2. HEALTH EFFECTS

2.1 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 chlorophenols. 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.

2.2 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 (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

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

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for chlorophenols. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

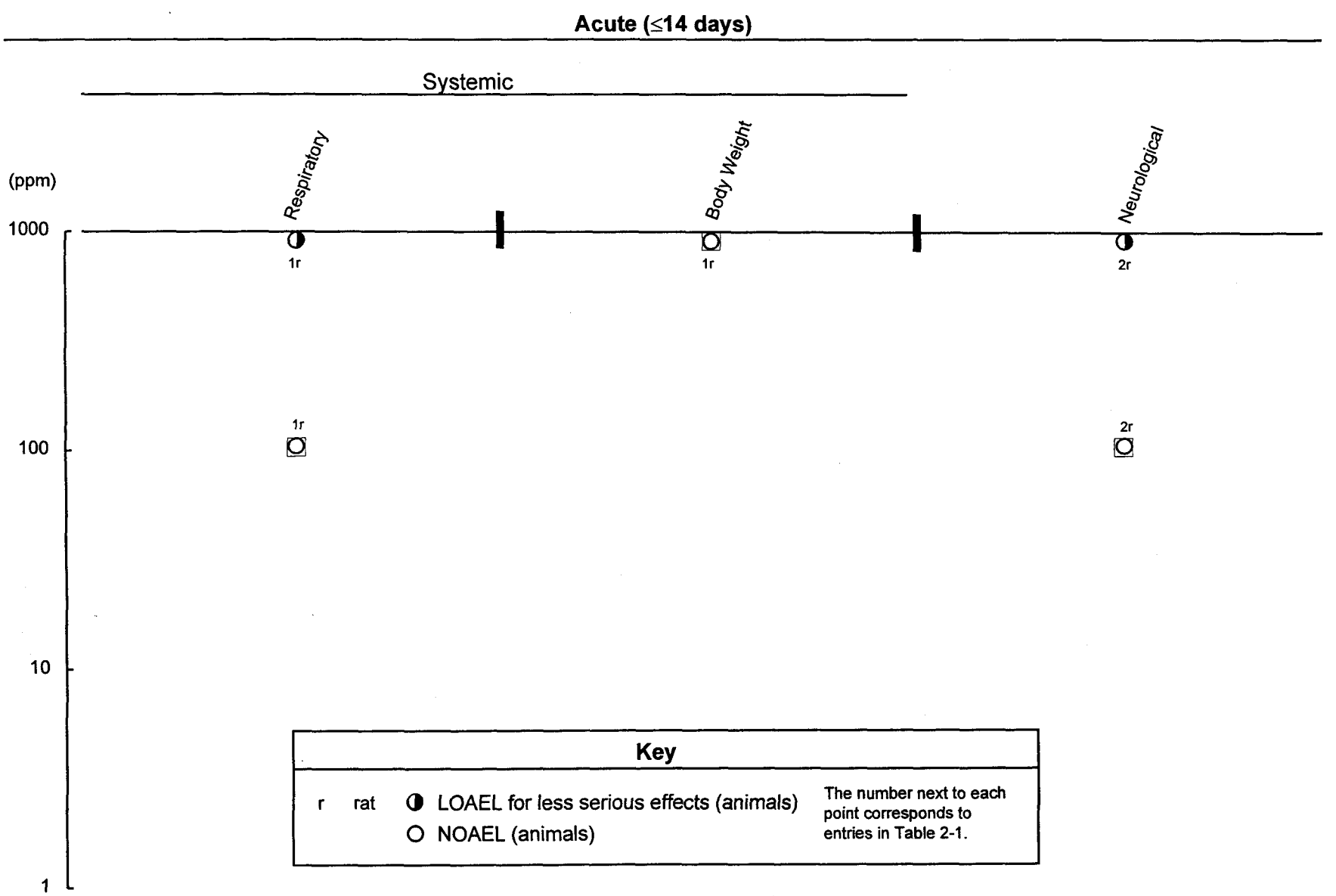
TABLE 2-1. Levels of Significant Exposure to Chlorophenols - Inhalation

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference/ Compound
ACUTE EXPOSURE							
Systemic							
1	Rat (Wistar)	4 hr	Resp	104	908 M (tachypnea 1/5 rats)		Duchosal and Biedermann 1991
							2-chlorophenol
			Bd Wt	908			
Neurological							
2	Rat (Wistar)	4 hr		104	908 (restlessness, hunched posture)		Duchosal and Biedermann 1991
							2-chlorophenol

[a] The number corresponds to entries in Figure 2-1.

Bd Wt = body weight; hr = hour(s); LOAEL= lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; Resp = respiratory

Figure 2-1. Levels of Significant Exposure to Chlorophenols - Inhalation



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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.

There are 19 isomers of the chlorophenols, each containing between 1 and 5 chlorines. All members of the series are chlorine derivatives of phenol, the simplest aromatic alcohol, i.e., hydroxybenzene. They possess both acute and chronic toxicity which varies with the number of chlorines present. However, this profile is concerned with only eight of these isomers, chosen on the basis of the following three criteria: (1) toxicity, (2) potential for human exposure, and (3) frequency of occurrence at NPL hazardous waste sites.

Because many of the isomers typically co-occur in the environment and have qualitatively (but not quantitatively) similar toxicological effects, they are combined into one profile to avoid repetition across multiple profiles. The isomers discussed include two monochlorinated compounds (2- and 4-chlorophenol, or 2-CP and 4-CP), one dichlorinated compound (2,4-dichlorophenol, or 2,4-DCP), two trichlorinated compounds (2,4,5- and 2,4,6-trichlorophenol, or 2,4,5-TCP and 2,4,6-TCP), and three tetrachlorinated compounds (2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachlorophenol, or 2,3,4,5-TeCP, 2,3,4,6-TeCP, and 2,3,5,6-TeCP). The information in the profile is organized by isomer (mono-, di-, tri-, tetrachlorophenols), and for each isomer the available data are then presented by duration (acute [14 days or less], intermediate [15 to 364 days], chronic [365 days or more]). In this text, the term "chlorophenols" will refer to any two or more of these eight isomers. The most commercially and toxicologically significant isomer, pentachlorophenol, is not included in this document because it is the subject of a separate profile.

2.2.1 Inhalation Exposure

2.2.1.l Death

Mortality studies of workers at phenoxy herbicide factories where exposure to chlorinated phenols (2,4,5-TCP, 2,4,6-TCP, and 2,4-DCP) occurred, have not shown increased mortality from any cause (Coggen et al. 1991; Ott et al. 1987). Additional occupational studies that focus on cancer-related deaths are discussed in Section 2.2.1.8. No studies were located regarding death in humans following inhalation exposure to any of the chlorophenols discussed in this profile.

Nose-only exposure of male and female Wistar rats to 2-CP for 4 hours to a concentration of 908 ppm (Duchosal and Biederman 1991) and whole-body exposure of Sprague-Dawley rats to 2-CP for 6 hours

at 620 ppm (Younger Labs 1975) did not result in any deaths. These studies are limited by a lack of experimental detail. Additional studies regarding lethality in animals following inhalation exposure to chlorophenols were not located.

2.2.1.2 Systemic Effects

No studies were located regarding musculoskeletal or renal effects in humans or animals after inhalation exposure to any of the eight chlorophenols discussed in this profile.

The limited studies examining systemic effects following inhalation exposure to chlorophenols are described below. The NOAEL and LOAEL values from the single reliable study are recorded in Table 2-1 and plotted in Figure 2-1.

Respiratory Effects. No studies were located regarding respiratory effects in humans after inhalation exposure to any of the eight chlorophenols discussed in this profile. Very limited studies of respiratory effects in workers exposed by inhalation to one or more of the chlorophenols in conjunction with other substances have been completed.

When compared to 260 unexposed referents, 281 workers involved in the production of sodium trichlorophenol and its derivatives for 18 years had no increased incidence of chronic bronchitis, chronic obstructive pulmonary disease, or altered measures of pulmonary function (Calvert et al. 1991). Exposure occurred 15 years before pulmonary function was examined. Because trichloro-phenols are rapidly cleared, serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which was produced as a contaminant in the manufacture of 2,4,5-TCP and its derivatives, was used to indicate that the workers had actually been exposed. The mean lipid adjusted TCDD serum concentration in exposed workers was 200 ppt relative to 7 ppt in the controls.

Occupational exposure of seven workers to an unspecified trichlorophenol isomer, in addition to other chemicals, by chronic inhalation was associated with adverse upper airway and chest symptoms (cough, chronic bronchitis, chest wheezing), altered pulmonary function (reduced expiratory flow rate of the lung, increased closing volume of the lung, increased elastic recoil pressure of the lung), and pulmonary lesions (interstitial densities) (Alexandersson and Hedenstierna 1982). The workers were exposed for 2-10 years and exposure concentrations were not well characterized. The study indicates that exposure concentrations were 0.003 mg/L (0.02 ppm) or less, and they may have varied considerably. The study was also limited

because of the small number of subjects (seven), which included three smokers. Therefore, it is not possible to determine whether the exposure to TCP alone induced the reported respiratory effects or whether smoking contributed to the effects. The authors (Alexandersson and Hedenstierna 1982) concluded that inhalation exposure to trichlorophenol may cause pulmonary dysfunction and possibly fibrosis following chronicduration exposure.

Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol reported upper respiratory tract irritation more frequently than unexposed workers (Kleinman et al. 1986). Tetrachlorophenol air concentrations ranged from 0.8 to 12.2 μg/m^3, and pentachlorophenol concentrations were below the limit of detection (0.5 μg/m^3).

Tachypnea was observed in one of five male rats exposed (nose-only) to 2-CP at 908 ppm for 4 hours (Duchosal and Biederman 1991). Tachypnea was not observed in any female rats exposed in the same manner. Dark red foci observed in the lungs (right caudal, median, or left lobe) of male and female rats exposed to 17 (2/5 males, 2/5 females) or 104 ppm (4/5 males, 2/5 females) were not found at 908 ppm (Duchosal and Biederman 1991). No controls were used in this study. The LOAEL for tachypnea and a NOAEL for respiratory effects identified in this limited study are presented in Table 2-l and Figure 2-l.

Cardiovascular Effects. Electrocardiograms were normal in three individuals who developed chloracne following occupational exposure (inhalation and dermal) to chlorophenols and other compounds during the manufacture of 2,4-DCP and 2,4,5-TCP (Bleiberg et al. 1964). No additional studies were located regarding cardiovascular effects in humans following inhalation exposure to any of the eight chlorophenols discussed in this profile.

No studies were located regarding cardiovascular effects in animals following inhalation exposure to any of the eight chlorophenols discussed in this profile.

Gastrointestinal Effects. The self-reported prevalence of gastrointestinal disease was not increased among 281 TCP production workers with elevated serum TCDD levels (Calvert et al. 1992). The workers had been exposed to a mixture of TCPs at least 15 years prior to the survey. However, the long time lag between exposure and examination of gastrointestinal symptoms may invalidate the study.

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No studies were located regarding gastrointestinal effects in animals after inhalation exposure to any of the eight chlorophenols discussed in this profile.

Hematological Effects. Clinical assessment of two patients occupationally exposed during the manufacture of 2,4-DCP- and 2,4,5-TCP-based herbicides revealed hematology and blood chemistry parameters (blood counts, bleeding and clotting time, serum bilirubin, blood urea nitrogen, and others) to be within normal ranges (Bleiberg et al. 1964).

No studies were located regarding hematological effects in animals after inhalation exposure to any of the eight chlorophenols discussed in this profile.

Hepatic Effects. Porphyria cutanea tarda has been reported in workers employed in the manufacture of 2,4-DCP and 2,4,5-TCP (Bleiberg et al. 1964). Exposure to chlorophenols and intermediates was probably through inhalation and dermal contact. Eleven cases of porphyria were identified, based on urinary porphyrin excretion, in a survey of 29 workers. Elevated serum transaminase levels and evidence of liver damage, e.g., regeneration of liver cells and hemofuscin (a brownish-yellow pigment that results from the decomposition of hemoglobin) deposition, were detected from liver biopsies in two cases that were studied in detail. Thus, the exposure was probably related to liver injury. Definitive conclusions regarding the connection between the porphyria or liver injury and exposure to chlorophenols in this group of workers cannot be made since the workers were exposed to a variety of chlorinated compounds, including a highly volatile chlorinated phenolic ether with six chlorines formed during the manufacturing process. The data provide an alert for potential human risk, however. Information on exposure to other liver toxicants, including the chronic ingestion of alcohol, was not obtained.

The results of a cross-sectional study of trichlorophenol production workers indicated an increased risk of elevated gamma-glutamyltransferase (GGT) activity in these workers (Calvert et al. 1992). GGT is a liver enzyme that is a potential marker of hepatobiliary disease. An interaction between alcohol consumption and exposure was related to increased GGT activity in these production workers (Calvert et al. 1992). However, the absence of increases in other hepatic enzymes may limit the diagnostic potential of the GGT findings in this study.

No studies were located regarding hepatic effects in animals following inhalation exposure to any of the eight chlorophenols discussed in this profile.

Dermal Effects. Chloracne, evidence of acquired porphyria cutanea tardia, hyperpigmentation, folliculitis, keratosis, and hirsutism have been observed in workers employed in the manufacture of 2,4-DCP- and 2,4,5-TCP-based herbicides (Bleiberg et al. 1964; Bond et al. 1989). As noted above in the discussion of hepatic effects, exposure to chlorophenols may have been through either inhalation or dermal contact or both. Furthermore, the subjects were exposed to several chlorinated compounds (e.g., dioxin) in addition to chlorophenols; therefore, the chloracne and other dermal effects cannot be ascribed specifically to chlorophenol exposure since chloracne is known to occur following exposure to TCDD.

No studies were located regarding dermal effects in animals following inhalation exposure to any of the eight chlorophenols discussed in this profile.

Ocular Effects. Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol reported eye irritation more frequently than unexposed workers (Kleinman et al. 1986). Tetrachlorophenol air concentrations ranged from 0.8 to 12.2 $\mu g/m^3$, and pentachlorophenol concentrations were below the limit of detection (0.5 $\mu g/m^3$). Industrial hygienists indicated that improvements in protective equipment were necessary at this mill, which suggests that ocular irritation could have resulted in part from contact with contaminated surfaces (e.g., hands, clothing).

No studies were located regarding ocular effects in animals following inhalation exposure to any of the eight chlorophenols discussed in this profile.

Body Weight Effects. No studies were located regarding body weight effects in humans following inhalation exposure to any of the eight chlorophenols discussed in this profile.

No changes in body weight were observed during the 15-day observation period after rats were exposed (nose-only) to 2-CP at 908 ppm for 4 hours (Duchosal and Biedermann 1991). No controls were included in this study. This NOAEL for body weight effects is recorded in Table 2-l and plotted in Figure 2-1.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans or animals after inhalation exposure to any of the eight chlorophenols discussed in this profile.

Dermal Effects. Chloracne, evidence of acquired porphyria cutanea tardia, hyperpigmentation, folliculitis, keratosis, and hirsutism have been observed in workers employed in the manufacture of 2,4-DCP- and 2,4,5-TCP-based herbicides (Bleiberg et al. 1964; Bond et al. 1989). As noted above in the discussion of hepatic effects, exposure to chlorophenols may have been through either inhalation or dermal contact or both. Furthermore, the subjects were exposed to several chlorinated compounds (e.g., dioxin) in addition to chlorophenols; therefore, the chloracne and other dermal effects cannot be ascribed specifically to chlorophenol exposure since chloracne is known to occur following exposure to TCDD.

No studies were located regarding dermal effects in animals following inhalation exposure to any of the eight chlorophenols discussed in this profile.

Ocular Effects. Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol reported eye irritation more frequently than unexposed workers (Kleinman et al. 1986). Tetrachlorophenol air concentrations ranged from 0.8 to 12.2 $\mu g/m^3$, and pentachlorophenol concentrations were below the limit of detection (0.5 $\mu g/m^3$). Industrial hygienists indicated that improvements in protective equipment were necessary at this mill, which suggests that ocular irritation could have resulted in part from contact with contaminated surfaces (e.g., hands, clothing).

No studies were located regarding ocular effects in animals following inhalation exposure to any of the eight chlorophenols discussed in this profile.

Body Weight Effects. No studies were located regarding body weight effects in humans following inhalation exposure to any of the eight chlorophenols discussed in this profile.

No changes in body weight were observed during the 15-day observation period after rats were exposed (nose-only) to 2-CP at 908 ppm for 4 hours (Duchosal and Biedermann 1991). No controls were included in this study. This NOAEL for body weight effects is recorded in Table 2-l and plotted in Figure 2-1.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans or animals after inhalation exposure to any of the eight chlorophenols discussed in this profile.

2.2.1.4 Neurological Effects

Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol reported headaches more frequently than unexposed workers (Kleinman et al. 1986). Monitoring of air and urinary concentrations of tetrachlorophenols suggested that exposure was principally through the skin.

Rats exposed for 4 hours to 908 ppm 2-CP using nose-only exposure showed restlessness, a hunched posture, and ruffled fur (Duchosal and Biedermann 1991). These effects were not observed at 104 ppm. The LOAEL and NOAEL for neurological effects is recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after inhalation exposure to any of the eight chlorophenols discussed in this profile.

2.2.1.6 Developmental Effects

No studies were located regarding developmental health effects in humans or animals after inhalation exposure to any of the eight chlorophenols discussed in this profile.

2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

A number of investigators have studied the potential association between chlorophenol-based pesticide production and carcinogenicity (Eriksson et al. 1981, 1990; Hardell et al. 1981; Hoar et al. 1986; Honchar and Halperin 1981; Kogevinas et al. 1992; Lynge 1985; Smith et al. 1984; Woods et al. 1987). Reports from Sweden indicate significantly increased relative risk ratios for soft tissue sarcomas (STS) and/or non-Hodgkin's lymphomas (NHLs) in exposed workers (Eriksson et al. 1981, 1990; Hardell et al. 1981). In a retrospective cohort study on Danish workers exposed to 2,4-DCP and 4- chloro-*o*-tolyloxy-acetic

The composite human results represent studies from a variety of occupational settings, with various degrees of exposure to chlorophenols, dioxins, intermediates, and final products, such as chlorophenoxy pesticides. The data are not sufficiently sensitive to support a relationship, per se, between any of the chlorophenol exposures and tumor incidence. However, taken in composite, the human study results do suggest a possible concern for increased tumorigenic risk in farm workers and production workers exposed to chlorophenols or their end-use products (Woods et al. 1987).

No studies were located regarding cancer in animals after inhalation exposure to any of the eight chlorophenols discussed in this profile.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

The lowest reported LD_{50} for a chlorophenol isomer was 89 mg/kg for male mice treated with 2,3,5,6 TeCP in ethanol (Ahlborg and Larsson 1978). The highest reported LD_{50} was 2,960 mg/kg for male rats treated with 2,4,5-TCP in corn oil (McCollister et al. 1961). The range of LD_{50} values indicates that the chlorophenols are slightly or moderately toxic according to the classification scheme of Hodge and Sterner (1949). Ahlborg and Larssen (1978) examined the acute oral toxicity of the TeCPs in both ethanol and propylene glycol. The LD_{50}s were higher when propylene glycol was used as the vehicle rather than ethanol (e.g., the LD_{50} for 2,3,4,6-TeCP in female mice was 131 when administered in ethanol and 735 mg/kg when administered in propylene glycol). The Ahlborg and Larssen (1978) study highlights the importance of vehicle effects in acute gavage studies, and because vehicles were different across studies and chlorophenol isomers, it is not possible to make definitive conclusions about which isomer is more toxic following a single oral dose.

In the only known toxicity study involving repeated dosing of monochlorophenols, groups of male and female ICR mice received daily gavage doses of 35, 69, or 175 mg/kg/day 2-CP in corn oil for 14 days. No exposure-related deaths occurred at the two lower treatment levels. All mice exposed at 175 mg/kg/day died,

suggesting a steep dose-response relationship between the mid- and high-treatment doses (Borzelleca et al.1985a).

In repeated-dose studies of 2,4-DCP in corn oil, 4 out of 34 pregnant Fischer-344 rats treated by gavage at 750 mg/kg/day on gestation days 6-15 died (Rodwell et al. 1989), while all non-pregnant rats treated with 2,000 mg/kg/day in the diet for 14 days survived (NTP 1989). Although pregnant rats may be more susceptible, the difference in effect may also be a result of differences in the rate of exposure between gavage and dietary dosing.

All rats and mice exposed to 2,4-DCP in the diet for 13 weeks at doses of 2,000 or 2,600 mg/kg/day survived (NTP 1989). However, all mice died when exposed to 5,200 mg/kg/day for 3 weeks (NTP 1989). In a 2-year study, decreased survival was not observed in rats fed 2,4-DCP in the diet at doses up to 440 mg/kg/day or in mice fed 2,4-DCP in the diet at doses up to 1,300 mg/kg/day for 103 weeks (NTP 1989).

No deaths were observed among rats treated by gavage (18 doses in olive oil) or in the diet with 2,4,5-TCP at doses up to 1,000 mg/kg/day for 90 days (McCollister et al. 1961). In addition, no deaths were observed in rabbits treated with 20 gavage doses of 500 mg/kg/day 2,4,5-TCP over 28 days (McCollister et al. 1961).

Deaths were observed during the first 4 weeks of treatment among female rats (3/40) and male rats (8/25) exposed to 2,4,6-TCP in corn oil by gavage for 11 weeks at 1,000 but not at 500 mg/kg/day (Blackbum et al.1986). The females were treated 2 weeks prior to pregnancy and then throughout gestation. No deaths were observed in rats treated by gavage with 2,4,6-TCP in corn oil at 720 mg/kg/day for 90 days (Bercz et al. 1990). In a 7-week dietary study, 1 of 5 rats died at 1,075 mg/kg/day and 4 of 10 mice died at 4,095 mg/kg/day, with no deaths observed at 735 mg/kg/day among rats or at 2,795 mg/kg/day among mice (NCI 1979). In a chronic study, no increased mortality trend was observed in rats or mice treated with 2,4,6-TCP in the diet at concentrations up to 500 mg/kg/day for 106-107 weeks for rats and 1,356 mg/kg/day for 105 weeks for mice (NCI 1979).

No deaths were observed in rats treated by gavage with 200 mg 2,3,4,6-TeCP/kg/day during gestation (RTI 1987) or among male and female rats treated at 200 mg/kg/day for 90 days (American Biogenics 1988). In both studies the 2,3,4,6-TeCP was given in olive oil.

The LD_{50} values and all reliable LOAEL values for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category for oral exposures to chlorophenols are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

Lung hemorrhaging occurred in rats treated with a single lethal gavage dose of 2,4-DCP (Wil Research Laboratories 1982). Nasal lesions were noted in male but not female rats exposed to 210 mg/kg/day for 103 weeks. Nasal lesions were not observed in mice fed as much as 1,300 mg/kg/day for the same exposure period (NTP 1989). This effect may, therefore, be specific to the male rat or may have been a result of aspiration while eating. Histopathological changes have not been observed in the lungs of rats or mice orally exposed to 2,4-DCP (Borzelleca et al. 1985a; NTP 1989), 2,4,5-TCP (McCollister et al. 1961), 2,4,6-TCP (Bercz et al. 1990; Blackburn et al. 1986; NCI 1979), or 2,3,4,6-TeCP (American Biogenics 1988).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

No change in heart weight was noted in mice fed 2,4-DCP at doses up to 230 mg/kg/day for 6 months (Kobayashi et al. 1972). Histopathological examinations of the heart have not revealed any effects in rats fed 2,4-DCP at 2,000 mg/kg/day or in mice fed 2,4-DCP at 2,600 mg/kg/day for 13 weeks (NTP 1989). Studies on rats exposed to 2,4-DCP at doses as high as 440 mg/kg/day and mice exposed to as much as 1,300 mg/kg/day for 103 weeks also showed no histological changes in the heart (NTP 1989).

Heart weight changes were not observed in rats treated with 18 gavage doses of 1,000 mg 2,4,5-TCP/kg, nor were.histological changes observed in the hearts of rats treated with 2,4,5-TCP in the diet at doses up to1,000 mg/kg/day for 98 days (McCollister et al. 1961).

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
	ACUTE EXPOSURE						
	Death						
1	Rat (Fischer-344)	10 d 1x/d Gd 6-15 (GO)				750 F (4/34 maternal deaths)	Rodwell et al. 1989 2,4-dichlorophenol
2	Rat (NS)	once (GO)				2960 M (LD50)	McCollister et al. 1961 2,4,5-trichloropheno
3	Mouse (CD-1 ICR)	14 d (GO)				175 (24/24 died)	Borzelleca et al. 1985a 2-chlorophenol
4	Mouse (CD-1 ICR)	once (GW)				345 F (LD50)	Borzelleca et al. 1985a, 1985b 2-chlorophenol
5	Mouse (CD-1 ICR)	once (GO)				1373 M (LD50)	Borzelleca et al. 1985a, 1985b 4-chlorophenol
6	Mouse (CD-1)	once (GO)				1276 M (LD50)	Borzelleca et al. 1985b, 1985c 2,4-dichlorophenol

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
7	Mouse (B6C3F1)	14 d (F)				5200 M (1/5 deaths)	NTP 1989
							2,4-dichlorophenol
8	Mouse (C57 black)	once (G)				400 F (LD50)	Ahlborg and Larsson 1978
							2,3,4,5-tetraCP
9	Mouse (C57 black)	once (G)				131 F (LD50)	Ahlborg and Larsson 1978
							2,3,4,6-tetraCP
10	Mouse (C57 black)	once (G)				89 M (LD50)	Ahlborg and Larsson 1978
							2,3,5,6-tetraCP
11	Gerbil (NS)	once (G)				533 F (LD50)	Ahlborg and Larsson 1978
							2,3,4,5-tetraCP
12	Gerbil (NS)	once (G)				698 F (LD50)	Ahlborg and Larsson 1978
							2,3,4,6-tetraCP
13	Gerbil (NS)	once (G)				979 F (LD50)	Ahlborg and Larsson 1978
							2,3,5,6-tetraCP

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
	Systemic						
14	Rat (Sprague-Dawley)	2 wk 2x/d (GO)	Hepatic	1.28 [b] M	2.58 M (hepatocytes: foamy cytoplasm, clustering of mitochondria and endoplasmic reticulum)		Phornchirasilp et al. 1989b 4-chlorophenol
15	Rat (Fischer-344/N)	14 d (F)	Bd Wt	500 M	1000 M (19% decrease in body weight)	2000 M (52% decrease in body weight)	NTP 1989 2,4-dichlorophenol
16	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic	400			Carlson 1978 2,4,5-trichlorophenol
17	Rat (Sprague-Dawley)	14 d 1x/d (GO)	Hepatic	400			Carlson 1978 2,4,6-trichlorophenol
18	Rat (Wistar)	once (GO)	Gastro	410	432 (mild necrosis)	632 (mucosal hyperemia of stomach, severe necrosis of intestine)	Hattula et al. 1981 2,3,4,6-tetraCP
			Musc/skel	632			
			Renal	632			

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
19	Mouse (CD-1 ICR)	14 d (GO)	Hemato	69			Borzelleca et al. 1985a 2-chlorophenol
			Hepatic	69			
			Renal	69			
			Bd Wt	35	69 (unspecified decreased body weight)		
20	Mouse (B6C3F1)	14 d (F)	Bd Wt	2600		5200 M (25% decrease body weight, reduced food intake)	NTP 1989 2,4-dichlorophenol
	Immuno/Lymphor						
21	Mouse (CD-1 ICR)	14 d (GO)		69			Borzelleca et al. 1985a 2-chlorophenol
	Neurological						
22	Rat (Fischer 344)	once (GO)		1000	2000 F (lethargy, ataxia, sensitivity to touch and sound, twitches)		Wil Research Laboratories, Inc. 1982 2,4-dichlorophenol
23	Mouse (CD-1 ICR)	14 d (GO)			35 (hyperactivity)		Borzelleca et al. 1985a 2-chlorophenol

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)		LOAEL (effect) Serious (mg/kg/day)		Reference/ Compound
24	Mouse (ICR)	2 doses 18 hr apart (GO)					1500	(central nervous system depression)	Kobayashi et al. 1972 2,4-dichlorophenol
25	Mouse (B6C3F1)	14 d (F)		2600			5200	(lethargy)	NTP 1989 2,4-dichlorophenol
	Developmental								
26	Rat (Sprague-Dawley)	once Gd 11 (G)		667 F	1000 F	(maternal toxicity: 10 g loss at 24 hrs compared to 0 in controls recovered by 72 hrs, no fetal toxicity noted)			Kavlock 1990 4-chlorophenol
27	Rat (Fischer-344)	Gd 6-15 1x/d (GO)		200	750	(fetal toxicity: delayed ossification, 3% reduced fetal body weights)			Rodwell et al. 1989 2,4-dichlorophenol
28	Rat (CD)	Gd 6-15 1x/d (GO)		25 F	100 F	(13% decrease in corrected maternal body weight gain; no fetal effects)	200 F	(26% decrease in corrected maternal body weight gain; no fetal effects)	RTI 1987 2,3,4,6-tetraCP

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
	INTERMEDIATE EXPOSURE						
	Death						
29	Rat (Long-Evans, hooded)	11 wk 5d/wk 1x/d (GO)				1000 M (8/25 died)	Blackburn et al. 1986 2,4,6-trichlorophenol
30	Mouse (B6C3F1)	3 wk (F)				5200 (20/20 died)	NTP 1989 2,4-dichlorophenol
31	Mouse (B6C3F1)	7 wk 7d/wk (F)				4095 (4/10 died)	NCI 1979 2,4,6-trichlorophenol
	Systemic						
32	Rat (Sprague-Dawley)	10 wk premating Gd 1-21 13 wk post-weaning (W)	Hepatic	3	30 (increased liver weight)		Exon and Koller 1985; Exon et al. 1984 2,4-dichlorophenol

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
33	Rat (Fischer-344/N)	13 wk (F)	Resp	2000			NTP 1989
							2,4-dichlorophenol
			Cardio	2000			
			Gastro	2000			
			Hemato	250 F		500 F (bone marrow atrophy: both erythroid and myeloid elements)	
			Musc/skel	2000			
			Hepatic	2000			
			Renal	2000			
			Endocr	2000			
			Derm	2000			
			Ocular	2000			
			Bd Wt	500	1000 M (20% reduction in body weight)		
34	Rat (Wistar)	98 d (F)	Resp	1000			McCollister et al. 1961
							2,4,5-trichlorophenol
			Cardio	1000			
			Hemato	1000			
			Hepatic	100	300 (mild centrilobular degeneration, focal necrosis)		
			Renal	100	300 (mild degenerative change in epithelium)		
			Endocr	1000			
			Bd Wt	300 F		1000 F (24% decrease in body weight gain)	

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
35	Rat Sprague-Dawley	90 d (GO)	Resp	720			Bercz et al. 1990
							2,4,6-trichlorophenol
			Cardio	720			
			Gastro	720			
			Hemato	720			
			Hepatic	80	240M (14 % increased relative liver weight)		
			Renal	240	720M (increased kidney weight, decreased urinary pH)		
			Endocr	720			
			Ocular	720			
			Bd Wt	720			
36	Rat (Long-Evans hooded)	11 wk 5d/wk 1x/d (GO)	Resp	1000 M			Blackburn et al. 1986
							2,4,6-trichlorophenol
			Cardio	1000 M			
			Hepatic	1000 M			
			Renal	1000 M			
			Endocr	1000 M			
			Bd Wt	1000 M			
37	Rat (Sprague-Dawley)	24-25 wk 7d/wk (W)	Hepatic	0.3	3 (15% increase in liver weight)		Exon and Koller 1985
							2,4,6-trichlorophenol
			Bd Wt	30			

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)		Serious (mg/kg/day)		Reference/ Compound
38	Rat (Fischer-344)	7 wk 7d/wk (F)	Hemato	1575	2300	(increase splenic hematopoiesis)			NCI 1979 2,4,6-trichlorophenol
			Hepatic	1575	2300M	(midzonal vacuolation of hepatocytes; 2/5)			
			Bd Wt	500	735	(11-16% decrease in body weight)	1075	(27% decrease in body weight)	
39	Rat (Sprague-Dawley)	90 d (GO)	Resp	200					American Biogenics Corp 1988 2,3,4,6-tetraCP
			Cardio	200					
			Gastro	200					
			Hemato	200					
			Musc/skel	200					
			Hepatic	25	100	(increased liver weights and centrilobular hypertrophy)			
			Renal	25	100	(increased kidney weights)			
			Endocr	200					
			Ocular	200					
			Bd Wt	100	200M	(body weight gain decreased by 11%)			
40	Rat (Wistar)	55 d, 7d/wk (GO)	Gastro	50	100	(focal necrosis of small intestine)			Hattula et al. 1981 2,3,4,6-tetraCP
			Musc/skel	100					
			Hepatic	10			50	(necrosis, thrombosed veins)	
			Bd Wt	100					

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
41	Mouse (ICR, ddN)	6 mo (F)	Cardio	230 M			Kobayashi et al. 1972
							2,4-dichlorophenol
			Hemato	230 M			
			Hepatic	100 M	230M (swelling of hepatic cells)		
			Renal	230 M			
			Bd Wt	230 M			
42	Mouse (B6C3F1)	13 wk (F)	Resp	2600			NTP 1989
							2,4-dichlorophenol
			Cardio	2600			
			Gastro	2600			
			Hemato	2600			
			Musc/skel	2600			
			Hepatic		325M (minimal hepatocellular necrosis 4/10)	2600 M (hepatocellular necrosis 10/10)	
			Renal	2600			
			Endocr	2600			
			Derm	2600			
			Ocular	2600			
			Bd Wt	1300	2600 (10-15% reduction in body weight)		
	Immuno/Lymphor						
43	Rat (Sprague-Dawley)	16 wk: Gd 1-21 ppd 1-91 (W)		50			Exon and Koller 1983, 1985
							2-chlorophenol

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
44	Rat (Sprague-Dawley)	15 wk premating Gd 1-21, 15 wk post-weaning (W)		0.3 c	3 (decreased delayed-type hypersensitivity)		Exon and Koller 1985; Exon et al. 1984 2,4-dichlorophenol
45	Rat (Sprague-Dawley)	24-25 wk 7d/wk 24hr/d (W)		3	30 (increased spleen weight)		Exon and Koller 1985 2,4,6-trichlorophenol
46	Rat (Sprague-Dawley)	90 d (GO)		200			American Biogenics Corp 1988 2,3,4,6-tetraCP
47	Mouse (CD-1)	90 d (W)		491 F			Borzelleca et al. 1985a 2,4-dichlorophenol
	Neurological						
48	Rat (Fischer-344/N)	13 wk (F)		1000	2000 (hunched posture)		NTP 1989 2,4-dichlorophenol
49	Rat (Sprague-Dawley)	90 d (GO)		200			American Biogenics Corp 1988 2,3,4,6-tetraCP

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)		LOAEL (effect) Serious (mg/kg/day)		Reference/ Compound
50	Mouse (B6C3F1)	13 wk (F)		2600					NTP 1989 2,4-dichlorophenol
	Reproductive								
51	Rat (Sprague-Dawley)	13 wk: 10 wk premating Gd 1-21 (W)		5			50	(increase in the percentage of stillborn pups; decrease in live litter size)	Exon and Koller 1982, 1985 2-chlorophenol
52	Rat (Sprague-Dawley)	13 wk: 10 wk premating Gd 1-21 (W)		3	30	(decreased mean litter size)			Exon and Koller 1985; Exon et al. 1984 2,4-dichlorophenol
53	Rat (Long-Evans hooded)	11 wk 5d/wk 1x/d (GO)		1000 M					Blackburn et al. 1986 2,4,6-trichlorophenol
54	Rat (Sprague-Dawley)	13 wk: 10 wk premating Gd 1-21 (W)		3			30	(decreased mean litter size)	Exon and Koller 1985 2,4,6-trichlorophenol
55	Rat (Sprague-Dawley)	90 d (GO)		200					American Biogenics Corp 1988 2,3,4,6-tetraCP

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
56	Mouse (CD-1)	90 d (W)		500 M			Seyler et al. 1984 2,4-dichlorophenol
	Developmental						
57	Rat (Sprague-Dawley)	31 wk: 10 wk, Gd 1-21 ppd 1-21 ad lib (W)		50			Exon and Koller 1981 2-chlorophenol
58	Rat (Sprague-Dawley)	16 wk: 3 wk (Gd 1-21) ppd 1-91 (W)		50			Exon and Koller 1983, 1985 2-chlorophenol
59	Rat (Sprague-Dawley)	13 wk: 10 wk premating Gd 1-21 (W)		30			Exon and Koller 1985; Exon et al. 1984 2,4-dichlorophenol
60	Rat (Long-Evans hooded)	2 wk 5d/wk 1x/d then Gd1-21 7d/wk 1x/d (GO)		100	500 (10-11% reduction in litter weight)		Blackburn et al. 1986 2,4,6-trichlorophenol

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
	CHRONIC EXPOSURE						
	Systemic						
61	Rat (Sprague-Dawley)	27 mo, 10 wk premating Gd 1-21 Ld 1-21 (W)	Hemato	50			Exon and Koller 1985 *2-chlorophenol*
62	Rat (Fischer-344)	103 wk (F)	Resp			210 M (nasal lesions; multifocal degeneration of respiratory epithelium)	NTP 1989 2,4-dichlorophenol
			Cardio	440 M			
			Gastro	440 M			
			Hemato	440 M			
			Musc/skel	440 M			
			Hepatic	440 M			
			Renal	440 M			
			Endocr	440 M			
			Derm	440 M			
			Ocular	440 M			
			Bd Wt	120 F	250 F (6-12% reduced body weight)		

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
63	Rat (Fischer-344)	107 wk 7d/wk (F)	Resp	500			NCI 1979
							2,4,6-trichlorophenol
			Cardio	500			
			Gastro	500			
			Hemato			250 M (bone marrow hyperplasia)	
			Hepatic	500			
			Renal	500			
			Endocr	500			
			Derm	500			
			Bd Wt		250 F (approximate 10% decrease in body weight)	500 F (approximate 29% decrease in body weight)	
64	Mouse (B6C3F1)	103 wk (F)	Resp	1300 M			NTP 1989
							2,4-dichlorophenol
			Cardio	1300 M			
			Gastro	1300 M			
			Hemato	1300 M			
			Musc/skel	1300 M			
			Hepatic	1300 M			
			Renal	1300 M			
			Endocr	1300 M			
			Ocular	1300 M			
			Derm	1300 M			
			Bd Wt	430 F	820 F (maximum 19% decrease in body weight relative to controls)		

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
65	Mouse (B6C3F1)	105 wk 7d/wk (F)	Resp	1300 M			NCI 1979
							2,4,6-trichlorophenol
			Cardio	1300 M			
			Gastro	1300 M			
			Hemato	1300 M			
			Hepatic			650 M (hepatic hyperplasia)	
			Renal	1356			
			Endocr	1356			
			Derm	1356			
			Bd Wt			658 F (approximately 24% decrease in body weight)	
	Immuno/Lymphor						
66	Rat (Fischer-334)	103 wk (F)		440 M			NTP 1989
							2,4-dichlorophenol
67	Rat (Fischer-344)	107 wk 7d/wk (F)		500			NCI 1979
							2,4,6-trichlorophenol
68	Mouse (B6C3F1)	103 wk (F)		1300 M			NTP 1989
							2,4-dichlorophenol

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
	Neurological						
69	Rat (Fischer-334)	103 wk (F)		440 M			NTP 1989 2,4-dichlorophenol
70	Rat (Fischer-344)	107 wk 7d/wk (F)		500			NCI 1979 2,4,6-trichlorophenol
71	Mouse (B6C3F1)	103 wk (F)		1300 M			NTP 1989 2,4-dichlorophenol
72	Mouse (B6C3F1)	105 wk 7d/wk (F)		1356 F			NCI 1979 2,4,6-trichlorophenol
	Reproductive						
73	Rat (Fischer-344)	103 wk (F)		440 M 250 F			NTP 1989 2,4-dichlorophenol
74	Rat (Sprague-Dawley)	107 wk 7d/wk (F)		500			NCI 1979 2,4,6-trichlorophenol

TABLE 2-2. Levels of Significant Exposure to Chlorophenols - Oral (continued)

Key to[a] figure	Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
75	Mouse (B6C3F1)	103 wk (F)		1300 M 820 F			NTP 1989 2,4-dichlorophenol
76	Mouse (B6C3F)	105 wk 7d/wk (F)		1300 M 1356 F			NCI 1979 2,4,6-trichlorophenol
	Cancer						
77	Rat (Fischer-344)	107 wk 7d/wk (F)				250 M (CEL: monocytic leukemia 23/50)	NCI 1979 2,4,6-trichlorophenol
78	Mouse (B6C3F1)	105 wk 7d/wk (F)				650 M (CEL: 7/47 hepatocellular carcinomas or adenomas)	NCI 1979 2,4,6-trichlorophenol

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an acute oral Minimal Risk Level (MRL) of 0.01 mg/kg/day for chlorophenols; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). This MRL, based on 4-CP, should be protective for all chlorophenols discussed in the profile, but could be overprotective.
[c]Used to derive an intermediate oral Minimal Risk Level (MRL) of 0.003 mg/kg/day for chlorophenols; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). This MRL, based on 2,4-DCP, should be protective for all chlorophenols discussed in the profile, but could be overprotective.

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s;) Derm = dermal; Endocr = endocrine; F= female; (F) = feed; (G) = gavage; Gastro = gastrointestinal; (GO) = gavage-oil; (GW) = gavage-water; Gd = gestation day; Hemato = hematological; hr = hour(s); Immuno/Lymphor =immunological/ lymphoreticular; Ld = lactation day; LD50 = 50% lethal concentration dose; LOAEL = lowest-observed-adverse-effect level; M= male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not stated; ppd = post parturition day; Resp = respiratory; tetraCP = tetrachlorophenol; (W) = water; wk = week(s); x = time(s)

Figure 2-2. Levels of Significant Exposure to Chlorophenols - Oral

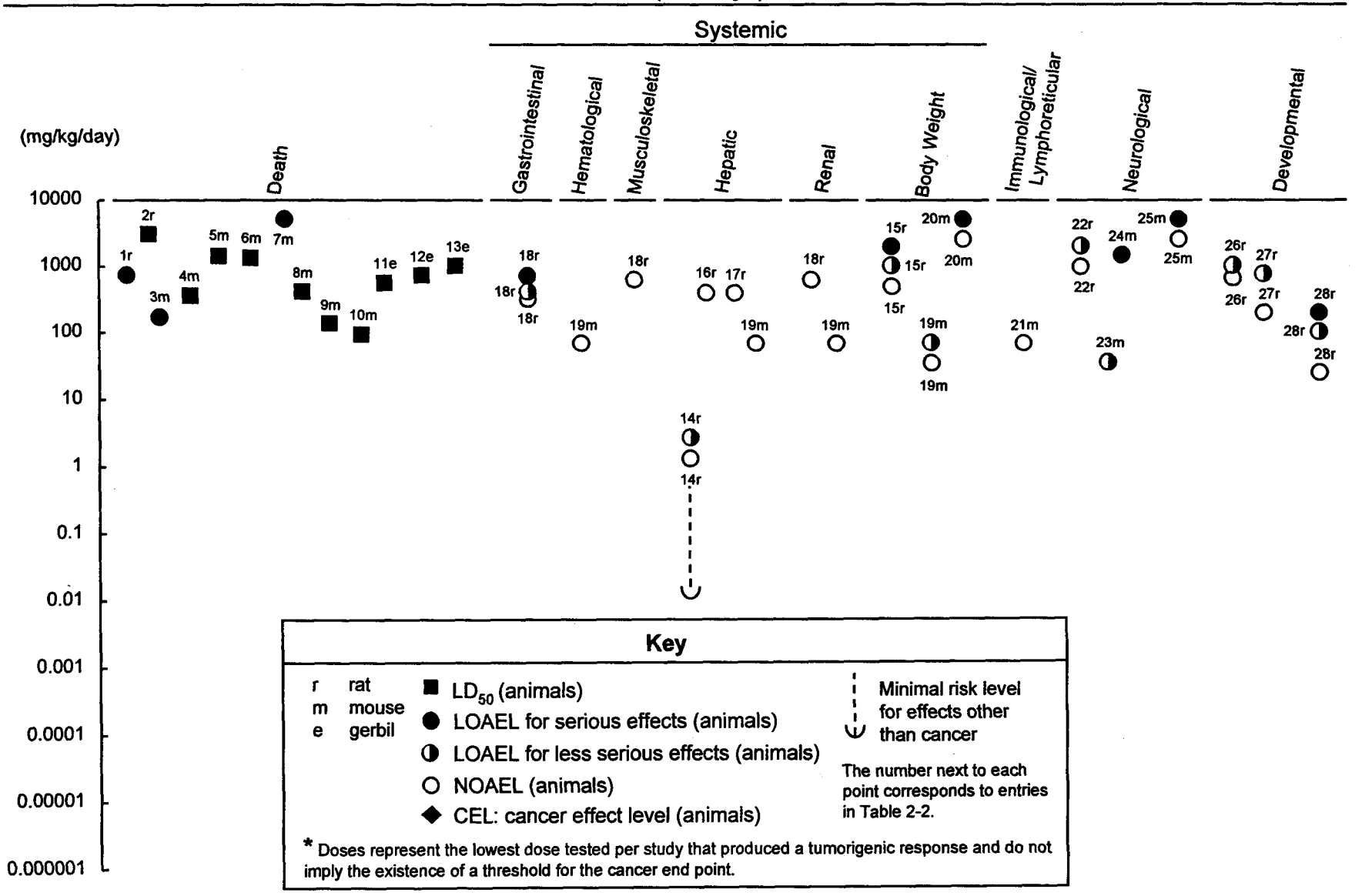


Figure 2-2. Levels of Significant Exposure to Chlorophenols - Oral (cont.)

Intermediate (15-364 days)

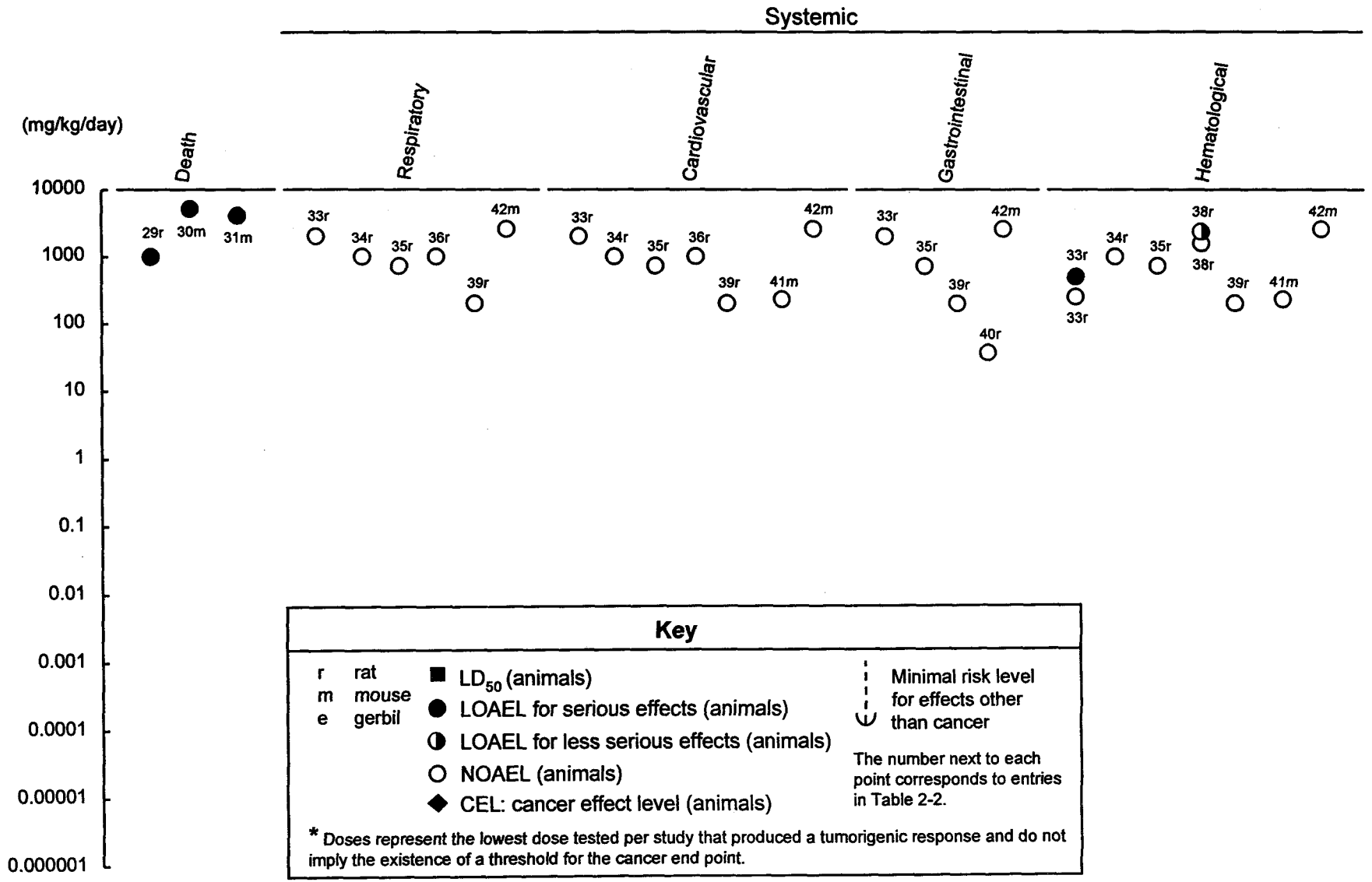


Figure 2-2. Levels of Significant Exposure to Chlorophenols - Oral (cont.)

Intermediate (15-364 days)

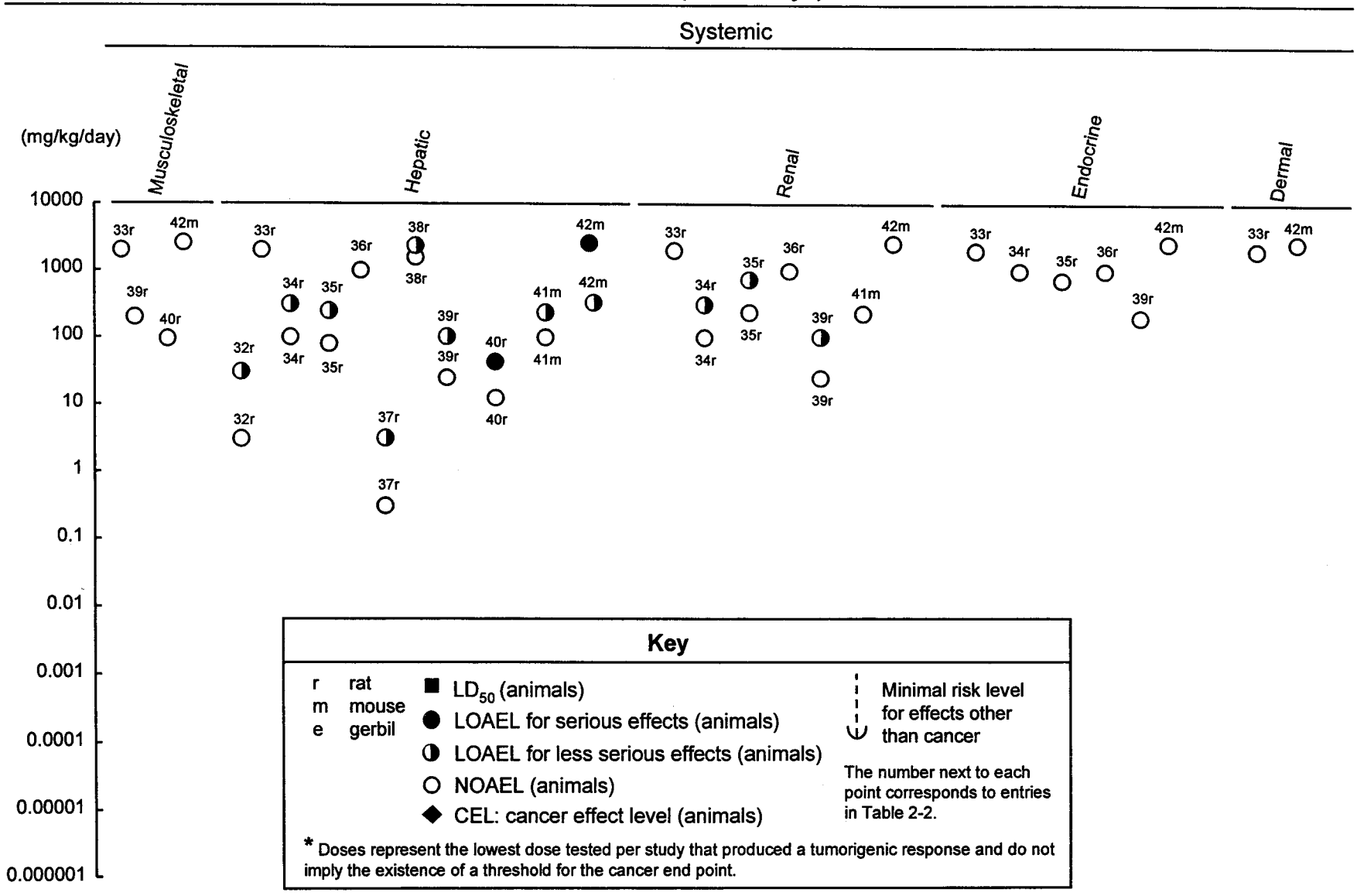


Figure 2-2. Levels of Significant Exposure to Chlorophenols - Oral (cont.)

Intermediate (15-364 days)

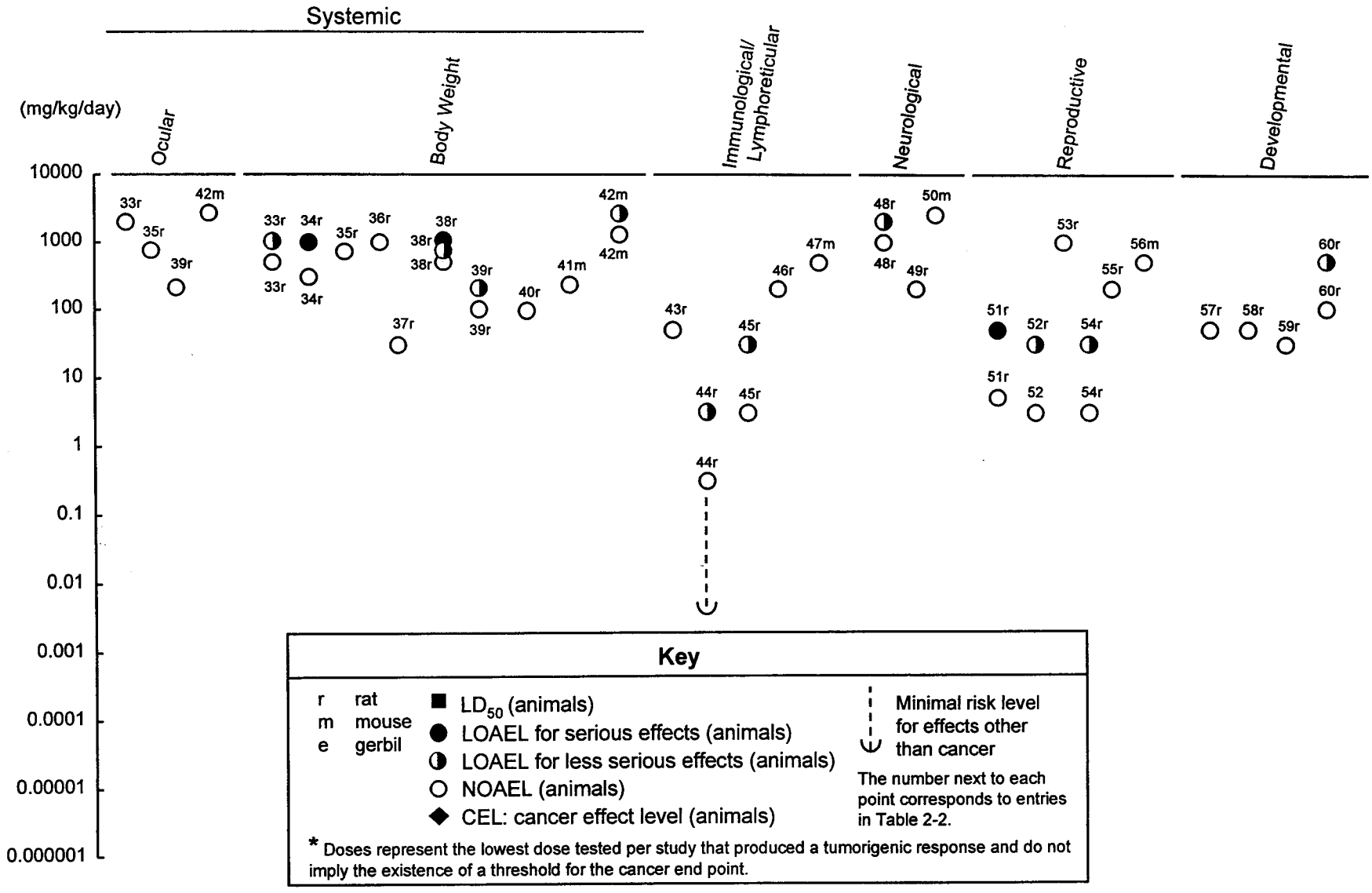


Figure 2-2. Levels of Significant Exposure to Chlorophenols - Oral (cont.)

Chronic (≥365 days)

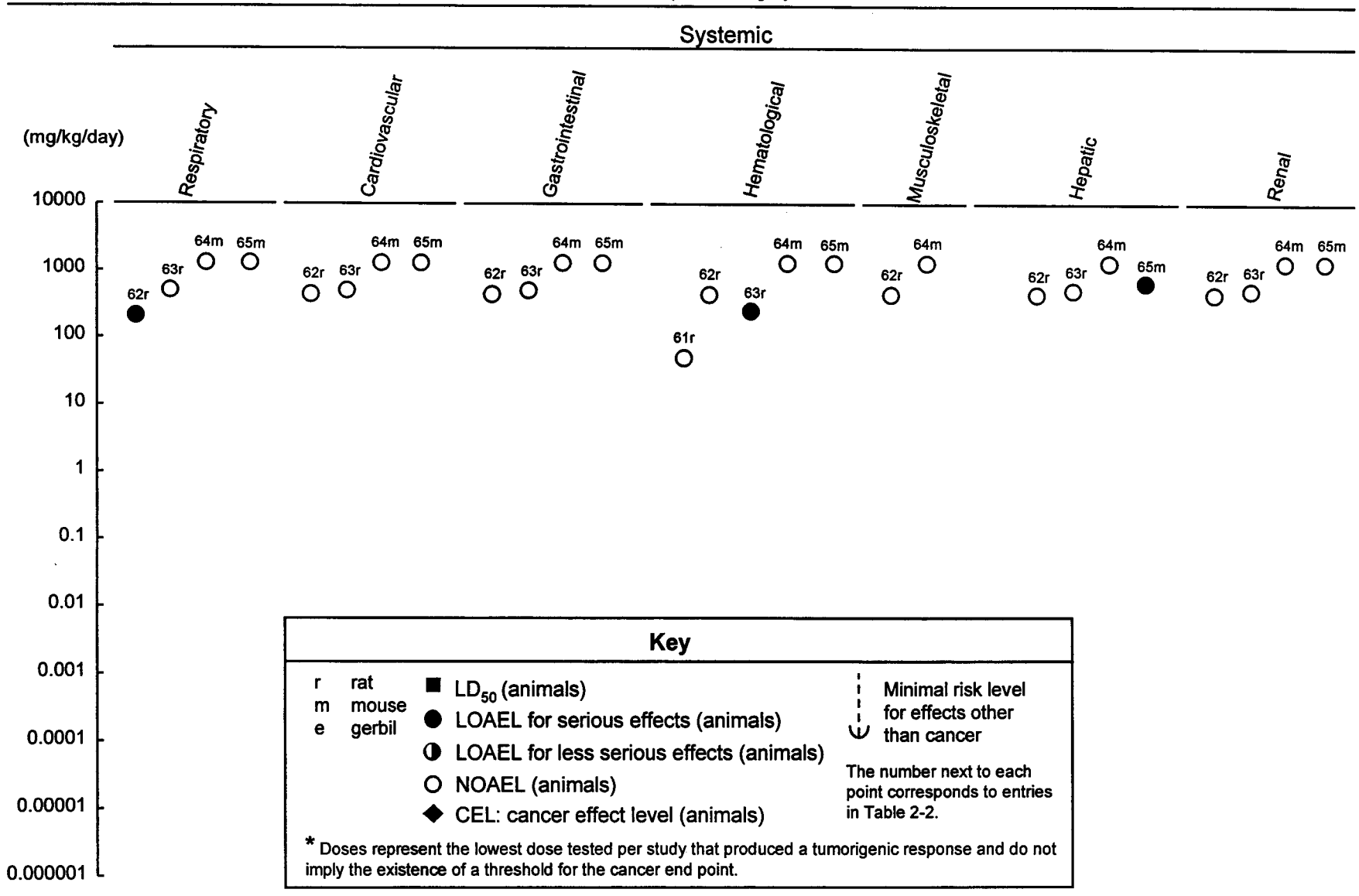
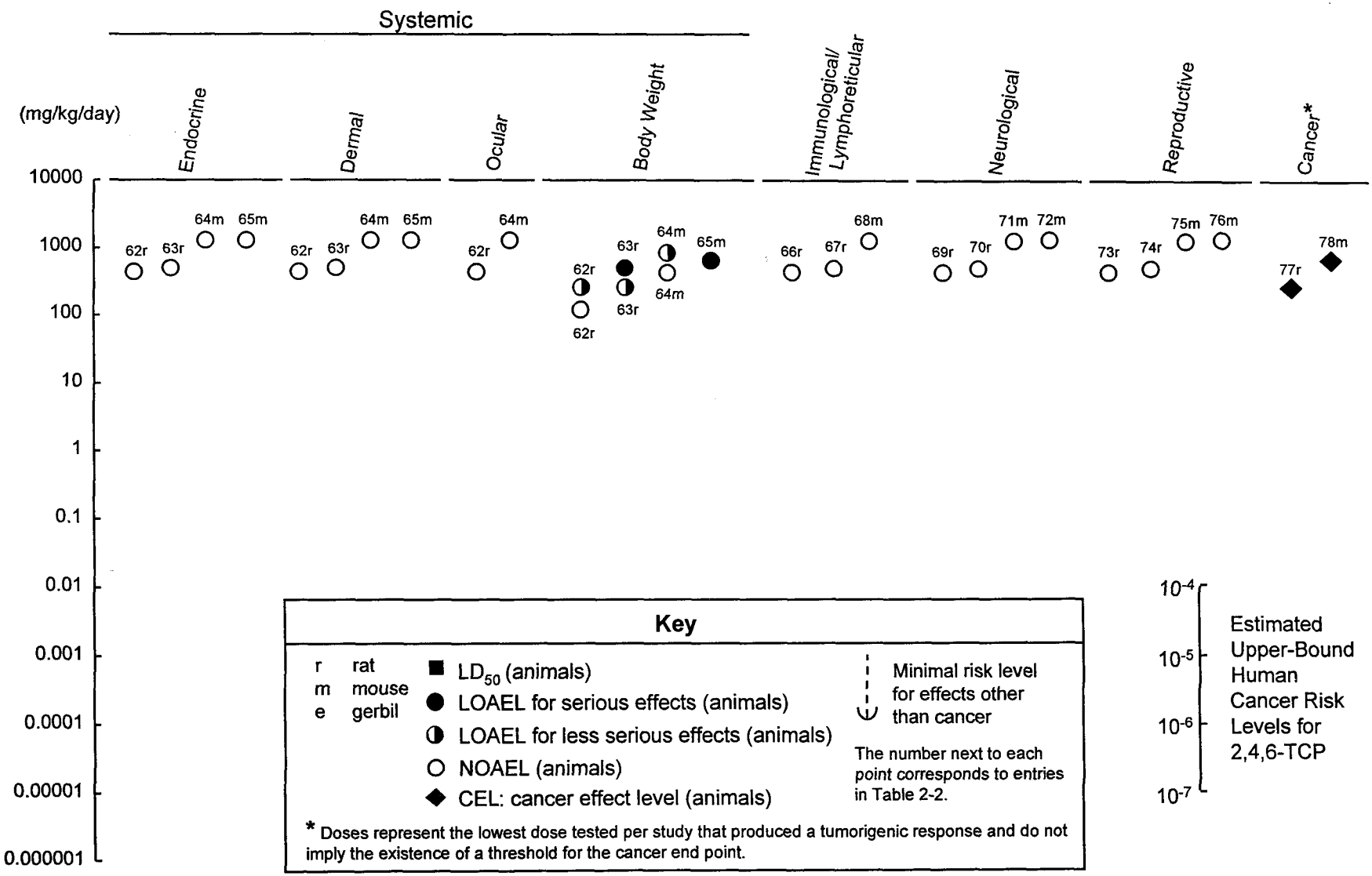


Figure 2-2. Levels of Significant Exposure to Chlorophenols - Oral (cont.)

Chronic (≥365 days)



Heart weight did not increase in rats exposed orally to 2,4,6-TCP over an intermediate (10 or 13 weeks) exposure period to doses as high as 1,000 mg/kg/day (Bercz et al. 1990; Blackburn et al. 1986). No treatment-related lesions were evident upon histopathologic examination of the hearts of rats and mice exposed to doses as high as 720 and 1,356 mg/kg/day of 2,4,6-TCP, respectively for 90 days (Bercz et al.1990) or 105 weeks (NCI 1979).

No changes in heart weight or histology were observed in rats treated with 2,3,4,6-TeCP for 90 days (American Biogenics 1988).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

Mild catarrhal enteritis was observed in female Sprague-Dawley albino rats given a single gavage dose of 316-5,000 mg/kg/day 2,4-DCP in corn oil and sacrificed 24 hours later (Henke and Lockwood 1978). No pathology reports were provided for rats that were sacrificed on day 7 or day 14. In another study, gross necropsy revealed reddened hind, stomach and intestines in Fischer-344 rats given a single gavage dose of 2,400 mg/kg/day 2,4-DCP in corn oil. Both studies demonstrated that this compound can be irritating to the gastrointestinal tract (Wil Research Laboratories 1982). The observation of gastrointestinal effects at lower doses in Sprague-Dawley compared to Fischer-344 rats suggests that Sprague-Dawley rats may be more sensitive to the acute gastrointestinal effects of 2,4-DCP. No significant histopathological changes were observed in the gastrointestinal tracts of Fischer-344 rats fed 2,000 mg/kg/day 2,4-DCP or mice fed 2,600 mg/kg/day 2,4-DCP for 13 weeks, or in rats fed 440 mg/kg/day 2,4-DCP or mice fed 1,300 mg/kg/day 2,4-DCP for 103 weeks (NTP 1989).

In a 90-day study, no significant histopathological changes were observed in the gastrointestinal tracts of rats treated by gavage with 2,4,6-TCP at 720 mg/kg/day (Bercz et al. 1990). Histopathologic examination of the stomach and intestines of rats and mice exposed to 2,4,6-TCP for 2 years at doses as high as 500 and 1,356 mg/kg/day, respectively, revealed no treatment-related lesions (NCI 1979).

Wistar rats administered a single gavage dose of 632 mg/kg 2,3,4,6-TeCP had mucosal hyperemia of the stomach and severe necrosis of the intestine (Hattula et al. 1981). At a dose of 432 mg/kg, mild necrosis was observed in the intestines of 1/10 rats, with no effects observed at 410 mg/kg. Focal necrosis of the small intestines was observed in Wistar rats treated by gavage for 55 days with 100 mg/kg/day 2,3,4,6- TeCP. No

effects were observed at 10 mg/kg/day (Hattula et al. 1981). In contrast, no histopathological changes were observed in the gastrointestinal tracts of Sprague-Dawley rats treated with 2,3,4,6-TeCP at 200 mg/kg/day for 90 days (American Biogenics 1988). 2,3,4,6-TeCP was administered in olive oil in both the Hattula et al. (1981) (concentrations not reported) and American Biogenics (1988) studies (maximum concentration of 20 mg/mL). Because olive oil was used as a vehicle for both studies, the differences in effects to the gastrointestinal tract may likely be due to the dissimilar dosing solution concentrations and rodent strains.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

Groups of 12 male and 12 female mice, administered once daily by gavage with up to 69 mg/kg/day 2-CP or up to 638 mg/kg/day 2,4-DCP for 14 days, showed no adverse effects on standard hematological parameters, including total and differential white blood cells, red blood cells, platelets, hematocrit, hemoglobin, and coagulation measures relative to unexposed controls (Borzelleca et al. 1985a). However, when groups of 20 male and 20 female mice were dosed with up to 383 mg/kg/day of 2,4-DCP (male), and 49 mg/kg/day (female) in drinking water for 90 days, the number of white blood cells was increased in the high-dose males (Borzelleca et al. 1985c). No changes in red or white blood cell counts were noted in mice exposed to 2,4-DCP at doses up to 230 mg/kg/day for 6 months (Kobayashi et al. 1972). After 13 weeks of prenatal exposure and up to 15 weeks of postnatal exposure to 2-CP in drinking water, rat weanlings showed no adverse effects on red cell count, hematocrit, mean corpuscular volume, white cell count, or hemoglobin concentration; the highest exposure dose was 50 mg/kg/day (Exon and Koller 1982). Chronic prenatal/postnatal exposure to either 50 mg/kg/day 2-CP or 30 mg/kg/day 2,4-DCP resulted in increased erythrocyte count, packed cell volume, and hemoglobin concentration. The increases for erythrocyte count and hemoglobin (>10%) were statistically significant ($p \leq 0.05$) (Exon and Koller 1985). However, the investigators suggested that the increase may be secondary to effects on liver enzymes or on hematopoietic stem cells and did not consider these effects biologically significant,

In an NTP study (NTP 1989), bone marrow atrophy was observed in male rats treated with 2,4-DCP in the diet at 1,000 mg 2,4-DCP/kg/day for 13 weeks and in female rats at 500 mg/kg/day. The atrophy resulted in depletion of both erythroid and myeloid elements, with no effects observed at 250 mg/kg/day. No hematological effects were noted in mice treated with 2,4-DCP in the diet for 13 weeks at doses up to 2,600 mg/kg/day or in rats or mice treated with 2,4-DCP for 103 weeks (rats, 440 mg/kg/day; mice, 1,300 mg/kg/day) (NTP 1989).

Treatment of rats with 2,4,5-TCP in the diet at doses up to 1,000 mg/kg/day for 98 days resulted in no changes in hematocrit, hemoglobin, or white blood cell counts(McCollister et al. 1961). Administration of up to 720 mg/kg/day 2,4,6-TCP to rats for 90 days resulted in no adverse effects on erythrocyte count, leukocyte count, corrected leukocyte count, hemoglobin, hematocrit, platelet count, or a differential analysis of leukocytes (Bercz et al. 1990). Rats exposed orally for 7 weeks to 2,4,6-TCP exhibited a "moderate to marked increase" in splenic hematopoiesis (NCI 1979). A high incidence of bone marrow hyperplasia and leukocytosis occurred in rats chronically exposed to 2,4,6-TCP in their diet at 250 mg/kg/day (NCI 1979). Further discussion of these hematological effects in rats can be found in Section 2.2.2.8. No hematological effects were evident in mice exposed chronically to 2,4,6-TCP in their diet at doses up to 1,300 mg/kg/day (NCI 1979).

Treatment of rats by gavage with doses of 200 mg/kg/day 2,3,4,6-TeCP for 90 days significantly ($p<0.05$) reduced hemoglobin and hematocrit in both sexes (American Biogenics 1988). Although the effects were statistically significant, the investigators did not consider the effects to be toxicologically significant because the group mean data were within the normal range of reference control data for the laboratory where the study was conducted. In addition, no gross or histopathologic evidence was found to support the decreases in hemoglobin and hematocrit.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

Ninety-day (up to 2,600 mg/kg/day) and 2-year (up to 1,300 mg/kg/day) exposure of rats and mice to 2,4-DCP did not result in any histopathological changes in the muscle or ribs (NTP 1989). Single dose and 55-day exposure to 2,3,4,6-TeCP produced no adverse histopathological effects on muscle in Wistar rats (Hattula et al. 1981). The highest single and intermediate-duration exposure levels were 632 mg/kg and 100 mg/kg/day, respectively (Hattula et al. 1981).

Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

Treatment of mice by gavage with 2-CP in corn oil at doses up to 69 mg/kg/day for 14 days resulted in a significant decrease in liver weights in females with no effects on serum glutamic-oxaloacetic transaminase (SGOT); serum glutamic-pyruvic transaminase (SGPT); liver microsomal proteins; cytochrome P-450;

cytochrome b5; or activities of liver aminopyrine demethylase, aniline hydroxylase, or arylhydrocarbon hydroxylase (Borzelleca et al. 1985a). The study authors did not consider the change in liver weight to be adverse because biologically or statistically significant compound-related adverse effects were not observed.

In Sprague-Dawley rats, twice daily administration of as little as 0.32 mg/kg 4-CP for 2 weeks (0.64 mg/kg/day) resulted in significant activation of hepatic enzymes including cytochrome P-450 (Phomchirasilp et al. 1989b). Microsomal protein and cytochrome P-450 levels were also elevated in the treated rats. The magnitude of increases over 2 weeks in liver microsomal protein and cytochrome P-450 content declined at doses above 0.64 mg/kg/day. Following additional experiments in which treatment was given two times per day, both a 2-week exposure to 2.58 mg/kg/day and an 8-week exposure to 0.64 mg/kg/day resulted in a foamy cytoplasm and the clustering of mitochondria and endoplasmic reticulum. The electron microscopic changes were not observed in the livers of rats treated at 1.28 mg/kg/day for 2 weeks. In separate studies, similar treatment doses of 4-CP had no effect on relative liver weights, microsomal zoxazolamine 6-hydroxylase activity, or measures of serum lipid and lipidlipoprotein concentrations, but did increase fasting glucose levels (Phomchirasilp et al. 1989a). Light microscopy was not reported in this study. Based on the electron microscopic changes following 2 weeks of exposure, 2.58 mg/kg/day is considered a LOAEL and 1.28 mg/kg/day is considered a NOAEL. As described in footnote "b" of Table 2-2, an acute duration oral MRL of 0.01 mg/kg/day was calculated for the chlorophenols based on 4-CP. The LOAEL for 4-CP was the lowest LOAEL among all the acute-duration LOAELs for all the chlorophenols discussed in this profile.

Sprague-Dawley rats dosed at 20 mg/kg/day of 2,4-DCP in the drinking water had increased liver weights (Exon et al. 1984), an effect that could indicate hyperplasia or enzyme induction. No histopathological changes were observed in the livers of Fischer-344 rats fed 2,4-DCP in the diet at doses up to 2,000 mg/kg/day for 13 weeks or 400 mg/kg/day for 103 weeks (NTP 1989). Liver weights or liver enzymes released to the serum were not measured in the NTP (1989) study. Mice fed 325 mg/kg/day of 2,4-DCP for 13 weeks had dose-related increases in hepatocellular necrosis (not further described) (NTP 1989). When mice were fed 383 or 230 mg/kg/day for 90 days or 6 months, respectively, no effects were noted on SGOT or SGPT activity (these enzymes are released into the bloodstream as a result of liver injury) (Borzelleca et al. 1985a; Kobayashi et al. 1972). One of 10 mice exposed to 230 mg/kg/day 2,4-DCP for 6 months had hepatocellular hyperplasia. No liver effects were observed at 100 mg/kg/day (Kobayashi et al.

1972). Diffuse syncytial alterations occurred in male mice given 800 mg/kg/day 2,4-DCP in the diet for 103 weeks (NTP 1989). The number of cells affected was small, and the affected cells were scattered within the histologic sections.

When guinea pigs were administered 40 mg/kg 2,4-DCP perorally 3 times a week for 2 weeks, lipid peroxidation was increased in the liver (Clerhata et al. 1996). A high intake of ascorbic acid (50 mg/animal/day) significantly decreased lipid peroxidation in the liver in comparison to guinea pigs with low ascorbic acid intake (2 mg/kg/day). 2,4-DCP accumulation was also decreased in the liver of animals with high ascorbic acid intake.

The pretreatment of rats with 2,4,5- or 2,4,6-TCP by gavage at doses up to 400 mg/kg/day for 14 days had no effect on ethylp-nitrophenylphosphonothionate detoxification (Carlson 1978). 2,4,5-TCP but not 2,4,6-TCP at 400 mg/kg/day decreased microsomal NADPH-reductase activity and cytochrome P-450 activity.

Histologic changes in the liver were not observed when rats were treated by gavage with 2,4,5-TCP in corn oil at doses up to 1,000 mg/kg/day for 18 or 24 days (McCollister et al. 1961). Slight pathologic changes, which were not further described, were noted in the livers of rabbits treated by gavage with 2,4,5-TCP in 5% gum acacia solution for 20 or 28 days (McCollister et al. 1961). Over a 98-day period, a dose of 300 mg/kg/day given to rats in the diet resulted in mild centrilobular degeneration and focal necrosis, with no effects observed at 100 mg/kg/day (McCollister et al. 1961).

Increased liver weight and midzonal vacuolation of hepatocytes were evident in rats exposed orally for 7 weeks to 2,300 mg/kg/day 2,4,6-TCP (NCI 1979). Increased relative liver weights were found in groups of male rats exposed to 240 and 720 mg/kg/day of 2,4,6-TCP for 90 days and groups of female rats exposed to 720 mg/kg/day of 2,4,6-TCP for 90 days (Bercz et al. 1990). No treatment-related histopathological evidence of tissue damage was noted. Clinical chemistry results included increased serum albumin and total protein concentrations, which the investigators attributed to either an altered hydration status or dysfunctional hepatic activity (Bercz et al. 1990). The investigators considered 240 mg/kg/day as a LOAEL for hepatic effects and the next lower dose, 80 mg/kg/day, as a NOAEL for acute duration exposure. In contrast, increased liver weight and histopathologic lesions were not evident in rats exposed to 2,4,6-TCP over intermediate or chronic periods at doses up to 1,000 and 500 mg/kg/day, respectively (Blackburn et al. 1986; NCI 1979).

Microscopic examination revealed hepatic hyperplasia and other signs of hepatocellular damage (e.g., liver cell abnormalities, focal areas of cellular alteration) in mice exposed chronically to 2,4,6-TCP in the diet at doses as low as 650 mg/kg/day (NCI 1979). It is possible these lesions were precursors of the hepatocellular adenomas and carcinomas also observed in this study. More information relating to these hepatic neoplasms can be found in Section 2.2.2.8.

Concentration-related increases in absolute liver weight occurred in rats exposed perinatally to 3 or 30 mg/kg/day 2,4,6-TCP for 15 weeks (Exon and Koller 1985). The investigators did not examine functional or anatomical hepatic parameters.

The different effects of 2,4,6-TCP in rats and mice may, in part, be a result of the different methodologies used for exposure, variations in experimental design, and/or possible differences in gastrointestinal absorption because of the nature of the vehicle. In the intermediate oral studies by Bercz et al. (1990) and Blackburn et al. (1986), 2,4,6-TCP was administered in corn oil by gavage. Interpretation of the Blackburn et al. (1986) data is further complicated by the investigators' failure to report sample sizes used in the statistical analysis. The NCI (1979) studies used administration of 2,4,6-TCP in the diet, while 2,4,6-TCP was administered in drinking water in the Exon and Koller (1985) study, therefore, a direct comparison is not very meaningful.

For both acute- (one dose) and intermediate-duration (55 days) administration of 2,3,4,6-TeCP in Wistar rats, the most severe effects occurred in the liver (Hattula et al. 1981). In the single dose study, various adverse histopathological effects occurred at unspecified dose levels up to a maximum dose of 632 mg/kg. Intermediate-duration (55 days) administration of 100 mg/kg/day resulted in both Level III (large confluative necroses with dilated and thrombosed veins) and Level II (bile duct proliferation, focal necrosis, and polymorphonuclear leukocyte infiltration) hepatic damage. At 50 mg/kg/day, 1 out of 10 rats showed Level III damage. The NOAEL for hepatic effects following 55 days of exposure was 10 mg/kg/day (Hattula et al. 1981). The number of animals used in the 55-day study was not stated.

In a study sponsored by the EPA (American Biogenics 1988), increased liver weights and centrilobular hypertrophy were observed in rats treated by gavage with 2,3,4,6-TeCP at 100 or 200 mg/kg/day for 90 days. No effects were observed at 25 mg/kg/day.

2. HEALTH EFFECTS

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

In mice, daily administration of 35 or 69 mg/kg/day 2-CP for 14 days had no adverse effects on measures of renal function, including blood urea nitrogen (BUN), total protein, albumin/globulin ratio, or electrolyte balance (Borzelleca et al. 1985a). No significant compound-related adverse effects were noted at necropsy. In the same study, a dose of 175 mg/kg/day was lethal to all exposed mice.

Except for renal tubular necrosis in mice that died following treatment with 2,4-DCP in the diet for 3 weeks at 5,200 mg/kg/day (NTP 1989), kidney effects have not been observed in animals treated with 2,4-DCP. Based on histological examinations, the reported NOAELs for kidney effects are 2,000 and 440 mg/kg/day for rats fed 2,4-DCP in the diet for 13 and 103 weeks, respectively (NTP 1989), and 230,2,600, and 1,300 for mice fed 2,4-DCP in the diet for 90 days, 13 weeks, and 103 weeks, respectively (Kobayashi et al. 1972; NTP 1989). Treatment of mice with 2,4-DCP in drinking water at doses up to 491 mg/kg/day had no effect on kidney weights or clinical chemistry values including urine protein, phosphorus, calcium, sodium, chloride, potassium, or creatinine levels (Borzelleca et al. 1985a). Histopathological examinations were not completed because the clinical chemistry was negative.

Treatment of rats with 2,4,5-TCP at 1,000 mg/kg/day by gavage for 18 days resulted in a significant increase in kidney weight, with no histopathologic changes or changes in BUN (McCollister et al. 1961). Slight pathologic changes (not further described) were observed in rabbits given 20 gavage doses of 100 or 500 mg/kg/day, with no effects noted at 10 mg/kg/day (McCollister et al. 1961). In a go-day study, 2,4,5-TCP administered in the diet at 300 mg/kg/day resulted in mild degenerative changes in the renal epithelium of the convoluted tubules and in proliferation of the interstitial tissue (McCollister et al. 1961). No kidney effects were observed at 100 mg/kg/day.

Administration of 720 mg/kg/day 2,4,6-TCP in corn oil by gavage for 90 days resulted in increased absolute and relative kidney weights in male, but not female, Sprague-Dawley rats and decreased urinary pH in both sexes. No other effects on clinical parameters of renal function were observed (Bercz et al. 1990). Renal weight did not increase in Long-Evans rats administered 2,4,6-TCP in corn oil by gavage at doses as high as 1,000 mg/kg/day for 11 weeks, 5 days per week (Blackburn et al. 1986). Strain differences and daily treatment as opposed to treatment five times per week may account for the differences in renal effects in the Bercz et al. (1990) and Blackburn et al. (1986) studies. No treatment-related lesions were evident upon

histopathologic examination of the kidney in rats and mice exposed to dietary 2,4,6-TCP for 2 years at doses as high as 500 and 1,356 mg/kg/day, respectively (NCI 1979).

A single dose or 55day exposure to 2,3,4,6-TeCP, at doses up to 632 mg/kg or 100 mg/kg/day, respectively, had no adverse effect on the histological appearance of the kidneys of rats (Hattula et al. 1981). Increased kidney weights without any histopathologic changes were observed in rats treated by gavage with 2,3,4,6-TeCP at 100 mg/kg/day for 90 days (American Biogenics 1988). No renal effects were observed at 25 mg/kg/day.

Endocrine Effects. No studies were located regarding endocrine effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

Histopathologic examinations did not reveal any changes in the endocrine glands (adrenals, pituitary, thyroid, pancreas) of rats or mice treated with 2,4-DCP in the diets at doses up to 2,000 (rats) or 2,600 (mice) mg/kg/day for 13 weeks, or at doses up to 440 (rats) or 1,300 (mice) mg/kg/day for 103 weeks (NTP 1989). Histopathologic changes of the adrenals were not observed in rats treated with 2,4,5-TCP in the diet at 1,000 mg/kg/day for 98 days (McCollister et al. 1961).

Female rats treated by gavage with 720 mg/kg/day of 2,4,6-TCP for 90 days had slightly, but statistically significant, elevated adrenal weights compared to untreated controls (Bercz et al. 1990). Because no histopathological changes were noted, this dose is considered a NOAEL. Adrenal gland weights were not increased in male rats treated by gavage with 2,4,6-TCP at 1,000 mg/kg/day for 11 weeks (Blackburn et al. 1986), providing further support that the adrenal glands are not a target of 2,4,6-TCP toxicity. However, differences between male and female rats could be due to endocrine differences between males and females. Histopathologic changes were not observed in the adrenal glands, thyroid, pancreas, or parathyroid glands in rats or mice treated with 2,4,6-TCP in the diet at doses of 500 (rats) or 1,356 (mice) mg/kg/day for 105 weeks (NCI 1979). Treatment of rats by gavage with 2,3,4,6-TeCP for 90 days at doses up to 200 mg/kg/day had no effect on the histologic appearance of the adrenal glands, pituitary, pancreas, or thymus (American Biogenics 1988).

Dermal Effects. No studies were located regarding dermal effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

Pregnant rats given 750 mg/kg 2,4-DCP by gavage experienced hair loss (Rodwell et al. 1989). No histological changes in the skin were found in rats or mice given as much as 2,000 or 2,600 mg/kg/day, respectively, for up to 13 weeks, nor for these same species fed up to 440 or 1,300 mg/kg/day for up to 103 weeks (NTP 1989). Upon histopathologic examination of the skin, no treatment-related effects were observed in rats or mice exposed chronically to oral doses of 2,4,6-TCP as high as 500 or 1,356 mg/kg/day, respectively (NCI 1979).

Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

Histopathologic examination of the eyes did not reveal any adverse effect in rats or mice either treated with 2,4-DCP (NTP 1989) in the diet or treated by gavage with 2,3,4,6-TeCP (American Biogenics 1988) for intermediate or chronic durations. Ophthalmoscopic examinations did not reveal any treatment-related effects in rats treated by gavage with 2,4,6-TCP at doses up to 720 mg/kg/day for 90 days (Bercz et al. 1990).

Body Weight Effects. No studies were located regarding body weight effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

In a 14-day study, both sexes of mice receiving 69 mg/kg/day 2-CP had unspecified body weight decrements (Borzelleca et al. 1985a); the NOAEL was 35 mg/kg/day. No effects on body weight were observed in rats treated with 2-CP in drinking water at doses of 50 mg/kg/day during gestation and lactation as well as 15-weeks postweaning (Exon and Koller 1981,1982). Single-day gestational exposure of gravid Sprague-Dawley rats to 1,000 mg/kg 4-CP resulted in a significant body weight loss (Kavlock 1990). By 72 hours after dosing, the body weight difference was no longer statistically significant, and lower levels did not produce any body weight gain inhibition in gravid Sprague-Dawley rats. The NOAEL for the body weight effect for 4-CP was 667 mg/kg/day. Additional results from this study are discussed in Section 2.2.2.6.

Studies with rats and mice fed 2,4-DCP for acute, intermediate, and chronic durations revealed dose-related decreases in food intake and body weight (NTP 1989). These effects are believed to be due to the bad taste of 2,4-DCP. Body weights were not affected in mice treated with 2,4-DCP in the diet at doses up to 230 mg/kg/day (Kobayshi et al. 1972) or in drinking water at doses up to 491 mg/kg/day (Borzelleca et al.1985a). To improve palatability in drinking water, Borzelleca et al. (1985a) used a 1:9 emulphor:water

solution which is a modified vegetable oil. Body weights of pregnant animals treated on gestation days 6-l5 were reduced at 375 but not 200 mg/kg/day (Rodwell et al. 1989).

Treatment of rats by gavage with 2,4,5-TCP for 18 or 24 days at 1,000 mg/kg/day had no effect on body weight (McCollister et al. 1961). In contrast, treatment with 2,4,5-TCP in the diet at 1,000 mg/kg/day for 90 days resulted in a 24% decrease in body weight gain in female but not in male rats (McCollister et al. 1961). No effects on food intake were measured.

Treatment of rats with 2,4,6-TCP by gavage at 1,000 mg/kg/day for 2 weeks before mating and throughout gestation resulted in reduced body weights through gestation day 14 (Blackburn et al. 1986). Body weights on gestation day 21 were not significantly different from those of the controls. No effect on body weight was observed in rats treated by gavage with 2,4,6-TCP at 1,000 mg/kg/day for 90 days (Bercz et al. 1990) or 11 weeks (Blackburn et al. 1986), suggesting that pregnant animals may be more sensitive to effects on body weight following treatment with 2,4,6-TCP. No effect on body weight was observed in mice treated with 2,4,6-TCP in drinking water at 30 mg/kg/day for 24-25 weeks (Exon and Koller 1985). Body weights were significantly reduced in rats treated with 2,4,6-TCP in the diet for 7 weeks at 735 but not at 500 mg/kg/day and 250 mg/kg/day for 105 weeks (NCI 1979). Body weights were also significantly decreased in mice fed 2,600 mg/kg/day 2,4,6-TCP in the diet for 7 weeks and at 658 mg/kg/day for 105 weeks (NCI 1979). No effects on body weight were observed in mice fed 1,300 mg/kg/day 2,4,6-TCP for 7 weeks (NCI 1979). Food intake data were not provided in the NCI (1979) study. The fact that 2,4,6-TCP affected body weight following dietary intake but had little effect at similar doses following gavage treatment suggests that 2,4,6-TCP may have caused the food to be less palatable and reduced food intake in mice at the concentrations used in the NCI (1979) study. Therefore, decreased body weight may be an effect of decreased food intake rather than an effect of 2,4,6-TCP treatment.

Body weight was significantly decreased in rats treated by gavage with 2,3,4,6-TeCP at 100 mg/kg/day (American Biogenics 1988) for 90 days, but not 100 mg/kg/day for 55 days (Hattula et al. 1981).

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after oral exposure to any of the eight chlorophenol isomers discussed in this profile.

Rats fed 50 mg/kg/day 2-CP for up to 16 weeks and mice fed 69 mg/kg/day 2-CP for 14 days showed no changes in humoral or cell-mediated immunological assays (Borzelleca et al. 1985a; Exon and Koller 1983,1985). Indices assessed in the Exon and Koller (1983, 1985) studies include antibody production, delayed type hypersensitivity, and phagocytic activity of peritoneal exudate cells. Female mice exposed to 69 mg/kg/day for 14 days had statistically significant decreases in spleen weight but no gross abnormalities in spleen morphology (Borzelleca et al. 1985a). Spleen and thymus weights were not significantly affected in rats that received 50 mg 2,4-DCP kg/day in drinking water for 16 weeks (Exon and Koller 1983, 1985). Perinatal exposure of young rats to 2-CP at doses up to 50 mg/kg/day produced no treatment-related effects on humoral or cell-mediated immunity, thymus weights, or spleen weights (Exon and Koller 1983, 1985).

Histopathological examination of lymph nodes, spleen, and thymus did not reveal any effects in rats or mice treated with 2,4-DCP in the diet at doses up to 2,000 (rats) and 2,600 mg/kg/day (mice) for 13 weeks, or 440 (rats) and 1,300 mg/kg/day (mice) for 103 weeks (NTP 1989). Bone marrow atrophy was observed in rats treated at 500 but not 250 mg/kg/day for 13 weeks (NTP 1989). Because both erythroid and myeloid elements were affected, this study is also discussed in Section 2.2.2.2 under Hematological Effects. No changes in spleen weight were observed in mice treated with 2,4-DCP in the diet at 230 mg/kg/day for 6 months (Kobayashi et al. 1972), and no changes in spleen or thymus weight were noted in mice treated with 2,4-DCP in the drinking water at doses up to 491 mg/kg/day for 90 days.

As shown in Table 2-2 and Figure 2-2, immune system effects have been reported in animals at low doses of 2,4-DCP. Decreased delayed-type hypersensitivity occurred in rats during 15-week-duration exposure to 3 mg/kg/day of 2,4-DCP in drinking water, and increased serum antibodies to key hole limpert nemocyanin were found in the blood of rats during similar exposures to 30 mg/kg/day (Exon and Koller 1985; Exon et al. 1984). Macrophage function, measured by the *in vitro* phagocytosis of sheep red blood cells, showed no effect from 2,4-DCP treatment. These results suggest that the immune system is quite sensitive to 2,4-DCP. No immune system effects occurred with exposure to 0.3 mg/kg/day (Exon et al. 1984). Based on the NOAEL of 0.3 mg/kg/day, an intermediate-duration oral MRL of 0.003 mg/kg/day was calculated for the chlorophenols as described in the footnote in Table 2-2. The LOAEL for 2,4-DCP was the lowest among all the intermediate-duration LOAELs for all the chlorophenols discussed in this profile.

No changes in spleen weight or histological appearance were observed in rats treated with 2,4,5-TCP in the diet at doses of 1,000 mg/kg/day for 98 days (McCollister et al. 1961) or in rats treated by gavage with 720 mg/kg/day 2,4,6-TCP for 90 days (Bercz et al. 1990). Spleen weights were significantly increased in rats exposed to 2,4,6-TCP in the drinking water both pre- and postnatally at doses of 30 mg/kg/day, while no significant effects on immune function (antibody levels, delayed-type hypersensitivity, macrophage numbers) were observed (Exon and Koller 1985). Treatment of rats and mice with 2,4,6-TCP in the diet for 2 years at doses up to 500 mg/kg/day for rats and 1,356 mg/kg/day for mice did not reveal any significant gross or histopathological changes in the spleen, lymph nodes, or thymus (NCI 1979).

Administration of a single gavage dose 632 mg/kg of 2,3,4,6-TeCP in Wistar rats resulted in "slight stasis" in the spleens of rats (Hattula et al. 1981); the toxicological significance of this finding is unknown. No histological changes were observed in the spleen, lymph nodes, or thymus of rats treated with 2,3,4,6-TeCP by gavage at doses up to 200 mg/kg/day for 90 days (American Biogenics 1988).

The highest NOAEL values and all reliable LOAEL values for immunological and lymphoreticular effects in rats and mice for each exposure duration are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to any of the eight chlorophenol isomers discussed in this profile.

In most acute animal studies involving 2-, 4-CP and 2,4-DCP exposure, a common syndrome of effects precedes death (Borzelleca et al. 1985a, 1985b; Kobayashi et al. 1972; Spencer and Williams 1950; Wil Research Laboratories 1982). This syndrome includes restlessness, tremors, convulsions, dyspnea and/or tachypnea, and collapse or coma. In many of these studies, the major effects associated with exposure to high doses of many phenolic compounds are myoclonic convulsions, or spasmodic twitching of a group of muscles. The relationship between chlorophenol exposure and the onset of convulsions is discussed further in Sections 2.4 and 2.5. In general, the sensitivity of these clinical signs (particularly convulsions) decreases with increasing chlorination.

In an LD_{50} study, single oral doses (unspecified) of 2-CP or 4-CP caused restlessness, motor weakness, tremors, convulsion, or central nervous system depression in rats and mice (Borzelleca et al. 1985a, 1985b).

The actual doses used in the study (Borzelleca et al. 1985b) were not stated. A single oral dose of 514 mg/kg 4-CP produced seizures immediately followed by death in male ICR mice (Phornchirasilp et al. 1989b). Single doses of 2-CP >300 mg/kg resulted in distress and twitching in rabbits (Spencer and Williams 1950). Administration of 4-CP produced similar effects at higher, unspecified doses. In male and female ICR mice, repeated administration of 35 and 69 mg/kg/day 2-CP for 44 days resulted in hyperactivity and decreased brain weight, respectively (Borzelleca et al. 1985a); although, the brain tissue appeared grossly normal (Borzelleca et al. 1985a).

Mice treated with 2,4-DCP in the diet at 5,200 mg/kg/day for 14 days were lethargic and 1 out of 5 males died (NTP 1989). Hunched posture was observed in rats treated with 2,4-DCP in the diet at 2,000 mg/kg/day for 13 weeks (NTP 1989) with no histopathological changes in the brain, sciatic nerve, or spinal cord. In mice treated with 2,4-DCP in the diet at doses up to 2,600 mg/kg/day for 13 weeks, no histopathological changes were observed in the brain, sciatic nerve, or spinal cord (NTP 1989). No effect on brain weight was observed in mice treated with 2,4-DCP in the drinking water at doses up to 491 mg/kg/day (Borzelleca et al.1985a). No clinical signs of neurological effects were reported in rats or mice fed doses up to 440 mg/kg/day for rats and 1,300 mg/kg/day for mice, and histopathologic examination of the brains of these animals did not reveal any effects (NTP 1989).

No changes in brain weight or histological appearance of the brain were observed in rats treated with 2,4,5-TCP in the diet at doses up to 1,000 mg/kg/day for 98 days (McCollister et al. 1961).

Histopathologic examination of the brain (cerebrum and cerebellum) of rats and mice exposed repeatedly to oral 2,4,6-TCP at doses as high as 720 and 1,356 mg/kg/day, respectively, revealed no treatment-related effects (Bercz et al. 1990; NCI 1979). Similarly, in Wistar rats exposed acutely to up to 632 mg/kg 2,3,4,6-TeCP, or repeatedly to 200 mg/kg/day 2,3,4,6-TeCP for 90 days, no histopathological effects in the brain were observed (American Biogenics 1988).

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

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to any of the eight chlorophenol isomers discussed in this profile.

A teratogenicity study in which pregnant rats were treated with 2,4-DCP by gavage on gestation days 6-15 at doses that caused maternal deaths and decreased body weight gain showed neither postimplantation loss nor changes in the numbers of resorptions and viable fetuses (Rodwell et al. 1989). No reproductive organ pathology was observed in rats or mice of either sex fed up to 2,000 or 2,600 mg/kg/day 2,4-DCP, respectively, for 13 weeks (NTP 1989). Reproductive organ pathology was also not observed in male rats fed 440 and female rats fed 250 mg/kg/day 2,4-DCP and male mice fed 1,300 and female mice fed 8,210 mg/kg/day 2,4-DCP for 2 years (NTP 1989). Sperm from male mice fed 500 mg/kg/day 2,4-DCP for 90 days in drinking water were not impaired in their ability to fertilize ova (Seyler et al. 1984).

Using identical experimental protocols, investigators have studied the reproductive effects of 2-CP, 2,4-DCP, and 2,4,6-TCP in Sprague-Dawley female rats (Exon and Koller 1985). Groups of rats received uncontaminated drinking water or one of three concentrations of a chlorophenol in drinking water, beginning at weaning and extending through mating and parturition. The total exposure duration for each group was approximately 13 weeks. The only consistent concentration-related effect observed in all three experiments was a marginal decrease ($p<0.10$) in litter size. In all cases, the individual conceptus, rather than the litter, was the unit of statistical analysis. For 2-CP, 2,4-DCP, and 2,4,6-TCP, the highest concentration in water corresponded to 50,30, and 30 mg/kg/day, respectively; these doses are considered LOAELs for reproductive effects. No significant reproductive effects were observed at 5 mg/kg/day 2-CP and 3 mg/kg/day for 2,5-DCP and 2,4,6-TCP.

In a study designed to look at reproductive function, Blackburn et al. (1986) treated female rats with 2,4,6-TCP by gavage at doses up to 1,000 mg/kg/day for 2 weeks before mating and throughout gestation and treated male rats with 2,4,6-TCP at doses up to 1,000 mg/kg/day for 10 weeks. The treated females were mated with untreated males, and the treated males were mated with untreated females. 2,4,6-TCP had no effects on breeding success, litter size, or litter survival when either sex was treated. Treatment of males had no effect on sperm count, motility, or morphology, nor were there any changes in weights of the testes, prostate, or seminal vesicles. Although treatment-related deaths occurred in both sexes at 1,000 mg/kg/day, this dose can be considered a NOAEL for 2,4,6-TCP reproductive effects in rats.

In 90-day studies, gavage treatment of rats with either 2,4,5-TCP at doses up to 1,000 mg/kg/day (McCollister et al. 1961) or with 2,4,6-TCP up to 720 mg/kg/day (Bercz et al. 1990) had no effect on the weight of the testes or ovaries. Treatment of rats with 2,4,6-TCP in the diet for 2 years had no effects on the histologic appearance of the testis and prostate or of the uterus or ovaries (NCI 1979).

In a study designed to examine developmental effects, pregnant rats were treated by gavage with 2,3,4,6-TeCP at doses up to 200 mg/kg/day on gestation days 6-l 5 (RTI 1987). An increased trend in percent preimplantation loss with dose suggested an effect on the process of implantation or early postimplantation viability. Because this study was not designed to examine the preimplantation/ implantation phase of reproduction, the investigators suggested that the effect requires confirmation. Therefore, a LOAEL or NOAEL for reproductive effects in female rats exposed to 2,3,4,6-TeCP is not clearly defined by this study. No histopathological changes were observed in the testes, ovaries, or uterus and cervix of rats treated by gavage with 2,3,4,6-TeCP at doses up to 200 mg/kg/day for 90 days (American Biogenics 1988).

The highest NOAEL values and all reliable LOAEL values are listed in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to any of the eight chlorophenol isomers discussed in this profile.

No significant changes in offspring body or liver weights were observed in rats treated with 2-CP in drinking water at doses up to 50 mg/kg/day throughout gestation and up to 91 days post partum (Exon and Keller 1981, 1983, 1985). Groups of 6-13 female Sprague-Dawley rats receiving a single dose of 333,667, or 1,000 mg/kg 4-CP on gestational day 11 showed no adverse changes in litter sizes, perinatal loss, pup weight, or litter biomass (Kavlock 1990). The only treatment-related effect was a transient decrease in maternal body weight at 1,000 mg/kg.

Oral exposure of pregnant rats to 750 mg/kg/day 2,4-DCP for 10 gestational days induced a slight decrease in fetal weight and a statistically significant delayed ossification of sternal and vertebral arches and led to a slight insignificant increase in early embryonic deaths (0.8/average litter controls; 1.2/litter 750 mg/kg/day) (Rodwell et al. 1989). Maternal death occurred at this dose level, indicating that 2,4-DCP was not selectively toxic to embryos or fetuses. The authors indicated that, although the number of deaths and fetal weights

differed from that of the concurrent controls, values were not different from the historical control data from their laboratory. No evidence of malformations in the offspring was found in this study. At 375 mg/kg/day, maternal body weight was reduced, with no effects observed at 200 mg/kg/day.

No effect on immune function parameters (antibody production, delayed type hypersensitivity response, phagocytic activity) was noted in 6-week-old rats treated with 2,4-DCP in the drinking water at doses up to 30 mg/kg/day throughout gestation (Exon and Koller 1985; Exon et al. 1984). Spleen weights were significantly increased at 30 mg/kg/day, although no histological changes in the spleen were observed.

Gavage administration of 650 mg/kg/day 2,4,5-TCP during organogenesis (days 6-15 of gestation) produced no fetotoxicity, malformations, or structural terata in the offspring of Sprague-Dawley rats (Chernoff et al. 1990). Treatment resulted in statistically insignificant increases in maternal lethality and decrements in maternal weight gain (Chernoff et al. 1990). In another developmental study, groups of mice received either a single gavage dose of 800-900 mg/kg 2,4,5-TCP on 1 day of gestation (any of gestation days S-15), or 250-300 mg/kg/day on any 3 days of gestation (gestation days 7-9, 10-12, or 13-15) (Hood et al. 1979). With the exception of a significant increase in the incidence of prenatal mortalities and resorptions in dams dosed on day fourteen, 2,4,5-TCP had no effect on resorption incidence or pup survival. 2,4,5-TCP administration did not affect mean fetal weight or the incidence of gross malformations, skeletal malformations, or cleft palates (Hood et al. 1979).

In a study designed to examine reproductive effects, a 10-11% decrease in litter weights was observed in litters of female rats treated by gavage with 2,4,6-TCP at 500 mg/kg/day for 2 weeks before mating and throughout gestation (Blackburn et al. 1986). No effects on litter weights were observed at 100 mg/kg/day, and no effects on survival to postnatal day 42 were observed. No effects on body weight were observed among offspring of male rats treated by gavage with 2,4,6-TCP at 1,000 mg/kg/day for 10 weeks before mating (Blackburn et al. 1986). Because comprehensive examinations of offspring were not completed, this study is not sufficient to conclude that developmental effects do not occur following exposure to 2,4,6-TCP.

Maternal exposure of rats to 500 mg/kg/day 2,4,6-TCP produced a transient reduction in the body weight of offspring (Blackbum et al. 1986). No developmental effects were noted in the offspring of female rats exposed to 2,4,6-TCP throughout gestation (Blackbum et al. 1986; Exon and Koller 1985). In addition, no developmental effects were noted in the offspring of male rats treated with 2,4,6-TCP and untreated females (Blackbum et al. 1986). These studies were limited by the lack of reporting on the number of animals from

which group means were calculated (Blackburn et al. 1986) and by a lack of reporting on maternal toxicity (Exon and Koller 1985).

In a developmental study in which female Sprague-Dawley rats orally received purified 2,3,4,6-TeCP throughout organogenesis, the only effect on the fetus was delayed ossification of the skull bones (Schwetz et al. 1974). The reported incidences were 14/173 (8%) and 18/104 (17%) at 0 and 30 mg/kg/day, respectively. When analyzed by litter, no statistical difference for delayed ossification was observed. Therefore, 30 mg/kg/day 2,3,4,6-TeCP is considered a NOAEL for developmental effects in rats. In a follow-up study, pregnant CD rats received 0,25, 100, or 200 mg/kg/day, in olive oil, every day during organogenesis (RTI 1987). Administration of the two highest doses resulted in corrected maternal body weight gain (dam body weight-gravid uterus weight) inhibitions of 13% and 26%, respectively, with no effects at 25 mg/kg/day. Measurement of food intake indicated that these effects were not related to decreased food consumption, Minor variations between dose groups in fetal malformation and aberrations were not dose related. The investigators also noted a dose-related trend for 2,3,4,6-TeCP-mediated effects on implantation or postimplantation viability. No further evidence of maternal or fetotoxic effects were observed (RTI 1987). Based on maternal toxicity, this study identifies 100 mg/kg/day as a LOAEL and 25 mg/kg/day as a NOAEL for developmental effects.

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

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to any of the eight chlorophenols discussed in this profile.

In ICR mice, daily gavage administration of 69 mg/kg/day 2-CP or 638 mg/kg/day 2,4-DCP in corn oil for 14 days did not increase sister chromatid exchange (SCE) rates in testicular or bone marrow cells (Borzelleca et al. 1985a). Further details were not provided. Ninety-day exposure,of mice to 2,4DCP in drinking water at doses up to 500 mg/kg/day also had no effect on SCE in bone marrow and testicular cells (Borzelleca et al. 1985a). A single gavage dose of 2,4,5-TCP (164 mg/kg), 2,4,6-TCP (164 mg/kg), or 2,3,4,6-TeCP (28 or 193 mg/kg) given to rats did not damage deoxyribonucleic acid (DNA) as measured by the fraction of DNA eluted from white blood cells or livers (Kitchin and Brown 1988).

Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to any of the eight chlorophenol isomers discussed in this profile.

In the one oral carcinogenicity study located, groups of Sprague-Dawley rats received prenatal, postnatal, or both pre- and postnatal exposure to 2-CP (Exon and Koller 1985). The exposure concentrations were 0,5, 50, and 500 ppm in drinking water (0, 0.5, 5, 50 mg/kg/day). Under all exposure conditions, 2-CP administration had no effect on the incidence, latency, or types of tumors relative to the untreated controls. Additional groups of gravid dams received ethylurea and nitrite, precursors of the carcinogenic initiator ethylnitrosourea (ENU), on gestation days 14 and 21. No consistent effects on either tumor incidence or latency occurred in rats treated with ENU and then treated either prenatally or postnatally with 2-CP. The groups of males receiving ENU and both prenatal and postnatal 2-CP had increased tumor incidence and decreased tumor latency relative to a control group receiving ENU only. The investigators indicate that the combined changes were marginally statistically significant ($p< 0.10$) in comparison to a group receiving the initiator ENU only. ENU-exposed female rats also exposed pre- and postnatally to 2-CP showed no consistent, concentration-related effects on either tumor incidence or latency (Exon and Koller 1985). Findings in the combined-exposure male treatment groups indicate that 2-CP may be either a cocarcinogen or a tumor promotor. However, an analysis of incidence and latency data suggests that the effects may not be concentration related. No effects on tumorigenicity were found in similar studies with 2,4-DCP given in drinking water at 0.3,3, or 30 mg/kg/day. It is not clear whether a maximum tolerated dose was achieved in these studies (Exon and Koller 1985).

Chronic carcinogenicity bioassays in rats and mice treated with 2,4-DCP in the diet at doses up to 440 mg/kg/day for rats and 1,300 mg/kg/day for mice did not provide any evidence that 2,4-DCP is carcinogenic (NTP 1989). In contrast, carcinogenicity bioassays with rats and mice provide evidence that chronic oral exposure to 2,4,6-TCP is associated with leukemia and liver cancer (NCI 1979). In male rats, chronic oral exposure to 2,4,6-TCP in the diet produced a significant dose-related increase in the incidence of monocytic leukemia (NCI 1979). An increased incidence of leukemia also occurred in female rats; however, the increase was not significant compared to the controls. In addition, leukocytosis and monocytosis as well as hyperplasia of the bone marrow were induced in treated male and female rats that did not develop

leukemia. In rats with leukemia, there were large numbers of circulating monocytes in the blood that ranged from well-differentiated monocytes to immature and blast forms. Monocytes were often observed in the liver, spleen, lymph tissue, and bone marrow and occasionally in the lungs, adrenals, and other organs.

In both male and female $B6C3F_1$ mice treated chronically with 2,4,6-TCP in the diet, a significant doserelated increase in the incidence of hepatocellular adenomas and carcinomas (not further described) was noted (NCI 1979). Liver damage, including individual liver cell abnormalities, focal areas of cellular alteration and focal and nodular areas of hyperplasia were commonly present in the treated mice. Significant limitations of this study included the failure to report the dioxin content of the 2,4,6-TCP formulation, changes in the dosing regimen of mice, and no testing of organ function. Another limitation was the failure to compare the incidence of liver tumors to historical controls as well as concurrent controls. Hepatocellular carcinoma has a high natural incidence in this strain of mouse which tends to vary from one study to the next.

A single oral dose of 2,4,6-TCP (200 mg/kg) did not significantly increase skin tumors in mice treated dermally with a tumor promoter (12-O-tetradecanoylphorbol-13-acetate [TPA]) relative to TPA alone, suggesting that 2,4,6-TCP does not act systemically as an initiator (Bull et al. 1986). Other studies also examined the possible carcinogenic effects of 2,4,6-TCP, but contained limitations that preclude a conclusion (Bionetics Research Labs 1968; Innes et al. 1969; Stoner et al. 1986). The limitations included early termination of the experiment (24 weeks) (Stoner et al. 1986), only one treatment group (Bionetics Research Labs 1968; Innes et al. 1969), a small number of treated animals (Bionetics Research Labs 1968; Innes et al. 1969), and a change in dosing regimen and method of exposure (Bionetics Research Labs 1968; Innes et al. 1969).

The Cancer Effect Levels (CELs) are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.3 Dermal Exposure

2.2.3.1 Death

A worker who splattered pure 2,4-DCP on portions of his right arm and leg while disposing of industrial waste collapsed and experienced a seizure within 20 minutes of the accident and died shortly thereafter. Postmortem examination revealed blood and urine 2,4-DCP concentrations of 24.3 and 5.3 mg/L, respectively. The identity of 2,4-DCP was confirmed by mass spectrometry. The investigators did not

estimate an absorbed dose (Kintz et al. 1992), but assuming a blood volume of 5 liters and a body weight of 70 kg, the dose would be approximately 2 mg/kg as a minimum. A screen for other drugs including ethanol, organic solvents, tranquilizers, and drugs of abuse was negative.

Limited data were located on the lethal effects of dermally applied chlorophenols in experimental animals. Results of a contract laboratory study indicate that the dermal LD_{50} of 2-CP in rabbits is between 1,000 and 1,580 mg/kg (Younger Lab 1975). Antemortem observations included increasing weakness, tremors, collapse, and coma. Gross necropsy in the rabbit studies indicated hemorrhage in the lungs, Liver discoloration, gastrointestinal inflammation, darkened spleens and kidneys, and enlarged gall bladders. The study data do not clearly indicate whether mortality resulted from any of these effects. Conclusions from this study are limited by small test groups and/or the lack of information regarding experimental methodology.

A dermal LD_{50} of 1,415 mg/kg has been reported for male rabbits exposed to 2,4-DCP for 24 hours (Carreon et al. 1980b). Because there were only two rabbits per dose group, the 95% confidence interval on this value is very large (236-8,455 mg/kg). Unoccluded dermal application of 2,000 mg/kg 2,3,4,5-TeCP or 2,3,5,6-TeCP resulted in 1 out of 20 and 2 out of 20 deaths, respectively, in Sprague-Dawley rats (Shen et al. 1983). The purity of each test compound was >99%. Because these preliminary studies indicated that the dermal LD_{50} values for 2,3,4,5-TeCP and 2,3,5,6-TeCP were greater than 2,000 mg/kg, further testing of these compounds was not completed. The LD_{50} for commercial tetrachlorophenol, consisting primarily of the 2,3,4,6-isomer (at least 90%), was 485 mg/kg in males and 565 mg/kg in females. Clinical signs preceding death for all tetrachlorophenol isomers included initial hyperactivity followed by hypoactivity, neuromuscular weakness, and convulsions (Shen et al. 1983).

The LD_{50} values and dermal doses of chlorophenols associated with death are recorded in Table 2-3.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, hematological, musculoskeletal, hepatic, or renal effects in humans or animals after dermal exposure to any of the eight chlorophenols discussed in this profile. The systemic effects that were observed after dermal exposure to chlorophenols are discussed below.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after dermal exposure to any of the eight chlorophenols discussed in this profile.

TABLE 2-3. Levels of Significant Exposure to Chlorophenols - Dermal

Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ Compound
ACUTE EXPOSURE						
Death						
Rat (Sprague-Dawley)	24 hr				2000 M (1/10 died)	Shen et al. 1983 2,3,4,5-tetraCP
Rat (Sprague-Dawley)	24 hr				485 M (LD50)	Shen et al. 1983 2,3,4,6-tetraCP
Rat (Sprague-Dawley)	24 hr				2000 F (2/10 died)	Shen et al. 1983 2,3,5,6-tetraCP
Rabbit (New Zealand albino)	24 hr				1580 (2/2 rabbits died)	Younger Labs 1975 2-chlorophenol
Rabbit (New Zealand albino)	24 hr				1414 M (LD50)	Carreon et al. 1980b 2,4-dichlorophenol
Systemic						
Mouse (dd)	6 hr	Derm	100 M			Dohi et al. 1989 4-chlorophenol

TABLE 2-3. Levels of Significant Exposure to Chlorophenols - Dermal (continued)

Species/ (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference/ *Compound*
Rabbit (NS)	once	Ocular		0.6 M (slight hyperemia)	1.2 M (severe hyperemia, edema, cloudiness of the cornea)	Harrison and Madonia 1971 4-chlorophenol
Rabbit (New Zealand albino)	24 hr	Derm		250 M (moderate to marked erythema, slight to marked edema and necrosis)		Carreon et al. *1980b* 2,4-dichlorophenol
Rabbit (New Zealand albino)	24 hr	Derm		200 F (moderate to marked erythema, edema, and necrosis)		Hencke and Lockwood 1978 2,4-dichlorophenol
Neurological						
Rabbit (New Zealand albino)	24 hr			250 M (lethargy)		Carreon et al. 1980b *2,4-dichlorophenol*

Derm = Dermal; F = female; hr =hour(s); LD50 = 50% lethal dose; LOAEL = lowest-observable-adverse-effect level; M = male; NOAEL = no-observable-adverse-effect level; NS = not stated

Diarrhea was observed in one of two female rabbits the day after a dermal exposure to a single dose of 398 mg/kg/day 2,4-DCP (Hencke and Lockwood 1978). This limited study suggests that either dermally applied 2,4-DCP, or the stress of being exposed to a skin irritant, can result in gastrointestinal effects in rabbits.

Dermal Effects. Chloracne and evidence of acquired porphyria, hyperpigmentation, and hirsutism have been observed in workers employed in the manufacture of 2,4-DCP- and 2,4,5-TCP-based herbicides (Bleiberg et al. 1964; Bond et al. 1989). The chloracne incidence was greatest in young employees exposed in trichlorophenol production and in chlorophenol production and finishing procedures (Bond et al. 1989). In this study, workers exposed to the highest concentration of the contaminant TCDD were at the greatest risk of developing chloracne.

The results of animal studies indicate that monochlorophenols are corrosive to epithelial tissue (Bioassay Systems 1981; Rhodia 1978). Severe effects have been reported at exposure levels of 242-2,000 mg/kg of 2-CP or 4-CP applied directly to rabbit skin. Corrosion (not further described) is typically accompanied by other signs of severe skin injury, including erythema, edema, and discoloration. A single dermal application of a lower dose (100 mg/kg) of 4-CP to one ear of a mouse did not increase ear weight relative to the untreated ear (Dohi et al. 1989). Because of the inadequacies of the test methodologies used, few conclusions regarding dose-response relationships or comparative isomeric potency can be made.

Dermal lesions were caused by a single direct application of as little as 200 mg/kg 2,4-DCP to bare abdominal skin of New Zealand White rabbits (Carreon et al. 1980a, 1980b; Hencke and Lockwood 1978; Younger Labs 1976). The dose-related dermal damage observed was described as mild-to-moderate erythema and mild-to-marked edema, followed by necrosis and scabbing. No NOAEL values were identified in these studies.

Dermal application of 20 mL/kg (32 g/kg) 2,3,4,5-TeCP on the shaved skin of female rats resulted in dermatosis associated with scar formation. Rats treated with the sodium hydroxide extracted fraction of 2,3,4,5-TeCP had no dermatological lesion, indicating that the adverse effects were attributable to the chlorophenol rather than contaminants, such as dioxins (Shen et al. 1983).

Ocular Effects. Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol reported eye irritation more frequently than unexposed workers (Kleinman et

al. 1986). The eye irritation was likely a direct effect of the tetrachlorophenols, resulting from contact with the airborne chemicals or contact with contaminated surfaces (e.g., hands, clothing).

Monochlorophenols produce effects ranging from slight hyperemia to severe corrosion when applied to the corneas of rabbits. Rabbits administered 0.6 mg/kg 4-CP (a 1% solution) showed slight hyperemia (Harrison and Madonia 1971). At 1.2 mg/kg, rabbits had more severe hyperemia with edematous swelling, corneal cloudiness, and exudation. The maximum response occurred 5 hours after application. Inflammation was no longer apparent at 96 hours. Severe discomfort and corrosion was reported to occur 1 minute after the application of 33 mg/kg undiluted 2-CP to rabbit eyes (Younger Labs 1975). Although the results are inadequate for an assessment of comparative potencies across isomers, the available data indicate that 2-CP and 4-CP produce rapid and severe cornea1 destruction at relatively low concentrations.

Severe cornea1 damage occurred in the eyes of rabbits after a single direct application of 0.1 mL 2,4-DCP (Hencke and Lockwood 1978). Careful washing of the eye 30 seconds after application did not prevent this damage.

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans following dermal exposure to any of the eight chlorophenols discussed in this profile.

The murine local lymph node assay, which is predictive of skin sensitization potential, was completed in mice treated with 2,4,5-TCP (Kimber and Weisberger 1991). A single dermal exposure of 50 mL of 2,4,5-TCP was applied on one shaved flank; 5 days later the mice were given 3 daily doses (140-560 mg/kg/day) applied to the ear. A positive response was observed at all doses, suggesting that 2,4,5-TCP can be a skin sensitizer. This study is limited since only three mice were used in each group and a statistical analysis of the data was not completed.

2.2.3.4 Neurological Effects.

An industrial waste worker who accidentally splashed pure 2,4-DCP on portions of his right arm and leg, experienced a seizure within 20 minutes of the exposure, and died shortly thereafter (Kintz et al. 1992). Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and

pentachlorophenol reported headaches more frequently than unexposed workers (Kleinman et al. 1986). Industrial hygiene observations of inadequate use of protective equipment to prevent skin exposure led the investigators to suggest that exposure was principally through the skin, with some possibility of oral ingestion.

Rabbits given single applications of 250 mg/kg 2,4-DCP or more became lethargic (Carreon et al. 1980a, 1980b; Younger Labs 1976), and two rabbits in the 2,000-mg/kg group and one in the 4,000-mg/kg group became anorexic (Carreon et al. 1980b). Small sample sizes weaken the validity of these data, but the lethargy observed in this study is in keeping with the signs of central nervous system depression seen in rats and mice orally exposed to 2,4-DCP. In a single-dose dermal study of the tetrachlorophenols in rats, clinical signs observed before death were hyperactivity, neuromuscular weakness, convulsions, and death (Shen et al. 1983). Both 2,3,5,6-TeCP and 2,3,4,5-TeCP that had dermal LD_{50} values >than 2,000 mg/kg were less toxic than 2,3,4,5-TeCP that had a dermal LD_{50} of 468 mg/kg in males and 565 mg/kg in females.

No NOAEL values were identified for neurological effects. The lowest LOAEL values for neurological effects in rabbits are recorded in Table 2-2.

2.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals after dermal exposure to any of the eight chlorophenol isomers discussed in this profile.

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to any of the eight chlorophenol isomers discussed in this profile.

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

Numerous authors have studied the possible relationship between occupational chlorophenol exposure and the expression of oncogenicity. For all of these studies, the workers were exposed by both the inhalation and dermal routes. The description and results of these studies were provided in Section 2.2.1.8.

Results of case-control studies have suggested increased risks for soft tissue sarcoma, malignant lymphoma, and acute myeloid leukemia in slaughterhouse workers exposed occupationally to a number of chemicals, including 2,4,6-TCP, by dermal exposure during the treatment of animal pelts (Pearce et al. 1988; Smith et al. 1984). Workers in these studies were also exposed to potentially oncogenic viruses (including bovine leukemia virus). Because of the confounding exposures to various agents, no conclusions can be made from these studies as to the causal agent for these cancers.

In 15-week mouse initiation-promotion studies, 2-CP and 2,4-DCP, but not 2,4,6-TCP, showed tumor promoting activity (Boutwell and Bosch 1959). One application of the known tumor initiator 9,10-dimethyl-1,2-benzanthracene (DMBA) to the middorsal region of mice was followed by twice weekly dermal applications of 25 μL of a 20% solution of either 2-CP, 2,4-DCP, or 2,4,6-TCP. Compared to DMBA treatment alone, 2-CP and 2,4-DCP increased the number of skin tumors, with no effect from 2,4,6-TCP exposure (Boutwell and Bosch 1959). In a study in which no initiator was used, 2-CP applied to the backs of mice twice per week for 12 weeks resulted in papillomas in 46% of the mice (Boutwell and Bosch 1959). No carcinomas were observed. The significance of these results is limited by the lack of appropriate vehicle control groups, irritation, and the reporting of only gross pathological effects (EPA 1980a).

2,4,6-TCP did not have initiating activity in an initiation-promotion study in mice (Bull et al. 1986). Mice were treated with a dermal dose of 200 mg/kg/day 2,4,6-TCP followed 2 weeks later by 20 weeks (3 times per week) of dermal 12-0-tetradecanoylphorbol-13-acetate (TPA) treatment.

2.3 TOXICOKINETICS

Tri- and tetrachlorophenol are rapidly absorbed and excreted following occupational exposure, which involves both the inhalation and dermal routes. Studies using human cadaver tissue also suggest rapid absorption after dermal application. Data on the absorption of chlorophenols by the oral route are limited

to animal studies. Based on the results of these studies and the physical properties of chlorophenols, the gastrointestinal absorption of chlorophenols should be rapid and virtually complete. Data are insufficient to quantitatively estimate the absorption rate or to compare absorption following administration in food versus administration in water.

Limited evidence from animal studies suggests rapid clearance of chlorophenols from all body tissues. Intravenous administration of 2,4-DCP to rats resulted in short-lived deposition in the kidney, liver, brain, and fat. The extent of plasma protein binding, which is a major determinant of both the body burden and elimination kinetics, increases with increasing chlorination. Increased plasma protein binding decreases the clearance rate of higher chlorinated phenols (Pekari et al. 1991).

Few systematic metabolic studies were located for chlorophenols. In general, rapid Phase II metabolism to glucuronide and sulfate conjugates seems to be the predominant route of metabolism. The relative proportion of these conjugates may be species-, dose-, and route-related. The most important Phase I metabolites are apparently quinone and semiquinone reactive intermediates. Prominent urinary metabolites after 2,4,6-TCP administration in rats are other trichlorophenols. In at least one *in vitro* study, no evidence of dioxin precursors was found.

After occupational exposure to chlorophenols in a lumber treatment facility, elimination rates were inversely proportional to the degree of chlorination probably because of increased plasma protein binding with increased chlorination. Elimination half-lives of 18 hours and 4.2 days were recorded for 2,4,6-TCP and 2,3,4,6-TeCP, respectively. Elimination occurred according to a two-compartment open model. In rats orally administered radiolabelled 2,4,6-TCP, 92.5% of the administered radioactivity appeared in the urine and 6.4% appeared in the feces within 3 days after exposure cessation.

2.3.1 Absorption

Absorption of chlorophenols has not been studied in children.

2.3.1.l Inhalation Exposure

The identification of 2,4,6-TCP and 2,3,4,6-TeCP in the serum and urine of workers exposed while treating lumber indicates that 2,4,6-TCP and 2,3,4,6-TeCP are absorbed through inhalation (Pekari et al. 1991). No

airborne chlorophenol concentrations were provided. Although inhalation exposure was possible, a study of pullers at a timber mill (Fenske et al. 1987) suggests that 95% of the estimated exposure is by the dermal route.

No studies were located regarding the absorption of chlorophenols in animals after inhalation exposure to any of the eight chlorophenol isomers discussed in this profile.

2.3.1.2 Oral Exposure

No studies were located regarding oral absorption in humans of any of the eight chlorophenol isomers discussed in this profile. These isomers have moderately high lipophilicity and pK_as >5.0; consequently, intestinal ahsorption should be favored (Ambre 1990). Based on chemical properties and on limited animal data, absorption through the gastrointestinal tract after oral intake in humans is expected to be both rapid and virtually complete.

The animal data indicating rapid and complete absorption are based solely on studies reporting recovery of all or most of the orally administered chlorophenols in the urine. Spencer and Williams (1950) recovered ≥100% of a single oral dose of 2- or 4-CP (emulsified in water) given to rabbits. Five days after three daily gavage treatments of rats with radiolabelled 2,4,6-TCP (vehicle not reported), 82.3% of the administered radioactivity was recovered in the urine (Korte et al. 1978). In a 15-day study of 25 μg/day radiolabelled 2,4,6-TCP, 92% of the administered radioactivity was recovered in the urine of the treated rats (Bahig et al. 1981).

2.3.1.3 Dermal Exposure

In vivo and *in vitro* data indicate that the chlorophenols are readily absorbed following dermal exposure. In an industrial accident, 20 minutes after a worker was splashed with a pure solution of 2,4-DCP on less than 10% of his body (arm and leg), he collapsed and shortly thereafter died (Kintz et al. 1992). Postmortem blood and urine concentrations of 2,4-DCP were 24.3 and 5.3 mg/L, respectively. Using a fluorescent tracer, and measures of urinary excretion of TeCP in lumber mill workers exposed to a wood preservative (20% TeCP, 3% pentachlorophenol, <0.4% other CPs), Fenske et al. (1987) estimated that 30-100% of the 2,3,4,6-TeCP deposited on the skin is absorbed. Absorption occurred through the hands and forearms despite the use of chemical-resistant gloves. Fenske et al. (1987) also indicate that the skin regions with

greatest exposure, the hands and forearms, were in frequent contact with wood so that abrasion may have reduced the barrier properties of the stratum corneum.

The results of diffusion experiments using hydrated human cadaver epidermis also indicate that the chlorophenols readily cross the skin at low concentrations. The permeability coefficients determined in excised human abdominal epidermis were 5 .5, 6.1, 10.0, and 9.9 cm mi*N*- $1x10^4$ for 2-CP, 4-CP, 2,4-DCP, and 2,4,6-TCP (Roberts et al. 1977). 2-CP and 4-CP were reported to damage the skin, determined by an increase in the permeability coefficient at aqueous concentrations of 0.8 and 0.75% (w/v), respectively, while no damage was observed with 2,4-DCP and 2,4,6-TCP at concentrations up to saturation. In a study using abdominal skin exposed to air, absorption of 2,3,4,6-TeCP over 24 hours was 33% from an aqueous medium (1.54% 2,3,4,6-TeCP) and 63% from a diesel-oil-based medium (0.96 2,3,4,6-TeCP) (Horstman et al. 1989). These values were determined by assuming that the amount of the applied dose that was not recovered from the skin's surface was the amount absorbed. The actual amounts recovered in the skin and receiving solutions were 9.5 and 3.9% for the aqueous- and oil-based medium, respectively. The authors attribute low recovery to difficulties in extracting 2,3,4,6-TeCP from the skin.

Dermal absorption can be inferred from *in vivo* animal studies resulting in death and/or adverse systemic effects following dermal exposure to 2-CP (Younger Labs 1975) and 2,4-DCP (Carreon et al. 1980a, 1980b; Hencke and Lockwood 1978; Younger Labs 1976).

Chlorophenols are also readily permeable in rodent skin *in vitro* preparations. At solution pHs between 5.0 and 5.74, the apparent 2-CP, 2,4-DCP, and 2,4,6-TCP permeability constants for a hairless mouse skin preparation over a concentration range of 0.05-0.5% varied from 0.14 to 0.36 cm/hour in whole skin and from 0.136 to 0.276 cm/hour in skin stripped of the stratum corneum (Huq et al. 1986). The investigators proposed that permeability is probably greater in the more highly vascularized human tissue because the extensive network of surface capillaries in humans reduces the thickness of the diffusional barrier. They further stated that dermally absorbed phenolic compounds are potentially more toxic than orally absorbed compounds because Phase II detoxification reactions are more rapid after oral exposure. In another *in vitro* diffusion study of 4-CP, 87.4 to 90.5% of the applied dose crossed rat epidermal preparations in 72 hours, indicating extensive absorption (Hughes et al. 1993). Those phenols (both chlorophenols and other substituted phenols) with log K_{ow}, values between 1.4 and 3.5 showed the greatest amount of permeability through the dermal membrane. Although specific data were not identified, dermal absorption of

chlorophenols should also be greater for the neutral acid form than for the phenolate anion as ions do not readily cross cell membranes.

2.3.1.4 Other Routes of Exposure

No studies were located regarding absorption in humans exposed to any of the eight chlorophenol isomers by other routes.

An experiment with rabbits indicated that 2,4,6-TCP is absorbed through the cornea to a minor degree following ocular application (Ismail et al. 1977).

2.3.2 Distribution

Distribution of chlorophenols has not been studied in children.

2.3.2.1 Inhalation Exposure

No studies were located regarding the tissue distribution in humans or animals exposed by inhalation to any of the eight chlorophenol isomers discussed in this profile.

2.3.2.2 Oral Exposure

No studies were located regarding the tissue distribution in humans exposed orally to any of the eight chlorophenol isomers discussed in this profile.

Chlorophenols do not appear to accumulate in animals following oral exposure. For example, liver 2-CP concentrations were 2.2,3.2, and 0.8 ppm, and kidney 2-CP concentrations were 2.6,2.4, and 2.2 ppm in female rats exposed to 2-CP in the drinking water for 16 weeks at 5,50, and 500 ppm, respectively (Exon and Koller 1982). The investigators did not provide an explanation for the low value (0.8 ppm) found in the livers of rats receiving the high dose and did not indicate whether these values were wet or dry weight concentrations. Radioactivity was not recovered in the liver, lung, and subcutaneous fat of rats given three daily gavage doses of radiolabelled 2,4,6-TCP (Korte et al. 1978) or in unspecified tissues of rats given radiolabelled 2,4,6-TCP by gavage for 15 days (Bahig et al. 1981).

The highest concentrations of 2,3,4,6-TeCP were found in the spleen followed by the kidneys and liver 24 hours after a single oral dose was given to rats (Hattula et al. 1981). In a 55day study in which rats were treated by gavage with 2,3,4,6-TeCP at 10,50, or 100 mg/kg/day, tissue levels, measured 24 hours after the last dose, were dose related. For all doses, the concentrations of 2,3,4,6-TeCP in the brain and muscle were lower than those found in the kidney, liver, and spleen. At the 100 mg/kg/day dose, the kidney had the highest 2,3,4,6-TeCP concentrations (5.1 ppm) followed by the spleen (3.2 ppm), liver (2.2 ppm), brain (1.2 ppm), and muscle (0.46 ppm) (Hattula et al. 1981). At the 10 mg/kg/day dose, 2,3,4,6-TeCP was not detected in the brain or muscle (detection limit not stated), while low levels were found in the spleen (0.04 ppm), kidney (0.03 ppm), and liver (0.01 ppm).

2.3.2.3 Dermal Exposure

Concentrations of 2,4-DCP were 24.3, 5.3, 18.7, and 1.2 mg/L in the blood, urine, bile, and stomach contents of a worker who collapsed (within 20 minutes) and died shortly after being splashed with pure 2,4-DCP on his right arm and leg (Kintz et al. 1992).

No studies were located regarding the tissue distribution in animals dermally exposed to any of the eight chlorophenol isomers discussed in this profile.

2.3.2.4 Other Routes of Exposure

A study in which laboratory animals were given intravenous 2,4-DCP provides some insight regarding distribution patterns anticipated in humans (Somani and Khalique 1982). Intravenously administered 2,4-DCP rapidly distributes to the kidney, liver, fat, and brain in rats, with the highest concentrations in the kidney and liver. Elimination from these tissues is also rapid; the elimination half-time for plasma is approximately 10 minutes (Somani and Khalique 1982). The results of *in vitro* binding studies using human serum proteins indicate that both 2,4-DCP and 2,4,6-TCP strongly bind to serum proteins, including albumin and globulin (Judis 1982). The percentage of the compound bound to albumin was slightly greater for 2,4,6-TCP (94.1%) than for 2,6-DCP (87.7%).

In rabbits, following ocular exposure, radiolabelled 2,4,6-TCP was distributed to various compartments of the eye (Ismail et al. 1977). At 30 minutes post exposure, the applied radioactivity was detected in the cornea (4%), aqueous humor (0.37%), lens (0.037%), iris (0.18%), choroid (0.04%), vitreous (0.01%), conjunctiva

(2.14%), limbus (0.96%), and sclera (0.35%). At 60 minutes post exposure the respective percentages were 2.4, 0.17, 0.03, 0.10, 0.13, 0.01, 2.49, 0.88, and 0.53%.

Peak concentrations of 2,4,6-TCP were observed in all tissues assayed (blood, liver, kidney, muscle, fat, and brain) 30 minutes after rats were given a single intraperitoneal injection of 25 mg/kg 2,4,6-TCP (Pekari et al. 1986). The highest concentration observed was in the kidneys, 329 ± 117 nmol/g tissue, a concentration approximately 2, 7, 10, 13, and 26 times the concentrations found in the blood, liver, fat, muscle, and brain, respectively.

2.3.3 Metabolism

Both human and animal studies indicate that sulfation and glucuronidation are the main metabolic pathways of chlorophenols. Among sawmill workers, virtually all the absorbed tri- and tetrachlorophenols were excreted in the urine as conjugated metabolites (Pekari et al. 1991). Sulfate conjugation was predominant.

A number of rabbit studies (Azouz et al. 1953; Bray et al. 1952a, 1952b; Spencer and Williams 1950) have shown that metabolism of the monochlorophenols is principally via conjugation. In the latter study, groups of 6 rabbits were treated by gavage with 171.3 mg/kg of 2-CP or 4-CP emulsified in water as a single dose. For both isomers, the 24-hour urine analysis indicated that between 78.1 and 88.3% of the administered dose was excreted as the glucuronide, and between 12.8 and 20.6% of the administered dose was excreted as the ethereal sulfate. A total of 101.7 and 101.1% of the administered 2-CP or 4-CP doses, respectively, was accounted for as urinary glucuronide and sulfate conjugates. Metabolism was further investigated in 4 rabbits, each treated by gavage with an average dose of 395 mg/kg/day of 4-CP. After 36 hours, 54.1% of the administered dose appeared in the urine as the glucuronide conjugate, and 10.4% of the administered dose appeared in the ethereal sulfate fraction. Only 0.1% of the administered dose was excreted as 4-chlorocatechol. The low total recovery (64.5%) in the latter experiment limits conclusions. Other rabbit studies indicated that chlorocatechols constituted only 1.5-4.5% of the administered doses of 300 mg/kg 2-CP or 500 mg/kg 4-CP (Azouz et al. 1953). In a limited study in dogs (Coombs and Hele 1926) about half of an oral dose of 2- or 4-CP was excreted in the urine as the ethereal sulphate. No evidence for metabolism to mercapturic acid was found.

In contrast to the study in dogs, Phornchirasilp et al. (1989a) has proposed that in mice 4-CP is metabolized by P-450 enzymes to intermediates that react with glutathione to form glutathionyl adducts. This pathway

was proposed based on the observation that 4-CP treatment of mice depleted liver thiol stores. The depletion of liver thiol stores was prevented by a P-450 inhibitor (SKP 525-A) suggesting that P-450 activity is required for this effect.

A study in rats found that glucuronides and other unspecified conjugates were formed following a single intravenous dose of 2,4-DCP (10 mg/kg) (Somani and Khalique 1982). One hour after dosing, only small amounts of 2,4-DCP were found in the tissues studied (plasma, liver, kidneys, fat, brain). Although other unspecified conjugates were found in the fat, glucuronide conjugates were not found in the fat at any time interval. Two minor metabolites of 2,4-DCP, both dichloromethoxy phenols, have been identified in studies using isolated perfused rat livers (Somani et al. 1984). The extent to which the dichloromethoxy phenols are formed *in vivo* has not been determined (Somani et al. 1984).

2,4-DCP has been shown to be metabolized into two major metabolites identified as 2-chloro-1,4-hydroxyquinone and 2-chloro- 1,4-benzoquinone by microsomal fractions and whole cells of yeast *Saccharomyces cerevisiae* expressing human cytochrome P-450 3A4 (Mehmood et al. 1997). Another metabolite, 1,2,4-hydroxybenzene, was also detected during biotransformation by whole cells but was not observed in microsomal fractions. Thus, human CYP3A4 can remove either or both chlorine atoms from the aromatic ring of 2,4-DCP molecule, forming 2-chloro-1,4-hydroxyquinone and 1,2,4-hydroxybenzene, respectively. 2-chloro-1,4-hydroxyquinone was probably acted on by dehydrogenase from yeast microsomes, forming 2-chloro-1,4-benzoquinone (Mehmood et al. 1997).

Little information was located on the metabolism of trichlorophenols. In general, 2,4,6-TCP undergoes biotic isomerization to other trichlorophenol isomers and conjugation with glucuronic acid (Bahig et al. 1981). Male rats eliminated 63% of a gavage dose of 2,4,6-TCP in the urine as 4 trichlorophenol isomers and 28% as conjugates. Three of the trichlorophenol isomers were identified as 2,4,6-TCP (parent compound), 2,3,6-TCP, and 2,4,5-TCP; the fourth isomer was not identified. Glucuronic acid accounts for approximately 80% of the conjugates detected in urine (Bahig et al. 1981).

In vitro studies using rat liver microsomes have shown that 2,4,5-TCP can be metabolized to 3,4,6-trichlorocatechol, 2,5-dichlorohydroquinone, and a dihydroxydichlorobenzene (not further characterized) (Butte et al. 1988; Juhl et al. 1991). Metabolites were also dimerized to a dihydroxyhexachlorobiphenyl, a dihydroxypentachlorodiphenyl ether, two hydroxypentachlorodiphenyl ethers, a hydoxyhexachlorodiphenyl ether, and a Hydroxyhexachlorodioxin or hydroxyhexachlorodiphenoquinone (Butte et al. 1988). Metabolites generated following incubation

of 2,4,6-TCP with rat liver S-9 fraction were 2,6-dichloro-1,4-hydroquinone and two isomers of hydroxypentachlorodiphenyl ether (Juhl et al. 1989). The 2,6-dichloro-1,4-semiquinone free radical was also identified. Although *in vivo* these metabolites may be minor, *in vitro* they were responsible for DNA damage (Juhl et al. 1989, 199l).

Metabolism of 2,4,6-TCP by the skin has not been detected (Huq et al. 1986). Therefore, Huq et al. (1986) have suggested that 2,4,6-TCP absorbed through the skin could be more toxic than a similar ingested dose because the ingested compound is partially converted to glucuronide conjugates.

In a study in rats, a majority (70%) of intraperitoneally administered 2,4,6-TCP detected in the blood was in conjugated form 30 minutes after dosing. The authors speculated that the chemical was conjugated with glucuronic acid (Pekari et al. 1986). The average percentage of the metabolites of 2,4,6-TCP conjugated in the blood over the course of the study was 83±11%.

A study of the metabolism of the TeCP isomers following intraperitoneal injection in rats, indicates that much of the dose is excreted in the urine unchanged (Ahlborg and Larrson 1978). Following treatment with 2,3,4,5- and 2,3,4,6-TeCP, a trichlorohydroquinone was identified in the urine as a minor metabolite. Following treatment with 2,3,5,6-TeCP, about 35% of the recovered dose (total recovery 98.7%) was tetrachloro-*p*-hydroquinone, while the remaining was the unchanged parent compound (Ahlborg and Larrson 1978).

Metabolism of chlorophenols has not been studied in children. In humans, activity of UDP-glucuronosyltransferase (responsible for glucuronide conjugates) does not reach adult levels until about 6-8 months of age, although the development of this activity is isoform specific. Activity of sulfotransferases (responsible for sulfate conjugates) seems to develop earlier, although again, it is isoform specific. The activity of some sulfotransferase isoforms may be greater than that of adults during infancy and early childhood (Leeder and Kearns 1997). Since chlorophenols are detoxified in the liver by conjugation with glucuronic acid and sulfate, the toxicity of chlorophenols may be different in children.

2.3.4 Excretion

Excretion of chlorophenols has not been studied in children.

2.3.4.1 Inhalation Exposure

After occupational exposure by combined dermal and inhalation routes to a chlorophenol dipping solution, maximal urinary concentrations were 1-11.8 μmol/L 3.4-17.3 μmol/L, and 0.2-0.9 μmol/L for tri-, tetra-, and pentachlorophenol, respectively (Pekari et al. 1991). Elimination half-lives were 18 hours, 4.2 days, and 16 days, respectively. The renal clearance rate of 2,3,4,6-TeCP was approximately five times faster than the clearance rate of pentachlorophenol; this finding reflects the increased plasma protein binding of the higher chlorinated compound (Pekari et al. 1991). The clearance rate of 2,4,6-TCP could not be calculated because of highly variable serum concentrations (Pekari et al. 1991).

No animal studies were located regarding the excretion of any of the eight chlorophenol isomers after inhalation exposure.

2.3.4.2 Oral Exposure

The limited available data indicate that orally administered monochlorophenols are rapidly absorbed and excreted in the urine, primarily as glucuronide and sulfate conjugates, in rats, rabbits, and dogs (Bray et al. 1952a, 1952b; Coombs and Hele 1926; Spencer and Williams 1950). Most of the administered dose is excreted in the urine within 24 hours. More comprehensive data, including the kinetics of tissue uptake and distribution, are limited to 4-CP (discussed below). Data are insufficient to identify differences in the excretion of monochlorophenol isomers between animal species.

At oral doses of 150-450 mg/kg, excretion of the glucuronide conjugate of orally administered 4-CP in rabbits followed first-order kinetics (Bray et al. 1952a). The velocity constant k_g, or the rate of glucuronide excretion relative to remaining body burden, was 0.41 $hour^{-1}$. The investigators noted that the value of k_g for 4-CP is apparently not related to the electron withdrawing influence of the substituent group (Bray et al.1952a).

Male rats administered radiolabelled 2,4,6-TCP by gavage for 3 days and observed for 5 days after dosing eliminated a total of 82.3% of the total dose in the urine and 22.2% in the feces (Korte et al. 1978). In a second study using male rats, radiolabelled 2,4,6-TCP was administered by gavage for 15 days, with sacrifice 3 days after administration ended. A total of 92.5% of the administered dose was excreted in the urine, and 6.4% was excreted unchanged in the feces (Bahig et al. 1981). Four trichlorophenol isomers

were detected in the urine and comprised 63% of the total urinary radioactivity. These isomers were the unchanged parent compounds, 2,3,6-TCP, 2,4,5-TCP, and an unidentified compound. The metabolites identified in the polar fraction were trichlorophenol conjugates with glucuronic acid; these products accounted for 28% of the radioactivity eliminated in the urine (Bahig et al. 1981). Free trichlorophenol was identified in the feces. The excretion of radioactivity declined rapidly after dosing ended. By the third day postexposure, only 4.3% of the radioactivity in a daily dose was detected in the urine and 1.9% was detected in the feces (Bahig et al.1981).

2.3.4.3 Dermal Exposure

As discussed in Section 2.3.4.1, combined dermal and inhalation exposure to a chlorophenol-containing wood treatment solution resulted in the urinary excretion of tri-, tetra-, and pentachlorophenol. Rate constants of elimination were inversely proportional to the extent of chlorination (Pekari et al. 1991).

No animal studies were located regarding the excretion of any of the eight chlorophenol isomers after dermal exposure.

2.3.4.4 Other Routes of Exposure

A study in rats showed rapid clearance from the kidney, liver, fat, brain, and plasma of both the parent compound and metabolites after intravenous administration of 10 mg/kg/day 2,4-DCP in an aqueous solution (Somani and Khalique 1982). Half-lives for 2,4-DCP and its conjugates ranged from 4 to 30 minutes in these tissues, with the highest values in kidney, followed by the liver, fat, plasma, and brain (Somani and Khalique 1982). No detectable amounts were found in the brain at 60 minutes. These data suggest that 2,4-DCP does not accumulate in body tissues and is quickly excreted.

In rats administered 2,4,6-TCP by intraperitoneal injection, the majority (70%) of 2,4,6-TCP associated radioactivity detected in the blood 30 minutes after dosing was in a conjugated form (Pekari et al. 1986). The authors speculated that it was conjugated with glucuronic acid. The biological half-life of conjugated 2,4,6-TCP was 1.4 hours in blood and ranged from 1.4 to 1.8 hours in other tissues. Elimination of approximately 90% of the administered dose in the urine occurred within 4-6 hours. Only trace amounts of trichlorophenol were detected in tissues 10 hours after dosing (Pekari et al. 1986).

Ahlborg and Larrson (1978) studied the urinary excretion of TeCPs in rats following intraperitoneal injection of a single dose. The slowest rate of excretion was observed following treatment with 2,3,4,5-TeCP. During the 72 hours after administration, about 60% of the dose was recovered in the urine; the majority of it was excreted unchanged. In contrast, following treatment with 2,3,4,6-TeCP, 95.9% of the dose was excreted in the urine in 72 hours, and 98.7% of the administered 2,3,5,6-TeCP was excreted in the urine within 24 hours after dosing. The investigators (Ahlborg and Larsson 1978) did not provide an explanation regarding the slower excretion of 2,3,4,5-TeCP compared to the excretion of 2,3,4,6-TeCP and 2,3,5,6-TeCP.

2.3.5 Physiologically based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

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

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen 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) is 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 2-3 shows a conceptualized representation of a PBPK model.

If PBPK models for chlorophenols 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.

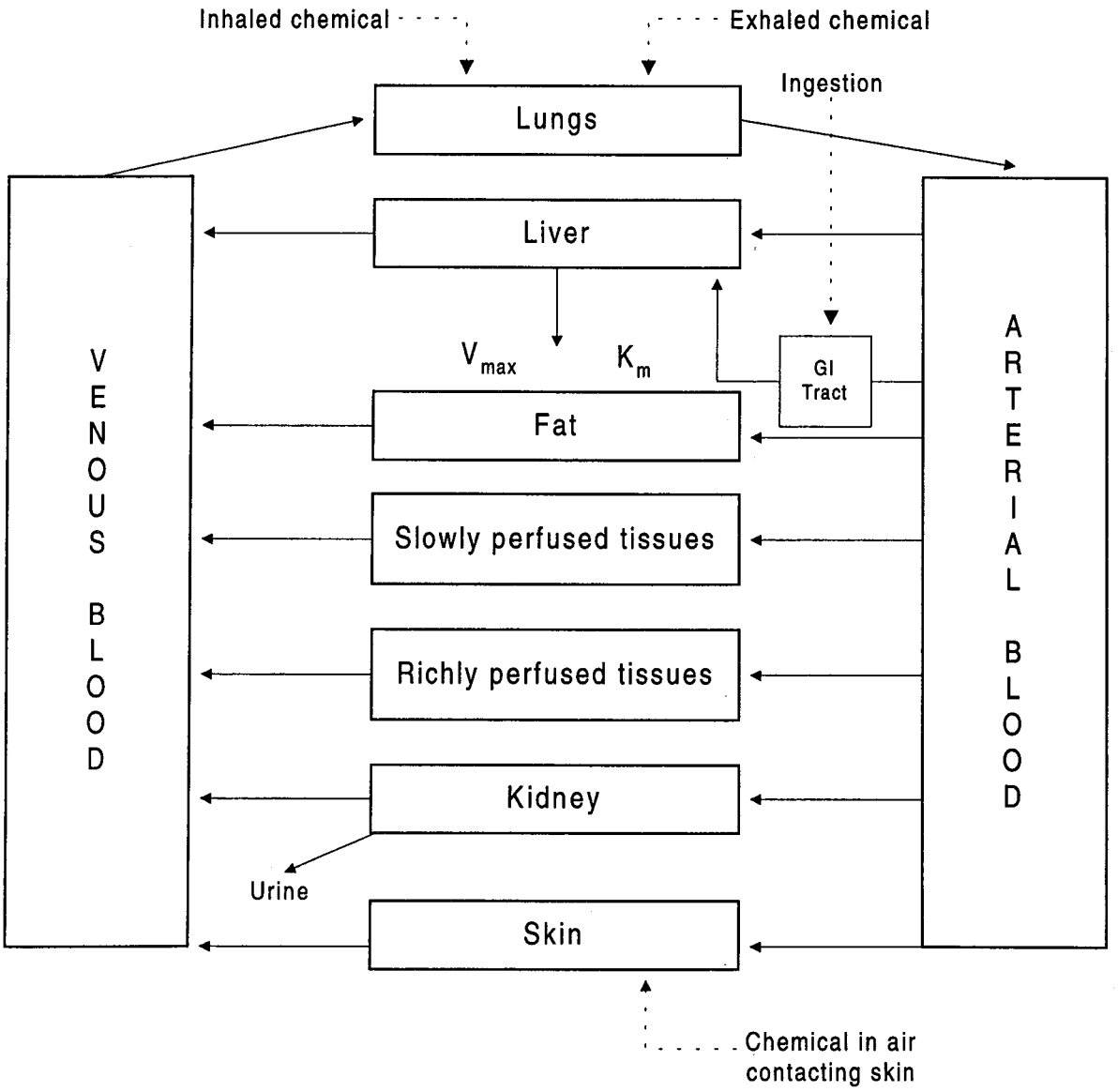
There are no PBPK models for chlorophenols.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

Chlorophenols have moderately high lipophilicity. They are weak organic acids with pK_a values that range from 5.4 to 8.9 (Shiu et al. 1994); consequently, absorption should be favored in the stomach and the intestine. Absorption through the gastrointestinal tract is by simple diffusion and is expected to be both rapid and virtually complete. The chlorophenols are also readily absorbed after dermal exposure. Dermal absorption should also be greater for the neutral acid form than for the phenolate anion as ions do not readily cross cell membranes. Dermally absorbed doses of chlorophenols are potentially more toxic than orally absorbed doses (Huq et al. 1986). The chlorophenols were not metabolized or conjugated during

Figure 2-3. 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.

their diffusive transport through the skin (Huq et al. 1986); however, they are partially converted to more easily eliminated, less toxic glucuronide conjugates after oral ingestions.

After a single oral dose of 2,3,4,6-TeCP to rats, the kidney had the highest tissue concentration, followed by the spleen, liver, brain, and muscle (Hattula et al. 1981). When administered intravenously to rats, 2,4-DCP rapidly distributes to the kidney, liver, fat, and brain, with the highest concentrations in the kidney and liver (Somani and Khalique 1982). 2,4-DCP and 2,4,6-TCP strongly bind to serum proteins, including albumin and globulin (Judis 1982).

2.4.2 Mechanisms of Toxicity

Chlorophenols uncouple mitochondrial oxidative phosphorylation and produce convulsions. Within 20 minutes of being accidentally splashed with 2,4-DCP on his right arm and leg, a worker experienced seizures, collapsed, and died shortly thereafter (Kintz et al. 1992). Lethargy, tremors, convulsions, and/or central nervous system depression have been reported in chlorophenol-exposed animals (Borzelleca et al. 1985a; Deichmann and Mergard 1948). Within the series including phenols and chlorinated phenols, convulsive effects decreased with increasing chlorination. Limited data were located on the mechanism of phenol- or chlorophenol-induced convulsions. Phenol administration in cats facilitated effects on central synaptic transmission at both excitatory and inhibitory synapses (Banna and Jabbur 1970). The authors proposed that certain phenols increase the amount of neurotransmitter released during synaptic transmission, resulting in convulsions. After intraperitoneal injection of several chlorophenols, convulsions predominated in those mice receiving the 2- and 4-CP compounds (Farquh&son et al. 1958). Because these compounds have pK values of 8.65 or higher and would not be in the ionic form at physiologic pH, the investigators attributed the observed effect to the chlorophenol rather than to the ion.

Particularly for the higher chlorophenols, the primary toxic mechanism associated with exposure is the uncoupling of mitochondrial oxidative phosphorylation (Farquharson et al. 1958; Weinbach and Garbus 1965). Although the kinetics of chlorophenol-induced uncoupling have primarily been studied in *in vitro* mitochondrial preparations, the associated metabolic effects (such as increased body temperature and dyspnea) have been verified *in vivo* (Farquharson et al. 1958). The ability of chlorophenols to uncouple oxidative phosphorylation increases with increasing chlorination. Toxic manifestations include central nervous system depression followed by increased respiration, hyperthermia, a blood pressure rise, progressive euromuscular weakness, and cyanosis. The results of a number of *in vitro* studies (Cascorbi and Ahlers

1989; Izushi et al. 1988; Mitsuda et al. 1963; Narasimhan et al. 1992; Shannon et al. 1991; Stockdale and Selwyn 1971) indicate a concentration-dependent triphasic effect of chlorophenols on phosphorylation and cellular respiration. At low concentrations, uncoupling produces stimulation of state 4 (resting state) respiration as a result of increased adenosine triphosphatase (ATPase) activity in the absence of a phosphate acceptor. Inhibition of state 3 (active) respiration is also observed. At moderate concentrations, resting respiration is neither stimulated nor inhibited. Significant inhibition of respiration, associated with a breakdown of the electron transport process and decreased ATPase activity, occurs at very high concentrations. These concentrations are also associated with mitochondrial swelling and disruption of the mitochondrial matrix structure. Investigators have cited two independent mechanisms to explain these effects on cellular metabolism. Uncoupling activity has been attributed to a protonophoric effect (a disruption of the energy gradient across the mitochondrial membrane resulting from distribution of chlorophenols in the phospholipid bilayer of the membrane), whereas inhibition of cellular respiration has been attributed to a direct action on intracellular proteins.

The results of these and other studies also illustrate that higher order chlorophenols have the greatest effects on cellular metabolism. In general, investigators have found that 2-CP and 4-CP are less than 7% as potent as tetrachlorophenol in uncoupling oxidative phosphorylation and inhibiting cellular respiration (Cascorbi and Ahlers 1989; Janik and Wolf 1992; Narasimhan et al. 1992; Weinbach and Garbus 1965). Within the chlorophenol series, two physicochemical parameters, the a-Hammett constant, a measure of electron withdrawing ability, and the octanol-water partition coefficient (log K_{ow}), accounted for 98% of the variability in the inhibition of ATPase activity (Cascorbi and Ahlers 1989).

Corrosive skin damage resulting from high concentration phenol exposure has been attributed to protein denaturation by protein-solute complexes (Roberts et al. 1977). In this study, various concentrations of 2-CP and 4-CP were applied to samples of human abdominal skin maintained in a diffusion chamber. The estimated threshold concentrations for damage (the aqueous concentration at which the transmembrane permeability coefficient began to increase) were 0.8% and 0.75%, respectively, for these two isomers. The investigators proposed that the extent of damage was related to the concentration of the solute partitioned into the stratum corneum, the diffusivity of the solute, and the pK of the applied phenolic compound.

In a study in rats, Kitchin and Brown (1988) examined the effects of single gavage doses of 2,4,5-TCP, 2,4,6-TCP, and 2,3,4,6-TeCP on markers of carcinogenic initiation (alkaline elution for DNA damage in liver and blood), promotion of carcinogenesis (ornithine decarboxylase activity in the liver), and hepatic cell damage

(serum alanine aminotransferase [SGPT] activity). At a dose one-fifth the LD_{50} (164 mg/kg for 2,4,5 and 2,4,6-TCP; 28 mg/kg for 2,3,4,6-TeCP), no effects were observed. 2,4,6-TCP and 2,3,4,6-TeCP were also tested at higher equimolar concentrations (2,4,6-TCP, 500 mg/kg; 2,3,4,6-TeCP, 193 mg/kg). At the high dose, both compounds resulted in a significant increase in liver ornithine decarboxylase activity. No effects on alkaline elution of DNA or on SGPT activity were observed, suggesting that 2,4,6-TCP and 2,3,4,6-TeCP were weak promoters.

2.4.3 Animal-to-Human Extrapolations

Extrapolating animal toxicity data to predict human risk from chlorophenol exposure appears to be reasonable because of the similarity in metabolic pathways and effects. However, the one case of human death following dermal exposure indicates that animals may be more resistant to the toxic effects of 2,4-dichlorophonol than humans.

2.5 RELEVANCE TO PUBLIC HEALTH

Issues relevant to children are explicitly discussed in 2.6 Children's Susceptibility and 5.6 Exposures of Children.

Overview.

Chlorophenols are used as intermediates in the production of dyes and chlorinated pesticides. Because of its biocidal properties, 4-CP is also used as a dental antiseptic. Runoff from pesticide degradation, contaminated food intake, and the chlorination of both drinking water and waste water are the environmental sources of human exposure to chlorophenols. A chlorophenol-containing waste site may result in groundwater contamination with subsequent introduction into the drinking water supplies. Dermal exposure can occur in occupational settings. Much lower levels of dermal exposure can occur through showering and bathing with water containing chlorophenols. In addition, the environmental dechlorination of the higher chlorophenols can result in exposure to the lower chlorophenols.

Exposed pesticide production workers may be at increased risk for soft tissue sarcoma, Hodgkin's disease, and non-Hodgkin's lymphoma. Although these results have been found in several occupational studies, the majority of studies find no, or only slightly, increased cancer risk associated with exposure. Possible

confounding variables in the positive studies included recall and selection bias. Another major health concern in workers is the development of chloracne, which is probably attributable to the presence of other chlorinated dioxins as contaminants in CP-containing pesticides.

The results of animal studies partially support the human data. 2,4,6-TCP was a carcinogen in 2-year rat and mouse studies. Other studies suggest that chlorophenols, rather than being initiators, may be tumor promoters. This is supported by genotoxicity data that suggest that the chlorophenols are not directly mutagenic. The EPA has classified 2,4,6-TCP as a class B2 carcinogen. Hepatic effects, ranging from enzyme induction to generalized necrosis, are also found in animals. Although 2,4-DCP has been shown to effect cell-mediated immunity in laboratory studies, the toxicological relevance of this finding to individuals exposed near hazardous waste sites is unknown. The higher chlorinated phenols have shown equivocal reproductive and developmental effects only at doses producing maternal toxicity. These findings indicate a possible concern for pregnant women exposed in the workplace or through contaminated environmental media. Observations of corrosion and death after skin application of high doses of chlorophenols reinforces the dermal health hazard concern for workers.

Minimal Risk Levels for Chlorophenols

Exposures to chlorophenols at hazardous waste sites are most likely to be to a mixture of chlorophenols rather than to a single compound. Unfortunately, no information regarding effects following exposure to a mixture of chlorophenols was identified. As a conservative approach, duration-specific MRLs for the chlorophenols as a class were developed based on the single compound with the lowest LOAEL and, therefore, should protect against effects following exposure to all chlorophenols as well as exposure to mixtures of chlorophenols if effects of multiple chlorophenols are additive.

Inhalation

The inhalation data for chlorophenols is limited to a single acute study of 2-CP in which rats were exposed (nose-only) to 17, 104, or 908 ppm for 4 hours (Duchosal and Biederman 1991). Restlessness and hunched posture were observed at the high concentration. Dark red foci were observed at the two lower concentrations.

but not at the high concentration. No controls were used in this study. Because of the lack of a clear doseresponse relationship and the absence of controls, this study was not considered appropriate for the derivation of an inhalation MRL.

Oral

- An MRL of 0.01 mg/kg/day has been derived for acute-duration oral exposure to chlorophenols. This MRL is based on electron microscopic changes (foamy cytoplasm and clustering of rnitochondria and endoplasmic reticulum) observed in the hepatocytes of rats treated with 4-CP at 2.58 mg/kg/day but not at 1.28 mg/kg/day (Phornchirasilp et al. 1989b). The rats were treated by gavage two times per day with the 4-CP in corn oil. There are no additional studies that examine liver effect following oral exposure to 4-CP.

- An MRL of 0.003 mg/kg/day has been derived for intermediate-duration oral exposure to chlorophenols. This MRL is based on a decrease in delayed type hypersensitivity observed in rats treated with 2,4-DCP in the drinking water throughout gestation and 10 weeks after weaning at 3 mg/kg/day but not at 0.3 mg/kg/day (Exon and Keller 1985; Exon et al. 1984).

Chronic

No chronic duration oral MRLs were derived for any of the chlorophenols because the NOAELs identified in the chronic studies were greater than LOAELs identified in the intermediate duration studies.

Death. No data were found on death following inhalation or ingestion of chlorophenols. The only report of death in humans following exposure to chlorophenols is a single case in which a man died shortly after being splashed on less than 10% of his body with 2,4-DCP (Kintz et al. 1992). Postmortem blood and urine concentrations were 24.3 and 5.3 mg/L, respectively. Most data from acute inhalation, oral, and dermal lethality animal studies suggest that chlorophenols are lethal only at high exposure levels. Acute lethality generally occurs at oral doses greater than 100 mg/kg (Ahlborg and Larsson 1978; Bercz et al. 1990; Blackburn et al. 1986; Borzelleca et al. 1985a, 1985b; Carreon et al. 1980a; Kobayashi et al. 1972; NTP 1989; Rodwell et al. 1989). The range of oral LD_{50} values, 89 mg/kg for male mice treated with 2,3,5,6- TeCP in ethanol (Ahlborg and Larsson 1978) to 2,960 mg/kg for male rats treated with 2,4,5-TCP in corn oil (McCollister et al. 1961), indicates that the chlorophenols are slightly or moderately toxic according to the

classification scheme of Hodge and Sterner (1949). The use of different vehicles in oral studies makes it difficult to draw any conclusions about which of the chlorophenols are the most toxic. For example, in studies of the TeCPs, oral LD_{50}s in rats were lower when the compounds were given in 40% ethanol rather than propylene glycol (Ahlborg and Larssen 1978).

More limited dermal LD_{50} data indicate a range of 485 mg/kg for 2,3,4,6-TeCP in rats (Shen et al. 1983) to 1,414 mg/kg/day for 2,4-DCP in rabbits (Carreon et al. 1980b). The range of LD_{50}s following intraperitoneal injection exposure was 48 mg/kg for 2,3,5,6-TeCP given to mice in 40% ethanol (Ahlborg and Larsson 1978) to 430 mg/kg for 2,4-DCP given to rats in olive oil (Farquharson et al. 1958).

Typical signs of severe acute intoxication include ataxia, fatigue, disorientation, tachycardia, and increased respiratory rate followed by dyspnea, myoclonic convulsions, and coma (Borzelleca et al. 1985a, 1985b; Farquharson et al. 1958; Kobayashi et al. 1972). Convulsions are a diagnostic feature of intoxication with certain phenolic compounds, including the lower chlorinated phenols. The mechanism of action of this effect is poorly understood. Furthermore, an adequate dose-response relationship for this end point has not been established. Convulsions have been reported after both oral and dermal administration, indicating that the effect is not route specific.

Given the high doses of chlorophenols required to produce death in laboratory animals, death in humans exposed through contaminated drinking water is unlikely. The taste and odor thresholds of the chlorophenols, which are in the ppb range (Burttschell et al. 1959), would make the likelihood of intoxication via drinking water quite remote because of the unpleasant taste and odor of the contaminated water. In the occupational setting, exposure to more highly concentrated chlorophenols could result in death following inhalation, oral, or dermal exposure as suggested by the case report by Kintz et al. (1992).

Systemic Effects. No data regarding the systemic toxicological effects of exposure to chlorophenols in humans, in isolation from other contaminants, were located in the literature. The discussion provided below will describe systemic effects in humans occupationally exposed to a variety of airborne contaminants (including chlorophenols), laboratory animal studies, and supporting data.

Respiratory Effects. In a small cross-sectional study on workers involved in 2,4,5,-TCP production, no adverse effects on pulmonary function or the incidence of pulmonary lesions were observed (Calvert et al. 1991). Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and

pentachlorophenol reported upper respiratory tract irritation more frequently than unexposed workers (Kleinman et al. 1986). Acute inhalation exposure of laboratory animals to monochlorophenols has resulted in hemorrhage in the lungs and tachypnea (Duchosal and Biedermann 1991). Rats and mice exposed to high oral doses of 2,4-DCP or 2,4,6-TCP had no adverse effects on lung weight or histopathology (Bercz et al. 1990; Blackburn et al. 1986; NCI 1979; NTP 1989).

The limited data are insufficient to predict the respiratory response of humans exposed either acutely or chronically to chlorophenols.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after exposure by any route to any of the eight chlorophenols discussed in this profile. Animal studies have not reported histological changes in the heart following exposure to 2,4-DCP, 2,4,5-TCP, or 2,4,6-TCP (Bercz et al 1990; Blackburn et al. 1986; NCI 1979; NTP 1989), providing limited evidence that these compounds are not cardiovascular toxicants.

Gastrointestinal Effects. Symptomology of gastrointestinal effects has not been reported in production workers exposed to trichlorophenols and other chlorinated organics (Calvert et al. 1992). Single gavage doses of 432 mg/kg or more 2,3,4,6-TeCP has produced intestinal cell necrosis in rats (Hattula et al. 1981). The results of other animal studies, however, did not indicate damage to the intestinal tract after exposure to large oral doses of chlorophenols (NCI 1979; NTP 1989).

Hematological Effects. Adequate data for the assessment of hematological effects in chlorophenol-exposed humans were not located. Results from oral acute- to chronic-duration animal studies indicate that 2-CP has no adverse effects on standard hematological end points at doses up to 50 mg/kg/day (Borzelleca et al. 1985a; Exon and Koller 1985). Consequently, under the conditions of these studies, the hematological system does not appear to be a target organ at moderately high exposure concentrations.

Results of chronic oral studies in rats indicate that various hematopoietic effects, such as bone marrow atrophy, hyperplasia, and leukocytosis, occur in rats exposed chronically to 500 mg/kg/day or more 2,4-DCP or 2,4,6-TCP (NCI 1979; NTP 1989). Furthermore, the results of the NCI (1979) studies indicate that 2,4,6- TCP is associated with an increased incidence of leukemia in rats.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans. Intermediate and chronic oral exposure of animals to 2,4-DCP (NTP 1989) or 2,3,4,6-TeCP (Hattula et al. 1981) did not result in any histopathologic changes in muscle.

Hepatic Effects. Chlorophenol-exposed manufacturing workers have been diagnosed with porphyria, elevated serum transaminase levels, regenerative hepatocellular activity, and hemofuscin deposition (Bleiberg et al. 1964). In another group of production workers, elevated GGT activity has been associated with an interaction between 2,4,5-TCP exposure and alcohol consumption (Calvert et al. 1992). These data indicate a concern for hepatic effects in individuals exposed to chlorophenols.

Acute exposure concentrations of up to 69 mg/kg/day 2-CP for 14 days in mice produced no changes in hepatic microsomal P-450 enzyme levels (Borzelleca et al. 1985a). In contrast, 2-week administration of 0.64 mg/kg/day 4-CP in Sprague-Dawley rats increased microsomal demethylase activities, microsomal protein, and cytochrome P-450 content (Phornchirasilp et al. 1989a). Electron microscopic changes in hepatocytes (foamy cytoplasm, clustering of mitochondria and endoplasmic reticulum) occurred at ≥ 2.58 mg/kg/day, and 1.28 mg/kg/day was considered a NOAEL. The discrepancies in results between these two studies may be related to species differences but is most likely due to a 10-fold difference in test material volume measured on a body weight basis.

Animal data regarding the hepatic effects of the higher chlorinated phenols are conflicting. Mouse studies indicate that chronic exposure to 2,4-DCP is associated with liver necrosis (NTP 1989). There is inconclusive evidence that 2,4-DCP causes hepatocellular hyperplasia in rats and mice and diffuse syncytial alterations in mice exposed for long periods. A possible mechanism for the observed diffuse syncytial alterations was demonstrated in an *in vitro* study (Onfelt 1987) in which 2,4-DCP interfered with normal cell division by disrupting spindle formation. Interference of 2,4-DCP with oxidative phosphorylation, as demonstrated in an *in vitro* study with isolated mitochondria (Stockdale and Selwyn 1971), may be a mechanism for any or all of these liver effects since it can deplete the energy stores available to affected cells.

Hepatic effects observed in rats and mice following intermediate and chronic oral exposure to 2,4,6-TCP include alterations in hepatocytes, increased liver weight, and hepatic hyperplasia (Exon and Koller 1985; NCI 1979). The latter effect may possibly be a precursor to hepatic adenomas and carcinomas also observed in mice chronically exposed to 2,4,6-TCP (NCI 1979). Exposure to mid- to high-oral doses of 2,4,6-TCP has resulted in increased relative liver weights (Bercz et al. 1990; NCI 1979) and midzonal

vacuolation of hepatocytes (NCI 1979). In intermediate-duration oral studies, administration of as low as 50 mg/kg/day was associated with massive hepatocellular necrosis and venous thrombosis in l/10 rats (Hattula et al. 1981).

The mechanism for the observed hepatic alterations is difficult to determine. Various hepatic microsomal drug-metabolizing enzymes were not induced in an acute study in which rats were given several intraperitoneal injections of 2,4,6-TCP (Denomme et al. 1983). Acute oral dosing with as much as 400 mg/kg had no effect on enzyme activities and protein levels that are indicative of either increased metabolic activity or hepatotoxicity (Carlson 1978). Hepatic ornithine decarboxylase activity, a potential marker of tumor promoting capabilities, was elevated over control levels only after administration of lethal doses of 2,4,6-TCP or 2,3,4,6-TeCP (Kitchin and Brown 1988). Despite the inability to specifically ascertain the mechanistic basis of observed hepatotoxic effects, the available *in vivo* data are adequate to indicate that individuals exposed to sufficient levels of chlorophenols near hazardous waste sites are potentially at risk for liver effects ranging from increased enzyme activity to generalized necrosis.

Renal Effects. No data on the renal effects of chlorophenols in humans were located. In animal studies, either no or mild renal effects are evident at oral exposure levels of 720-2,600 mg/kg/day (Bercz et al. 1990; Blackburn et al. 1986; NCI 1979; NTP 1989). Renal tubular necrosis occurred only at lethal exposure concentrations (NTP 1989). The current data suggest that renal toxicity may only occur at doses unlikely in occupational or environmental settings.

Endocrine Effects. There are no reports of endocrine effects in humans occupationally exposed to chlorophenols. Histopathological changes have not been observed in endocrine glands (adrenal gland, pituitary, thyroid, parathyroids, pancreas) in animals exposed to chlorophenols for intermediate and chronic durations, providing limited evidence that endocrine glands are not a target of chlorophenol toxicity (American Biogenics 1988; Bercz et al. 1990; Blackburn et al. 1986; McCollister et al. 1961; NCI 1979; NTP 1989). An *in vitro* study examining the binding of chlorophenols to human transthyretin (a carrier of thyroid hormone) found that increasing chlorination resulted in greater affinity for the thyroxine (T4) binding site of the carrier (van den Berg 1990). The affinities of 2-CP, 2,4,5-TCP, and 2,4,6-TCP compared to T4 were 0.004, 0.15, and 0.33, respectively. Tetrachlorophenols were not tested. Based on the results of this *in vitro* study, van den Berg (1990) suggests that chlorophenols may reduce plasma T4 levels through competition with T4 binding on transthyretin and other T4 carriers (e.g., thyroid binding globulin, albumin).

Dermal Effects. Chloracne and evidence of acquired porphyria cutanea tarda, hyperpigmentation, and hirsutism have been observed in factory workers who were concurrently exposed to chlorophenols in phenoxy-based herbicides and, potentially, to dioxins (TCDD) (Bleiberg et al. 1964; Bond et al. 1989). In at least one study, chloracne incidence was positively correlated with TCDD exposure (Bond et al. 1989). The results of dermal studies with rats and rabbits indicate that chlorophenols can produce various effects, ranging from mild erythema to severe corrosion of the skin (Bioassay Systems 1981; Carreon et al. 1980a, 1980b; Hencke and Lockwood 1978; Rhodia 1978; Shen et al. 1983). The reports describing dermal effects are very limited and do not provide details of the effects observed; additionally, dose-response relationships are not clearly defined. The lowest dose resulting in direct dermal effects was 242 mg/kg/day 2-CP applied to the skin of rabbits (Bioassay Systems 1981).

Dermal exposure may be a special concern because wood treatment workers had measurable tetrachlorophenol levels on the hands and volar surface of the forearm, despite the use of gloves when handling timber (Fenske et al. 1987).

Ocular Effects. Eye irritation was reported among lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol (Kleinman et al. 1986). The eye irritation was likely a direct effect of the tetrachlorophenols. The results of ocular toxicity studies in rabbits indicate that 2-CP concentrations as low as 1% can result in mild hyperemia (Rhodia 1978). The extent of tissue damage is concentration-related. Application of a 2% solution resulted in severe hyperemia with edema and cloudy swelling (Rhodia 1978), while administration of undiluted 2-CP produced edema with severe tissue erythema and corrosion (Younger Labs 1975). Severe cornea1 damage in rabbits occurred after the direct application of 0.1 mL 2,4-DCP in the eye (Hencke and Lockwood 1978). Consequently, moderately high vapor concentrations of 2-CP, 2,4-DCP, and possibly other chlorophenols in industry or at hazardous waste sites have the potential to produce ocular irritation and/or damage in unprotected individuals.

Body Weight Effects. Effects on body weight have not been reported in humans occupationally exposed to chlorophenols. Body weight loss has been observed in animals exposed to chlorophenols following acute, intermediate, and chronic duration exposure (American Biogenics 1988; Borzelleca et al. 1985a; Kavlock 1990; McCollister et al. 1961; NCI 1979; NTP 1989; Rodwell et al. 1989). When chlorophenols are administered in the diet, changes in body weight may be in part a result of decreased food intake because of reduced palatability (NTP 1989), although body weight decreases have also been observed following gavage administration (American Biogenics 1988). The lowest dose associated with body weight decrease was

20 mg 2,3,4,6-TeCP given to rats by gavage for 90 days (American Biogenics 1988). The observation that a TeCP has the greatest effect on body weight is consistent with the observation that TeCPs are more potent in uncoupling oxidative phosphorylation than the monochlorophenols (Cascorbi and Ahlers 1989; Narasimhan et al. 1992).

Immunological and Lymphoreticular Effects. No human data were located regarding immunological or lymphoreticular effects of chlorophenols. The results of one animal study indicate that neither humoral nor cell-mediated immunity is affected by oral exposure to 2-CP at doses up to 50 mg/kg/day (Exon and Koller 1985). In contrast, combined pre- and postnatal exposure to 2,4-DCP at 3 mg/kg/day both stimulated antibody production and inhibited a delayed type hypersensitivity response (Exon and Koller 1985). Combined pre- and postnatal exposure to 2,4,6-TCP in drinking water at doses up to 30 mg/kg/day did not significantly affect humoral or cell-mediated immunity in rats (Exon and Koller 1985). As discussed under hematological effects, bone marrow atrophy affecting both erythroid and myeloid elements has been observed in rats treated with 2,4-DCP in the diet at 500 mg/kg/day for 13 weeks (NTP 1989). Histological examination of lymph nodes, spleen, and thymus in chlorophenol-exposed animals has not revealed any effects in intermediate and chronic duration studies (McCollister et al. 1961; NCI 1979; NTP 1989).

Because of limited animal data and knowledge regarding the toxicological significance of many immunological findings, the relevance to public health of immunological effects following exposure to the chlorophenols cannot be determined specifically. However, animal evidence indicates that 2,4-DCP may have an effect on the immune system.

Neurological Effects. Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol reported headaches more frequently than unexposed workers (Kleinman et al. 1986). In animals exposed to chlorophenols by the inhalation, oral, or dermal routes, convulsions (a sign of exposure to high doses of some phenolic compounds) decrease with increasing chlorination. In oral and dermal acute lethality studies, convulsions were noted only as antecedents of death (Borzelleca et al. 1985a, 1985b; Deichmann and Mergard 1948). Consequently, adequate information about dose-response relationships or isomeric potencies were not determined. The mechanisms for these effects are not known, although interference with oxidative energy metabolism in the central nervous system has been suggested, based on *in vitro* studies with rat brain and nerve tissues (Farquharson et al. 1958). After intraperitoneal injection in mice, the median doses producing convulsions were 99 and 116 mg/kg, respectively, for 2-CP and 4-CP (Angel and Rogers 1972). Although this administration route has limited relevance for human exposure, the

data do suggest that humans exposed at hazardous waste sites via drinking water are likely to be at minimal risk of developing convulsions.

Reproductive Effects. No data were located regarding the reproductive effects of chlorophenols in humans. Studies on the reproductive toxicity of chlorophenols in rats suggest that 2-CP, 2,4-DCP, and 2,4,6-TCP, administered on days 6 through 15 of gestation, may reduce liter size (Exon and Koller 1985). However, at the highest dose tested (about 30 mg/kg/day 2,4-DCP and 2,4,6-TCP, and 50 mg/kg/day 2-CP) litter sizes were reduced compared to controls only at the $p \leq 0.1$ level. A study designed to assess the reproductive toxicity of 2,4,6-TCP found no effect on male or female reproductive functions at doses that caused death and reduced body weight (Blackburn et al. 1986). An increasing trend for preimplantation loss was observed in pregnant rats treated with 2,3,4,6-TeCP on gestation days 6-15 (RTI 1987). Because this study was not designed to examine the preimplantation/ implantation phase of reproduction, the issue of whether 2,3,4,6-TeCP affects implantation still needs to be resolved. Histological changes in the testes or ovaries have not been observed in rats or mice exposed to 2,4-DCP, 2,4,5- or 2,4,6-TCP, or 2,3,4,6-TeCP in intermediate- and chronic-duration studies (American Biogenics 1988; Bercz et al. 1990; McCollister et al. 1961; NCI 1979; NTP 1989).

Developmental Effects. No data were located regarding developmental effects in humans following exposure to chlorophenols. The results of developmental studies in animals do not clearly show a selective effect on development at doses lower than those causing maternal toxicity. At maternally toxic doses (750 mg/kg/day) reduced fetal body weight and delayed ossification were observed in the offspring of rats treated with 2,4-DCP on gestation days 6-15 (Rodwell et al. 1989). No developmental effects were noted in rats treated with 2,3,4,6-TeCP even at a dose that caused a 26% reduction in maternal body weight gain. The lack of a specific developmental effect for 2-CP, 2,4,6-TCP (Fu et al. 1990), 4-CP, and 2,3,4,5-TeCP (Mayura et al. 1991) is supported by studies using the *in vitro* hydra assay which showed that the compounds were equally toxic to adult and developing organisms.

In vitro exposure of rat embryos indicates that the chlorophenols can directly affect development. Exposure of rat embryos to 6 mM 4-CP or 2,3,4,5-TeCP resulted in a significant decrease in crown-rump length, somite number, and DNA and protein content (Mayura et al. 1991). The effects of 2,3,4,5-TeCP on development were much greater than 4-CP. 2,3,4,5-TeCP but not 4-CP also resulted in a significant reduction in yolk sac diameter. 4-CP exposure (195-781 nM) of rat embryos reduced measures of growth (somite number, crown rump length, DNA content) and increased structural defects (hind limb bud absence,

hypoplasia of first arch, tail defects) (Oglesby et al. 1992). The effects of 4-CP were ameliorated by coculture with hepatocytes, suggesting that 4CP, rather than a metabolite, was responsible for the effects on development.

In summary, the *in vivo* data suggest marginal effects of chlorophenol exposure on maternal and fetal toxicity and no evidence of teratogenicity. The more highly chlorinated compounds may present the greatest health risk to pregnant women exposed occupationally or through contaminated drinking water.

Genotoxic Effects. Studies regarding genotoxic effects in humans were not available. 2-CP and 4-CP have been tested in one *in vivo* and several *in vitro* genotoxicity assays. Acute administration of up to 69 mg/kg/day 2-CP in mice was not associated with any changes in bone marrow or testicular sister chromatid exchange (SCE) frequency (Table 2-4). Similarly, oral administration of up to 638 mg/kg/day (14 days) or 500 mg/kg/day (90 days) had no effect on SCEs in the testes or bone marrow (Borzelleca et al. 1985a). No further details were provided.

In mammalian *in vitro* systems, 2-CP induced slight-to-moderate increases in c-mitosis (indicating disturbances of the spindle function) and aneuploidy in cultured Chinese hamster lung cells (Onfelt 1987; see Table 2-5). The increase in aneuploidy, compared to controls, was statistically significant ($p<0.025$) by the chisquare test. The results of prokaryotic genotoxicity assays for 2-CP and 4-CP were primarily negative for mutagenicity. In standard *Salmonella typhimurium* reverse mutation assays with strains TA98, TA100, TA1535, TA1537, and TA1538, treatment generally did not produce an increased number of revertants (DeMarini et al. 1990; Haworth et al. 1983; Rapson et al. 1980). Negative findings occurred both in the presence and absence of metabolic activation. In one study, 4-CP had a marginally positive response in strain TA1537 (Seuferer et al. 1979). Neither 2-CP nor 4-CP, either with or without the S9 protein fraction, showed positive gene expression in a *umu* test system (Ono et al. 1992; Sakagami et al. 1988). 2-CP and 4-CP were negative in a prophage induction assay with *Escherichia coli* (DeMarini et al. 1990). 4-CP induced an increased number of revertants in *S. typhimurium* strains TA97 TA98, TAlOO, and TA104 (Strobe1 and Grummt 1987). The effects were most pronounced in strain TA97, in the presence of metabolic activation. Interpretation of these data is confounded by the absence of concentration-effect relationships.

2,4-DCP was negative for SCE induction in the testes and bone marrow after drinking water administration in rats (Borzelleca et al. 1985a; see Table 2-4). As shown in Table 2-5, the compound was mostly negative for mutagenic activity in *S. typhimurium* assays (Haworth et al. 1983; Probst et al. 1981; Rapson et al. 1980;

Table 2-4. Genotoxicity of Chlorophenols *In Vivo*

Species (test system)	End point	Exposure route	Results	Reference	Isomer (purity)
Mammalian systems:					
Mouse (testes and bone marrow)	Sister chromatid exchange	Oral	–	Borzelleca et al. 1985a	2-CP (98+%)
Mouse (testes and bone marrow)	Sister chromatid exchange	Drinking water	–	Borzelleca et al. 1985a	2,4-DCP (98+%)
Mouse (spot test)	Point mutation	Drinking water	–	Borzelleca et al. 1985a	2,4-DCP (98+%)
Rat (alkaline elution WBC and liver DNA)	DNA damage	Oral	–	Kitchin and Brown 1988	2,4,5-TCP (99%)
Rat (alkaline elution WBC and liver DNA)	DNA damage	Oral	–	Kitchin and Brown 1988	2,4,6-TCP (98%)
Rat (alkaline elution WBC and liver DNA)	DNA damage	Oral	–	Kitchin and Brown 1988	2,3,4,6-TeCP (92%)
Insect system:					
Drosophila melanogaster (sex-linked recessive lethal text)	Recessive lethal	Feeding	–	Valencia et al. 1985	2,4,6-TCP (NS)[a]
D. melanogaster (sex-linked recessive lethal text)	Recessive lethal	Injection	–	Valencia et al. 1985	2,4,6-TCP (NS)[a]

[a]The authors reported that "practical grade" 2,4,6-trichlorophenol was tested; however, the purity of this material was not specified (Valencia et al. 1985).

– = negative result; DNA = deoxyribonucleic acid; NS = not specified; WBC = white blood cells

Table 2-5. Genotoxicity of Chlorophenols *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Isomer (purity)
Prokaryotic organisms:	Mutation	–	–	Haworth et al. 1983	2-CP (NS)
Salmonella typhimurium TA98, TA100, TA1535, TA1537 (preincubation assay)					
S. typhimurium TA100 (plate incorporation)	Mutation	NS	–	Rapson et al. 1980	2-CP ("pure")
S. typhimurium TA1535/pSK1002 (induction of DNA repair genes)	Mutation	–	–	Ono et al. 1992	2-CP (NS)
Escherichia coli WP2s(λ) (microsuspension)	Prophage induction	–	–	DeMarini et al. 1990	2-CP (reagent grade)
S. typhimurium TA97, TA98, TA100, TA104 (plate incorporation)	Mutation	+	+	Strobel and Grummt 1987	4-CP (NS)
S. typhimurium TA98, TA100, TA1535, TA1537 (preincubation assay)	Mutation	–	–	Haworth et al. 1983	4-CP (NS)
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538 (plate incorporation)	Mutation	NS	+/–	Seuferer et al. 1979	4-CP (NS)
S. typhimurium TA1535/pSK1002 (induction of DNA repair genes)	Mutation	NS	–	Rapson et al. 1980	4-CP ("pure")
S. typhimurium TA1535/pSK1002 (induction of DNA repair genes)	Mutation	–	–	Sakagami et al. 1988	4-CP (reagent grade)
E. coli WP2s(λ) (microsuspension)	Prophage induction	–	–	DeMarini et al. 1990	4-CP (NS)

Table 2-5. Genotoxicity of Chlorophenols *In Vitro* (*continued*)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Isomer (purity)
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	DeMarini et al. 1990	4-CP (practical grade)
E. coli WP2s(l) (microsuspension)	Prophage induction	+	–	DeMarini et al. 1990	2,4-DCP (99%)
S. typhimurium TA1535/pSK1002 (induction of DNA repair genes)	Mutation	–	+	Ono et al. 1992	2,4-DCP (NS)
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Rasanen et al. 1977	2,4-DCP (NS)
S. typhimurium TA87, TA100, TA1535, TA1537, TA1538	Mutation	–	–	Simmon et al. 1977	2,4-DCP (NS)
S. typhimurium TA100	Mutation	–	–	Rapson et al. 1980	2,4-DCP ("pure")
S. typhimurium C3076, D3052, G46, TA98, TA1000, TA1535, TA1537, TA1538	Mutation	–	–	Probst et al. 1981	2,4-DCP (NS)
S. typhimurium TA98, TA100, TA1535, TA1537 (preincubation assay)	Mutation	+	–	Haworth et al. 1983; NTP 1989	2,4-DCP (NS)
S. typhimurium TA1535/pSK1002 (induction of DNA repair genes)	Mutation	+	+	Ono et al. 1992	2,4,5-TCP (NS)
E. coli WP2s(l) (microsuspension)	Prophage induction	+	–	DeMarini et al. 1990	2,4,5-TCP (99%)
S. typhimurium TA98, TA100, TA102, TA104	Mutation	–	–	George et al. 1992	2,4,5-TCP ("purified")
λ prophage induction	Mutation	–	+	George et al. 1992	2,4,5-TCP ("purified")

Table 2-5. Genotoxicity of Chlorophenols *In Vitro* (*continued*)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Isomer (purity)
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Rasanen et al. 1977	2,4,5-TCP (NS)
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Rasanen et al. 1977	2,4,5-TCP (NS)
E. coli WP2s(l) (microsuspension)	Prophage induction	+	+	DeMarini et al. 1990	2,4,6-TCP (practical grade)
S. typhimurium TA1535/pSK1002 (induction of DNA repair genes)	Mutation	–	+	Ono et al. 1992	2,4,6-TCP (NS)
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Rasanen et al. 1977	2,4,6-TCP (NS)
S. typhimurium TA100 (plate incorporation assay)	Mutation	NS	–	Rapson et al. 1980	2,4,6-TCP ("pure")
S. typhimurium TA100, TA1535, TA1537 (preincubation assay)	Mutation	–	–	Haworth et al. 1983	2,4,6-TCP (NS)
S. typhimurium TA98, TA100, TA1537 (plate incorporation assay)	Mutation	–	–	Kinae et al. 1981	2,4,6-TCP (NS)
Bacillus subtilis H-17, M-45 (rec assay)	DNA damage	NS	+	Kinae et al. 1981	2,4,6-TCP (NS)
E. coli WP2s(l) (microsuspension)	Prophage induction	+	–	DeMarini et al. 1990	2,3,4,5-TeCP (98%)
S. typhimurium TA97, TA98, TA100, TA1535 (preincubation assay)	Mutation	–	–	Zeiger et al. 1988	2,3,4,5-TeCP (>99%)
E. coli WP2s(l) (microsuspension)	Prophage induction	+	–	DeMarini et al. 1990	2,3,4,6-TeCP (unknown)

Table 2-5. Genotoxicity of Chlorophenols *In Vitro* (*continued*)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Isomer (purity)
S. typhimurium TA1535/pSK1002 (induction of DNA repair genes)	Mutation	+	+	Ono et al. 1992	2,3,4,6-TeCP (NS)
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Rasanen et al. 1977	2,3,4,6-TECP (NS)
S. typhimurium TA97, TA98, TA100, TA1535 (preincubation assay)	Mutation	–	–	Zeiger et al. 1988	2,3,4,6-TeCP (>86%)
E. coli WP2s(l) (microsuspension)	Prophage induction	–	–	DeMarini et al. 1990	2,3,5,6-TeCP (99%)
S. typhimurium TA97, TA98, TA100, TA1535 (preincubation assay)	Mutation	–	–	Zeiger et al. 1988	2,3,5,6-TeCP (97%)
Eukaryotic organisms:					
Chinese hamster V79 cells	Chromosomal aberrations	NS	+	Onfelt 1987	2-CP ("purified")
Chinese hamster V79 cells	Mutation	NS	–	Hattula and Knuutinen 1985	2,4-DCP (NS)
Chinese hamster and rat cells (V79 cells cocultured with primary rat hepatocytes; hepatocyte-mediated assay)	Mutation	–	NS	Hattula and Knuutinen 1985	2,4-DCP (NS)
Chinese hamster V79 cells	Chromosomal aberrations	NS	+	Onfelt 1987	2,4-DCP ("purified")

Table 2-5. Genotoxicity of Chlorophenols *In Vitro* (*continued*)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Isomer (purity)
Adult rat hepatocytes	*Unscheduled DNA* synthesis	*NS*	+	*Probst et al. 1981*	*2,4-DCP* (NS)
Chinese hamster ovary cells	Chromosomal aberrations	+	+	Armstrong et al. 1993	2,4,5-TCP (>99%)
Chinese hamster V79 cells	Mutation	NS	–	Jansson and Jansson 1992	2,4,6-TCP (99.7%)
	Chromosomal aberrations	NS	+		
Saccharomyces cerevisiae MP-1 (plate suspension assay)	Mutation	–	+	Fahrig et al. 1978	2,4,6-TCP (99%)
S. cerevisiae MP-1 (plate suspension assay)	Mitotic crossing over	NS	–	Fahrig et al. 1978	2,4,6-TCP (99%)
S. cerevisiae MP-1 (plate suspension assay)	*Mitotic gene* conversion	*NS*	–	Fahrig et al. 1978	2,4,6-TCP (99%)
Chinese hamster and rat cells (hamster V79 cells cultured with primary rat hepatocytes; hepatocyte-mediated assay)	Mutation	–	NS	Hattula and Knuutinen 1985	2,4,6-TCP (NS)
Chinese hamster V79 cells	Mutation	NS	+	Hattula and Knuutinen 1985	2,4,6-TCP (NS)
Mouse (cultured lymphoma L5178Y tk+/– cells; forward mutation assay)	Mutation	NS	+	McGregor et al. 1988	2,4,6-TCP (NS)
Chinese hamster ovary cells	Sister chromatid exchanges and chromosomal aberrations	NS	–	Galloway et al. 1987	2,4,6-TCP (NS)
Chinese hamster ovary cells	Chromosomal aberrations	+	+	Armstrong et al. 1993	2,4,6-TCP (>99.7%)

Table 2-5. Genotoxicity of Chlorophenols *In Vitro* (*continued*)

Species (test system)	End point	Results: With activation	Results: Without activation	Reference	Isomer (purity)
Chinese hamster V79 cells	Mutation	NS	+	Hattula and Knuutinen 1985	2,3,4,6-TeCP (NS)

– = negative result; + = positive result; +/– = borderline mutagen; DNA = deoxyribonucleic acid; NS = not specified

Rasanen et al. 1977; Simmon et al. 1977) but was positive with activation in a prophage induction assay (DeMarini et al. 1990) and positive without activation in a umu test system (Ono et al. 1992). In mammalian cells (Table 2-5), 2,4-DCP was negative for gene mutation in Chinese hamster V79 cells (Hattula and Knuutinen 1985) but produced chromosomal aberrations in Chinese hamster V79 cells (Onfelt 1987) and induced unscheduled DNA synthesis in rat hepatocytes (Probst et al. 1981).

2,4,5-TCP did not increase DNA damage in mice given a single oral dose (164 mg/kg) as indicated by alkaline elution of DNA from white blood cells and the liver (Kitchin and Brown 1988). 2,4,5-TCP was predominantly negative in standard S. typhimurium reversion bioassays (George et al. 1992; Rasanen et al. 1977) but was positive both with and without activation in a *umu* test system (Ono et al. 1992) and with (DeMarini et al. 1990) and without activation (George et al. 1992) in prophage induction assays (Table 2-5). 2,4,5-TCP did induce chromosome aberrations in Chinese hamster ovary cells both with and without metabolic activation (Armstrong et al. 1993).

2,4,6-TCP has been evaluated for genotoxicity in a variety of *in vitro* and in viva assays. As summarized in Tables 2-4 and 2-5, the results of these various assays have been both positive and negative, with the majority of studies reporting negative results. According to Kitchin and Brown (1988), short-term *in vitro* tests with chlorinated phenols often show weakly positive or no effects. Five different assays have reported positive results. *In vitro,* 2,4,6-TCP has demonstrated genotoxic activity without metabolic activation in bacteria *(Bacillus subtilis),* yeast *(Saccharomyces cervisiae),* and mammalian cells (Chinese hamster V79 cells, mouse lymphoma L5178Y TK +/- cells) (Fahrig et al. 1978; Hattula and Knuutinen 1985; Kinae et al. 1981; McGregor et al. 1988). *In vivo,* 2,4,6-TCP has demonstrated genotoxic activity in somatic cells of mice in the spot test (Fahrig et al. 1978). 2,4,6-TCP has tested positive for chromosomal aberrations in Chinese hamster ovary cells both with and without metabolic activation (Armstrong et al. 1993). Positive results for mutations in Chinese hamster V-79 cells reported by Hattula and Knuutinen (1985) were in contrast to the negative results reported by Jansson and Jansson (1992). Additional negative results occurred in bacteria *(S. typhimurium* without activation), yeast *(S. cervisie),* and mammalian ovary cells (Fahrig et al. 1978; Galloway et al. 1987; Haworth et al. 1983; Kinae et al. 1981; Lawlor et al. 1979; Rasanen et al. 1977). *In vivo* tests using insect systems *(Drosophila melanogaster)* were also negative (Valencia et al. 1985).

An increase in DNA damage was not observed in the white blood cells or livers of mice given a single oral dose of 2,3,4,6-TeCP (193 mg/kg) (Kitchin and Brown 1988). 2,3,4,6-TeCP did test positive for mutation in Chinese hamster V79 cells (Hattula and Knuutinen 1985). All three tetrachlorophenol isomers have

tested negative for mutation in standard *S. typhimurium* strains (Rasanen et al. 1977; Zeiger et al. 1988). 2,3,4,5- and 2,3,4,6-TeCP, but not 2,3,5,6-TeCP, tested positive in a prophage induction assay (DeMarini et al. 1990), and 2,3,4,6-TeCP was positive both with and without activation in *a umu* test system (Ono et al. 1992).

The preponderance of the evidence from *in vivo* (Borzelleca et al. 1985a; Kitchen and Brown 1988; Valencia et al. 1985) and *in vitro* studies with prokaryotes (DeMarini et al. 1990; George et al. 1992; Haworth et al. 1983; Kinae et al. 1979; Ono et al. 1992; Probst et al. 1981; Rapson et al. 1980; Rasanen et al. 1977; Sakagami et al. 1988; Simmon et al. 1977; Zeiger et al. 1988) suggests that as a class the chlorophenols are not directly mutagenic. In contrast, a more limited number of *in vitro* studies with eukaryotic cells have generally been positive for chromosomal aberrations (Armstrong et al. 1993; Jansson and Jansson 1992; Onfelt 1987; Probst et al. 1981) suggesting that chromosome malsegregation may be the mechanism of genotoxicity for the chlorophenols. The lack of effect in the *in vivo* studies may be a result of the rapid urinary excretion of chlorophenols in these single-dose studies (Borzelleca et al. 1985a; Kitchin and Brown 1988).

Cancer. Numerous cohort and case-control studies of wood finishing and chlorophenoxy herbicide workers exposed to higher chlorophenols are available (Coggon et al. 1991; Eriksson et al. 1981, 1990; Hardell et al. 1987; Hoar et al. 1986; Honchar and Halperin 1981; Kogevinas et al. 1992; Lynge 1985; Ott et al. 1980; Pearce et al. 1988; Smith et al. 1984; Woods et al. 1987). The results of these studies vary, but some suggest a relationship between chlorophenol exposure and increased incidence of soft-tissue sarcomas, lung cancers, malignant lymphomas, non-Hodgkin's lymphomas, and nasal/nasopharyngeal cancers. The conclusions from these studies are limited by small cohort sizes, coexposure to other contaminants (including TCDD), and the lack of adequate control groups.

Chronic oral studies of 2,4-DCP in rats and mice (Exon and Koller 1985; NTP 1989) have not resulted in any significant carcinogenic effect, even following both pre- and postnatal exposure (Exon and Koller 1985). A positive result in a dermal initiation promotion study suggests that 2,4-DCP can act as a promoter (Boutwell and Bosch 1959).

The data available are inadequate regarding cancer in humans following exposure to 2,4,6-TCP. Animal data suggest that humans may be at risk of cancer following exposure to 2,4,6-TCP (NCI 1979). A significant dose-related increase in the incidence of leukemia occurred in male rats chronically exposed to

2,4,6-TCP in the diet (NCI 1979). Both female and male mice treated chronically with 2,4,6-TCP in the diet had significantly increased incidences of hepatocellular carcinomas and adenomas when compared to the controls (NCI 1979).

The carcinogenicity of 2,4,6-TCP following both intraperitoneal and subcutaneous exposure has been evaluated in studies with mice. No significant increase in the incidence of pulmonary tumors was noted in mice given repeated intraperitoneal injections of 2,4,6-TCP over an intermediate exposure period and followed over a 24-week observation period (Stoner et al. 1986). No significant increase in the incidence of injection-site tumors or systemic tumors was observed in mice 18 months after a single subcutaneous injection of 2,4,6-TCP (Bionetics Research Labs 1968). These intraperitoneal and subcutaneous studies are limited because the duration of exposures was less than lifetime. In addition, although the intraperitoneal and subcutaneous routes of administration are valuable research methods, their relevance to human exposure pathways is limited.

IARC (1987) considers chlorophenols as a group to have limited evidence for human carcinogenicity (group 2B). The Department of Health and Human Services (NTP 1994) considers that 2,4,6-TCP may reasonably be anticipated to be a carcinogen. Based on the positive NCI (1979) cancer bioassays with rats and mice, EPA (IRIS 1994) has classified 2,4,6-TCP as a B2 agent (probable human carcinogen). This category applies to those chemical agents for which there is sufficient evidence of carcinogenicity in animals and inadequate evidence of carcinogenicity in humans. EPA (IRIS 1994) has calculated a cancer potency factor (q_1* or slope factor) for 2,4,6-TCP of 0.02 (mg/kg/day) for both oral and inhalation exposure. This cancer potency factor is equivalent to a drinking water unit risk value and an inhalation unit risk value of 3.1×10^{-7} $(\mu g/L)^{-1}$ and 3.1×10^{-6} $(\mu g/m^3)^{-1}$, respectively (IRIS 1994). The drinking water concentration of 2,4,6-TCP associated with an excess lifetime cancer risk of 10^{-4}, 10^{-5}, and 10^{-6} is 300 μg/L, 30 μg/L , and 3 μg/L, respectively. The air concentration of 2,4,6-TCP associated with an excess lifetime cancer risk of 10^{-4}, 10^{-5}, and 10^{-6} is 30 $\mu g/m^3$, 3 $\mu g/m^3$, and 0.3 $\mu g/m^3$, respectively (IRIS 1994).

The mechanism(s) by which 2,4,6-TCP induces cancer in animals are not known. However, it has recently been suggested that 2,4,6-TCP causes cancer either by suppressing the immune system, by acting as a weak clastogen, and/or by acting as a weak initiator or promoter of carcinogenesis (Kitchin and Brown 1988). According to Kitchin and Brown (1988), two positive results (direct V-79, *Bacillus subtilus)* support the idea that 2,4,6-TCP may be an initiator. However, three negative results in the Ames test, hepatocyte mediated V-79, and alkaline elution *in vivo* fail to support the idea that 2,4,6-TCP is an initiator. 2,4,6-TCP was also

not an initiator following a single oral, dermal, or subcutaneous dose given to mice followed by 20 weeks of three times per week skin applications of the promotor 12-*O*-tetradecanoylphorbol-13-acetate (Bull et al. 1986). Supporting evidence for 2,4,6-TCP as a promotor includes the observation that ornithine decarboxylase and cytochrome P-450 induction occurs at high doses.

2.6 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 due to 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 5.6 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 pre-natal and post-natal 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 (Widdowson and Dickerson 1964; Foman et al. 1982; Owen and Brozek 1966; Altman and Dittmer 1974; Foman 1966). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns and 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 (feeder and Kearns 1997; Komori 1990; Vieira et al. 1996; NRC 1993). Whether differences in xenobiotic metabolism make the child more or less susceptible also depend 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 the newborn who has a low glomerular filtration rate and has not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; West et al. 1948; NRC 1993). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer 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 while others may decrease susceptibility to the same chemical. For example, the fact that infants breathe more air per kilogram of body weight than adults may be somewhat counterbalanced by their alveoli being less developed, so there is a disproportionately smaller surface area for absorption (NRC 1993).

No direct information is available regarding the health effects of chlorophenols observed in children. However, health effects observed in adults are also expected to be of potential concern in children. Although no direct information is available on the effects of chlorophenols on the developmental process in humans, studies in animals indicate few developmental effects. No significant changes in offspring body or liver weights were observed in rats treated with 2-CP in drinking water at doses up to 50 mg/kg/day throughout gestation and up to 91 days post par-turn (Exon and Koller 1981, 1985). No adverse changes in litter sizes, perinatal loss, pup weight, or litter biomass were observed when female rats received a single dose of 4-CP as high as 1,000 mg/kg on gestational day 11 (Kavlock 1990). Oral exposure of pregnant rats to a maternal toxic dose of 750 mg/kg/day 2,4-DCP for 10 gestational days induced a slight decrease in fetal weight and a statistically significant delayed ossification of sternal and vertebral arches and led to a slight insignificant increase in early embryonic deaths (Rodwell et al. 1989). No effects on immune function parameters were observed in 6-week old rats treated with 2,4-DCP in the drinking water at doses up to 30 mg/kg/day throughout gestation (Exon and Koller 1985; Exon et al. 1984). Gavage administration of 650 mg/kg/day 2,4,5-TCP during organogenesis (days 6-15 of gestation) produced no fetotoxicity, malformations, or structural terata in the offspring of rats (Chemoff et al. 1990). Administration of 800-900 mg/kg 2,4,5-TCP in mice on 1 day of gestation or 250-300 mg/kg/day on any 3 days of gestation had no effect on resorption incidence, pup survival, mean fetal weight, gross malformations, skeletal malformation, or cleft palates (Hood et al. 1979). Similarly, maternal exposure of rats to 500 mg/kg/day 2,4,6-TCP only produced a

transient reduction in the body weight of offspring (Blackburn et al. 1986). When female Sprague-Dawley rats orally received purified 2,3,4,6-TeCP throughout organogenesis, the only effect on the fetus was delayed ossification of the skull bones (Schwetz et al. 1974). However, this effect was not statistically significant when analyzed by litters. In a follow-up study in pregnant rats receiving 0,25, 100, or 200 mg/kg/day every day during organogenesis, the two highest doses resulted in inhibition of maternal body weight gain. There was also a dose-related trend for 2,3,4,6-TeCP-mediated effects on implantation or postimplantation viability. Chlorophenols do not appear to be teratogenic in animals (Rodwell et al. 1989; Exon and Koller 1981; Schwetz et al. 1974).

Prior maternal exposure to chlorophenols is unlikely to affect the fetus or a nursing neonate. The relative rapid metabolism and excretion of chlorophenols (Keith et al. 1980) should limit their potential to accumulate in maternal tissue; chlorophenols do not appear to accumulate in animals after oral exposure (Korte et al. 1978; Bahig et al. 1981). The accumulation of 2-CP in tissues of dams was found to be minimal (Exon and Koller 1982). However, 2,3,4,6-TeCP was detected in adipose tissues from people not occupationally exposed to chlorophenols (Mussalo-Rauhamaa et al. 1989), probably due to the relatively higher octanolwater partition coefficient of TeCP. Chlorophenols and/or their metabolites might cross the placenta as it has been shown in a reproductive study in rats that transplacental exposure to 2-CP can be feto- or embryotoxic at a high dose, resulting in a significant increase in the number of still births (Exon and Koller 1982), although it is possible that these could be indirect effects of the fetus. In another study, an increase in delayed ossification of the fetal skull bones was observed when pregnant Sprague-Dawley rats were treated with TeCP, supporting that chlorophenols might cross the placenta. No studies are available that measured chlorophenols in breast milk.

As a class, the chlorophenols are not directly genotoxic although a limited number of *in vitro* studies with eukaryotic cells have been positive for chromosomal aberrations. There is no information to indicate that parental exposure may affect children via damage to germ cells.

Metabolism of chlorophenols has not been studied in children. However, sulfation and glucuronidation are the main metabolic pathways for chlorophenols in both human and animal studies. The conjugated metabolites are then eliminated in urine. In humans, activity of UDP-glucuronosyltransferase (responsible for glucuronide conjugates) does not reach adult levels until about 6-8 months of age, although the development of this activity is isoform specific. Activity of sulfotransferases (responsible for sulfate conjugates) seems to develop earlier, although again it is isoform specific. The activity of some sulfotransferase isoforms may be

greater than that of adults during infancy and early childhood (Leeder and Kearns 1997). In rats, while sulfation is almost at adult levels at birth, UDP-glucuronosyltransferase activity towards different xenobiotics varies with maturation (Weiss 1960; Young and Lietman 1978). It is possible that chlorophenols might be eliminated at a slower rate in children, resulting in increased susceptibility of children to their toxicity.

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to chlorophenols are discussed in Section 2.7.1.

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 chlorophenols are discussed in Section 2.7.2.

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 2.9, "Populations That Are Unusually Susceptible."

2.7.1 Biomarkers Used to Identify or Quantify Exposure to Chlorophenols

There are no biomarkers of exposure or effect that have been validated in children or adults exposed as children. The only known biomarkers of chlorophenol exposure are the hydrolyzed urinary extracts of the parent compounds and dechlorinated derivatives. However, these extracts are not unique to chlorophenol exposure. For example, conjugated forms of higher chlorophenols have been observed after laboratory administration of hexachlorocyclohexanes (Engst et al. 1976; Koransky et al. 1975), indicating that urinary chlorophenol levels are not specific to chlorophenol exposure. Similarly, the presence of chlorophenols or their metabolites in urine is not necessarily diagnostic for chlorophenol exposure because these compounds are also detectable in urine after exposure to certain other pesticides, such as lindane (Karapally et al. 1973), VC-13 (Shafik et al. 1973), 2,4-dichloro-phenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid (Hill et al. 1989). Finally, metabolic dechlorination of higher chlorophenols to lower chlorophenols occurs under some conditions (Renner and Mucke 1986). Studies to determine the importance of these processes on urinary chlorophenol formation, as either conjugated or unconjugated metabolites, have not been conducted. Consequently, the value of assessing urinary chlorophenol concentrations as measures of potential exposure in workers or residents near hazardous waste sites cannot currently be determined.

2.7.2 Biomarkers Used to Characterize Effects Caused by Chlorophenols

No unique biomarkers of effects are available for chlorophenols. As discussed in Section 2.2, the clinical signs associated with high acute levels of monochlorophenol administration include myoclonic convulsions (Angel and Rogers 1972; Borzelleca et al. 1985a, 1985b; Farquharson et al. 1958) and dermal and ocular lesions (Bioassay Systems 1981; Rhodia 1978; Younger Labs 1975). Both myoclonic convulsions and epithelial tissue corrosion are commonly observed after exposure to numerous phenolic compounds and are therefore not necessarily diagnostic of monochlorophenol exposure. Increasing chlorination results in clinical signs of metabolic derangement, such as hyperthermia and blood pressure decrements (Angel and Rogers 1972; Farquharson et al. 1958). These clinical signs are not specific to chlorophenols; they occur

following exposure to any agent that uncouples mitochondrial oxidative phosphorylation such as nitrophenol (Ahlborg and Thunburg 1980). Other effects of chlorophenols, including effects on the immune system (Exon et al. 1984) and on reproduction (Exon and Koller 1985), are also not specific to chlorophenols.

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

2.8 INTERACTIONS WITH OTHER SUBSTANCES

Data regarding the interaction of chlorophenols with other chemical substances in humans or animals were not located. Substances that result in effects similar to those for the chlorophenols have the potential to interact with these compounds. For example, chlorophenols may interact with other carcinogens, promoters, neurotoxic agents, and liver, renal, dermatologic, and ocular toxins.

Factors interfering with Phase II conjugation reactions would inhibit the detoxification of chlorophenols. The results of recent experimentation indicate that the polycyclic aromatic hydrocarbon (PAH), 3-methylcholanthrene (3-MC), stimulates the glucuronidation of phenolic substrates through the induction of glucuronylsyltransferase (Jansen et al. 1992; Wishart 1978a, 1978b). The enzymes induced have a spectrophotometric peak of 448 nanometers (cytochrome P-448) and are characteristically distinct from the phenobarbital-type induced enzymes that have an absorbance maximum at 450 nanometers. These findings suggest that other toxic and/or carcinogenic PAHs, such as benzo(a)pyrene, can significantly enhance the metabolism of phenols. The relationship between PAH particulate and solid material, commonly associated with incinerators and hazardous waste sites, and chlorophenol metabolism has not been studied. In general, the ability of another chemical to affect the toxicity of chlorophenols may depend on its affinity for the cytochrome P-448 substrate binding site.

Chlorophenols are toxic to the liver. Exposure to hepatotoxic drugs such as acetaminaphen (Tylenol®) and chlorophenols may result in additive effect. However, there are no studies that indicate such interaction.

Using an *in vitro* rat liver microsomal preparation, Arrhenius et al. (1977) noted that 2,4-DCP, 2,4,6-TCP, and 2,3,4,6-TeCP in the concentration range of 0.03-3 mM shifted the metabolism of aromatic amines from C-oxygenation to n-oxygenation. The carcinogenic metabolites of aromatic amines can be formed by M-

oxygenation. Therefore, Arrhenius et al. (1977) suggested that the chlorophenols should be considered as possible synergists for the carcinogenicity of aromatic amines.

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to chlorophenols than will most persons exposed to the same level of chlorophenols in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters may result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the preexisting compromised function of target organs (including effects or clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly, with declining organ function, and the youngest of the population, with immature and developing organs, will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.7, Populations With Potentially High Exposure.

No specific population that would be particularly susceptible to chlorophenol intoxication has been identified. Because of the extensive hepatic conjugation and renal clearance of these compounds, individuals with liver or kidney dysfunction may be the most sensitive population. The results of recent studies indicate that individuals with cirrhosis of the liver (Macdonald et al. 1992; Ohta 1991) or hepatitis (Ohta 1991) show impaired Phase II conjugation. Chronic renal failure is associated with the inability to clear conjugated metabolites, resulting in elevated, steady-state whole body concentrations of glucuronide and sulfate metabolites (Martin et al. 1991). Patients with acute tubular necrosis, with or without cirrhosis, show markedly elevated urinary β-glucuronidase concentrations (Solis-Herruzo et al. 1986) and, theoretically, a high body burden of unconjugated metabolites. These data suggest that chlorophenol-exposed individuals with preexisting liver or kidney disease may be at increased risk from exposure.

Individuals with Gilbert's disease or Crigler-Najjar syndrome, inherited deficiencies of bilirubin UDPglucuronyl transferase (UGT), may have increased sensitivity to the effects of chlorophenol exposure (de Morais and Wells 1988; de Morais et al. 1992). Considerable progress in understanding the genetic control of these abnormalities has recently been made (Jansen et al. 1992). Patients with Type I Crigler-Najjar syndrome may be at the greatest risk following exposure to phenolic compounds. This form of the syndrome

is characterized by unconjugated hyperbilirubinemia and apparently results from a deficiency in the 3-Mcinducible form of the phenolic UGT. Defects in cytochrome P-450 induction occur in Type II (partial hyperbilirubinemia) Crigler-Najjar syndrome and Gilbert disease; consequently, these patients may not be as sensitive to increased plasma concentrations of chlorophenol.

Cigarette smokers are at potentially increased risk from chlorophenol exposure (Alvares 1978; Bock et al. 1987). The incomplete combustion products of smoking, such as PAHs, induce P-448 metabolism, resulting in the potential formation of reactive metabolites and an increased conjugation rate (see Sections 2.3.3 and 2.7). Alternatively, PAHs may accelerate the detoxification of chlorophenol. Consequently, definitive statements about the relationship between cigarette smoking and chlorophenol metabolism cannot be made at the present time.

Evidence from rat studies (Exon et al. 1985) suggests that at least one part of the immune system (delayed hypersensitivity) is sensitive to 2,4-DCP. Persons with immune system deficiencies, therefore, may be more susceptible to the adverse effects of 2,4-DCP exposure.

Fetuses or neonates may also be at increased risk. In humans, activity of UDP-glucuronosyltransferase (responsible for glucuronide conjugates) does not reach adult levels until about 6-8 months of age, although the development of this activity is isoform specific. Activity of sulfotransferases (responsible for sulfate conjugates) seems to develop earlier, although again, it is isoform specific. The activity of some sulfotransferase isoforms may be greater than that of adults during infancy and early childhood (Leeder and Kearns 1997). This conclusion is also supported by data from rat hepatic bioassays in which UGT activities toward phenolic substrates reached adult levels between days 16 and 20 of gestation (Wishart 1978a). Enzymatic activity toward bilirubin is negligible during this gestational period (the “late foetal” group), but surges between gestation day 20 and postnatal day 2 (the “postnatal” group). The differences in fetal and neonatal detoxification systems, compared to the mature organism, may result in a slower elimination of the chlorophenols, which may serve to increase the toxicity of these compounds.

Further discussion of the susceptibility of children is in Section 2.6 Children’s Susceptibility.

2.10 METHODS FOR REDUCING TOXIC EFFECTS

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

2.10.1 Reducing Peak Absorption Following Exposure

Following high-dose oral exposure to chlorophenols, administration of water for dilution may be warranted (Bronstein and Currance 1988). Although Ellenhorn and Barceloux (1988) recommend the use of ipecac/lavage, activated charcoal, and cathartics for gastric contamination from chlorophenols, Bronstein and Currance (1988) recommend against emesis because of possible aspiration into the lungs. This is more important for the liquid chlorophenols than for the solid species.

Specific decontamination procedures for chlorophenols after skin or eye contact are not available. However, general approaches for minimizing absorption can be extrapolated from literature on phenol or pentachlorophenol. After dermal contact, rinsing with water (Gosselin et al. 1984) or washing with soap (Bronstein and Currance 1988) may be the procedures of choice. The case reported by Kintz et al. (1992) in which death occurred in a worker dermally exposed to 2,4-DCP on less than 10% of his body indicated that washing with water may not be sufficient, especially if contaminated clothing is not removed. Attempts to decontaminate phenols with alcohol, vegetable oils, glycerin, or polyethylene glycol, with or without methanol, have met with variable success (Gosselin et al. 1984). After flushing a contaminated eye with water, irrigation is sometimes used. This technique is suggested for adults with an intact lid who have no evidence of edema (Bronstein and Currance 1988). For children, irrigation of each eye with normal saline, using large bore intravenous tubing, is sometimes recommended (Bronstein and Currance 1988). In more advanced cases, the use of proparacaine hydrochloride may follow eye irrigation (Bronstein and Currance 1988).

2.10.2 Reducing Body Burden

The limited experimental data suggest that orally administered chlorophenols are rapidly conjugated and excreted in the urine (see Sections 2.3.3 and 2.3.4). The efficacy of drug therapy to accelerate Phase II

detoxification reactions is not known. For example, supplying substrates for glucuronidation and sulfur conjugation may increase the excretion of the chlorophenols. Based on studies involving pentachlorophenol exposure, the utility of either exchange transfusions or forced diuresis is equivocal (Robson et al. 1969; Young and Haley 1978).

A high intake of ascorbic acid has been shown to reduce accumulation of 2,4-DCP and decrease liquid peroxidation in the liver of guinea pigs compared to animals with low ascorbic acid intake (Derhata et al. 1996).

2.10.3 Interfering with the Mechanism of Action for Toxic Effects

The mechanism of toxic action for 2-CP or 4-CP is not known. Although these chemicals may be weak uncouplers of oxidative phosphorylation, inconsistent findings of increased metabolic rate after exposure suggest that this mechanism may not be the mechanism of greatest consequence (Angel and Rogers 1972; Farquharson et al. 1958; Weinbach and Garbus 1965). Convulsive seizures, of unknown origin, are the most characteristic clinical signs of acute overdose (Angel and Rogers 1972; Borzelleca et al. 1985a). Treatment for the prevention of seizures includes the common anticonvulsant sequence of diazepam, phenytoin, and phenobarbital (Bronstein and Currance 1988; Ellenhorn and Barceloux 1988). The use of anticonvulsants on infants and children must be closely monitored to prevent overdosage and toxic effects of drugs.

No methods to directly reduce the adverse effects of chlorophenol-induced stimulation of mitochondrial respiration were located.

2.11 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 chlorophenols 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 chlorophenols.

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.

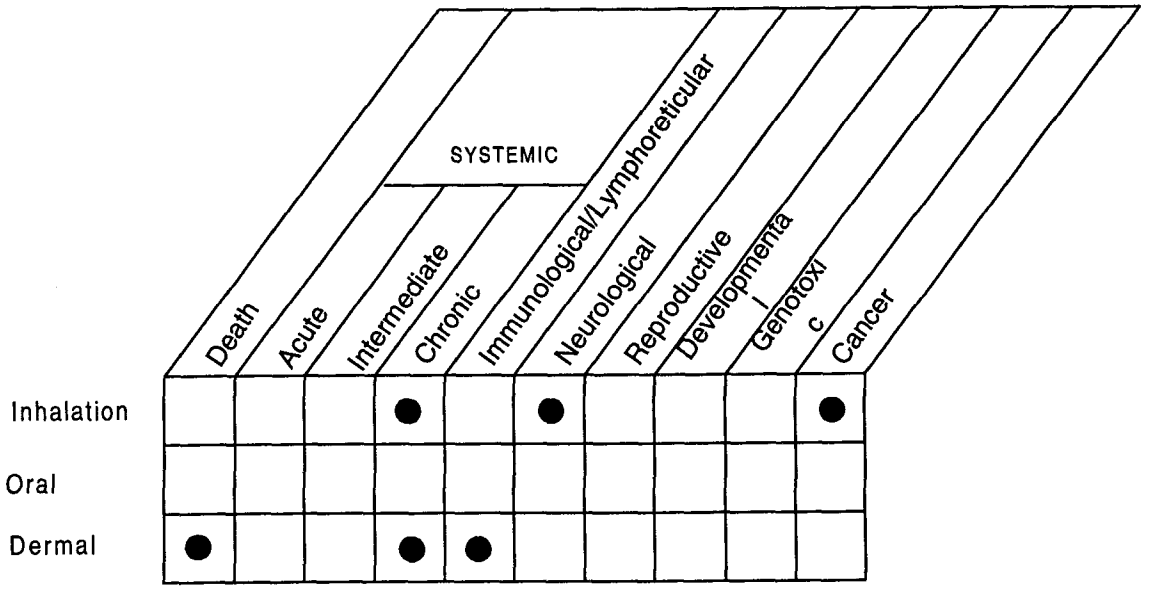
2.11.1 Existing Information on Health Effects of Chlorophenols

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chlorophenols are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of chlorophenols. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 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.

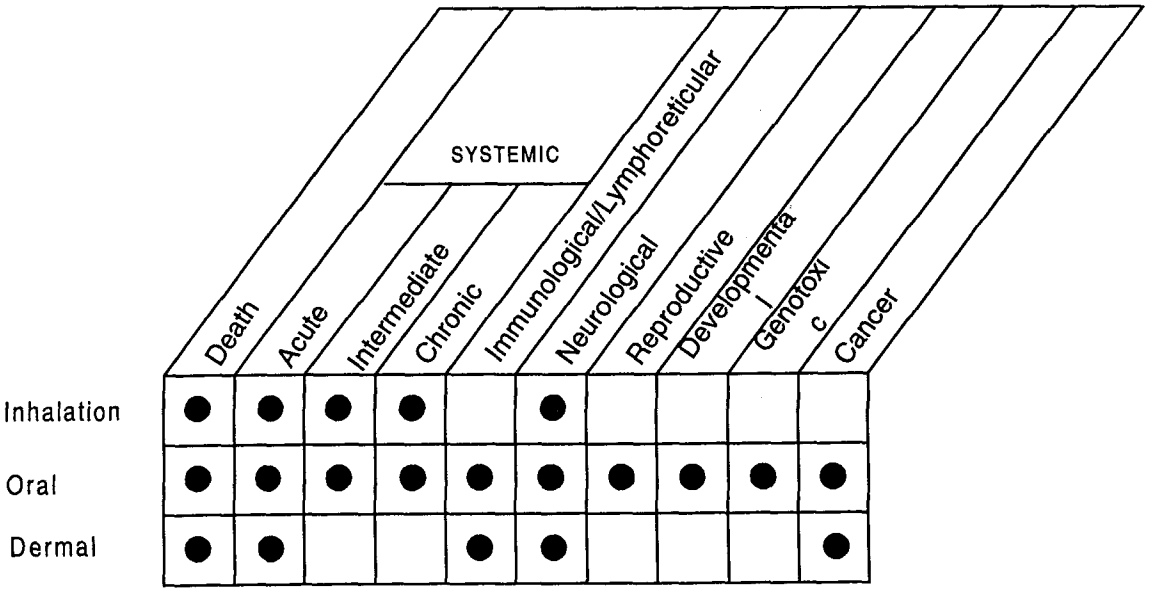
Humans are potentially exposed to chlorophenols occupationally, through municipal solid waste combustion, and as a result of the disinfection of drinking water. The chlorophenol by-products of manufacturing activities may be the greatest single source of concern at NPL hazardous waste sites. Workers in phenoxy pesticide production, wood preservation, dye manufacturing, and alcohol denaturation may be at some risk from chlorophenol exposure. Exposure potential in most of these industries is greater for the higher chlorinated phenols, which, based on the results of animal studies, are more toxic than the dichlorophenols and the monochlorophenols (Borzelleca et al. 1985a). Results of human studies involving exposure to higher chlorophenols suggest that occupational dermal exposure is a more significant concern than inhalation exposure (Kleinman et al. 1986).

Except for the single death following dermal 2,4-DCP exposure (Kintz et al. 1992) no health effects data for humans exposed exclusively to chlorophenols were located. Available occupational data indicate that chemicals involved in the production of phenoxy-based herbicides, and/or the end-use products themselves, may be associated with the development of cancers of the hematopoietic and lymphatic systems (Eriksson et al. 1981; Hardell et al. 1981). Positive results for carcinogenicity do not show a consistent trend across

Figure 2-4. Existing Information on Health Effects of Chlorophenols



Human



Animal

● Existing Studies

worker groups and cannot be specifically associated with a single contaminant or group of contaminants (including trichlorophenols). No epidemiological evidence of adverse health effects associated with drinking chlorophenol-contaminated water was located.

Most of the available animal data, which include several recent, well-conducted studies, have involved the oral route of administration. After acute exposure, the higher chlorophenols, in particular, have effects on basal metabolism that are typical of uncouplers of mitochondrial oxidative phosphorylation. Experimental results suggest that the acute toxicity of tetrachlorophenols is the greatest, followed by the monochlorophenols and then the trichlorophenols, with the dichlorophenols being the least toxic isomers (Borzelleca et al. 1985a). The adverse effects sometimes reported after repeated exposures do not indicate a well-defined toxic syndrome. Intermediate to high chlorophenol doses have been associated with marginal effects on the immune, lymphoreticular, reproductive, and developmental systems, and a range of effects on the liver. Further research to help define the experimental conditions and mechanisms associated with these noncarcinogenic findings, and how they may relate to chlorophenol-exposed humans, is required. 2,4,6-TCP is an animal carcinogen that has produced leukemia in rats and hepatocellular carcinoma and adenomas in mice (NCI 1979). Furthermore, other chlorophenols have tumor promoting capabilities.

2.11.2 Identification of Data Needs

Acute-Duration Exposure. Information from animal studies indicates that most chlorophenols produce lethality in experimental animals following a single-dose oral exposure of 89-5,000 mg/kg (Ahlborg and Larsson 1978; Borzelleca et al. 1985a; Kobayashi et al. 1972; NTP 1989; Vernot et al. 1977). The limited inhalation and dermal data tend to corroborate these findings (Carreon et al. 1980a; Duchosal and Biedermann 1991). The most characteristic clinical sign after lethal or high sublethal doses of monochlorophenols is convulsions (Borzelleca et al. 1985a, 1985b). Physiological changes associated with the uncoupling of oxidative phosphorylation increase with the increasing degree of chlorination (Cascorbi and Ahlers 1989; Farquharson et al. 1958; Izushi et al. 1988; Mitsuda et al. 1963; Narasimhan et al. 1992; Shannon et al. 1991; Stockdale and Selwyn 1971). Following acute oral exposure, the order of toxicity as indicated by LD_{50}s was tetrachlorophenols > monochlorophenols > dichlorophenols > trichlorophenols (Borzelleca et al. 1985a; Deichmann and Mergard 1948). Maternal toxicity, but not fetal toxicity, was observed in rats treated by gavage with 2,4-DCP at 100 mg/kg/day on gestation days 6-15 (Rodwell et al. 1989). No maternal or fetal effects were observed at 25 mg/kg/day. The lowest acute oral LOAEL for the chlorophenols that was identified was 2.58 mg/kg/day for electron microscopic changes in hepatocytes

(foamy cytoplasm, clustering of mitochondria, and endoplasmic reticulum) that were not observed at 1.28 mg/kg/day (Phornchirasilp et al. 1989a). Based on the NOAEL of 1.28 mg/kg/day for liver effects following 4-CP exposure, an acute-duration oral MRL of 0.01 mg/kg/day was calculated for the chlorophenols. Data are insufficient and are needed for the derivation of an acute inhalation MRL for the chlorophenols. Additional experiments to assess dose-response relationships are needed because of reporting deficiencies and methodological differences to obtain data for an inhalation MRL. These data are needed to assess risk to workers exposed during accidental releases. The report of a worker who died after splattering pure 2,4-DCP on portions of his right arm and leg while disposing industrial wastes (Kintz et al. 1992) demonstrates the toxicity of chlorophenols via dermal exposure. More comprehensive dermal toxicity studies analyzing both acute toxicity incidence and localized effects are also needed. Finally, additional studies examining the liver effects following oral exposure to chlorophenols other than 4-CP are needed to confirm that the MRL based on 4-CP exposure is truly protective of liver effects following exposure to these other compounds.

Intermediate-Duration Exposure. Occupational studies have typically involved individuals exposed to chlorophenols for intermediate- or chronic-duration periods. The results of these studies are described under the section Chronic-Duration Exposure and Cancer. Intermediate-duration oral administration of chlorophenols to experimental animals generally produces no effects or marginal effects on systemic, immunological, reproductive, and developmental end points (Bercz et al. 1990; Borzelleca et al. 1985a; Carlson 1978; Exon and Koller 1982, 1983, 1985; Hattula et al. 1981). Immunological effects (a decrease in delayed type hypersensitivity) appears to be the most sensitive target of 2,4-DCP toxicity (Exon and Koller 1985; Exon et al. 1984), and an intermediate oral MRL (0.003 mg/kg/day) based on a NOAEL for immunological effects has been derived for the chlorophenols. Data are insufficient and are needed for the derivation of an intermediate-duration exposure. The liver is the organ most consistently affected by the chlorophenols. Effects noted include enzyme induction (Phornchirasilp et al. 1989a), increased liver weight (Bercz et al. 1990; Exon and Koller 1985), hypertrophy (American Biogenics 1988), and centrilobular degeneration and focal necrosis (Hattula et al. 1981; McCollister et al. 1961). Data on intermediate-duration dermal exposure of chlorophenols in humans or animals are not available and are needed.

Additional studies examining the immunological effects following oral exposure to chlorophenols other than 2,4-DCP are needed to confirm that the intermediate-duration MRL based on 2,4-DCP exposure is truly protective of liver effects following exposure to the other compounds. Mechanistic and toxicokinetic studies may provide useful information about the rate of formation and time course of potential intermediates

associated with chlorophenol-induced hepatotoxicity. Further development of dose-response relationships, with respect to biomarkers of hepatic injury, is needed. These data may provide information about both mechanism of action and potential adverse effects expected at the exposure concentrations associated with NPL sites. Additional experiments to assess dose-response relationship are needed to obtain an intermediate inhalation MRL. Studies focusing on organ function, in addition to histopathological analysis, would be particularly important. Because the higher chlorinated compounds, in particular, contain toxic impurities (particularly dioxins and dibenzofurans), the impurities of each test compound should be minimized since the effects of the contaminants could override the effects from the substance tested. Studies specifically designed to examine the interaction of dioxins and dibenzofurans with chlorophenols should also be completed.

Chronic-Duration Exposure and Cancer. Dermal or inhalation exposure to the chlorophenols used in phenoxy herbicide production may be associated with cancer induction (Eriksson et al. 1981, 1990; Hardell et al. 1981; Hoar et al. 1986). Most investigators, however, have found only weak trends or no evidence of an association (Coggon et al. 1991; Kogevinas et al. 1992; Lynge 1985; Pearce et al. 1988; Smith et al. 1984; Woods et al. 1987). Many of the latter studies included a multinational cohort analysis of workers involved in similar production processes. Current experimental methodology is not sufficiently sensitive to determine those exposure factors, if any, that may be associated with the expression of cancer. Other investigators have observed a possible association between chlorophenol exposure in production workers and the onset of hepatic abnormalities, including porphyria (Bleiberg et al. 1964; Calvert et al. 1992). These results suggest the possibility of biomonitoring individuals living near hazardous waste sites for serological evidence of hepatic dysfunction. No data were located regarding the chronic effects of oral chlorophenol exposure in humans.

Available chronic studies with rats and mice, and evidence of clastogenicity, have indicated that 2,4,6-TCP may produce carcinogenicity in animal models through mechanisms other than direct gene mutation (Armstrong et al. 1993; Jansson and Jansson 1992; NCI 1979). Limited experimental data on mouse skin and orally exposed rats suggest that 2-CP and 2,4-DCP may have tumor promoting capabilities, but are not complete carcinogens (Boutwell and Bosch 1959; Exon and Koller 1985). Additional animal studies designed to determine the conditions under which chlorophenols induce cancer, including supporting evidence for a clastogenic or epigenetic role, are needed. Because of significant information gaps, data are not sufficient to determine a chronic-duration MRL for chlorophenols by either the oral or inhalation route of exposure. Such data should be obtained so that chronic-duration MRLs for chlorophenols can be calculated.

Genotoxicity. There are no human studies on the genotoxicity of the eight chlorophenols. In general, chlorophenols have been negative for mutagenicity in most prokaryotic assays (George et al. 1992; Haworth et al. 1983; Lawlor et al. 1979; Rapson et al 1980; Seuferer et al. 1979; Zeiger et al. 1988). Further mutagenicity studies in these test systems are not needed. Neither 2-CP nor 2,4-DCP induced an increased incidence of SCEs in mouse testicular or bone marrow cells (Borzelleca et al. 1985a). In other eukaryotic tests, 2,4,6-TCP has been associated with structural chromosomal aberrations in both somatic and germ cells (Armstrong et al. 1993; Jansson and Jansson 1992). Evidence of mutational activity in both *in vivo* (Fahrig et al. 1978) and *in vitro* (Fahrig et al. 1978; Hattula and Knuutinen 1985; McGregor et al. 1988) assays is also available for 2,4,6-TCP. The implications of these findings for cancer induction needs further research, including the use of both mammalian cell cultures and additional *in vivo* clastogencity assays in mammals.

Reproductive Toxicity. No occupational or epidemiological studies of potential reproductive effects in exposed individuals are available. Only one animal study comprehensively addresses the reproductive effects of chlorophenols. Blackburn et al. (1986) did not find reproductive effects in male or female rats exposed to 2,4,6-TCP by gavage at doses that caused other systemic effects (e.g., decreased body weight gain). There is limited evidence that 2-CP, 2,4-DCP, and 2,4,6-TCP may reduce litter sizes when administered to rats in drinking water (Exon and Koller 1985). This effect was significant only at $p \leq 0.1$ and was observed at doses that caused other effects (e.g., increased liver weights, decreased delayed-type hypersensitivity). An increase in early embryo loss is suggested by a teratology study of 2,3,4,6-TeCP (RTI 1987). However, the rats were dosed on gestation days 6-15 and not earlier in gestation; thus, further studies regarding the effect of 2,3,4,6- TeCP on pre- and early implantation are needed. No data are available on the reproductive toxicity of chlorophenols after inhalation or dermal exposure in humans or animals.

Developmental Toxicity. Pregnant women may be exposed to chlorophenols occupationally, through the drinking water, or by living near a hazardous waste site. No data are currently available to assess the potential effects of postimplantation exposure on the developing offspring of these women. Results from animal studies showed minor effects occurring at doses that are maternally toxic (Blackburn et al. 1986; Exon and Koller 1985; Rodwell et al. 1989; RTI 1987; Schwetz et al. 1974). Frank teratogenic effects have not been observed following exposure of animals during organogenesis. No data are available on the developmental toxicity of chlorophenols after inhalation or dermal exposure in humans or animals.

Mechanistic studies indicated that cultured hepatocytes ameliorated the adverse developmental effects associated with *in vitro* 4-CP exposure (Oglesby et al. 1992). This finding is apparently attributable to

increased rates of detoxification in the hepatocyte cell cultures. In addition, results from mammalian embryo assay indicate that monochlorophenols are not potent developmental toxicants or teratogens (Mayura et al. 1991). *In vivo* studies involving exposure around the implantation period may help corroborate these *in vitro* data. Studies on developmental effects of postnatal exposure will also be useful.

Immunotoxicity. No experimental data involving the immunotoxic effects of human exposure to chlorophenols are available. Oral studies in rats suggest that a low dose of 2,4-DCP (3 mg/kg/day) is associated with a decreased delayed-type hypersensitivity response and an increased humoral immune response with decreased thymus weights following intermediate exposure (Exon et al. 1984). Both erythroid and myeloid elements of the bone marrow are depleted after oral administration with 500 mg/kg/day of 2,4-DCP for 13 weeks in rats (NTP 1989). Oral administration of other chlorophenols has been associated with elevated spleen weights but not with significant effects on humoral or cell-mediated components (Borzelleca et al. 1985a; Exon and Koller 1983, 1985). The implications of these findings for individuals exposed near NPL hazardous waste sites can only be fully assessed with *in vivo* functional tests of immunocompetence, graft rejection, or immunosurveillance in appropriate animals. Additional animal studies are also needed to provide dose-response and/or threshold information. No data are available on the immunotoxicity of chlorophenols after inhalation exposure in animals. Single dermal exposure of 50 mL of 2,4,5-TCP on one shaved flank of mice indicated 2,4,5-TCP can be a skin sensitizer (Kimber and Weisberger 1991). More data on immunotoxicity of chlorophenols by all the routes of exposure are needed.

Neurotoxicity. Within 20 minutes of being accidentally splashed with 2,4-DCP on his right arm and leg, a worker experienced seizures, collapsed, and died shortly thereafter (Kintz et al. 1992). At single oral doses >300 mg/kg monochlorophenols and dichlorophenols, chlorophenols can produce a variety of neurological effects, including tremors, myoclonic convulsions, a hunched posture, dyspnea, collapse, and coma (Borzelleca et al. 1985a, 1985b; Duchosal and Biedermann 1991; Kobayashi et al. 1972; Phornchirasilp et al. 1989b; Spencer and Williams 1950; Wil Research Laboratories 1982). Convulsions are common after high-dose (intraperitoneal dose of 99 mg/kg) 2-CP administration and apparently are less frequent with increasing halogen substitution on the phenol ring (Angel and Rogers 1972). Other investigators have not observed either clinical or histopathological signs of neurological dysfunction after intermediate-duration exposure to doses up to 1,300 mg/kg/day of 2,4-DCP, trichlorophenols, or tetrachlorophenols (Bercz et al. 1990; NCI 1979; NTP 1989). Acute administration of monochlorophenols has reportedly been associated with hyperactivity and decreased brain weight (Borzelleca et al. 1985a). However, the investigators provided no detailed results, and the conclusions remain questionable. As acute high-dose exposures resulting from

accidents are possible, further studies regarding the dose-response relationship of neurotoxic effects following exposure to chlorophenols are needed. The need is greatest for studies on neurotoxicity following dermal exposure.

Epidemiological and Human Dosimetry Studies. As indicated above, accurate human dosimetry studies may not be possible because environmental and occupational chlorophenols typically exist only in association with other chlorinated organics. Consequently, it would be difficult to ascribe any observed health effect to a single chemical or a single group of isomers. Additional studies in workers, such as sawmill employees, that are exposed specifically to chlorophenols are needed. Careful monitoring of chlorophenol air concentrations and skin exposure combined with kinetic measures of urinary output for specific isomers may provide important correlative data for human dosimetry.

Biomarkers of Exposure and Effect

Exposure. Currently, no specific biomarkers for chlorophenols are known. The presence of chlorophenols or their metabolites in urine is not necessarily diagnostic for chlorophenol exposure because these compounds are also detectable in urine after exposure to other pesticides (Hill et al. 1989; Karapally et al. 1973; Shafik et al. 1973) and hexachlorocyclohexanes (Engst et al. 1976; Koransky et al. 1975). Well-designed animal metabolism studies involving the kinetics of urinary chlorophenol conjugate excretion after exposure to a battery of chlorinated organics are needed to provide data regarding the usefulness of urinary measures as biomarkers of exposure for individuals living near hazardous waste sites.

Effect. No carcinogenic or noncarcinogenic effect in exposed humans has been associated with a specific chlorophenol or a group of chlorophenols. With the possible exceptions of convulsions following monochlorophenol exposure (Angel and Rogers 1972; Borzelleca et al. 1985a, 1985b; Farquharson et al. 1958), clearly defined end points of toxicity in animals are not known. Furthermore, these end points that are associated with chlorophenol exposure are not specific to chlorophenols. When in direct contact with skin and eyes, the chlorophenols demonstrate varying degrees of corrosiveness (Bioassay Systems 1982; Rhodia 1978; Younger Labs 1975). Other effects of chlorophenols, including effects on the immune system (Exon et al. 1984) and reproduction (Exon and Koller 1985), are also not specific to chlorophenols.

Further studies designed to identify specific biomarkers of effects of chlorophenols would facilitate medical surveillance of exposed populations leading to early detection of potentially adverse health effects and possible treatment.

Absorption, Distribution, Metabolism, and Excretion. Studies in sawmill workers indicate that trichlorophenol and tetrachlorophenol are well absorbed during occupational exposure (Fenske et al. 1987; Pekari et al. 1991). The dermal route had a greater absorption potential than the inhalation route. The investigators determined that elimination half-lives were directly proportional to the degree of chlorination for the tri-, tetra-, and pentachlorophenols. Concentrations of 2,4-DCP were measured in the blood, urine, bile, and stomach contents of a worker who collapsed (within 20 minutes) and died shortly after being splashed with pure 2,4-DCP on his right arm and leg (Kintz et al. 1992). Attempts to attain additional data in humans are limited by ethical considerations and by the fact that measurements of chlorophenol levels in the blood and urine of manufacturing workers may not be specific for chlorophenol exposure.

The limited amount of data available from animal oral studies indicates that these chemicals are rapidly absorbed, conjugated to polar metabolites in the liver and kidney, and excreted in the urine (Bahig et al. 1981; Bray et al. 1952a, 1952b; Korte et al. 1978; Spencer and Williams 1950). Absorption also occurs after *in vivo* (Carreon et al. 1980a, 1980b; Hencke and Lockwood 1978) and *in vitro* (Hughes et al. 1993; Huq et al. 1986) dermal application. Rate constants for *in vivo* preparations were not located. Tissue burden is apparently short-lived, with plasma elimination half-lives of approximately 10 minutes after intravenous 2,4-DCP administration (Somani and Khalique 1982), and peak tissue concentrations occurring approximately 30 minutes after intraperitoneal 2,4,6-TCP dosing (Pekari et al. 1986). Plasma protein binding is significant after administration of the higher chlorophenols; in humans, the extent of binding increases with the increasing degree of chlorination (Pekari et al. 1991).

Studies concerning the inhalation absorption and oral absorption of chlorophenols from different media (e.g., water, soil) and the effect of ionization on dermal absorption are needed for estimating exposure at a hazardous waste site.

More systematic information about absorption and elimination kinetics are needed. Furthermore, time series studies using radiolabelled chlorophenols would help identify residence times in individual organs, which may provide insight into potential target organs in human populations.

Semiquinones and quinones may be potentially toxic but short-lived metabolites after oral exposure (Juhl et al. 1991; Phornchirasilp et al. 1989b). The extent and type of Phase II detoxification reactions is apparently species- and isomer-related (Bahig et al. 1981; Bray et al. 1952a, 1952b; Pekari et al. 1986; Somani and Khalique 1982; Spencer and Williams 1950). Broad-based experimentation to determine dose-effect relationships for hepatic adaptive and toxic effects are needed. Part of this experimentation may involve estimation of the rate constants for the formation of both potentially toxic intermediates and Phase II conjugates. Metabolic studies for determining rate differences in conjugate formation between oral, inhalation, and dermal exposure may also suggest route-specific differences in the expression of toxicity.

Comparative Toxicokinetics. Toxicokinetic studies with chlorophenols have been conducted in humans, mice, rats, rabbits, and dogs (Azouz et al. 1953; Bray et al. 1952a, 1952b; Exon and Koller 1982; Fenske et al. 1987; Hattula et al. 1981; Phomchirasilp et al. 1989a; Somani and Khalique 1982; Spencer and Williams 1950). The results of these studies provide a limited profile of toxicokinetics information after oral exposure. These results do not adequately characterize the metabolic rate differences between the various isomers. More comprehensive toxicokinetic studies using radiolabelled isomers administered at several dose levels in two rodent species and one or more nonrodents are needed. These data may be supplemented by hepatic and renal biopsy data and urinary metabolite analysis obtained in exposed individuals.

Methods for Reducing Toxic Effects. The P-448 inducer 3-MC, and possibly other PAHs, apparently increase the Phase 11 conjugation rates of phenolic substrates (Jansen et al. 1992; Wishart 1978a, 1978b). This observation implies that certain chemicals may decrease the body burden of chlorophenols by accelerating the elimination process. Despite this capability, PAH-induced P-448 metabolism may actually increase the extent of hepatic injury through the formation of metabolites capable of undergoing covalent binding (Arrhenius et al. 1977). Research into the development of therapeutic agents that accelerate chlorophenol elimination through Phase II detoxification reactions, but that do not produce bioreactive metabolites (such as semiquinones), may be advisable.

Children's Susceptibility No data are available on the health effects of chlorophenols on exposed children. Since the metabolic enzymes for detoxification may have age dependent expression, there is a need for such data.

There is inadequate experimental evidence to evaluate whether pharmacokinetics of chlorophenols are different in children. There are also limited data to show whether chlorophenols are stored in maternal tissues. There are no direct data on whether chlorophenols cross the placenta or accumulate in breast milk.

There is no experimental evidence to evaluate whether metabolism of chlorophenols or their mechanism of action is different in children. However, in humans, activity of UDP-glucuronosyltransferase (responsible for glucuronide conjugates) does not reach adult levels until about 6-8 months of age, although the development of this activity is isoform specific. Activity of sulfotransferases (responsible for sulfate conjugates) seems to develop earlier, although again, it is isoform specific. The activity of some sulfotransferase isoforms may be greater than that of adults during infancy and early childhood (Leeder and Kearns 1997). It will be helpful to have data on the metabolism and mechanism of action of chlorophenols in children to determine whether children are more vulnerable than adults to the health effects from exposure to chlorophenols. There are no biomarkers of exposure or effect that have been validated in children or adults exposed as children. There are no data to determine whether there are any interactions with other chemicals that are unique to children or whether interactions observed in adults occur in children.

Child health data needs relating to exposure are discussed in 5.8.1 Data Needs: Exposures of Children.

2.11.3 On-going Studies

Dr. Corwin Hansch of Pomona College in California is developing quantitative structure-activity relationships for a variety of chlorinated organics, including the monochlorophenols. The principal end point of concern is teratogenicity in mice and rats. This work is currently being prepared for publication.

No other information regarding on-going research on the health effects of chlorophenols in humans or animals were located.