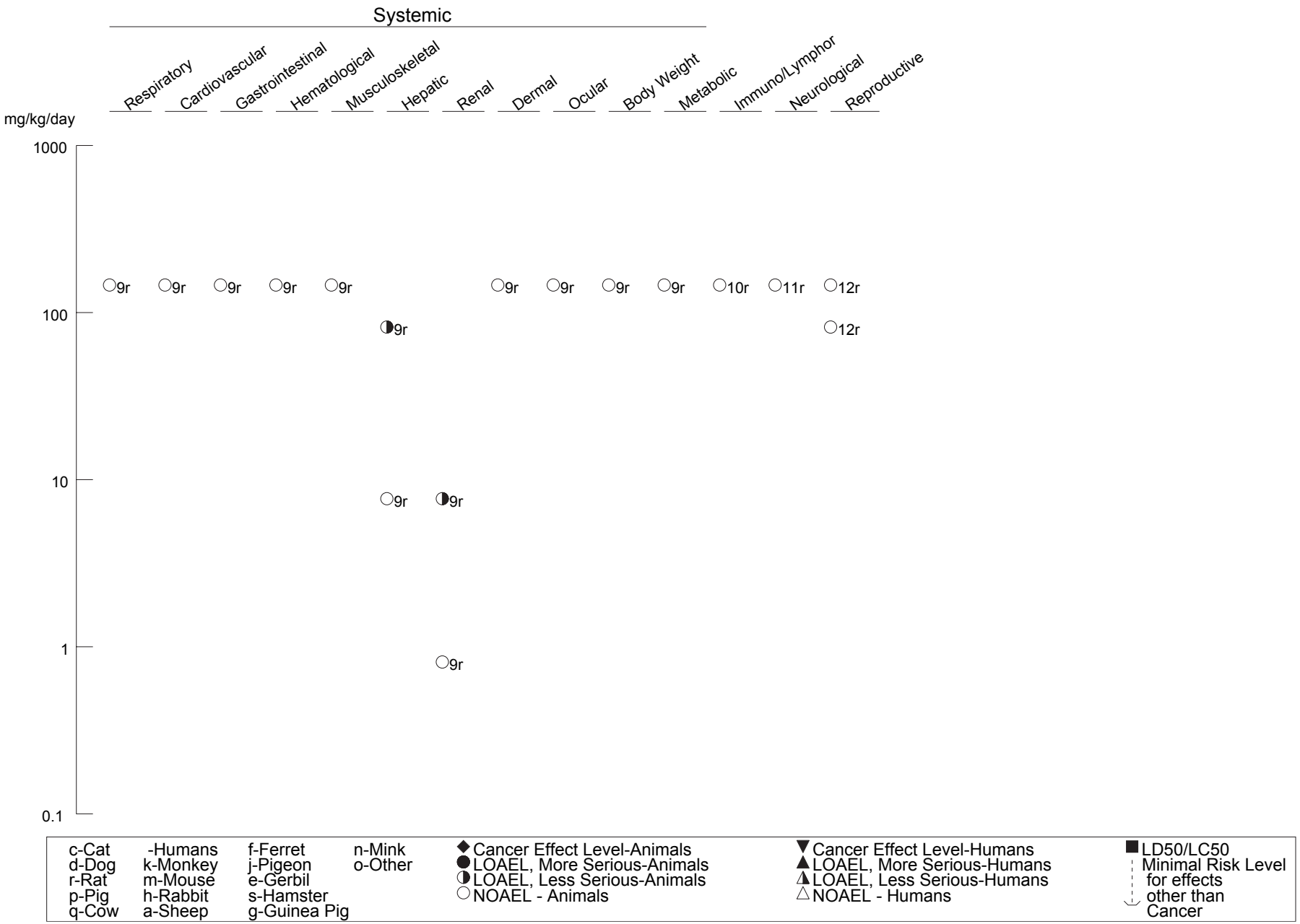


Figure 3-5 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)



studies, there were also no significant histological alterations in the lungs, trachea, or bronchi from rats exposed via the diet to up to 101 mg/kg/day (Côté et al. 1988) or 150.6 mg/kg/day (CMA 1989) 1,2,4-trichlorobenzene. A 13-week dietary study in mice reported no significant histological alterations in the lungs or trachea from animals dosed with up to 1,345 mg/kg/day 1,2,4-trichlorobenzene (Hiles 1989). Chronic-duration exposure to 1,2,4-trichlorobenzene also did not induce significant histological alterations in the lungs and trachea from rats dosed via the diet with up to 81.4 mg/kg/day 1,2,4-trichlorobenzene (Moore 1994a) or from mice dosed with up to 572.6 mg/kg/day 1,2,4-trichlorobenzene (Moore 1994b).

Black et al. (1988) also studied the 1,2,3- and 1,3,5- isomers and reported no significant effects in the lungs, bronchi, and trachea of pregnant rats dosed by gavage with up to 600 mg/kg/day of either isomer on Gd 6–15 and sacrificed on Gd 22. Similar results were reported in rats treated with up to 113 mg/kg/day 1,2,3-trichlorobenzene or 146 mg/kg/day 1,3,5-trichlorobenzene in the diet for 13 weeks (Côté et al. 1988).

Cardiovascular Effects. No significant gross or microscopic alterations were observed in the heart of rats dosed by gavage with up to 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3- or 1,3,5-trichlorobenzene on Gd 6–15 and sacrificed on Gd 22 (Black et al. 1988). Similar results were reported regarding the heart and aorta from rats treated with up to 101 mg/kg/day 1,2,4-trichlorobenzene, 113 mg/kg/day 1,2,3-trichlorobenzene, or 146 mg/kg/day 1,3,5-trichlorobenzene in the diet for 13 weeks (Côté et al. 1988), or 150.6 mg/kg/day 1,2,4-trichlorobenzene for 14 weeks (CMA 1989). Treatment of mice with up to 1,345 mg/kg/day 1,2,4-trichlorobenzene for 13 weeks via the diet did not significantly alter the gross or microscopic appearance of the heart or aorta (Hiles 1989). Treatment of rats with up to 81.4 mg/kg/day 1,2,4-trichlorobenzene or mice with up to 572.6 mg/kg/day 1,2,4-trichlorobenzene through the diet for 104 weeks did not induce gross or microscopic changes in the heart (Moore 1994a, 1994b).

Gastrointestinal Effects. No significant gross or microscopic alterations were reported in the gastrointestinal tract of rats dosed by gavage with up to 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3- or 1,3,5-trichlorobenzene on Gd 6–15 and sacrificed on Gd 22 (Black et al. 1988). Similar results were reported in rats treated with up to 101 mg/kg/day 1,2,4-trichlorobenzene, 113 mg/kg/day 1,2,3-trichlorobenzene, or 146 mg/kg/day 1,3,5-trichlorobenzene in the diet for 13 weeks (Côté et al. 1988), or 150.6 mg/kg/day 1,2,4-trichlorobenzene for 14 weeks (CMA 1989). Examination of the gastrointestinal tract from mice dosed with up to 1,345 mg/kg/day 1,2,4-trichlorobenzene for

13 weeks via the diet did not reveal significant treatment-related gross or histological alterations (Hiles 1989). No gross or histological alterations were reported in the gastrointestinal tract from rats or mice dosed via the diet with up to 81.4 or 572.6 mg/kg/day 1,2,4-trichlorobenzene, respectively for 104 weeks (Moore 1994a, 1994b).

Hematological Effects. Changes in hematological parameters have been reported in some acute- and intermediate-duration studies. However, since the reductions in hemoglobin and hematocrit reported in some of these studies are within the normal range for these parameters, they are not listed as LOAELs in Table 3-2; instead, the highest doses tested are listed as NOAELs for hematological effects.

Treatment of pregnant rats with 150 or 300 mg/kg/day 1,2,4-trichlorobenzene during Gd 6–15 resulted in reductions in hemoglobin (6–7%) and hematocrit (6%) (Black et al. 1988). In the same study, doses of 300 and 600 mg/kg/day 1,2,3-trichlorobenzene reduced hemoglobin by 6–7% and doses of 600 mg/kg/day reduced the hematocrit by approximately 6%. Rats treated with 600 mg/kg/day 1,3,5-trichlorobenzene showed reduction in hemoglobin and hematocrit of approximately 10–11% (Black et al. 1988). In another acute-duration study in rats, doses of up to 40 mg/kg/day 1,2,4-trichlorobenzene by gavage for 14 days did not affect hemoglobin or hematocrit levels (Carlson and Tardiff 1976).

Some intermediate-duration dietary studies have reported mild hematological changes following exposure to 1,2,4-trichlorobenzene. For example, treatment of male rats with 133.7 mg/kg/day, but not 45.6 mg/kg/day, 1,2,4-trichlorobenzene in the diet for 14 weeks reduced mean erythrocyte count (5%), hemoglobin (1.2%), and hematocrit (6%) (CMA 1989). In female rats, doses of 150.6 mg/kg/day reduced hemoglobin and hematocrit by 3.6–4%. Platelets were increased in males dosed with 133.7 mg/kg/day. In a similar study, administration of doses of up to 82 mg/kg/day to male rats and 101 mg/kg/day to females did not alter hematological parameters (Côté et al. 1988). No significant hematological changes were observed in male mice dosed with up to 1,222 mg/kg/day 1,2,4-trichlorobenzene for 13 weeks (Hiles 1989). In the same study, female mice dosed with 1,345 mg/kg/day 1,2,4-trichlorobenzene had lower (5–9%) hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin; since the these changes occurred only in females, the investigator (Hiles 1989) did not consider them to be treatment-related.

Administration in the diet of up to 113 mg/kg/day 1,2,3-trichlorobenzene or 146 mg/kg/day 1,3,5-trichlorobenzene to rats for 13 weeks did not alter hematological parameters (Côté et al. 1988).

In the chronic-duration oral studies with 1,2,4-trichlorobenzene, cellular morphology and leukocyte differential were monitored on weeks 52, 78, and at termination (Moore 1994a, 1994b). The results showed no significant treatment-related alterations in the parameters monitored in rats dosed through the diet with up to 81.4 mg/kg/day or in mice dosed with up to 572.6 mg/kg/day 1,2,4-trichlorobenzene. No evidence of leukemia was seen in either study.

Musculoskeletal Effects. No significant gross or histological alterations were reported in skeletal muscle from pregnant rats dosed with up to 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3-trichlorobenzene or 1,3,5,-trichlorobenzene on Gd 6–15 and sacrificed on Gd 22 (Black et al. 1988).

Similar results were reported in rats dosed via the diet with up to 150.6 mg/kg/day 1,2,4-trichlorobenzene, 113 mg/kg/day 1,2,3-trichlorobenzene, or 146 mg/kg/day 1,3,5-trichlorobenzene for 3 months (CMA 1989; Côté et al. 1988). An intermediate-duration dietary study in mice dosed with up to 1,345 mg/kg/day 1,2,4-trichlorobenzene for 13 weeks also did not find morphological alterations in bone or skeletal muscle (Hiles 1989). Chronic-duration studies did not find morphological alterations in bone or skeletal muscle from rats or mice dosed with up to 81.4 or 572.6 mg/kg/day 1,2,4-trichlorobenzene via the diet, respectively, for 104 weeks (Moore 1994a, 1994b).

Hepatic Effects. Acute-, intermediate-, and chronic-duration oral studies in animals indicate that the liver is a target for trichlorobenzenes. An acute-duration study with 1,2,4-trichlorobenzene described intense necrosis and fatty change in rats following administration of 500 mg/kg/day 1,2,4-trichlorobenzene by gavage for 10 days (Rimington and Ziegler 1963). In a 14-day gavage study in male rats, 1,2,4-trichlorobenzene induced a dose-related increase in relative liver weight (all doses, 15.3% at 10 mg/kg/day, 28.9% at 40 mg/kg/day) (Carlson and Tardiff 1976). Moderate hepatocellular hypertrophy was reported in the liver from pregnant rats following administration of 360 mg/kg/day for 4 days; the NOAEL for this effect was 120 mg/kg/day (Kitchin and Ebron 1983). In another developmental study, mild hepatic changes consisting of increased periportal cytoplasmic eosinophilia and mild anisokaryosis of hepatocellular nuclei were reported in pregnant rats dosed with 150 and 300 mg/kg/day 1,2,4-trichlorobenzene on Gd 6–15 (Black et al. 1988). The same was reported in pregnant rats dosed with 300 and 600 mg/kg/day 1,2,3- or 1,3,5-trichlorobenzene. Because no quantitative histological data were presented in the latter study, this information is not listed in Tables 3-3, 3-4, and 3-5. Histological alterations in the liver were almost always accompanied by increases in liver weight.

In a 90-day study in male rats, gavage administration of 1,2,4-trichlorobenzene (10, 20, 40 mg/kg/day) increased relative liver weight 14% in high-dose rats after a 30-day recovery period; however, no significant histological alterations were seen in the liver (Carlson and Tardiff 1976). Dietary administration of ≥14.6 mg/kg/day 1,2,4-trichlorobenzene to male rats or ≥52.5 mg/kg/day to females for 14 weeks induced dose-related increases in absolute and relative liver weights (CMA 1989). Microscopic examination of the liver revealed an increased incidence of centrilobular hepatocyte hypertrophy in male rats dosed with ≥45.6 mg/kg/day 1,2,4-trichlorobenzene and in female rats dosed with 150.6 mg/kg/day; the corresponding NOAELs were 14.6 and 52.5 mg/kg/day (CMA 1989). Centrilobular hepatocyte hypertrophy was used as the basis for derivation of an intermediate-duration oral MRL for 1,2,4-trichlorobenzene. An additional intermediate-duration dietary study reported significant increases in relative liver weight in male rats dosed with 78–82 mg/kg/day of each trichlorobenzene isomer (Côté et al. 1988). The investigators stated that most treated groups (doses ranged from approximately 0.1 to 140 mg/kg/day) showed mild-to-moderate increases in cytoplasmic volume and anisokaryosis of hepatocytes mostly in perivenous and midzone areas. High-dose rats showed aggregated basophilia as well as widespread midzonal vacuolation due to fatty infiltration. However, since only a qualitative description of the histological changes was provided, it is impossible to determine with certainty NOAELs or LOAELs for the lesions; therefore, they are not listed in Tables 3-3, 3-4, or 3-5, but the organ weight changes are.

A significant increase in absolute and relative liver weight was reported in male mice dosed via the diet with ≥850 mg/kg/day 1,2,4-trichlorobenzene and female mice dosed with ≥1,183 mg/kg/day 1,2,4-trichlorobenzene for 13 weeks (Hiles 1989). These changes in liver weight correlated with microscopic changes characterized by hepatocellular cytomegaly with karyomegaly and multinucleation complex with adjacent hepatocellular compression, atrophy, anisocytosis, vacuolar degeneration, and necrosis. The NOAELs for these alterations were 67 and 86 mg/kg/day in males and females, respectively. In addition, changes in clinical chemistry that were considered treatment-related consisted of higher total protein in mid-dose males (850 mg/kg/day) and high-dose males (1,222 mg/kg/day) and females (1,345 mg/kg/day), increased albumin and globulin in high-dose males and females, increased serum alanine aminotransferase (ALT) in mid-dose males and in high-dose males and females, and increased sorbitol dehydrogenase (SDH) in mid- and high-dose males and females. According to the investigators, the higher protein was probably caused by dehydration.

In a 104-week dietary study with 1,2,4-trichlorobenzene in rats, doses of 66.5 mg/kg/day in males and 81.4 mg/kg/day in females produced significant increases in absolute and relative liver weight at termination (Moore 1994a). The corresponding NOAELs were 19.4 and 23.5 mg/kg/day. Significant

increases in the incidence of liver lesions were reported in rats that received the highest doses, 66.5 mg/kg/day in males and 81.4 mg/kg/day in females. The histological alterations consisted of hepatocyte hypertrophy, focal cystic degeneration, and diffuse fatty change. The increased incidence of hepatocyte hypertrophy in male rats served as the basis for derivation of a chronic-duration oral MRL for 1,2,4-trichlorobenzene. In a similar study in mice, doses ≥21 mg/kg/day 1,2,4-trichlorobenzene induced a dose-related increase in absolute and relative liver weight in males, whereas doses ≥100.6 mg/kg/day 1,2,4-trichlorobenzene increased relative liver weight (Moore 1994b). In females, doses ≥26.3 mg/kg/day also increased absolute and relative liver weight. Histological examination of the liver showed a significant increase in the incidence of centrilobular hepatocytomegaly in males dosed with ≥101 mg/kg/day 1,2,4-trichlorobenzene. The neoplastic effects in mice are described in Section 3.2.2.7, Cancer.

In addition to inducing changes in liver weight and morphological alterations in the liver, 1,2,4-trichlorobenzene has been shown to be a potent inducer of phase I and phase II metabolic enzymes (Ariyoshi et al. 1975a, 1975b; Carlson and Tardiff 1976; Kato and Kimura 2002; Kato et al. 1988, 1993; Kitchin and Ebron 1983). For example, administration of ≥10 mg/kg/day 1,2,4-trichlorobenzene to rats by gavage for 14 days resulted in dose-related increases in cytochrome c reductase, cytochrome P-450, glucuronyltransferase, EPN detoxification, and azoreductase (Carlson and Tardiff 1976). Extending the dosing period to 90 days resulted in smaller increases in enzyme activities, except for glucuronyltransferase activity, which was reduced relative to controls (Carlson and Tardiff 1976). In rats, 1,2,4-trichlorobenzene also induced ALA synthetase, the rate-limiting enzyme in the biosynthesis of heme, which is consistent with the development of porphyria in rats administered 1,2,4- trichlorobenzene (Rimington and Ziegler 1963). For example, doses of 250–500 mg/kg/day significantly increased the urinary excretion of coproporphyrin and, to a smaller extent, the excretion of uroporphyrins. Liver uroporphyrin was also increased by 1,2,4-trichlorobenzene; liver coproporphyrin and protoporphyrin were increased in rats showing marked porphyrinuria. Studies by Kato and coworkers (Kato and Kimura 2002; Kato et al. 1988, 1993) have shown that both the induction of drug-metabolizing enzymes and ALA synthetase are not due to 1,2,4-trichlorobenzene itself but its metabolite 2,3,5-trichlorophenyl methyl sulfone. Further information on the mechanism of porphyria can be found in Section 3.5, Mechanisms of Action.

Renal Effects. No gross or microscopic alterations were reported in the kidneys from pregnant rats dosed on Gd 6–15 with up to 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3-trichlorobenzene or 1,3,5-trichlorobenzene and sacrificed on Gd 22 (Black et al. 1988).

In a 13-week dietary study in rats with trichlorobenzenes, the three isomers increased relative kidney weight in male rats in doses ranging between 78 and 82 mg/kg/day (no data on females were presented), but only 1,3,5-trichlorobenzene reportedly induced morphological alterations in the kidneys (Côté et al. 1988). The changes were characterized by eosinophilic inclusion, enlargement and anisokariosis of the epithelial lining cells, and hyperplasia of renal tubular epithelial cells. Only the changes associated with the highest dose levels, 78–82 mg/kg/day, were considered to be biologically significant by the investigators. Since only a qualitative description of the histology was provided, NOAELs and LOAELs for histological changes are not presented in Tables 3-3, 3-4, and 3-5, but the organ weight changes are. In a 14-week dietary study with 1,2,4-trichlorobenzene in rats, doses ≥45.6 mg/kg/day in males and ≥52.5 mg/kg/day in females significantly increased relative kidney weight, but morphological alterations were seen only in high-dose males (133.7 mg/kg/day) and consisted of dilated tubules, granular casts, hyaline droplets, and interstitial nephritis (CMA 1989). BUN was also significantly elevated in high-dose males and females (150.6 mg/kg/day). Mice administered up to 1,345 mg/kg/day 1,2,4-trichlorobenzene via the diet for 13 weeks did not show significant gross or microscopic alterations in the kidneys (Hiles 1989).

In the 104-week dietary studies with 1,2,4-trichlorobenzene in rats and mice, kidney alterations were reported only in rats (Moore 1994a). Gross necropsy at termination showed increases incidence of kidney abnormalities in mid- (19.4 mg/kg/day) and high-dose (66.5 mg/kg/day) males. Microscopically, there was an increased incidence in marked renal papilla mineralization in high-dose males and in transitional renal cell hyperplasia, also in males; only the latter effect showed dose-response. In addition, there was an increase in severity of chronic nephropathy in males. In mice, there were no significant gross or microscopic alterations in the kidneys following dosing with up to 572.6 mg/kg/day 1,2,4-trichlorobenzene (Moore 1994b).

Endocrine Effects. The only relevant information from acute-duration exposures is that from the study by Black et al. (1988) in which pregnant rats were administered trichlorobenzenes by gavage on Gd 6–15 and were sacrificed on Gd 22. No significant gross or microscopic alterations were reported in the pituitary, parathyroid, adrenals, or pancreas from dams following doses of up to 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3-trichlorobenzene or 1,3,5-trichlorobenzene. However, mild thyroid histopathology was reported in rats dosed with ≥300 mg/kg/day of each isomer; the NOAEL in each case was 150 mg/kg/day. The alterations were described as reduction of follicle size and increased epithelial height accompanied by cytoplasmic vacuolization. Since no quantitative data were presented, NOAELs and LOAELs for thyroid effects are not presented in Tables 3-3, 3-4, and 3-5.

Côté et al. (1988) examined the same organs in a 13-week study in Sprague-Dawley rats and reported similar results. Morphological alterations were limited to the thyroid and were described as mild and appeared to be more severe in males. Reported changes consisted of reduction in follicular size, increased epithelial height from flattened cuboidal cells to columnar shape, and reduced colloid density. Changes in the high-dose groups (78–82 mg/kg/day) varied from mild to moderate. Since no quantitative data were presented, NOAELs and LOAELs for histological alterations in the thyroid are not listed in Tables 3-3, 3-4, and 3-5. In contrast, CMA (1989) did not find significant histological alterations in the thyroid from Fisher-344 rats dosed with up to 150.6 mg/kg/day 1,2,4-trichlorobenzene in the diet for 14 weeks. Whether this can be attributed to differences in sensitivity between rat strains is unknown. No significant alterations were reported in endocrine glands from mice dosed via the diet with up to 1,345 mg/kg/day 1,2,4-trichlorobenzene for 13 weeks (Hiles 1989) or from rats or mice dosed with up to 81.4 or 572.6 mg/kg/day 1,2,4-trichlorobenzene, respectively, for 104 weeks (Moore 1994a, 1994b).

Dermal Effects. None of the studies that examined the skin of animals following oral administration of trichlorobenzenes reported treatment-related gross or microscopic alterations. These include the acute developmental study by Black et al. (1988) (300 mg/kg/day 1,2,4-trichlorobenzene; 600 mg/kg/day 1,2,3-trichlorobenzene and 1,3,5-trichlorobenzene), a 13-week dietary study (Côté et al. 1988) (78–82 mg/kg/day), and 104-week dietary studies with 1,2,4-trichlorobenzene in rats (Moore 1994a) (81.4 mg/kg/day) and mice (Moore 1994b) (572.6 mg/kg/day).

Ocular Effects. Examination of the eyes of animals exposed orally to trichlorobenzenes did not show treatment-related gross or histological alterations. These include the acute developmental study by Black et al. (1988; 300 mg/kg/day 1,2,4-trichlorobenzene; 600 mg/kg/day 1,2,3-trichlorobenzene and 1,3,5-trichlorobenzene), 3-month dietary studies in rats (Côté et al. 1988; 78–82 mg/kg/day, and CMA 1989; 150.6 mg/kg/day), a 13-week dietary study with 1,2,4-trichlorobenzene in mice (Hiles 1989; 1,345 mg/kg/day), and 104-week dietary studies with 1,2,4-trichlorobenzene in rats (Moore 1994a; 81.4 mg/kg/day) and mice (Moore 1994b; 572.6 mg/kg/day).

Body Weight Effects. Almost all studies of trichlorobenzenes monitored body weight of the animals. Pregnant rats administered 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3-trichlorobenzene on Gd 6–15 gained less weight than controls, but quantitative data for these isomers were not provided (Black et al. 1988). However, administration of 600 mg/kg/day 1,3,5-trichlorobenzene resulted in a reduction in weight gain of 34% relative to controls on Gd 22 (Black et al. 1988). In another

developmental study, doses of 360 mg/kg/day 1,2,4-trichlorobenzene on Gd 9–13 induced weight loss; 120 mg/kg/day had no significant effect (Kitchin and Ebron 1983). Weight loss was also reported in a study in rats dosed by gavage with 500 mg/kg/day 1,2,4-trichlorobenzene for 10 days or with 780 mg/kg/day 1,2,3-trichlorobenzene for 7 day (Rimington and Ziegler 1963). None of these studies provided information on food consumption.

Fifteen days of dosing with 730 mg/kg/day 1,2,4-trichlorobenzene by gavage resulted in loss of appetite and weight loss in rats (Rimington and Ziegler 1963). Neither food consumption nor weight gain was significantly affected in rats dosed via the diet with up to 101 mg/kg/day 1,2,4-trichlorobenzene or 146 mg/kg/day 1,3,5-trichlorobenzene for 13 weeks (Côté et al. 1988). Similar results were reported in rats dosed through the diet with up to 150.6 mg/kg/day 1,2,4-trichlorobenzene for 14 weeks (CMA 1989). Male rats treated with 78 mg/kg/day 1,2,3-trichlorobenzene for 13 weeks gained 10.2% less weight than controls (Côté et al. 1988); the NOAEL was 7.6 mg/kg/day. Final body weight was significantly reduced in male mice dosed with 1,222 mg/kg/day 1,2,4-trichlorobenzene (9%) and in female mice dosed via the diet with 1,345 mg/kg/day 1,2,4-trichlorobenzene (8.3%) for 13 weeks (Hiles 1989). Cumulative body weight gain was significantly reduced in males dosed with 67 mg/kg/day 1,2,4-trichlorobenzene (27%), in males dosed with 1,222 mg/kg/day 1,2,4-trichlorobenzene (40%), and in females dosed with 1,345 mg/kg/day 1,2,4-trichlorobenzene (33%); these changes were associated with significant reductions in food consumption throughout the study.

Final body weight of rats dosed through the diet with up to 81.4 mg/kg/day 1,2,4-trichlorobenzene for 104 weeks was not significantly different than controls (Moore 1994a). Final body weight of male mice dosed with 519.9 mg/kg/day 1,2,4-trichlorobenzene in the diet for 104 weeks was 16% lower than controls; food consumption in this group was reduced during the first 52 weeks of the study (Moore 1994b). Final body weight was not significantly affected in female mice dosed with up to 572.6 mg/kg/day 1,2,4-trichlorobenzene. The NOAEL for body weight changes in male mice was 100.6 mg/kg/day.

Metabolic Effects. Administration of up to 300 mg/kg/day 1,2,4-trichlorobenzene or up to 600 mg/kg/day 1,2,3- or 1,3,5-trichlorobenzene to pregnant rats on Gd 6–15 did not significantly alter the concentration of electrolytes or glucose in blood (Black et al. 1988).

None of the intermediate-duration studies with trichlorobenzenes reported adverse metabolic effects. Treatment of rats with dietary doses of up to 150.6 mg/kg/day or mice with up to 1,345 mg/kg/day

1,2,4-trichlorobenzene for 3 months had no significant effect on serum electrolyte or glucose levels (CMA 1989; Côté et al. 1988; Hiles 1989). Similar results were reported in rats dosed via the diet with up to 113 mg/kg/day 1,2,3-trichlorobenzene or 146 mg/kg/day 1,3,5-trichlorobenzene (Côté et al. 1988).

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after oral exposure to trichlorobenzenes.

Information from studies in animals is limited to evaluations of the gross and microscopic morphology of lymphoreticular organs and tissues. In pregnant rats that were exposed to up to 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3-trichlorobenzene or 1,3,5-trichlorobenzene on Gd 6–15, there were no significant morphological changes in the spleen or thymus on Gd 22 (Black et al. 1988).

Intermediate-duration studies showed that exposure of rats to up to 150.6 mg/kg/day 1,2,4-trichlorobenzene, 113 mg/kg/day 1,2,3-trichlorobenzene, or 146 mg/kg/day 1,3,5-trichlorobenzene, or mice to up to 1,345 mg/kg/day 1,2,4-trichlorobenzene in the diet for 3 months did not alter the gross or microscopic morphology of the spleen, thymus, or lymph nodes (CMA 1989; Côté et al. 1988). Similar results were reported in rats and mice dosed with up to 81.4 and 572.6 mg/kg/day 1,2,4-trichlorobenzene, respectively, in the diet for 104 weeks (Moore 1994a, 1994b).

These values are presented as NOAELs for lymphoreticular effects in Table 3-3, 3-4, and 3-5 and are plotted in Figures 3-3, 3-4, and 3-5.

3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to trichlorobenzenes.

No gross or histological alterations were observed in the brain and peripheral nerves from rats exposed to up to 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3-trichlorobenzene or 1,3,5-trichlorobenzene on Gd 6–15 and sacrificed on Gd 22 (Black et al. 1988).

Côté et al. (1988) examined the brain, spinal cord, and sciatic nerve from rats following a 13-week exposure period via the diet to doses of up to 101 mg/kg/day 1,2,4-trichlorobenzene, 113 mg/kg/day

1,2,3-trichlorobenzene, or 146 mg/kg/day 1,3,5-trichlorobenzene and reported no significant gross or microscopic alterations in those tissues. Similar results were reported in rats dosed via the diet with up to 150.6 mg/kg/day 1,2,4-trichlorobenzene for 14 weeks (CMA 1989) and in mice dosed with up to 1,345 mg/kg/day 1,2,4-trichlorobenzene for 13 weeks (Hiles 1989). Dietary exposure of rats and mice to up to 81.4 and 572.6 mg/kg/day 1,2,4-trichlorobenzene, respectively, in the diet for 104 weeks also did not induce histological alterations in the brain, spinal cord, or sciatic nerve (Moore 1994a, 1994b).

The NOAELs for morphological changes in the central and peripheral nervous systems are listed in Tables 3-3, 3-4, and 3-5 and are plotted in Figures 3-3, 3-4, and 3-5.

3.2.2.5 Reproductive Effects

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

Examination of the ovaries and uteri from rats dosed by gavage with up to 300 mg/kg/day 1,2,4-trichlorobenzene or 600 mg/kg/day 1,2,3-trichlorobenzene or 1,3,5-trichlorobenzene on Gd 6–15 and sacrificed on Gd 22 did not show significant gross or microscopic alterations (Black et al. 1988).

No treatment-related morphological alterations were reported in the reproductive organs from male or female rats dosed via the diet with 1,2,4-trichlorobenzene (82 mg/kg/day males, 101 mg/kg/day females), 1,2,3-trichlorobenzene (78 mg/kg/day males, 113 mg/kg/day females), or 1,3,5-trichlorobenzene (82 mg/kg/day males, 146 mg/kg/day females) for 13 weeks (Côté et al. 1988). No alterations were noted in the reproductive organs from male and female rats dosed via the diet with up to 133.7 and 150.6 mg/kg/day 1,2,4-trichlorobenzene, respectively, for 14 weeks (CMA 1989) or male and female mice dosed with up to 1,222 and 1,345 mg/kg/day, respectively, for 13 weeks (Hiles 1989).

Two-year dietary studies with 1,2,4-trichlorobenzene in rats (66.5 mg/kg/day males, 81.4 mg/kg/day females) and mice (519.9 mg/kg/day males, 572.6 mg/kg/day females) also did not find gross or microscopic alterations in the reproductive organs (Moore 1994a, 1994b).

Robinson et al. (1981) conducted a multi-generation reproductive study in rats in which the F0 and F1 generations were exposed to 1,2,4-trichlorobenzene via the mother's milk until weaning and then directly through their drinking water. At approximately 90 days of age in the F0 and F1 generations, the rats were

mated to produce the subsequent generation. The results showed that treatment of males and females with up to 33 or 53.6 mg/kg/day 1,2,4-trichlorobenzene, respectively, did not affect fertility. The doses correspond to the intake of test material by the F0 generation at 83 days of age and were estimated by the investigators.

The NOAELs for reproductive organs histology and the NOAEL for fertility from Robinson et al. (1981) are presented in Tables 3-3, 3-4, and 3-5 and are plotted in Figures 3-3, 3-4, and 3-5.

3.2.2.6 Developmental Effects

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

Black et al. (1988) examined the developmental effects of trichlorobenzenes in Sprague-Dawley rats. Rats were administered 0, 75, 150, or 300 mg/kg/day 1,2,4-trichlorobenzene or 0, 150, 300, or 600 mg/kg/day 1,2,3-trichlorobenzene or 1,3,5-trichlorobenzene by gavage in corn oil on Gd 6–15. Dams were sacrificed on Gd 22, and the uterus and ovaries were removed. Fetuses were examined grossly for birth defects and were also examined for skeletal and visceral anomalies. Also, entire fetuses were fixed, stained, and examined microscopically. Gestational exposure to the trichlorobenzenes did not significantly alter the number of pregnancies, fetal weight, litter size, resorptions, or dead fetuses, or the incidences of skeletal and visceral anomalies. However, fetuses from the 150 mg/kg/day 1,2,4-trichlorobenzene group and all groups exposed to 1,3,5-trichlorobenzene showed histological alterations in the lenses of the eye consisting of central areas of cellular disorientation and disaggregation with ballooning and granular degeneration. The investigators stated that autolysis and incomplete preservation made examination of other fetal tissues difficult, but there did not appear to be any significant treatment-related changes. In an additional study, pregnant rats were dosed with 0 or 360 mg/kg/day 1,2,4-trichlorobenzene on Gd 9–13 and were sacrificed on Gd 14 (Kitchin and Ebron 1983). Treatment did not increase resorptions or induce significant embryolethality or teratogenicity; however, it significantly retarded development as measured by reduced head length, crown-rump length, somite number, and protein content. It should be noted that dams administered 360 mg/kg/day 1,2,4-trichlorobenzene lost considerable body weight, which probably contributed to the delayed development of the offspring.

A series of studies were conducted with 1,2,4-trichlorobenzene in which pregnant mice were administered 0 or 130 mg/kg/day 1,2,4-trichlorobenzene by gavage on Gd 8–12 (Chernoff and Kavlock 1983; Gray and

Kavlock 1984; Gray et al. 1986). Treatment with 1,2,4-trichlorobenzene had no significant effect on average litter weight, pup viability, or growth. Testing of the pups in a figure 8 maze for reactive locomotor activity levels on postnatal days 22, 58, and 200 showed no significant differences between pups from treated groups and controls. Examination of female pups at age 30 days showed no significant effect on percent vaginal patency. Breeding of the F1 pups resulted in no significant effects on percent pregnant, age at parturition, F2 litter size, or abnormalities in the F2 generation. Necropsy of F1 males at about 250 days of age showed no significant effects on body weight and weight of the liver, testes, seminal vesicles, or right kidneys.

In the multi-generation reproductive study conducted by Robinson et al. (1981), treatment with 1,2,4-trichlorobenzene did not affect neonates' weight, litter size, or viability during the pre-weaning period in any generation. In addition, there were no treatment-related effects on locomotor activity in the F1 or F2 generation rats. Of the organs weighed in the pups (liver, lungs, heart, kidneys, adrenals, and gonads, as well as seminal vesicles in males), only the adrenals were affected by 1,2,4-trichlorobenzene. Absolute weight of the adrenals of high-dose F0 and F1 males and females were significantly increased relative to controls (7–12%), although with no clear dose-response relationship. Microscopic examination of liver and kidneys from F1 rats showed no histological damage. Results from blood chemistry tests in F0 and F1 rats did not reveal any treatment-related alterations. The dose levels of 1,2,4-trichlorobenzene at which the increase in adrenal weight were observed in F0 males and females were estimated by the investigators to be 33 and 53.6 mg/kg/day, respectively.

NOAELs and LOAELs for developmental effects are presented in Tables 3-3, 3-4, and 3-5 and are plotted in Figure 3-3, 3-4, and 3-5.

3.2.2.7 Cancer

Two long-term bioassays are available for 1,2,4-trichlorobenzene. Groups of F-344 rats (50/sex/group) were fed a diet containing 0, 100, 350, or 1,200 ppm 1,2,4-trichlorobenzene for 104 weeks (Moore 1994a). The diet provided doses of 0, 5.6, 19.4, or 66.5 mg/kg/day to males and 0, 6.9, 23.5, or 81.4 mg/kg/day to females. In the study in mice, groups of B6C3F_1 mice (50/sex/group) were fed a diet containing 0, 150, 700, or 3,200 ppm 1,2,4-trichlorobenzene (98.9% pure) for 104 weeks (Moore 1994b). The diet provided doses of 0, 21, 100.6, or 519.9 mg/kg/day to males and 0, 26.3, 127, or 572.6 mg/kg/day to females. Parameters evaluated in both studies included mortality (twice daily), clinical signs, body weight, and food consumption (weekly for 16 weeks and every 4 weeks thereafter),

hematology (week 52 and 78 for cellular morphology and leukocyte differential, from control and high-dose groups), organ weight (at termination, brain, brainstem, liver, kidneys, testes, and epididymis), and necropsy and histopathology findings (at termination, all major organs and tissues).

There was no evidence of treatment-related increases in the incidences of neoplasia in rats. In mice, treatment with 1,2,4-trichlorobenzene resulted in significant early mortality in both high-dose male and female groups. Percent survival on week 105 was 90, 88, 82, and 10% in males and 78, 76, 84, and 0% in females from the control, low-, mid-, and high-dose mice, respectively. Histological examination of tissue and organs from both unscheduled deaths and terminal sacrifice showed significantly increased incidence of hepatocellular carcinoma in mid- and high-dose groups (males: 8/50, 5/50, 27/50, and 50/50; females: 1/50, 1/50, 28/50, and 46/50). Neoplasms in other organs showed comparable incidences between the control and treated groups.

EPA classified 1,2,4-trichlorobenzene in Group D: not classifiable as to human carcinogenicity (IRIS 2010), or as a chemical for which there is "Inadequate Information to Assess Carcinogenic Potential" according to the Guidelines for Carcinogen Risk Assessment (EPA 2005a). EPA's classification was done in 1988 and was last revised in 1991.

3.2.3 Dermal Exposure

3.2.3.1 Death

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

A dermal LD_{50} of 6,139 mg/kg was reported for 1,2,4-trichlorobenzene in Sprague-Dawley rats (Brown et al. 1969). This value is presented in Table 3-6. The investigators noted that low doses caused depression of activity, whereas lethal doses induced extensor convulsions, and that all deaths occurred within 5 days of exposure. Dermal LD_{50} values of 300 and 305 mg/kg were estimated for 1,2,4-trichlorobenzene in mice applied doses ranging from 122 to 769 mg/kg (Yamamoto et al. 1978). During the 7-day observation period, no deaths occurred in the group dosed with 122 mg/kg 1,2,4-trichlorobenzene. No information was located regarding 1,2,3-trichlorobenzene or 1,3,5-trichlorobenzene.

Application of 0.5 mL of undiluted 1,2,4-trichlorobenzene to a shaven area of the back of guinea pigs 6 hours/day, 5 days/week for 3 weeks resulted in the death of an unspecified number of guinea pigs; the time of death was also not specified (Brown et al. 1969).

3.2.3.2 Systemic Effects

The highest NOAEL values and all LOAEL values from each study for systemic effects in each species and duration category are recorded in Tables 3-6, 3-7, and 3-8.

No studies were located regarding systemic effects in humans after dermal exposure to trichlorobenzenes.

Respiratory Effects. Application of technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin of rabbits in doses of up to 450 mg/kg 5 days/week for 4 weeks did not induce histological alterations in the respiratory tract including the nasal turbinates (Rao et al. 1982).

Cardiovascular Effects. No histological alterations were observed in the heart from rabbits administered 0.2 mL of a 100% solution of technical-grade 1,2,4-trichlorobenzene (97 mg/kg) to the ear 3 times/week for 13 weeks (Powers et al. 1975). Similar results were reported in rabbits following application of technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin in doses of up to 450 mg/kg, 5 days/week for 4 weeks (Rao et al. 1982).

Gastrointestinal Effects. Application of technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin of rabbits in doses of up to 450 mg/kg 5 days/week for 4 weeks did not induce histological alterations in the gastrointestinal tract, including the nasal turbinates (Rao et al. 1982).

Hematological Effects. No treatment-related hematological alterations were reported in rabbits following application of technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin in doses of up to 450 mg/kg, 5 days/week for 4 weeks (Rao et al. 1982).

Musculoskeletal Effects. Histological examination of skeletal and bone from rabbits administered technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin in doses of up to 450 mg/kg, 5 days/week for 4 weeks did not show treatment-related effects (Rao et al. 1982).

Hepatic Effects. In an acute lethality study in mice applied single doses of 1,2,4-trichlorobenzene ranging from 123 to 769 mg/kg onto the skin, mice that survived the highest dose showed congestion and necrosis of the liver (Yamamoto et al. 1978). Guinea pigs that died following application of 0.5 mL of

Table 3-6 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL				Reference Chemical Form	Comments
				Less Serious		Serious			
ACUTE EXPOSURE									
Death									
Rat (Sprague-Dawley)	once (NS)					6139 B mg/kg	(LD50)	Brown et al.1969 1,2,4-trichlorobenzene	
Mouse (CD-1)	once					300 M mg/kg	(LD50)	Yamamoto et al. 1978 1,2,4-trichlorobenzene	
						305 F mg/kg	(LD50)		
Systemic									
Mouse (CD-1)	once	Hepatic	591 B mg/kg			769 B mg/kg	(liver congestion and necrosis)	Yamamoto et al. 1978 1,2,4-trichlorobenzene	
Mouse (CD-1)	once	Dermal		70 B %volume	(erythema)			Yamamoto et al. 1978 1,2,4-trichlorobenzene	
Gn Pig (albino)	3 wk	Dermal		0.05 M mL	(moderate to severe skin irritation in older guinea pigs)			E.I. Dupont 1971 1,2,4-trichlorobenzene	No to mild irritation was reported in young guinea pigs; TCB was 75% or 95% v/v
Rabbit (NS)	3 d 6 hr/d	Dermal		2 B mL	(spongiosis, acanthosis, parakeratosis)			Brown et al.1969 1,2,4-trichlorobenzene	

Table 3-6 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Immuno/ Lymphoret							
Gn Pig (albino)	3 wk		0.05 M mL			E.I. Dupont 1971 1,2,4-trichlorobenzene	NOAEL is for skin sensitization; applied TCB was 95% solution
INTERMEDIATE EXPOSURE							
Death							
Gn Pig (NS)	3 wk 5 d/wk 1 x/d 6 hr/d				0.5 B mL/day (death of unspecified number of g. pigs)	Brown et al.1969 1,2,4-trichlorobenzene	Neither number of g. pigs that died nor time of death provided)
Systemic							
Gn Pig (NS)	3 wk 5 d/wk 1 x/d 6 hr/d	Hepatic		0.5 B mL/day (necrotic foci in the liver)		Brown et al.1969 1,2,4-trichlorobenzene	
		Dermal		0.5 B mL/day (spongiosis, acanthosis, parakeratosis)			
Rabbit (NS)	3 wk 5 d/wk 1 x/d 6 hr/d	Dermal		1 B mL/day (spongiosis, acanthosis, parakeratosis)		Brown et al.1969 1,2,4-trichlorobenzene	

Table 3-6 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
Rabbit (New Zealand)	13 wk 3 x/wk	Cardio	97 B mg/kg				Powers et al. 1975 1,2,4-trichlorobenzene	Effects increased in severity as the dose increased to 97 mg/kg
		Hepatic	97 B mg/kg					
		Renal	97 B mg/kg					
		Dermal		4.8 B mg/kg	(slight skin redness and scaling with desquamation)			
		Bd Wt	97 B mg/kg					

Table 3-6 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL		Reference Chemical Form	Comments
				Less Serious	Serious		
Rabbit (New Zealand)	4 wk 5 d/wk 1 x/d	Resp	450 B mg/kg			Rao et al. 1982 1,2,4-trichlorobenzene	NOAELs are for histopathology or organs and tissues
		Cardio	450 B mg/kg				
		Gastro	450 B mg/kg				
		Hemato	450 B mg/kg				
		Musc/skel	450 B mg/kg				
		Hepatic	450 B mg/kg				
		Renal	450 B mg/kg				
		Endocr	450 B mg/kg				
		Dermal		30 B mg/kg	(slight gross and histological damage at application site)		
		Ocular	450 B mg/kg				
		Bd Wt	450 B mg/kg				

Table 3-6 Levels of Significant Exposure to 1,2,4-Trichlorobenzene - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form	Comments
Immuno/ Lymphoret							
Gn Pig (NS)	3 wk 3 d/wk		0.1 %volume			Brown et al.1969 1,2,4-trichlorobenzene	No skin sensitization was observed
Rabbit (New Zealand)	13 wk 3 x/wk		97 B mg/kg			Powers et al. 1975 1,2,4-trichlorobenzene	NOAEL is for gross and microscopic alterations in the spleen
Rabbit (New Zealand)	4 wk 5 d/wk 1 x/d		450 B mg/kg			Rao et al. 1982 1,2,4-trichlorobenzene	NOAEL is for histopathology of lymphoreticular organs
Reproductive							
Rabbit (New Zealand)	4 wk 5 d/wk 1 x/d		450 B mg/kg			Rao et al. 1982 1,2,4-trichlorobenzene	NOAELs are for histopathology or organs and tissues
CHRONIC EXPOSURE							
Systemic							
Mouse (CD-1)	104 wk 2 x/wk	Dermal		30 B %volume	(keratinization of epidermis, inflammation)	Yamamoto et al. 1982 1,2,4-trichlorobenzene	The applied amount was 0.03 mL
		Bd Wt	60 B %volume				

B = both; Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = Female; Gastro = gastrointestinal; Gn Pig = guinea pig; Hemato = hematological; hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s)

Table 3-7 Levels of Significant Exposure to 1,2,3-Trichlorobenzene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
ACUTE EXPOSURE								
Systemic								
Rabbit (NS)	once	Ocular		10 %volume	(slight conjunctival irritation and trace of corneal injury)		Dow Chemical 1956 1,2,3-Trichlorobenzene	
Rabbit (NS)	7 d 1 x/d	Dermal		100 %volume	(slight reddening of the skin to slight exfoliation after 7 applications)		Dow Chemical 1956 1,2,3-Trichlorobenzene	

d = day(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified; x = time(s)

Table 3-8 Levels of Significant Exposure to 1,3,5-Trichlorobenzene - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form	Comments
				Less Serious		Serious		
ACUTE EXPOSURE								
Systemic								
Rabbit (New Zealand)	once	Dermal		500 mg	(mild skin irritation)		Jorgenson et al. 1976 1,3,5-Trichlorobenzene	
Rabbit (New Zealand)	once	Ocular		100 mg	(mild, transitory eye irritation)		Jorgenson et al. 1976 1,3,5-Trichlorobenzene	
Immuno/ Lymphoret								
Gn Pig (Hartley)	once		0.1 M %volume				Jorgenson et al. 1976 1,3,5-Trichlorobenzene	No skin sensitization was observed.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

undiluted 1,2,4-trichlorobenzene to a shaven area of the back for 6 hours/day, 5 days/week for 3 weeks showed necrotic foci in the liver (Brown et al. 1969).

No histological alterations were observed in the liver from rabbits administered 0.2 mL of a 100% solution of technical-grade 1,2,4-trichlorobenzene (97 mg/kg) to the ear 3 times/week for 13 weeks (Powers et al. 1975). Application of technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin of rabbits in doses of up to 450 mg/kg, 5 days/week for 4 weeks did not induce histological alterations in the liver; grossly, however, the liver of rabbits treated with 450 mg/kg did show slight pallor (Rao et al. 1982).

Renal Effects. No histological alterations were reported in the kidneys from rabbits applied 0.2 mL of a 100% solution of technical-grade 1,2,4-trichlorobenzene (97 mg/kg) to the ear 3 times/week for 13 weeks (Powers et al. 1975). No histological alterations were reported in the kidneys from rabbits following application of technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin in doses of up to 450 mg/kg, 5 days/week for 4 weeks (Rao et al. 1982). However, urinalysis revealed a slight but significant increase in urinary coproporphyrin in high-dose males on day 24 of the study, which was considered only a slight or questionable effect of treatment.

Endocrine Effects. Microscopic examination of the pituitary gland, pancreas, adrenal gland, and thyroid and parathyroid glands from rabbits administered technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin in doses of up to 450 mg/kg, 5 days/week for 4 weeks showed no significant treatment-related alterations (Rao et al. 1982).

Dermal Effects. Application of 1,2,4-trichlorobenzene in concentrations of 70–100% to the skin of mice produced erythema, but histological examination of the skin showed no remarkable change (Yamamoto et al. 1978). Application of 0.05 mL of a 75% solution of 1,2,4-trichlorobenzene to the shaved intact shoulder of guinea pigs for 24 hours produced no to mild irritation in young animals, but moderate to severe irritation in older guinea pigs (E.I. DuPont 1971). In a repeated treatment study, 1 mL of undiluted 1,2,4-trichlorobenzene was applied in a patch of lint to the shaved skin of rabbits for 6 hours during 3 consecutive days (Brown et al. 1969). Seven days after the first application, histological examination of the skin showed spongiosis, acanthosis, and parakeratosis.

Repeated applications of a 10% solution of 1,2,3-trichlorobenzene to the abraded belly of rabbits caused questionable irritation and edema that healed normally (Dow Chemical 1956). When the solution was

applied to intact skin, essentially no response was observed except for slight exfoliation after six applications. Application of undiluted 1,2,3-trichlorobenzene to the intact skin induced slight reddening to slight exfoliation after seven applications. However, in the abraded area, the undiluted chemical produced moderate reddening of the skin, slight edema, and trace of necrosis; healing was described as ordinary (Dow Chemical 1956).

In a study with 1,3,5-trichlorobenzene, 500 mg of the chemical was applied to the abraded or intact skin of six rabbits (Jorgenson et al. 1976). The test sites were covered and 24 hours later, the excess compound was removed and the sites were scored for erythema and edema. All treated rabbits showed erythema and edema at the 24-hour reading, and only three rabbits showed erythema at the 72-hour reading. The chemical was considered mildly irritating.

Intermittent application of 0.5 mL of undiluted 1,2,4-trichlorobenzene to guinea pigs or 1 mL to rabbits for 3 weeks produced spongiosis, acanthosis, and parakeratosis in both species (Brown et al. 1969). Rabbits applied 0.2 mL of a 5% solution of technical-grade 1,2,4-trichlorobenzene (4.8 mg/kg) onto the ventral surface of the ear 3 times/week for 13 weeks showed slight redness with slight scaling and desquamation that did not increase in severity after 39 exposures (Powers et al. 1975). Rabbits applied 25 or 100% solutions (24 or 97 mg/kg) showed moderate to severe irritation characterized by slight to severe erythema, severe scaling, desquamation and encrustation with slight enlargement of the follicles, some hair loss, and scarring. There was no evidence of acne form dermatitis.

A study was conducted in rabbits in which the animals were applied technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin in doses of 0, 30, 150, or 450 mg/kg, 5 days/week for 4 weeks (Rao et al. 1982). At termination, the skin of all treated rabbits showed localized effects considered slight at the low- and mid-dose levels and moderate at the high-dose level. The effects consisted of an area where regrowing fur was matted by a white bran-like scale, slight thickening of the skin, fissures which progressed to erosions and shallow ulcers, and erythema. Increasing dose increased the affected area. Microscopically, the skin site showed changes including inflammation, focal erosion and ulcers, and accumulation of inflammatory cells with varying degrees of exudation. Some rabbits showed slight superficial edema with slight fibrosis.

Painting 0.03 mL of a 30% solution of 1,2,4-trichlorobenzene 2 times/week for 2 years induced thickening and keratinization of the epidermis followed by inflammation (Yamamoto et al. 1982).

Ocular Effects. Instillation of a 10% solution of 1,2,3-trichlorobenzene to the eyes of rabbits followed by washing with water produced marked pain, slight conjunctival irritation, and trace of corneal injury (Dow Chemical 1956). The corneal injury almost completely healed in 24 hours, and irritation almost completely healed in 48 hours. In the unwashed eye, there was marked pain, conjunctival irritation and no corneal injury; complete healing occurred in 24 hours. In unwashed eyes instilled with undiluted 1,2,3-trichlorobenzene, there was a trace of pain, slight conjunctival irritation, and no corneal injury; healing occurred in 48 hours. The same was found in the washed eye, but healing occurred in 24 hours.

In a study with 1,3,5-trichlorobenzene, an amount of 100 mg of the chemical was applied into the eyes of rabbits (Jorgenson et al. 1976). Some rabbits had the compound washed out at 30 seconds or 5 minutes after application, or did not have the compound washed out. The eye was graded for ocular lesions at 24, 48, 72, and 96 hours. There was no corneal damage in rabbits that underwent washing, but minor damage was observed in the no-wash group. The cornea returned to normal within 3 days. Occasional circumcorneal injection was seen in some rabbits, but all effects on the iris disappeared by posttreatment day 4. Conjunctival responses occurred in all treated rabbits and generally involved varying degrees of redness, chemosis, and discharge. The eyes of the rabbits in the 30-second wash group returned to normal within 24 hours, but the 5-minute wash group was not normal until day 3, and the no-wash group did not return to normal until day 7. The investigators concluded that 1,3,5-trichlorobenzene produced mild transitory eye irritation.

Histological examination of the eyes from rabbits administered technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin in doses of up to 450 mg/kg, 5 days/week for 4 weeks showed no significant treatment-related alterations (Rao et al. 1982).

Body Weight Effects. Application of 0.2 mL of a 100% solution of technical-grade 1,2,4-trichlorobenzene (97 mg/kg) to the ear of rabbits 3 times/week for 13 weeks did not affect body weight (Powers et al. 1975). Application of technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin of rabbits in doses of up to 450 mg/kg, 5 days/week for 4 weeks did not significantly affect body weight (Rao et al. 1982).

3.2.3.3 Immunological and Lymphoreticular Effects

No histological alterations were reported in the spleen from rabbits administered 0.2 mL of a 100% solution of technical-grade 1,2,4-trichlorobenzene (97 mg/kg) to the ear 3 times/week for 13 weeks

(Powers et al. 1975). Similar results were reported for the spleen, thymus, and lymph nodes from rabbits following application of technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin in doses of up to 450 mg/kg, 5 days/week for 4 weeks (Rao et al. 1982).

Tests for skin sensitization conducted with 1,2,4-trichlorobenzene or 1,3,5-trichlorobenzene in guinea pigs were negative (Brown et al. 1969; E.I. DuPont 1971; Jorgenson et al. 1976).

3.2.3.4 Neurological Effects

Microscopic examination of the brain, spinal cord, and peripheral nerve from rabbits applied technical-grade 1,2,4-trichlorobenzene onto a shaved area of the back skin in doses of up to 450 mg/kg, 5 days/week for 4 weeks showed no significant treatment-related alterations (Rao et al. 1982).

3.2.3.5 Reproductive Effects

Application of technical-grade 1,2,4-trichlorobenzene to a shaved area of the back skin of male and female rabbits in doses of up to 450 mg/kg, 5 days/week for 4 weeks did not induce gross or microscopic alterations in the reproductive organs (Rao et al. 1982).

3.2.3.6 Developmental Effects

No studies were located that assessed developmental effects in humans or animals following dermal exposure to trichlorobenzenes.

3.2.3.7 Cancer

A 2-year cancer study was conducted with 1,2,4-trichlorobenzene in mice (Yamamoto et al. 1982). Groups of Slc:ddY mice (75/sex/group) were painted with 0.03 mL of a 30 or 60% solution of 1,2,4-trichlorobenzene 2 times/week for 104 weeks. There was high mortality both in the treated and control groups, beginning on week 30. At week 83, <10% of treated females and <15% of treated males survived. The main causes of death were respiratory infections, amyloidosis, and tumors. Histological alterations in tissues appeared more prevalent in the high-dose group, but the number of mice examined was not indicated. Tumors developed in the lungs, kidneys, stomach, urinary bladder, mammary gland, and skin in both treated and control groups. There was no indication of the time to first tumor appearance or whether the tumors were all found in different animals or were multiple tumors in the same animal.

Skin tumors were classified as squamous cell carcinoma, papilloma, and fibroma. This study is inadequate for assessing the potential carcinogenicity of 1,2,4-trichlorobenzene following dermal exposure.

3.3 GENOTOXICITY

For the most part, genotoxicity data for trichlorobenzenes have provided negative evidence of mutagenicity in *in vitro* tests with prokaryotic organisms (i.e., *Salmonella typhimurium*) and positive evidence of deoxyribonucleic acid (DNA) damage at concentrations that were generally also cytotoxic in mammalian cell systems *in vitro*. *In vivo* studies suggest that trichlorobenzenes are clastogenic. The role, if any, that these effects may play in the liver carcinogenicity of 1,2,4-trichlorobenzene in mice (Moore 1994b) is unknown. Tables 3-9 and 3-10 provide a summary of genotoxicity data for these test systems.

In vitro Exposure Studies. As shown in Table 3-9, *in vitro* assays of gene mutation in various strains of *S. typhimurium* provided mostly negative results, regardless of the presence or absence of metabolic activation in the incubation medium (Ethyl Corp. 1975; Haworth et al. 1983; Jorgenson et al. 1976; Kubo et al. 2002; Lawlor et al. 1979; Nohmi et al. 1985; Ono et al. 1992; Schoeny et al. 1979).

Studies with mammalian cell systems *in vitro* assessed mainly clastogenicity, cytotoxicity, and DNA damage, and for the most part, yielded positive results. A series of experiments conducted by Fratello et al. (1997) showed 1,2,3-trichlorobenzene and 1,2,4-trichlorobenzene to induce DNA damage as assessed by detecting loss of DNA fragment by means of cytofluorimetric analysis. Since the preparations used (Chinese hamster V79 cells) were devoid of cytochrome P-450 activity, the DNA damage was attributed to the parent compound rather than to a toxic metabolite. 1,3,5-Trichlorobenzene was considerably less cytotoxic than the other two isomers (Fratello et al. 1997). A similar study also found 1,2,4-trichlorobenzene to be cytotoxic in Chinese hamster ovary cells by inhibiting protein and DNA synthesis (Garret and Lewtas 1983). 1,2,4-Trichlorobenzene produced positive results in tests for cytotoxicity in rat hepatocytes and was positive in a transformation assay in rat liver epithelial (ARL) cells in the absence of metabolic activation, but at concentrations that were toxic to the cells (Shimada et al. 1983). Conversely, in an hepatocyte primary culture (HPC)/DNA repair assay conducted by Shimada et al. (1983), 1,2,4-trichlorobenzene was not genotoxic to rat hepatocytes in the absence of metabolic activation.

Table 3-9. Genotoxicity of Trichlorobenzenes *In Vitro*

Species (test system)	Compound	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:					
Salmonella typhimurium, TA98, TA100, TA1535, TA1537	1,2,3-TCB	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium, TA98, TA100	1,2,3-TCB	Gene mutation	–	–	Kubo et al. 2002
S. typhimurium, TA98, TA100, TA2637	1,2,3-TCB	Gene mutation	–	–	Nohmi et al. 1985
S. typhimurium, TA1535/pSK1002	1,2,3-TCB	Gene mutation	–	–	Ono et al. 1992
S. typhimurium, TA98, TA100, TA1535, TA1537	1,2,4-TCB	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium, TA98, TA100	1,2,4-TCB	Gene mutation	–	–	Kubo et al. 2002
S. typhimurium, TA98, TA100, TA2637	1,2,4-TCB	Gene mutation	–	–	Nohmi et al. 1985
S. typhimurium, TA1535/pSK1002	1,2,4-TCB	Gene mutation	–	+	Ono et al. 1992
S. typhimurium, TA98, TA100, TA1535, TA1537	1,2,4-TCB	Gene mutation, microsomal assay	–	–	Schoeny et al. 1979
S. typhimurium, TA98, TA100, TA1535, TA1537, TA1538	1,2,4-TCB	Gene mutation	–	–	Ethyl Corp. 1975
Saccharomyces cerevisiae D3	1,2,4-TCB	Gene mutation, mitotic recombination assay	–	–	Ethyl Corp. 1975
S. typhimurium, TA98, TA100, TA1535, TA1537	1,3,5-TCB	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium, TA98, TA100, TA2637	1,3,5-TCB	Gene mutation	–	–	Nohmi et al. 1985
S. typhimurium, TA1535/pSK1002	1,3,5-TCB	Gene mutation	–	–	Ono et al. 1992
S. typhimurium, TA98, TA100, TA1535, TA1537, TA1538	1,3,5-TCB	Gene Mutation	–	–	Jorgenson et al. 1976

Table 3-9. Genotoxicity of Trichlorobenzenes *In Vitro*

Species (test system)	Compound	End point	Results: With activation	Results: Without activation	Reference
Escherichia coli, WP2uvrA$^-$	1,3,5-TCB	Reverse gene mutation	–	–	Jorgenson et al. 1976
E. coli, W3110 and p3478	1,3,5-TCB	DNA repair assay	No data	–	Jorgenson et al. 1976
Bacillus subtilis, H17 and M45	1,3,5-TCB	DNA repair assay	No data	–	Jorgenson et al. 1976
S. cerevisiae D3	1,3,5-TCB	Mitotic recombination	(+)	(+)	Jorgenson et al. 1976
Mammalian cells:					
Chinese hamster lung (CHL) cells	1,2,3-TCB	Chromosomal aberrations	–	–	McElroy et al. 2003
Chinese hamster ovary (CHO) cells	1,2,3-TCB	Chromosomal aberrations	–	–	McElroy et al. 2003
Chinese hamster V79 cells	1,2,3-TCB	Cytotoxicity, neutral red uptake assay	No data	+	Fratello et al. 1997
Chinese hamster V79 cells	1,2,3-TCB	Cell replication (Colony forming ability)	No data	+	Fratello et al. 1997
Chinese hamster V79 cells	1,2,3-TCB	DNA damage	No data	+	Fratello et al. 1997
Chinese hamster V79 cells	1,2,4-TCB	Cytotoxicity, neutral red uptake assay	No data	+	Fratello et al. 1997
Chinese hamster V79 cells	1,2,4-TCB	Cell replication (Colony forming ability)	No data	+	Fratello et al. 1997
Chinese hamster V79 cells	1,2,4-TCB	DNA damage	No data	+	Fratello et al. 1997
CHO cells	1,2,4-TCB	Cytotoxicity	No data	+	Garrett and Lewtas 1983
Rat hepatocyte (male F344 Fischer)	1,2,4-TCB	Gene mutation, HPC/DNA repair assay	No data	–	Shimada et al. 1983
Rat hepatocyte (male F344 Fischer)	1,2,4-TCB	Cytotoxicity	No data	+	Shimada et al. 1983
Rat liver epithelial (ARL) cells (male F344 Fischer)	1,2,4-TCB	Gene mutation, Transformation Assay	No data	+	Shimada et al. 1983
Chinese hamster V79 cells	1,3,5-TCB	Cytotoxicity, neutral red uptake assay	No data	(+)	Fratello et al. 1997

Table 3-9. Genotoxicity of Trichlorobenzenes *In Vitro*

Species (test system)	Compound	End point	Results		Reference
			With activation	Without activation	
Chinese hamster V79 cells	1,3,5-TCB	Cell replication (Colony forming ability)	No data	(+)	Fratello et al. 1997
Chinese hamster V79 cells	1,3,5-TCB	DNA damage	No data	(+)	Fratello et al. 1997

+ = positive result; (+) = weakly positive result; — = negative result; DNA = deoxyribonucleic acid; HPC = hepatocyte primary culture; TCB = trichlorobenzene

Table 3-10. Genotoxicity of Trichlorobenzenes *In Vivo*

Species (test system)	Compound	End point	Results	Reference
Mouse (male NMR, 5/dose)	1,2,3-TCB	Chromosomal aberrations, micronucleus assay	+	Mohtashamipur et al. 1987
Mouse (male Swiss CD-1)	1,2,3-TCB	Chromosomal aberrations, micronucleous assay	+	Parrini et al. 1990
Mouse (male NMR, 5/dose)	1,2,4-TCB	Chromosomal aberrations, micronucleus assay	+	Mohtashamipur et al. 1987
Mouse (male Swiss CD-1)	1,2,4-TCB	Chromosomal aberrations, micronucleous assay	+	Parrini et al. 1990
Mouse (male NMR, 5/dose)	1,3,5-TCB	Chromosomal aberrations, micronucleus assay	+	Mohtashamipur et al. 1987
Mouse (male Swiss CD-1)	1,3,5-TCB	Chromosomal aberrations, micronucleous assay	+	Parrini et al. 1990
Drosophila melanogaster	1,3,5-TCB	Gene mutation, sex-linked recessive lethal test	–	Zimmering et al. 1985

+ = positive result; – = negative result; TCB = trichlorobenzene

1,2,4-Trichlorobenzene did not induce chromosomal aberrations in Chinese hamster lung (CHL) or ovary (CHO) cells in assays conducted with and without metabolic activation (McElroy et al. 2003).

In vivo Exposure Studies. There were few studies available for review that examined the potential *in vivo* genotoxicity of trichlorobenzenes; however, as seen in Table 3-10, the results of the available studies were mostly positive. Intraperitoneal administration of either one of the trichlorobenzene isomers to male NMRI mice in doses ranging from 210 to 1,700 mg/kg resulted in dose-related increases in micronuclei in polychromatic erythrocytes of the femoral bone marrow 30 hours after the first of two injections (Mohtashamipur et al. 1987). A similar study in male Swiss CD1 mice also reported increases in micronuclei frequency in the femoral bone marrow 6 hours after a second intraperitoneal injection of 500–650 mg/kg of either one of the trichlorobenzene isomers (Parrini et al. 1990). There were no significant differences among the trichlorobenzenes in either study. In a sex-linked recessive lethal test, 1,3,5-trichlorobenzene was negative for mutagenesis in *Drosophila melanogaster* (Zimmering et al. 1985).

3.4 TOXICOKINETICS

Results from studies of orally exposed animals indicate that trichlorobenzene isomers are rapidly and extensively absorbed, widely distributed, and quickly eliminated. Urinary excretion of radiolabeled 1,2,3-, 1,2,4-, and 1,3,5-trichlorobenzene has illustrated that at least 50–80% of the administered radioactivity is absorbed and excreted in rats within 24 hours of dose administration. Urinary elimination of metabolites is the major route of elimination, with fecal elimination representing a minor pathway. In bile duct-cannulated rats, significant enterohepatic circulation of metabolites has been demonstrated. Results from rat studies indicate that, once absorbed, all three isomers are widely distributed throughout the body and are detectable in tissue within 0.5 hours of dosing. Tissues with the highest peak concentrations following oral administration included fat, skin, liver, kidneys, bladder, and the gastrointestinal tract. Identification of metabolites in urine, feces, and bile following exposure of rabbits, rats, and monkeys to the individual isomers indicates that the parent compounds are metabolized to phenolic compounds through arene oxide intermediates, which are conjugated to glutathione, glucuronic acid, or sulfates before elimination in the urine, feces, or bile. Some evidence is available suggesting that conjugation with glutathione predominates in rats, whereas glucuronidation predominates in monkeys. Enzymes involved in the proposed metabolic schemes for each isomer have not been definitively identified. However, the initial formation of arene oxides and phenols is likely to be catalyzed by

cytochrome P450 (CYP) oxygenases. The relevance of the animal toxicokinetic information for humans has not been established because, as indicated below, there are no human data.

Studies describing the absorption, distribution, metabolism, and elimination of 1,2,3-, 1,2,4-, and 1,3,5-trichlorobenzene following oral exposure in humans, as well as inhalation and dermal exposure in humans and animals, are not available.

3.4.1 Absorption

No information was located regarding absorption of trichlorobenzenes in humans following any route of exposure.

3.4.1.1 Inhalation Exposure

Quantitative data on the absorption of trichlorobenzenes following inhalation exposure are not available for animals. However, indirect evidence that 1,2,4-trichlorobenzene is absorbed through inhalation can be inferred from minor liver effects in rats following inhalation exposure (Kociba et al. 1981).

3.4.1.2 Oral Exposure

Evidence from elimination studies in animals indicate that trichlorobenzene isomers are rapidly and extensively absorbed through the gastrointestinal tract in animals.

1,2,3-Trichlorobenzene. Radiolabeled 1,2,3-trichlorobenzene has been detected in both the urine and feces of rats following oral exposure. Specifically, 92% of administered radioactivity was eliminated through the urine and feces within 24 hours of administration of a single 10 mg/kg gavage dose of ^{14}C-labeled 1,2,3-trichlorobenzene to male Sprague-Dawley rats (Chu et al. 1987). Overall excretion increased to 95% at 48 hours post ingestion. The percentage of the administered radioactivity in urine alone accounted for 56 and 59% of the total ingested dose at 24 and 48 hours, respectively. Therefore, assuming that none of the radioactivity excreted in the feces was absorbed, at least 59% of the administered dose was absorbed. These results are also consistent with as much as 95% of the administered dose may have been absorbed if all radiolabeled material excreted in the feces had first been absorbed and excreted through the biliary system.

1,2,4-Trichlorobenzene. Within 7 days after receiving a dose of 50 mg/kg of ^{14}C-labeled 1,2,4-trichlorobenzene, male Wistar rats excreted 66 and 17% of the radioactivity in urine and feces, respectively, suggesting that at least 66% of the administered dose was absorbed (Tanaka et al. 1986). Radioactivity in expired air accounted for 2.1% of the absorbed dose. Excretion in all three excreta reached a peak on day 3, indicating rapid absorption and elimination. Measurements of biliary excretion for 4 days in bile-cannulated rats showed that radioactivity in bile accounted for 45% of the administered dose (Tanaka et al. 1986). Bakke et al. (1992) reported that >60% of a 21 mg/kg dose of ^{14}C-1,2,4-trichlorobenzene was excreted in bile, while 21% was excreted in the urine of bile-cannulated male Sprague-Dawley rats within 24 hours. Findings from this study indicate an 81% absorption rate following oral exposure to 1,2,4-trichlorobenzene. In control non-cannulated rats, 70 and 9% of the radioactivity was excreted within 24 hours in urine and feces, respectively (Bakke et al. 1992). Following 7 consecutive days of oral dosing of male Sprague-Dawley rats with 181.5 mg/kg/day ^{14}C-1,2,4-trichlorobenzene, radioactivity in urine was still detectable 21 days after the first oral administration; total radioactivity detected in urine accounted for approximately 72% of the administered dose (Smith and Carlson 1980). Radioactivity in the feces was not detectable past day 15 and accounted for only 4% of the administered dose. Thus, between 72 and 76% of the administered dose was absorbed in this study.

^{14}C-Labeled material was excreted by both monkeys and rats following oral exposure to a 10 mg/kg dose of ^{14}C-labeled 1,2,4-trichlorobenzene. By 24 hours after dosing, female Rhesus monkeys had excreted about 40% of the administered radioactivity in the urine and <1% in the feces. Male albino rats, however, excreted 84% of the administered radioactivity in the urine and 11% in the feces by 24 hours. With intravenous administration of 10 mg/kg ^{14}C-labeled 1,2,4-trichlorobenzene, monkeys eliminated about 22% of the administered radioactivity in urine within 24 hours (none was detected in feces), whereas radioactivity in 24-hour urine and feces in rats accounted for 78 and 7% of the administered dose, respectively (Lingg et al. 1982). These data suggest that differences in elimination rates between rats and monkeys may be due to species differences in metabolic rate or elimination rate, rather than absorption rate.

1,3,5-Trichlorobenzene. The excretion of radioactivity derived from 1,3,5-trichlorobenzene in both the urine and feces of rats has also been monitored following oral exposure. Within 24 hours of a single 10 mg/kg dose of ^{14}C-labeled 1,3,5-trichlorobenzene to male Sprague-Dawley rats, 82% of radioactivity was eliminated through the urine and feces (Chu et al. 1987). Overall excretion increased to 89% at 48 hours post ingestion. The percentage of administered radioactivity in urine accounted for 47 and 50% at 24 and 48 hours, respectively. Thus, assuming that none of the radioactivity excreted in the feces was

absorbed, at least 50% of the administered dose was absorbed. These results are also consistent with as much as 89% of the administered dose may have been absorbed if all radiolabeled material excreted in the feces had first been absorbed and excreted through the biliary system.

3.4.1.3 Dermal Exposure

No quantitative data were located on the absorption of trichlorobenzenes following dermal exposure of animals. Theoretical predictive models based on chemical and physical properties, however, have indicated that 1,2,4-trichlorobenzene has a significant potential for dermal absorption (Fiserova-Bergerova et al. 1990). Specifically, the findings of this study suggest that dermal exposure to 1,2,4-trichlorobenzene is expected to raise the biological levels of this isomer 30% above those occurring during inhalation exposure to threshold limit values. 1,2,4-Trichlorobenzene absorption can also be inferred from evidence of systemic toxicity in animals following dermal exposure (Brown et al. 1969; Yamamoto et al. 1978).

3.4.2 Distribution

1,2,3- and 1,3,5-Trichlorobenzene were detected in autopsies of Canadian citizens at median levels of 1.9 and 1.1 ng/g, respectively, and at maximum levels of 9.1 and 3.7 ng/g, respectively (Mes 1992). Levels were below the detection limits in blood samples. 1,2,4-Trichlorobenzene was detected in human follicular fluid at a mean concentration of 214 pg/mL for patients undergoing *in vitro* fertilization in Canada (Younglai et al. 2002). Trichlorobenzenes detected in the general population are the result of inhalation of ambient air and ingestion of food and drinking water contaminated with trichlorobenzenes. No specific information was located regarding distribution of trichlorobenzenes in children.

3.4.2.1 Inhalation Exposure

No data on the distribution of trichlorobenzenes following inhalation exposure were located for animals.

3.4.2.2 Oral Exposure

Trichlorobenzene isomers are readily distributed throughout bodily tissues in animals. However, the level of distribution and length of retention vary between isomers. Several studies have been conducted with ^{14}C-labeled trichlorobenzenes. It should be noted that generally in these studies no distinction can be

made between parent compound, metabolites, and recycled carbon incorporated into body macromolecules.

1,2,3-Trichlorobenzene. Radioactivity was present in the blood and tissues of male Sprague-Dawley rats 0.5 hours following gavage administration of 10 mg/kg ^{14}C-labeled 1,2,3-trichlorobenzene (Chu et al. 1987). Tissue concentrations peaked at 24 hours with very high concentrations appearing in the gastrointestinal tract (2,180 ppb), liver (277 ppb), fat (1,920 ppb), kidney (399 ppb), and bladder (284 ppb). Seven days after dosing, radioactivity in the brain, muscle, testes, and seminal vesicles were no longer detectible. Radioactivity was nearly undetectable in the liver, fat, and skin by 56 days after dosing (Chu et al. 1987). Chu et al. (1987) reported gas chromatography (GC) data that indicated that most of the radioactivity in skin, liver, and fat was the parent compound, whereas that in muscle and kidney was predominantly more polar metabolites.

1,2,4-Trichlorobenzene. Radioactivity was found in the blood and tissues of male Sprague-Dawley rats 0.5 hours after oral dosing with 10 mg/kg ^{14}C-labeled 1,2,4-trichlorobenzene (Chu et al. 1987). Levels peaked around 4 hours and declined thereafter. High concentrations remained present in the bladder (1,280 ppb), kidney (1,160 ppb), fat (4,260 ppb), skin (243 ppb), liver (680 ppb), and adrenal glands (850 ppb) at 24 hours. Seven days after dosing, radioactivity was no longer detectable in the brain, spleen, muscle, testes, seminal vesicles, epididymis, or prostate. Most tissues showed levels of radioactivity barely distinguishable from background levels 28 days after dosing (<10 ppb except adrenals with 40 ppb) (Chu et al. 1987). Sprague-Dawley rats given a daily oral dose of 181.5 mg/kg ^{14}C-labeled 1,2,4-trichlorobenzene for 7 consecutive days showed the highest initial concentration of radioactivity in the adrenal glands (Smith and Carlson 1980). This level declined rapidly and no radioactivity could be detected 11 days after dosing. Abdominal fat had the highest concentrations on day 1 (2,033 dpm/g) and maintained detectable concentrations (~20% of day 1 level) through the remainder of the 16-day observation period. The liver also maintained detectable concentrations (1,075 dpm/g on day 1 and 317 dpm/g on day 19) throughout the entire observation period (Smith and Carlson 1980). High levels of radioactivity were found in adipose tissue and skin (81 and 15% of administered dose, respectively) 12 hours following administration of a single 50 mg/kg oral dose of ^{14}C-labeled 1,2,4-trichlorobenzene to male Wistar rats. Radioactivity was also detectable in muscle and intestine at this time (~8 and 5% of administered dose). By 168 days after dosing, radioactivity was virtually undetectable in all examined tissues (Tanaka et al. 1986).

1,3,5-Trichlorobenzene. An early study by Parke and Williams (1960) indicated a wide distribution of 1,3,5-trichlorobenzene following a 500 mg/kg dose in rabbits. Specifically, 13% of the administered dose was detected in the feces, 23% in the gut, 5% in the pelt, 5% in the depot fat, and 22% in the carcass 8 days after administration. A later study revealed radioactivity in the blood and tissues 0.5 hours after oral dosing of male Sprague-Dawley rats with 10 mg/kg of ^{14}C-labeled 1,3,5-trichlorobenzene (Chu et al. 1987). As with the other isomers, levels of radioactivity peaked at 1 day after administration. The peak concentrations in tissues with the highest concentrations showed the following order: fat (8,960 ppb) > gastrointestinal tract > salivary gland > adrenal ≈ bladder > liver > kidney > pancreas > epididymis > prostate > skin > lung > seminal vesicle (410 ppb). Peak tissue concentrations were generally higher following administration of 1,3,5-trichlorobenzene compared with 1,2,3- and 1,2,4-trichlorobenzene (Chu et al. 1987). Chu et al. (1987) reported GC data that indicated that most of the radioactivity in skin, liver, and fat was the parent compound, whereas that in muscle and kidney was predominantly more polar metabolites. Côté et al. (1988) found an accumulation of trichlorobenzene isomers in the fat and liver of rats, indicating that 1,3,5-trichlorobenzene accumulated at a higher level in these tissues than 1,2,4- and 1,2,3-trichlorobenzene when presented at 1,000 ppm in food for 13 weeks. The levels of isomer in the fat (15.5–76 ppm in males; 7.8–49 ppm in females) were one order of magnitude higher than those found in the liver (1.4–4.3 ppm in males; 0.73–1.9 ppm in females).

Trichlorobenzenes have been identified in human breast milk; therefore, infants may also be potentially exposed through breast feeding (see Section 6.6, Exposures of Children).

3.4.2.3 Dermal Exposure

Quantitative data on the distribution of trichlorobenzenes following dermal exposure were not located for humans or animals.

3.4.3 Metabolism

There is no information regarding the metabolism of trichlorobenzenes in humans following exposure by any route or regarding the metabolism of trichlorobenzenes in animals following inhalation or dermal exposure. Several studies have examined and identified urinary metabolites following oral, intraperitoneal, or intravenous exposure of rabbits (Jondorf et al. 1955; Kohli et al. 1976; Parke and Williams 1960), rats (Bakke et al. 1992; Lingg et al. 1982), and monkeys (Lingg et al. 1982). Identified metabolites are consistent with the initial formation of phenolic intermediates (indicative of arene oxide intermediates), which become conjugated with glutathione or glucuronic acid prior to excretion in the

urine or bile. A comparison of rat and monkey urinary metabolites indicated that glutathione conjugation was the predominant pathway in rats, whereas glucuronidation was predominant in monkeys (Lingg et al. 1982). Figure 3-6 diagrams hypothesized metabolic pathways of trichlorobenzene isomers. To date, enzymes involved in the proposed steps have not been definitively established, but based on analogy to benzene and other halogenated benzenes, cytochrome P450 isozymes likely catalyze the initial formation of phenols through arene oxides.

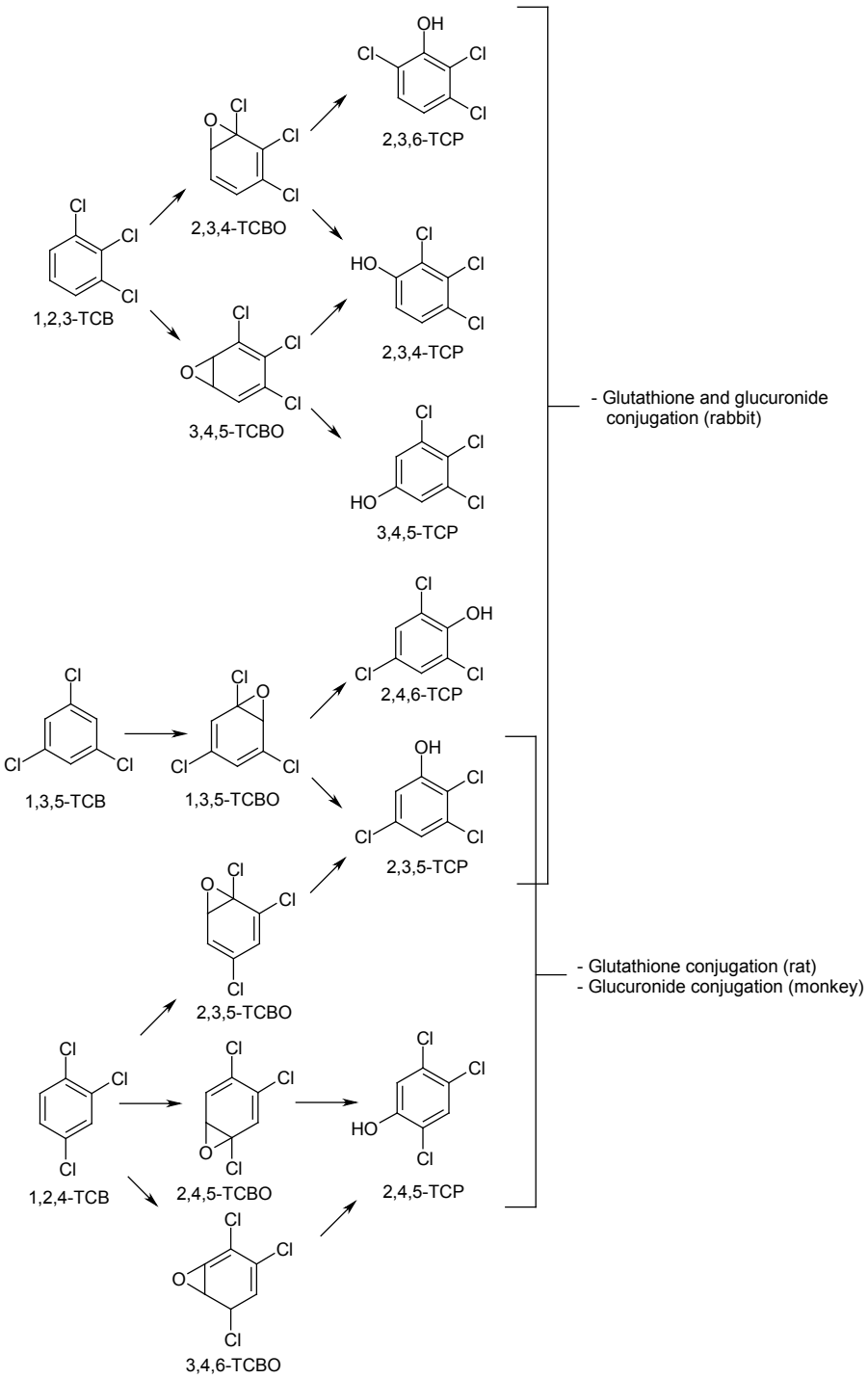
1,2,3-Trichlorobenzene. Jondorf et al. (1955) identified urinary metabolites of all three isomers in Chinchilla rabbits given a single oral 500 mg/kg dose. Spectrophotometric analysis indicated that 1,2,3-trichlorobenzene was the most rapidly metabolized of the three isomers. This isomer was mostly metabolized to 2,3,4-trichlorophenol, but lesser amounts of 3,4,5-trichlorophenol and 3,4,5-trichloro-catechol were also detected. Of the administered dose, 62% was excreted in urine as oxygen conjugates containing glucuronic and sulphuric acids within 5 days. Major metabolite excretions rose to a maximum on the first day after dosing and were no longer detectable in rabbit urine after 5 days.

Kohli et al. (1976) identified urinary metabolites of trichlorobenzene isomers following a single 60–75 mg/kg intraperitoneal injection in male rabbits, finding similar results as those reported by Jondorf et al. (1955). Specifically, GC and mass spectrometry (MS) revealed the major metabolite of 1,2,3-trichlorobenzene to be 2,3,4-trichlorophenol with 2,3,6- and 3,4,5-trichlorophenol appearing at lower levels.

1,2,4-Trichlorobenzene. The only relevant information regarding metabolism of 1,2,4-trichlorobenzene in humans is that incubation of 1,2,4-trichlorobenzene with microsomes derived from cell lines expressing human CYP1A1, CYP1A2, CYP3A4, CYP2E1, and CYP2D6 showed that CYP2E1had the highest enzymatic activity towards the chemical (Bogaards et al. 1995). The investigators also reported that in microsomal preparations from 22 human livers, CYP2E1 was the major enzyme involved in the formation of 2,3,5-trichlorophenol and 2,3,4-trichlorophenol from 1,2,4-trichlorobenzene, whereas CYP3A4 was responsible for the formation of 2,3,6-trichlorophenol.

In Chinchilla rabbits given a single 500 mg/kg dose by gavage, 1,2,4-tricholorobenzene was primarily metabolized to 2,4,5- and 2,3,5-trichlorophenol, with 5-day urinary metabolites of the isomer consisting of 38% glucuronic and sulphuric acid conjugates (Jondorf et al. 1955). Similar findings were obtained in a study examining urinary metabolites of trichlorobenzene isomers following a single 60–75 mg/kg intraperitoneal injection in male rabbits (Kohli et al. 1976). This study indicated that the primary

Figure 3-6. Hypothesized Metabolic Pathways for Trichlorobenzene Isomers Through Arene Oxide Intermediates



TCB = trichlorobenzene; TCBO = trichlorobenzene oxide; TCP = trichlorophenol

Source: Adapted from Kohli et al. (1976), with information from Bakke et al. (1992), Lingg et al. (1982), and Parke and Williams (1960).

metabolites of 1,2,4-trichlorobenzene were 2,3,5- and 2,4,5-trichlorophenol, while those of 1,3,5-trichlorobenzene were 2,3,5- and 2,4,6-trichlorophenol.

Further studies of 1,2,4-trichlorobenzene metabolism monitored urinary metabolites from rats and monkeys following oral or intravenous administration of single 10 mg/kg doses (Lingg et al. 1982). Although this study revealed similar metabolites as those previously observed in rabbits, some species-specific differences in conjugated metabolites were apparent. In rats, 60–62% of the urinary metabolites consisted of the mercapturic acids 2,3,5- and 2,4,5-N-acetyl-S-(trichlorophenyl)-L-cysteine 24 hours after dose administration. This finding suggests that conjugation with glutathione is the major metabolic pathway and that arene oxides, such as 3,4,6-trichlorobenzene oxide, are likely reactive metabolic intermediates in rats. In the urine of monkeys, isomeric glucuronides of 3,4,6-trichloro-3,5 cyclo-hexadiene accounted for between 48 and 61% of urinary metabolites. Sulfur-containing metabolites were not found in the urine of monkeys following oral dosing with 10 mg/kg, indicating that conjugation of metabolic intermediates to glucuronic acid, not glutathione conjugation, is important in the monkey.

A later study in bile-duct cannulated male Sprague-Dawley rats reported that over 60% of a 21 mg/kg oral dose of ^{14}C-1,2,4-trichlorobenzene was excreted in bile, while 21 and 2% were excreted in the urine and feces, respectively, within 24 hours (Bakke et al. 1992). In intact rats, about 70 and 9% of the administered dose were excreted in the urine and feces, respectively, within 24 hours. In urine from cannulated rats, the major identified metabolites were consistent with catabolism following glutathione conjugation of phenolic intermediates. *S*-(trichlorophenyl)-*N*-(acetyl) cysteine was the major urinary metabolite identified; *S*-(dichloro-hydroxyphenol)-*N*-(acetyl) cysteine (another mercapturic acid), trichlorothiophenol, and trichlorophenol were present at lesser concentrations. Only trace levels of glucuronides and sulphate esters were detected. Bile showed a wider range of metabolites consistent with catabolism of glutathione conjugates and relatively more trichlorothiophenol compared with urine. A single 3.5 mg intraperitoneal injection of ^{14}C-2,4,5-trichlorothiophenol resulted in excretion of 17% of the administered dose as *S*-glucuronide and 36% as *S*-(methylsulphonyl-dichlorophenyl)-mercapturic acid, metabolites not found in the excrement of rats dosed with 1,2,4-trichlorobenzene. These findings led to the suggestion that trichlorothiophenols are not major intermediates or end-products of enzymatic metabolism of trichlorobenzene in rats, but were formed from enterohepatic circulation via intestinal flora. This was later confirmed by Kato et al. (1993) who suggested that the formation of methylsulfonyl metabolites of 1,2,4-trichlorobenzene involves the initial biliary secretion of 1,2,4-trichlorobenzene metabolites into the intestinal tract, followed by metabolism by intestinal microflora and the absorption of secondary metabolites into the systemic circulation.

The metabolism of 1,2,4-trichlorobenzene *in vitro* has also been investigated. Incubation of 1,2,4-trichlorobenzene with liver microsomes from male rats pretreated with dexamethasone (DEX) showed the formation of trichlorohydroquinone in addition to various trichlorophenols (Den Besten et al. 1991). Trichlorohydroquinone, which appeared to be formed as a result of secondary metabolism, was found to covalently interact with microsomal protein. Both the conversion and the covalent binding of 1,2,4-trichlorobenzene were mediated by cytochrome P-450 as shown by the dependence on the presence of NADPH and the inhibitory action of metyrapone. The addition of glutathione reduced the covalent binding almost completely through the formation of water soluble conjugates. Pretreatment of the rats with different inducers resulted in preferential formation of different trichlorophenols. Induction with DEX resulted in preferential formation of 2,3,6-trichlorophenol, whereas other inducers preferentially produced 2,4,5-trichlorophenol. 2,4,6-Trichlorophenol was a minor metabolite in all microsomal suspensions, whereas 2,3,4-trichlorophenol and 2,3,5-trichlorophenol were formed only in trace amounts. Adding DNA to the microsomal suspension resulted in covalent binding of 1,2,4-trichlorophenol with the DNA, although to a much lesser extent than with proteins.

1,3,5-Trichlorobenzene. Following oral administration of a 500 mg/kg dose of 1,3,5-trichlorobenzene in Chinchilla rabbits, only one phenol was detected in the urine (2,4,6-trichlorophenol), and only 23% of the administered dose was excreted as conjugates of glucuronic and sulfuric acids within 5 days (Jondorf et al. 1955). A subsequent study by Parke and Williams (1960) further detailed the metabolism of 1,3,5-trichlorobenzene in Chinchilla rabbits given a single 500 mg/kg dose by gavage. Within the first 3 days after dosing, 2,4,6-trichlorophenol and some minor monochlorophenols were excreted in the urine. 2,4,6-Trichlorophenol continued to be excreted from days 4 through 9 with the addition of 4-chlorophenol elimination. The principal urinary metabolites identified in urine collected from male rabbits following an intraperitoneal injection of 300 mg 1,3,5-trichlorobenzene were 2,3,5- and 2,4,6-trichlorophenol (Kohli et al. 1976). A third more polar metabolite was identified, but insufficient material was available to determine the structure of the compound by MS.

3.4.4 Elimination and Excretion

No information was located regarding elimination and excretion of trichlorobenzenes in humans following any route of exposure.

3.4.4.1 Inhalation Exposure

No data were found on the elimination and excretion of trichlorobenzenes following inhalation exposure in animals.

3.4.4.2 Oral Exposure

All three isomers of trichlorobenzene have been shown to be rapidly and efficiently eliminated following oral exposure, principally via metabolites in the urine in rabbits (Jondorf et al. 1955) and rats (Bakke et al. 1992; Chu et al. 1987), although enterohepatic biliary circulation has been demonstrated in bile-cannulated rats (Bakke et al. 1992).

1,2,3-Trichlorobenzene. Jondorf et al. (1955) illustrated the rapid elimination of 1,2,3-trichlorobenzene in Chinchilla rabbits administered a 500 mg/kg oral dose. Five days after dosing, 78% of the administered dose had been excreted as trichlorophenols and 62% as oxygen conjugates in urine, with no trace of the isomer found in feces. In rats, 92% of ^{14}C-labeled 1,2,3-trichlorobenzene was eliminated through the urine and feces within 24 hours of a single 10 mg/kg gavage dose (Chu et al. 1987). Overall excretion increased to 95% at 48 hours post ingestion. The percentage of the radiolabeled 1,2,3-isomer in urine alone accounted for 56 and 59% at 24 and 48 hours, respectively. Data for radioactivity in tissues measured at 0.5, 1, 2, and 4 hours and 1, 2, 7, 14, 28, and 56 days after dose administration were fit to a two-compartment elimination model; estimated half-lives were 9.2 and 145 hours for the first and second compartments, respectively.

1,2,4-Trichlorobenzene. Chinchilla rabbits excreted 42% of a single 500 mg/kg oral dose of 1,2,4-trichlorobenzene as trichlorophenols 5 days after administration (Jondorf et al. 1955). This same study also noted that 38% of the administered dose was excreted in the urine as oxygen conjugates during the same time frame. A later study in rats dosed with 50 mg/kg ^{14}C-labeled 1,2,4-trichlorobenzene revealed 66 and 17% excretion of radioactivity in urine and feces, respectively, 7 days post administration (Tanaka et al. 1986). Radioactivity in expired air consisted of 2.1% of the absorbed dose. Excretion in all three excreta reached a peak on day 3. Biliary excretion accounted for 45% of the radioactivity. The authors considered the enterohepatic circulation of trichlorobenzene metabolites in the body as a possible explanation for the difference in biliary and fecal excretion rates. Bakke et al. (1992) reported that >60% of a 21 mg/kg dose of ^{14}C-1,2,4-trichlorobenzene was excreted in bile, while 21% was excreted in the urine of bile-cannulated Sprague-Dawley rats within 24 hours. In control rats without bile cannulation,

70 and 9% of the administered radioactivity was excreted within 24 hours in the urine and feces, respectively (Bakke et al. 1992).

Following a longer period of exposure, 181.5 mg/kg/day of ^{14}C-labeled 1,2,4-trichlorobenzene for 7 consecutive days, Smith and Carlson (1980) found that the urinary and fecal excretion of the isomer-derived radioactivity in Sprague-Dawley rats peaked during the first 3 days of dosing and declined rapidly thereafter. Radioactivity in the feces was not detectable past day 15 and accounted for only 4% of the administered dose. Urinary excretion, however, displayed detectable radioactivity 21 days after the first oral administration. Radioactivity in the urine accounted for approximately 72% of the administered dose.

Showing variations between species, Lingg et al. (1982) measured excretion rates in both monkeys and rats given a 10 mg/kg oral dose of ^{14}C-labeled 1,2,4-trichlorobenzene. By 24 hours after dosing, monkeys had excreted 40% of the administered dose in the urine. Less than 1% was found in the feces. Rats excreted 84% of the oral dose via the urine by 24 hours; 11% was found in feces. Following intravenous administration of 10 mg/kg ^{14}C-labeled 1,2,4-trichlorobenzene, monkeys eliminated about 22% of the administered radioactivity in urine and none in the feces 24 hours after exposure. Rats, on the other hand, excreted 78 and 7% of the administered dose in urine and feces at 24 hours, respectively. Regardless of the route of exposure, rats excreted radioactivity at a rate roughly 2–3 times faster than monkeys following administration of ^{14}C-labeled 1,2,4-trichlorobenzene.

1,3,5-Trichlorobenzene. In Chinchilla rabbits dosed with a single 500 mg/kg dose by gavage, 9% of the administered dose was excreted as trichlorophenols in the urine during the 5 days after dosing (Jondorf et al. 1955), while 23% was excreted as oxygen conjugates. A follow-up study indicated that only 4 and 14% of a 500 mg/kg dose were eliminated as phenols 8 and 9 days after dosing, respectively (Parke and Williams 1960). A much higher level of excretion, 82% of ^{14}C-labeled 1,3,5-trichlorobenzene, was found in the urine and feces of Sprague-Dawley rats within 24 hours of a single 10-mg/kg gavage dose (Chu et al. 1987). Overall excretion increased to 89% at 48 hours post ingestion. The percentage of the radiolabeled 1,3,5-isomer in urine alone accounted for 47 and 50% at 24 and 48 hours, respectively. Data for radioactivity in tissues measured at 1, 2, 7, 14, 28, and 56 days after dose administration were fit to a two-compartment elimination model; estimated half-lives were 8 and 67.5 hours for the first and second compartments, respectively (Chu et al. 1987).

3.4.4.3 Dermal Exposure

No data on the elimination and excretion of trichlorobenzenes following dermal exposure were located for animals.

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

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

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 et al. 1987; Andersen and Krishnan 1994). 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 parametrization, (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 3-7 shows a conceptualized representation of a PBPK model.

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

No PBPK/PD models have been developed for trichlorobenzenes.

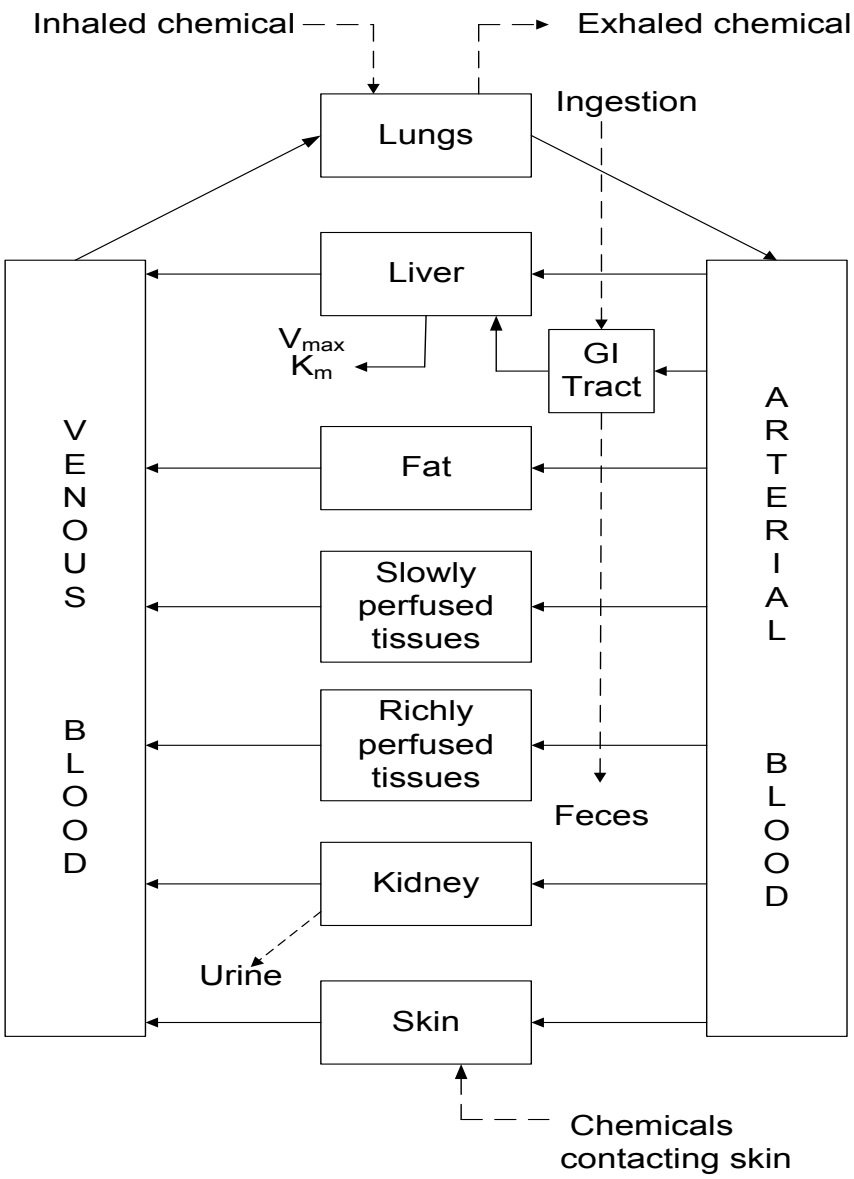
3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Studies in animals indicate that trichlorobenzenes are rapidly and efficiently absorbed through the gastrointestinal tract. However, the specific mechanisms by which this occurs have not been evaluated. Given that they are fairly soluble in lipids, it is reasonable to assume that absorption will occur through passive diffusion. No specific information was located regarding mechanisms of inhalation or dermal absorption. In addition, no relevant information was located regarding mechanisms of transport of trichlorobenzenes (or metabolites) in the blood, distribution to tissues, or storage in tissues.

The role of metabolism on the liver effects of 1,2,4-trichlorobenzene, specifically enzyme induction and alterations in heme metabolism, has been examined by Kato and coworkers (Kato and Kimura 2002; Kato et al. 1988, 1993). Intraperitoneal injection of the metabolite 2,3,5-trichlorophenyl methyl sulfone to

Figure 3-7. 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

male Wistar rats resulted in significant increases in the activities of aminopyrine N-demethylase and cytochrome P-450 content in liver microsomes, reaching a maximum at 48–72 hours after the injection (Kato et al. 1993). Significant increases in aniline hydroxylase and cytochrome b_5 contents were also observed at 12–120 hours and 24–120 hours, respectively. The rise and fall in enzyme activities correlated with the rise and fall of the hepatic concentration of 2,3,5-trichlorophenyl methyl sulfone rather than with the hepatic concentration of 1,2,4-trichlorobenzene. This was consistent with the estimated elimination half-lives from the liver of 5.2 hours and 37.8 hours for 1,2,4-trichlorobenzene and 2,3,5-trichlorophenyl methyl sulfone, respectively. In this study, 2,4,5-trichlorophenyl methyl sulfone was found to be a weaker inducer, although an earlier study had shown 2,4,5-trichlorophenyl methyl sulfone to induce a component of the microsomal electron transport system and also uridine diphosphate-glucuronyltransferase, a phase II metabolic enzyme (Kato et al. 1988). Overall, these findings strongly suggest that the inducing effect of 1,2,4-trichlorobenzene on liver microsomal drug-metabolizing enzymes is not due to the parent compound, but to its methysulfonyl metabolite 2,4,5-trichlorophenyl methyl sulfone.

1,2,4-Trichlorobenzene has been shown to induce ALA synthetase, the rate-limiting enzyme in the biosynthesis of heme (Ariyoshi et al. 1975a, 1975b) and also heme oxygenase, the rate-limiting enzyme in the degradation of heme (Kato and Kimura 2002). Administration of 1,2,4-trichlorobenzene or 2,3,5-trichlorophenyl methyl sulfone to male Wistar rats resulted in significant increases in ALA synthetase activity, but only 1,2,4-trichlorobenzene induced heme oxygenase (Kato and Kimura 2002). In both cases, there were significant increases in the contents of cytochrome P-450, which paralleled increases in total heme content. In rats with reduced liver glutathione levels by pretreatment with buthionine-(*S*,*R*)-sulfoximine (BSO) and dosed with 1,2,4-trichlorobenzene, 2,3,5-trichlorophenyl methyl sulfone was present in the liver at much lower concentrations than in rats not pretreated with BSO. In addition, 1,2,4-trichlorobenzene did not induce ALA synthetase in BSO-treated rats, but 2,3,5-trichlorophenyl methyl sulfone did, suggesting that the induction of ALA synthetase in rats dosed with 1,2,4-trichlorobenzene is not due to 1,2,4-trichlorobenzene, but to 2,3,5-trichlorophenyl methyl sulfone. Kato and Kimura (2002) suggested that the prolonged induction of ALA synthetase by 2,3,5-trichlorophenyl methyl sulfone results in excess heme biosynthesis, which overrides the destruction of heme that results from the induction of heme oxygenase by 1,2,4-trichlorobenzene or a metabolite other than 2,3,5-trichlorophenyl methyl sulfone. This is consistent with the induction of porphyria in rats dosed with 1,2,4-trichlorobenzene (i.e., Rimington and Ziegler 1963).

No studies were located that examined the effect of dose on metabolism or excretion pathways of trichlorobenzenes.

3.5.2 Mechanisms of Toxicity

The toxicity of trichlorobenzenes does not appear to be route-dependent, and the liver appears to be the main target regardless of the duration of exposure.

The mechanism(s) of liver toxicity induced by trichlorobenzenes has not been elucidated, but it probably involves arene oxide intermediates which form during the initial transformation to trichlorophenols. As mentioned above, 1,2,4-trichlorobenzene, and presumably the other isomers, induce a number of drug-metabolizing enzymes in the liver of rats which will affect the biotransformation of other chemicals. Whether or not this will result in increased toxicity of other xenobiotics will depend on whether the toxicity of the other chemical is due to the parent compound or to a metabolite. Also, as mentioned above, exposure to 1,2,4-trichlorobenzene induced porphyria in rats by inducing ALA synthetase and thus increasing heme production. Trichlorobenzenes and other chlorinated benzenes induce nephropathy in the male rat by a mechanism that involves a series of events beginning with an excessive accumulation of hyaline droplets. This appears to be unique to the male rat and is not relevant for human risk assessment. A study of 1,2,4-trichlorobenzene with liver microsomes from male rats *in vitro* reported the formation of trichlorohydroquinone as a result of secondary metabolism (Den Besten et al. 1991). Trichloro-hydroquinone was found to covalently interact with microsomal protein and with added DNA. Whether or not this plays a role in the *in vivo* toxicity of 1,2,4-trichlorobenzene is unknown.

3.5.3 Animal-to-Human Extrapolations

There is virtually no information on health effects of trichlorobenzenes in humans, so the animal species that is the most appropriate model for human exposure is not known. The liver is the target for trichlorobenzenes in animals, and rats appear to be more sensitive than other species. Based on information from effects of other chlorinated benzenes in humans, and from limited information on the metabolism of 1,2,4-trichlorobenzene by microsomal preparations from human livers that indicated that cytochrome P-450 enzymes might be involved in the metabolism of trichlorobenzenes, it is reasonable to suggest that excessive exposure to trichlorobenzenes might induce liver effects such as porphyria in humans.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Thomas and Colborn (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC) which in 1998 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 terms *endocrine modulators* have also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There is no evidence from the reproductive and developmental studies, summarized in Sections 3.2.2.5 and 3.2.2.6, respectively, that suggests that trichlorobenzenes affect the neuroendocrine axis.

The only relevant information that was located regarding *in vitro* tests is that 1,2,4-trichlorobenzene and 1,2,3-trichlorobenzene tested negative for estrogenic activity in a reporter gene expression assay using yeast cells (Nishihara et al. 2000). A substance was considered positive when its activity was more than 10% of the activity of 10^{-7}M 17β-estradiol.

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).

No studies were located that described health effects in children following exposure to trichlorobenzenes. Also, no studies were located that compared the health effects of these compounds in young and adult animals to ascertain potential age-related differences in susceptibility.

Standard developmental toxicity studies in animals do not suggest that trichlorobenzenes are embryotoxic or teratogenic or that they alter the development of young animals. The only effects reported in a study with 1,2,4-trichlorobenzene in rats were the presence of microscopic alterations in the lenses of the eye of fetuses from dams treated with 150 mg/kg/day 1,2,4-trichlorobenzene on Gd 6–15 and sacrificed on Gd 22 (Black et al. 1988). However, no lesions were observed in fetuses from dams dosed with 300 mg/kg/day 1,2,4-trichlorobenzene. This lesion was also observed in fetuses from dams dosed with 150, 300, or 600 mg/kg/day 1,3,5-trichlorobenzene; however, since no quantitative data were presented, it is not known whether the incidences were dose-related. Another gestational exposure study reported retarded development of the fetuses from rats dosed with 360 mg/kg/day 1,2,4-trichlorobenzene on Gd 9–13 and sacrificed on Gd 14 (Kitchin and Ebron 1983). This dose level was lethal to two out nine dams and induced significant weight loss in dams that survived, which may have contributed to the delayed development of the fetuses. In studies of pregnant mice dosed with 0 or 130 mg/kg/day 1,2,4-trichlorobenzene on Gd 8–12, the chemical did not affect pup's viability or growth, or offspring's locomotor activity or fertility to produce a second generation (Chernoff and Kavlock 1983; Gray and Kavlock 1984; Gray et al. 1986).

Trichlorobenzenes have been identified in breast milk; therefore, infants may also be potentially exposed through breast feeding (see Section 6.6, Exposures of Children). No information was located regarding the pharmacokinetics of these compounds in children, regarding biomarkers of exposure or effect for these compounds in children, or regarding interactions with other chemicals in 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), 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 exposures 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 trichlorobenzenes 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 trichlorobenzenes 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 Trichlorobenzenes

Trichlorobenzenes have been detected in blood (Bristol et al. 1982; Mes 1992; Pellizzari et al. 1985a), adipose tissue (Mes 1992), and exhaled breath (Pellizzari et al. 1985b) and their presence in the body can be used as biomarkers of exposure to trichlorobenzenes. However, metabolites of trichlorobenzenes, such as trichlorophenols, cannot be used as specific biomarkers for exposure to trichlorobenzenes because they can also be generated from the metabolism of other chlorinated compounds such as the pesticide lindane (Agency for Toxic Substances and Disease Registry 2005).

3.8.2 Biomarkers Used to Characterize Effects Caused by Trichlorobenzenes

No specific biomarker of effects could be identified from the very limited information regarding humans exposed to trichlorobenzenes (see Section 3.2.1.2, Systemic Effects). Based on the existing information regarding the effects of trichlorobenzenes in animals, it is difficult to envision a health condition that could be attributed solely to exposure to trichlorobenzenes.

3.9 INTERACTIONS WITH OTHER CHEMICALS

No studies were located regarding interactions among trichlorobenzenes and limited data were found regarding interactions between trichlorobenzenes and other chemicals. Since trichlorobenzenes are inducers of liver microsomal enzymes (Ariyoshi et al. 1975a, 1975b; Carlson and Tardiff 1976; Kato and Kimura 2002; Kato et al. 1988, 1993; Kitchin and Ebron 1983), they will affect the metabolism of other compounds. For example, gavage administration of 1,2,4-trichlorobenzene at 600 mg/kg/day to rats for 14 days significantly decreased hexobarbital sleeping time (Carlson and Tardiff 1976). In another study, 1,2,4-trichlorobenzene increased the LD_{50} values for malathion, malaoxon, parathion, and paraoxon in mice (Townsend and Carlson 1981). In the same study, 1,2,4-trichlorobenzene protected against the decrease in brain cholinesterase induced by malathion, but not against reductions in liver, plasma, or RBC cholinesterase. The protection afforded by 1,2,4-trichlorobenzene correlated well with increased carboxylesterase activity. These examples can be considered inhibitory types of interactions.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

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

A specific target for trichlorobenzene toxicity in humans exposed to these compounds has not been identified, but it is reasonable to assume that the liver could be a main target based on studies in animals. Therefore, individuals with compromised liver function may represent a susceptible population. No information was located regarding whether or not children represent a group unusually susceptible to trichlorobenzenes.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to trichlorobenzenes. 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 trichlorobenzenes. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. No texts were identified that provide specific information about treatment following exposures to trichlorobenzenes.

3.11.1 Reducing Peak Absorption Following Exposure

The following information has been extracted from HSDB (2010). Methods for reducing peak absorption of trichlorobenzenes include gut dilution with water or milk and eye irrigation with water or sterile saline. Activated charcoal is not recommended, as it may promote vomiting and make endoscopic evaluation difficult. If exposure occurs via the dermal route, contaminated clothing should be removed and affected areas should be washed with soap. Exposure to fresh air or oxygen treatment is recommended to reduce absorption after inhalation exposure to trichlorobenzenes.

3.11.2 Reducing Body Burden

No information was located regarding reducing body burden of trichlorobenzenes following exposure to these chemicals.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Studies in animals indicate that isomers of trichlorobenzene enhance xenobiotic metabolism via the induction of numerous hepatic drug-metabolizing enzymes including cytochromes c and P-450, glucuronyltransferase, glutathione S-transferase, and microsomal proteins (Ariyoshi et al. 1975a, 1975b; Kato and Kimura 2002; Kato et al. 1988, 1993). Also induced by 1,2,4-trichlorobenzene is ALA synthetase, the rate-limiting enzyme in heme biosynthesis (Ariyoshi et al. 1975a, 1975b), which is the cause of hepatic porphyria in rats treated with 1,2,4-trichlorobenzene (Rimington and Ziegler 1963). Studies suggest that both the induction of hepatic microsomal drug-metabolizing enzymes and ALA synthetase may not be mediated directly by 1,2,4-trichlorobenzene, but by its metabolite 2,3,5-trichlorophenyl methyl sulfone (Kato and Kimura 2002; Kato et al. 1988, 1993). Since 2,3,5-trichlorophenyl methyl sulfone results from the conjugation of glutathione with a 1,2,4-trichlorobenzene hydroxyl derivative, theoretically, reducing glutathione levels would prevent, at least in part, the effects of 1,2,4-trichlorobenzene.

3.12 ADEQUACY OF THE DATABASE

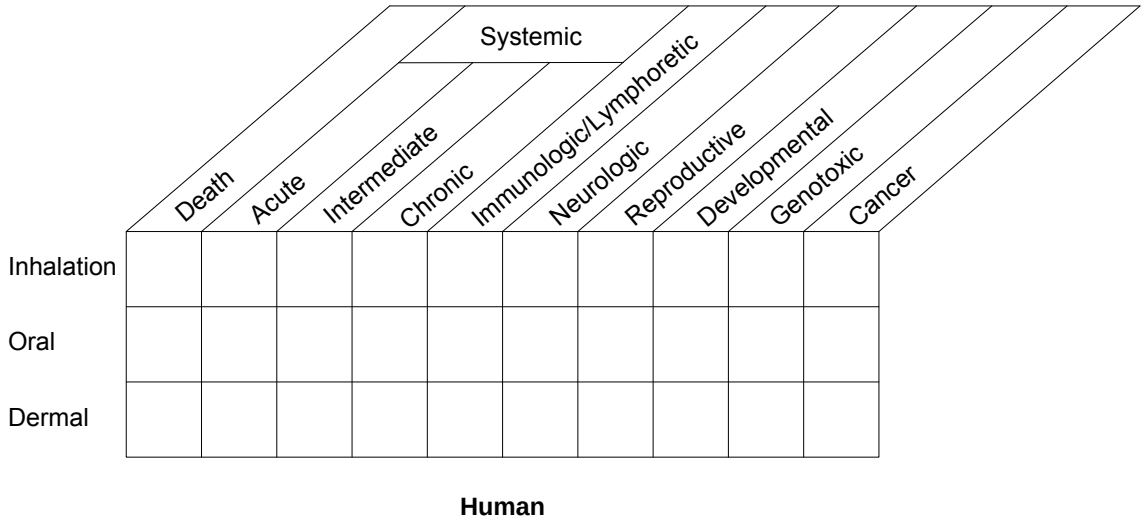
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of trichlorobenzenes is available. Where adequate information 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 trichlorobenzenes.

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.

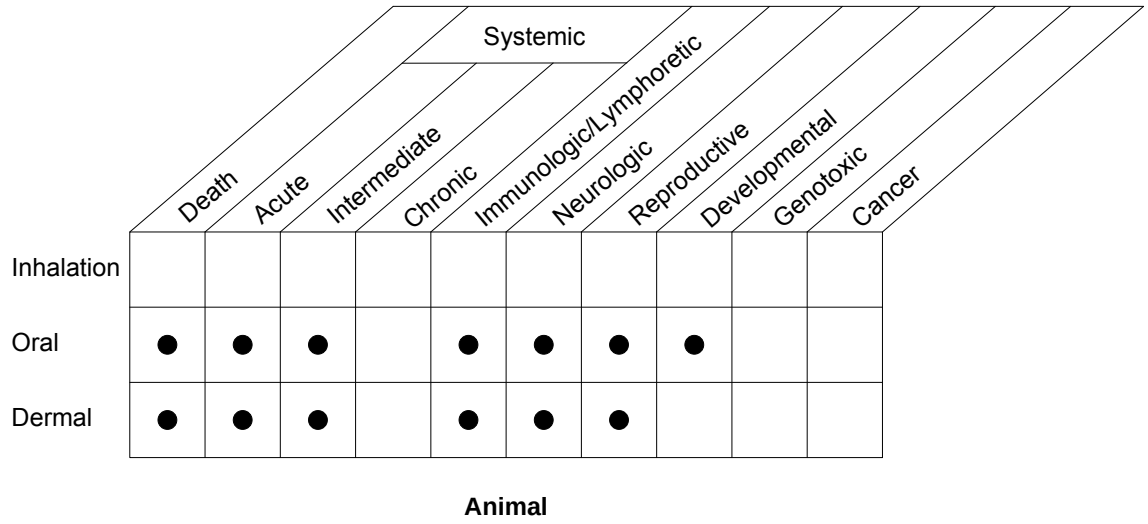
3.12.1 Existing Information on Health Effects of Trichlorobenzenes

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to 1,2,3- ,1,2,4-, and 1,3,5-trichlorobenzenes are summarized in Figures 3-8, 3-9, and 3-10, respectively. The purpose of these figures is to illustrate the existing information concerning the health effects of

Figure 3-8. Existing Information on Health Effects of 1,2,3-Trichlorobenzene



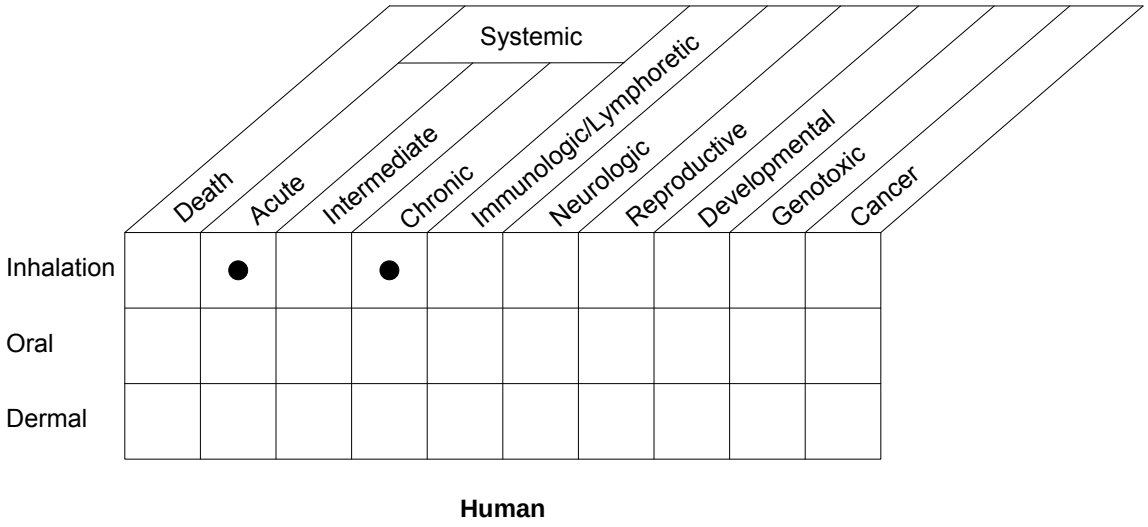
Human



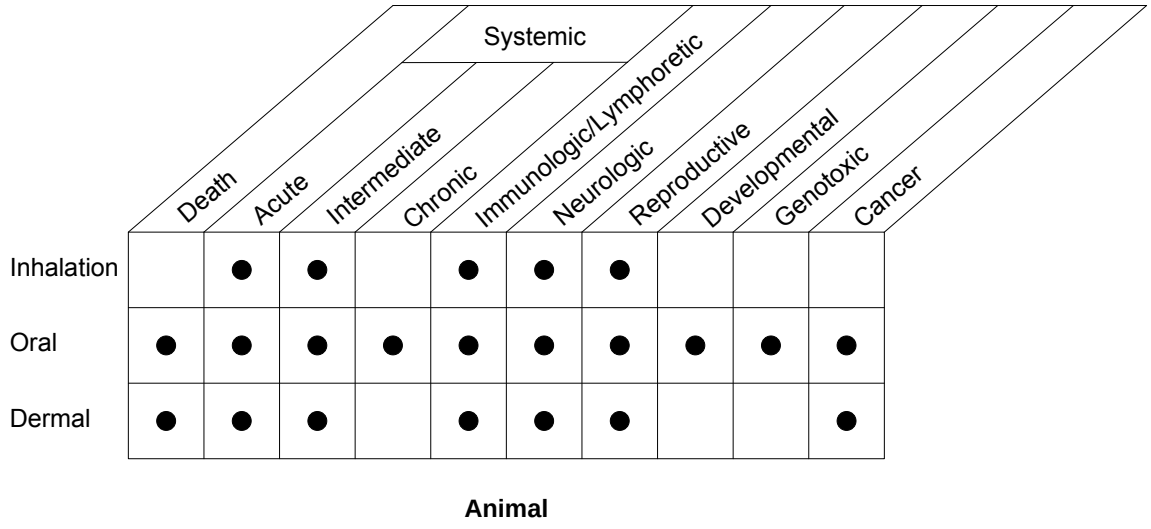
Animal

● Existing Studies

Figure 3-9. Existing Information on Health Effects of 1,2,4-Trichlorobenzene



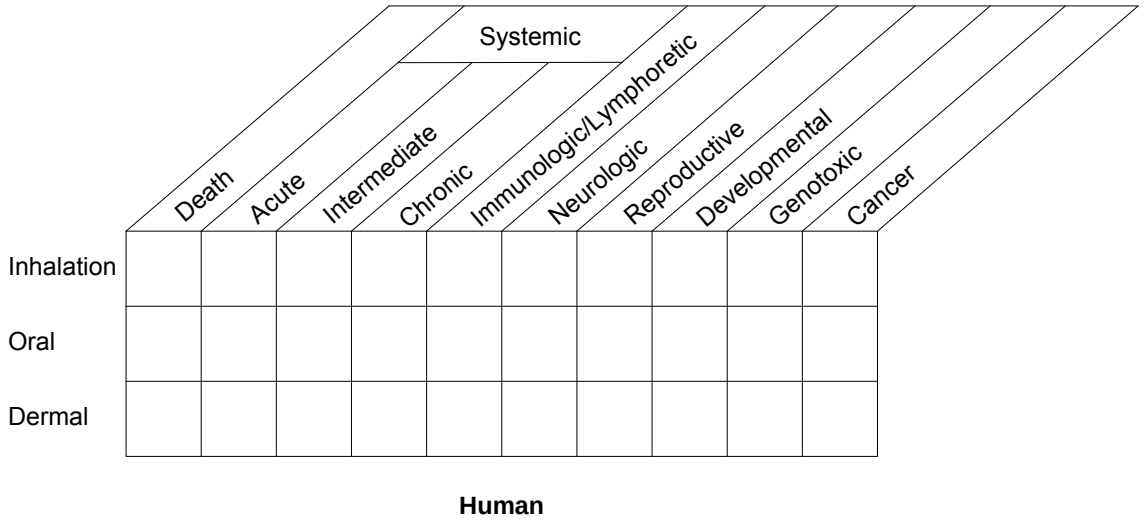
Human



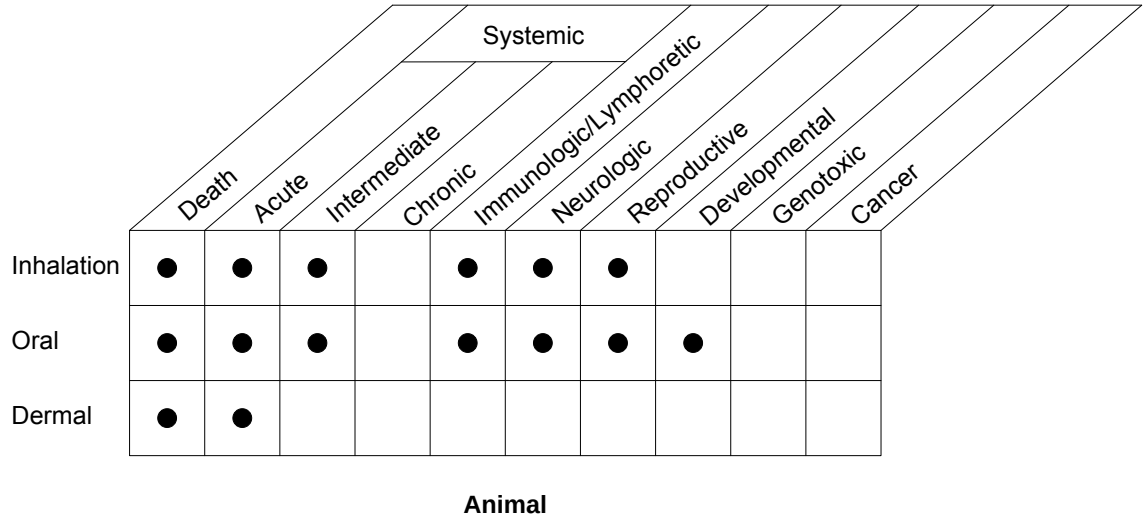
Animal

● Existing Studies

Figure 3-10. Existing Information on Health Effects of 1,3,5-Trichlorobenzene



Human



Animal

● Existing Studies

trichlorobenzene. Each dot in the figures 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.

There is virtually no information regarding health effects in humans exposed to trichlorobenzenes. Information is available regarding health effects in animals exposed orally and dermally to each one of the three trichlorobenzene isomers and exposed by inhalation to 1,2,4-trichlorobenzene and 1,3,5-trichlorobenzene. 1,2,4-Trichlorobenzene has been the most widely studied of the three isomers. Chronic-duration studies in animals are available only for 1,2,4-trichlorobenzene and only via the oral route of exposure. Studies in animals showed that the liver is a target for trichlorobenzene toxicity, particularly in rats. The kidney was also affected in male rats, but this effect appears to be a unique response of male rats to exposure to a variety of organic chemicals and not relevant to humans. 1,2,4-Trichlorobenzene was evaluated for carcinogenicity in rats and mice; this isomer induced malignant liver tumors in mice. Trichlorobenzenes induced transitory skin and eye irritation when applied onto the skin or instilled into the eyes of animals.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. No acute-duration studies were located in humans exposed by inhalation to trichlorobenzenes that could be used for derivation of an acute-duration MRL for this route. A review of the literature indicates that an adult male who inhaled trichlorobenzene for several hours during the repair of a pump suffered massive hemoptysis, and that some trichlorobenzene production workers developed chloroacne (IPCS 1991). Citing an unpublished source, ACGIH (2001) stated that minimal eye and throat irritation could occur in some people exposed to 3–5 ppm 1,2,4-trichlorobenzene. No relevant inhalation studies were located of animals exposed to 1,2,4-trichlorobenzene or 1,3,5-trichlorobenzene, and no information was located for 1,2,3-trichlorobenzene. A decision to conduct studies in animals for possible derivation of acute-duration inhalation MRLs for trichlorobenzenes has to be made after evaluating the likelihood that exposure to high concentrations of these chemicals for short periods of time will occur. No information was located regarding oral toxicity of trichlorobenzenes in humans. Aside from studies that provided information on lethal doses of trichlorobenzenes in animals (Brown et

al. 1969; Côté et al. 1988; Jorgenson et al. 1976), there are few studies available that examined the effects of acute oral exposure to these compounds. A developmental study in rats exposed to the three trichlorobenzene isomers during Gd 6–15 conducted histological evaluations of tissues and organs from the dams sacrificed on Gd 22 (Black et al. 1988). The liver and possibly the thyroid appeared to be targets for trichlorobenzenes; however, no quantitative data were presented, so NOAELs or LOAELs could not be defined and the study could not be used for MRL derivation. A 14-day gavage study with 1,2,4-trichlorobenzene in rats by Carlson and Tardiff (1976) identified the liver as a target for this isomer based on increases in liver weight and enzyme induction, but did not provide data regarding histopathology of the liver; therefore, it was considered inadequate for MRL derivation. While additional studies that provide adequate data for establishing dose-response relationships for acute exposure to the trichlorobenzenes would be valuable, particularly for liver effects, it is unlikely that acute oral exposure to high amounts of trichlorobenzenes will occur in humans. Studies in animals are available indicating that trichlorobenzenes are mild to moderate skin and eye irritants (Brown et al. 1969; Dow Chemical 1956; E.I. Dupont 1971; Jorgenson et al. 1976; Yamamoto et al. 1978). Additional acute-duration dermal studies do not seem necessary at this time.

Intermediate-Duration Exposure. No studies were located regarding health effects in humans exposed to trichlorobenzenes for intermediate duration periods by any route of exposure. Intermediate-duration inhalation studies in animals are available for 1,2,4-trichlorobenzene in various animal species (Coate et al. 1977; Gage 1970; Kociba et al. 1981) and for 1,3,5-trichlorobenzene in rats (Sasmore et al. 1983). The results showed that exposure to up to 100 ppm 1,2,4-trichlorobenzene for up to 26 weeks or 130 ppm 1,3,5-trichlorobenzene for 13 weeks had no significant effect on hematological and clinical chemistry tests or on histological appearance of tissues and organs. Although there is suggestive evidence from some studies that the liver might be a target for 1,2,4-trichlorobenzene, inadequacies in the studies (no quantitative data, too few animals) precluded derivation of an intermediate-duration inhalation MRL for this isomer. No intermediate-duration inhalation study for 1,2,3-trichlorobenzenes was located; however, there is no reason to believe that the results would have been different than those obtained for 1,2,4-trichlorobenzene or 1,3,5-trichlorobenzene. It should be noted that the exposure levels used in the animal studies are several orders of magnitude higher than those monitored in outdoor air in the United States in 2008 (EPA 2010a; see Section 6.4, Levels Monitored or Estimated in the Environment). Additional inhalation studies in animals do not seem necessary at this time. Wide-scope intermediate-duration oral studies in animals are available for 1,2,4-trichlorobenzene in rats (CMA 1989; Côté et al. 1988) and mice (Hiles 1989) and for 1,2,3-trichlorobenzene and 1,3,5-trichlorobenzene in rats (Côté et al. 1988). These studies identified the liver as the main target for trichlorobenzenes. Kidney effects were

also reported in male rats, but this response was characterized by hyaline droplet accumulation and was unique to the male and considered not relevant for human risk assessment. The studies by CMA (1989) in rats and Hiles (1989) in mice provided quantitative histological data, and the former was used to derive an intermediate-oral MRL for 1,2,4-trichlorobenzene. The study with 1,2,3-trichlorobenzene and 1,3,5-trichlorobenzene in rats (Côté et al. 1988) reported quantitative changes in liver weight and histological alterations in the liver and thyroid in treated groups of rats. However, quantitative histological data were not provided and, therefore, NOAELs and LOAELs for histological alterations could not be defined; thus, the study was considered inadequate for derivation of intermediate-duration oral MRLs for 1,2,3- and 1,3,5-trichlorobenzene. Additional studies that provide quantitative histological data would be valuable to define better points of departure for MRL derivation than organ weight. Intermediate-duration dermal studies are available for 1,2,4-trichlorobenzene (Brown et al. 1969; Powers et al. 1975; Rao et al. 1982). For the most part, effects were limited to the site of application of the chemical. Additional dermal studies with 1,2,4-trichlorobenzene do not seem necessary. Although no intermediate-dermal studies were located for 1,2,3-trichlorobenzene or 1,3,5-trichlorobenzene, it is unclear what key new information such studies would provide.

Chronic-Duration Exposure and Cancer. No studies were located regarding health effects in humans exposed chronically to trichlorobenzenes. Health evaluations of workers exposed to trichlorobenzenes may have been conducted, but none were identified in the literature reviewed. No chronic-duration inhalation animal studies are available for trichlorobenzenes. Since chlorobenzenes have been detected in outdoor air from cities in the United States (EPA 2010a), the general population is exposed to these chemicals by inhalation. However, as mentioned above, the exposure levels monitored in that study were several orders of magnitude lower than those used in intermediate-duration inhalation studies in animals which did not induce significant health effects. Therefore, the value of conducting chronic-duration inhalation studies with exposure concentrations around environmental levels is questionable. There are chronic-duration oral studies with 1,2,4-trichlorobenzene in rats (Moore 1994a) and mice (Moore 1994b). These studies reported adverse histological effects in the liver from rats and mice and also renal lesions in male rats. The liver effects in rats served as basis for the derivation of a chronic-duration oral MRL for 1,2,4-trichlorobenzene.

There are no studies of cancer in humans exposed to trichlorobenzenes. Both the chronic-duration oral study with 1,2,4-trichlorobenzene in rats (Moore 1994a) and mice (Moore 1994b) evaluated the animals for tumors and reported that 1,2,4-trichlorobenzene increased the incidence of hepatocellular carcinoma in both male and female mice. Additional cancer studies for this isomer do not appear necessary, but oral

toxicity/carcinogenicity studies for 1,2,3-trichlorobenzene and 1,3,5-trichlorobenzene seem warranted since the general population is exposed to these isomers through the consumption of fish and other foods (see Section 6.4.4, Other Environmental Media).

Genotoxicity. Trichlorobenzenes were not mutagenic in *in vitro* tests with prokaryotic organisms with or without metabolic activation (Haworth et al. 1983; Jorgenson et al. 1976; Kubo et al. 2002; Nohmi et al. 1985; Ono et al. 1992; Schoeny et al. 1979). It is unclear what key information additional studies would provide. 1,2,3-Trichlorobenzene and 1,2,4-trichlorobenzene were cytotoxic to mammalian cells in studies conducted in Chinese hamster V79 cells *in vitro*, a system that lacks metabolic activation capacity, indicating that the effect was due to the parent compound (Fratello et al. 1997). Other studies *in vitro* with mammalian cells also showed 1,2,4-trichlorobenzene to be cytotoxic (Garrett and Lewtas 1983; Shimada et al. 1983). Fratello et al. (1997) suggested that the toxicity of these chemicals was related to their ability to penetrate/perturbate the cellular membranes, but further studies examining this issue would be valuable. Limited studies of the genotoxicity of trichlorobenzenes *in vivo*, mostly micronuclei assays, indicate that these compounds are clastogenic (Mohtashamipur et al. 1987; Parrini et al. 1990). Studies that evaluate whether metabolites of trichlorobenzenes, such as methylsulfones, thought to be responsible for the induction of drug-metabolizing enzymes and alterations in heme metabolism (Kato and Kimura 2002; Kato et al. 1993) could provide insight into the mechanism of action of trichlorobenzenes.

Reproductive Toxicity. There is no information regarding reproductive effects in humans exposed to trichlorobenzenes. A multi-generation reproductive study in rats exposed to 1,2,4-trichlorobenzene in the drinking water is available (Robinson et al. 1981). In that study, 1,2,4-trichlorobenzene did not affect fertility. None of the intermediate-duration oral, inhalation, or dermal studies or the chronic-duration oral studies conducted with trichlorobenzenes reported treatment-related histological alterations in the reproductive organs of male and female animals (Black et al. 1988; CMA 1989; Coate et al. 1977; Côté et al. 1988; Hiles 1989; Kociba et al. 1981; Moore 1994a, 1994b; Rao et al. 1982; Sasmore et al. 1983). Additional studies with 1,2,4-trichlorobenzene or multi-generation studies with 1,2,3-trichlorobenzene or 1,3,5-trichlorobenzene do not seem warranted at this time.

Developmental Toxicity. No information was located regarding developmental effects in humans exposed to trichlorobenzenes. The three trichlorobenzene isomers have been tested for potential developmental toxicity in rats (Black et al. 1988; Kitchin and Ebron 1983; Robinson et al. 1981); 1,2,4-trichlorobenzene has also been tested in mice (Chernoff and Kavlock 1983; Gray and Kavlock 1984; Gray et al. 1986). In all these studies, 1,2,4-trichlorobenzene was administered by oral gavage

during pregnancy. Kitchin and Ebron (1983) reported retarded development of the fetuses from rats dosed with 360 mg/kg/day 1,2,4-trichlorobenzene; this dose level also induced significant weight loss in the dams, which may have contributed to the slower fetal development. In the studies in pregnant mice dosed with up to 130 mg/kg/day 1,2,4-trichlorobenzene on Gd 8–12, the chemical did not affect pup's viability or growth, or offspring's locomotor activity or fertility to produce a second generation. In the study by Black et al. (1988) with the three trichlorobenzene isomers, the only significant effect reported was the presence of microscopic alterations in the lenses of the eye of fetuses from rats treated with 150 mg/kg/day; no lesions were observed in fetuses from dams dosed with 75 or 300 mg/kg/day 1,2,4-trichlorobenzene. This lesion was not observed in fetuses from dams dosed with 150, 300, or 600 mg/kg/day 1,2,3-trichlorobenzene, but occurred in fetuses from dams administered 150, 300, or 600 mg/kg/day 1,3,5-trichlorobenzene; however, because no quantitative data were presented, it is not known whether the incidences were dose-related. It seems important to try to duplicate these findings and/or test a different animal species, and to also perform quantitative analyses of the results to obtain dose-response relationships. Since the toxicity of trichlorobenzenes does not seem to be route-dependent, developmental studies by the inhalation and dermal routes do not appear necessary.

Immunotoxicity. No information was located regarding immunological effects in humans exposed to trichlorobenzenes. The information available from studies in animals is limited to results of evaluations of the gross and microscopic morphology of lymphoreticular organs and tissues in some inhalation, oral, and dermal studies conducted with trichlorobenzenes (Black et al. 1988; CMA 1989; Coate et al. 1977; Côté et al. 1988; Hiles 1989; Kociba et al. 1981; Moore 1994a, 1994b; Powers et al. 1975; Rao et al. 1982; Sasmore et al. 1983). For the most part, no significant alterations have been reported. Tests for skin sensitization conducted with 1,2,4-trichlorobenzene or 1,3,5-trichlorobenzene in guinea pigs were negative (Brown et al. 1969; E.I. DuPont 1971; Jorgenson et al. 1976). No studies were located that examined immunocompetence in animals exposed to trichlorobenzenes. Since it is not uncommon to find that subtle changes in immunological parameters occur at exposure levels or doses of chemicals lower than (sometimes much lower) those that produce systemic toxicity, it would be useful to conduct screening studies (Tier I) to assess, for example, cell-mediated and humoral-mediated immunity in rodents exposed to trichlorobenzenes.

Neurotoxicity. No studies were located regarding neurological effects in humans exposed to trichlorobenzenes. Tremors and convulsions were reported in rats and mice administered lethal doses of 1,2,4-trichlorobenzene or 1,3,5-trichlorobenzene (Brown et al. 1969; Jorgenson et al. 1976). Several inhalation, oral, and dermal intermediate- and chronic-duration studies in animals examined the gross and

microscopic appearance of the brain, spinal cord, and peripheral nerve and reported no significant alterations (Black et al. 1988; CMA 1989; Coate et al. 1977; Côté et al. 1988; Hiles 1989; Kociba et al. 1981; Moore 1994a, 1994b; Rao et al. 1982; Sasmore et al. 1983). One intermediate-duration inhalation study in monkeys exposed to 1,2,4-trichlorobenzene conducted operant behavior tests in the animals throughout the exposure period and reported no exposure-related alterations (Coate et al. 1977). The data available suggest that the nervous system is not a sensitive target for trichlorobenzenes, but there has not been extensive testing of neurological parameters in animals during prolonged exposure to trichlorobenzenes.

Epidemiological and Human Dosimetry Studies. No epidemiological studies were identified for trichlorobenzenes. It is likely that health evaluations of workers exposed to 1,2,4-trichlorobenzene during the production of this chemical have been conducted at some point, but no data were located in the literature available for review. Studies in animals indicate that the main target for trichlorobenzenes is the liver. Therefore, should a population be identified as being exposed to high levels of trichlorobenzenes, liver function should be monitored with the appropriate tests.

Biomarkers of Exposure and Effect.

Exposure. A biomarker of exposure is an exogenous substance, or its metabolite(s) or the product of the interaction between a xenobiotic agent and some target molecule or cell that is measured within a compartment of an organism (e.g., measurement of the parent compound or its metabolite(s), DNA adducts, etc.).

Body burdens of trichlorobenzenes do not necessarily indicate that exposure to trichlorobenzenes occurred or is occurring because they can also be generated in the body from the metabolism of higher chlorinated benzenes such as tetrachlorobenzenes, pentachlorobenzene, hexachlorobenzene, or the pesticide lindane. Studies of the metabolism of trichlorobenzenes in workers exposed solely to trichlorobenzenes are needed to elucidate a metabolite profile which might include a metabolite that is not produced following exposures to other chlorinated benzenes. This would allow the differentiation between exposures to trichlorobenzenes from exposures to other chlorinated benzenes.

Effect. For the purpose of this data need, a biomarker of effect is a measurable biochemical, physiological, or other alteration within an organism that, depending on the magnitude, can be recognized as an established or potential health impairment or disease.

There is virtually no information regarding health effects of trichlorobenzenes in humans, so no specific effect of exposure has been identified. Studies in animals have identified the liver as the main target for trichlorobenzenes toxicity. Even if this were the case in humans exposed to trichlorobenzenes, similar liver alterations can be produced by exposure to many other chemicals. Health evaluations of trichlorobenzene workers may provide useful information regarding health effects that may be specific to exposure to these substances.

Absorption, Distribution, Metabolism, and Excretion. There is virtually no information regarding toxicokinetics of trichlorobenzenes in humans. Studies in animals have shown that trichlorobenzenes are readily absorbed through the gastrointestinal tract following oral exposure (Bakke et al. 1992; Chu et al. 1987; Tanaka et al. 1986), but no information is available regarding absorption through the lungs or the skin. Since trichlorobenzenes are present in outdoor air in cities in the United States (EPA 2010a), absorption studies in animals exposed by inhalation would provide useful information.

Radioactivity derived from the labeled trichlorobenzenes was found widely distributed in tissues of rats and rabbits following administration of single doses of ^{14}C-trichlorobenzenes (Chu et al. 1987; Parke and Williams 1960). Elimination half-lives of 1,2,4-trichlorobenzene from the blood, liver, and kidneys from male rats given a single intraperitoneal injection of the chemical were 5.8, 5.2, and 6.2 hours, respectively (Kato et al. 1993). Elimination half-lives for the other isomers are not available. While short-term studies showed that trichlorobenzenes did not accumulate in tissues, information from repeated-dosing studies is lacking. One 13-week dietary study reported that 1,3,5-trichlorobenzene accumulated at higher levels in fat and liver from rats than 1,2,3-trichlorobenzene or 1,2,4-trichlorobenzene; the levels in fat were one order of magnitude higher than those measured in the liver (Côté et al. 1988).

The only relevant information regarding metabolism of trichlorobenzenes in humans is that in microsomal preparations from human livers, CYP2E1 was the major enzyme in the formation of 2,3,4-trichlorophenol and 2,3,5-trichlorophenol, while CYP3A4 was responsible for the formation of 2,3,6-trichlorophenol (Bogaards et al. 1995). The metabolism of trichlorobenzenes in animals exposed orally and parenterally has been fairly well studied, but no information is available following inhalation or dermal exposure. The specific enzymes involved in the initial formation of phenolic intermediates are not known, but probably involve cytochrome P-450 isozymes. The role of metabolism in the toxicity of 1,2,4-trichlorobenzene has been studied (Kato and Kimura 2002; Kato et al. 1993), but no information is available for the other two

trichlorobenzene isomers. A study in which 1,2,4-trichlorobenzene was incubated *in vitro* with microsomes isolated from the liver of male rats reported the formation of quinone metabolites, which bound covalently with microsomal protein and with exogenously added DNA (Den Besten et al. 1991). Further studies are needed to examine whether this can also occur *in vivo* and to determine the role that these metabolites may play in the toxicity of trichlorobenzenes.

Data are available on the elimination and excretion of metabolites of the three trichlorobenzene isomers following single or short-term oral exposure to these chemicals (Chu et al. 1987; Jondorf et al. 1955; Lingg et al. 1982; Parke and Williams 1960; Tanaka et al. 1986). Further information regarding route of excretion in relation to increasing dose levels in single dose studies and in relation to duration of exposure would be useful.

Comparative Toxicokinetics. There are no data regarding toxicokinetics of trichlorobenzenes in humans. Studies in animals have reported qualitative differences in metabolite production and disposition between species. For example, glutathione conjugation was the predominant pathway in rats, whereas glucuronidation was predominant in monkeys (Lingg et al. 1982). The same study also showed that rats excreted 1,2,4-trichlorobenzene-derived radioactivity in the urine 2–3 times faster than monkeys. However, since there are no toxicokinetic data in humans, the animal species that is the most appropriate model for humans is unknown. Analyses of the urine of workers exposed to trichlorobenzenes would provide valuable information.

Methods for Reducing Toxic Effects. No specific methods for the mitigation of effects of acute exposure to trichlorobenzenes were located other than measures to support vital functions. No information was located concerning mitigation of effects of lower-level or longer-term exposure to trichlorobenzenes. This, in part, may reflect the fact that no population has been identified as having been subjected or currently undergoing exposure to excessive amounts of trichlorobenzenes. Therefore, it is difficult to design studies or methods for reducing toxic effects if no significant health effects have been reported to date.

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.

It is not known whether children are more or less susceptible to the effects of exposure to trichlorobenzenes than adults because there are no studies that specifically addressed this question. There is no information on whether the developmental process is altered in humans exposed to trichlorobenzenes. For the most part, studies in rats and mice did not show trichlorobenzenes to be embryotoxic or teratogenic (Black et al. 1988; Chernoff and Kavlock 1983; Gray and Kavlock 1984; Gray et al. 1986; Kitchin and Ebron 1983). However, Black et al. (1988) reported lesions in the lenses of the eye of fetuses from rats dosed with 1,2,4-trichlorobenzene and 1,3,5-trichlorobenzene on Gd 6–15 and sacrificed on Gd 22. Since no quantitative data were shown, the shape of the dose-response relationship could not be verified. Replication of these results would be useful. The information available does not suggest that trichlorobenzenes have endocrine-disrupting ability, but this issue has not been systematically studied.

There are no data to evaluate whether pharmacokinetics of trichlorobenzenes in children are different from adults. There is no information on whether trichlorobenzenes can cross the placenta, but they have been detected in human breast milk, so they could be transferred to newborns (Mes et al. 1993; Newsome et al. 1995). There are no data to permit an evaluation of whether metabolism of trichlorobenzenes is different in children than in adults.

Research into the development of sensitive and specific biomarkers of exposures and effects for trichlorobenzenes would be valuable for both adults and children. There are no data on the interactions of trichlorobenzenes with other chemicals in children. There are no pediatric-specific methods to reduce peak absorption, body burdens, or to interfere with the mechanisms of action of trichlorobenzenes. Based on the information available, it is reasonable to assume that the supportive methods recommended for maintaining vital functions in adults will also be applicable to children.

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

3.12.3 Ongoing Studies

No ongoing studies pertaining to trichlorobenzenes were identified in Toxline (2013).