2. HEALTH EFFECTS

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 of the toxicology of chlorinated dibenzo-*p*-dioxins (CDDs).

CDDs are a class of related chlorinated hydrocarbons that are structurally similar. The basic structure is a dibenzo-*p*-dioxin (DD) molecule comprised of two benzene rings joined via two oxygen bridges at adjacent carbons on each of the benzene rings. There are eight homologues of CDDs, monochlorinated through octachlorinated. Each homologous class contains one or more isomers or congeners. The family of CDDs contains 75 congeners—2 monochlorodibenzo-*p*-dioxins (MCDD), 10 dichlorodibenzo-*p*-dioxins (DCDD), 14 trichlorodibenzo-*p*-dioxins (TrCDD), 22 tetrachlorodibenzo-*p*-dioxins (TCDD), 14 pentachlorodibenzo-*p*-dioxins (PeCDD), 10 hexachlorodibenzo-*p*-dioxins (HxCDD), 2 heptachlorodibenzo-*p*-dioxins (HpCDD), and a single octachlorodibenzo-*p*-dioxin (OCDD). The seven 2,3,7,8-chlorine substituted congeners are the most toxic CDD congeners, with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) being one of the most toxic and most extensively studied. This compound is often called "TCDD" or merely "dioxin" in the popular literature. **Chlorinated dibenzofurans (CDFs) are structurally and toxicologically related chemicals as are certain “dioxin-like” PCBs; the reader is encouraged to consult the toxicological profile for CDFs (ATSDR 1994) and the toxicological profile for PCBs (ATSDR 1996) for information on the health effects associated with exposure to these groups of chemicals.**

2.1 HUMAN STUDIES

This section presents information on human health effects, including those known to be associated and those possibly associated with exposure to CDDs (primarily 2,3,7,8-TCDD). Since limited data exist to assign a specific route of exposure (inhalation, oral, dermal) to human studies, the information in this section is organized by health effects—death, systemic, immunological, neurological, developmental, reproductive, 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).

The human studies discussed in this section are of populations known to reside or work in environments with above-background levels of CDDs and related compounds. Data on health effects in humans following exposure to CDDs have come from studies on accidental, occupational, and residential exposure and from studies on the use of 2,3,7,8-TCDD-contaminated pesticides. Because a number of these studies examined several end points, brief descriptions of these CDD-exposed populations are included in this section. Several factors complicate the interpretation of data regarding health effects in humans following exposure to CDDs; these include incomplete exposure data, concomitant exposure to other compounds, and a small number of participants, which limits the statistical power of the study to detect adverse health effects. Many of the studies on health outcomes following exposure to 2,3,7,8-TCDD and related compounds did not monitor exposure levels or internal dose. Surrogates of exposure were often used to identify potentially exposed populations and the level of exposure; some of the more commonly used surrogates include chloracne (a dermal condition generally indicative of appreciable exposure), potential exposure to phenoxy herbicides known to be contaminated with 2,3,7,8-TCDD, living in the vicinity of an accidental release of substances containing CDDs and related compounds, or an area with CDD-contaminated soil. Some of the more recent studies have used blood lipid CDD levels as a measure of internal dose in order to quantify exposure in individuals. In many of these studies, serum 2,3,7,8-TCDD levels were measured a number of years after exposure termination. CDDs are highly persistent lipophilic compounds which are resistant to biodegradation and have a great potential to bioaccumulate. Thus, a single chemical analysis of blood or adipose tissue represents a measure of past cumulative exposure to CDDs. With the assumptions of first-order kinetics for the elimination of 2,3,7,8-TCDD and an elimination half-life of 7–12 years, it is possible to extrapolate or adjust the serum or adipose tissue lipid concentration of 2,3,7,8-TCDD back to the time of the original excess exposure which may have occurred many years earlier, if the time of original exposure is known. Body burden or total dioxin amount can then be calculated from the serum 2,3,7,8-TCDD levels using the assumption that the concentration of 2,3,7,8-TCDD in serum lipid is in equilibrium with total body lipid 2,3,7,8-TCDD concentrations and that in an average adult 22% of the body weight is lipid. Body burdens were calculated (see Table 2-1) for the human studies reporting serum (or tissue) lipid 2,3,7,8-TCDD concentrations. If only current serum 2,3,7,8-TCDD levels were reported, then half-life-adjusted serum levels and body burdens were calculated using a half-life of 8.5 years (Michalek et al. 1996) and 22% body fat for a 70 kg adult (DeVito et al. 1995). A number of studies have calculated half-life-adjusted serum 2,3,7,8-TCDD levels; in these cases, the reported half-life adjusted serum 2,3,7,8-TCDD levels were used to estimate body burdens. Egeland et al. (1994), Jansing and Korff (1994), and Wolfe et al. (1995) calculated half-life adjusted serum 2,3,7,8-TCDD levels using a half-life of approximately 7 years. The 7.1-year half-life was derived from a study of 36 veterans involved in Operation Ranch Hand (Pirkle

Table 2-1. Health Effects in Humans Associated with Estimated 2,3,7,8-TCDD Body Burdens

Exposure duration	Effect	Current serum levels[a] (pg/g lipid)		Estimated levels at the time of exposure termination[b]		Elimination half-life (years)	Reference
		Mean	Range	Serum level (pg/g lipid)	Body burden[c] (ng/kg)		
<1	Chloracne in children	19144	828-56,000	NA	2876[d]	NA	Mocarelli et al. 1991
<1	No increased risk of spontaneous abortion	NR	>10	>110[e]	>24	7.1	Wolfe et al. 1995
≥15	No increased risk of clinical gastrointestinal disease	220	NR	1,900[f]	493	NR	Calvert et al. 1992
≥15	No increased risk of clinical hepatic disease	220	NR	1,900[f]	493	NR	Calvert et al. 1992
NR	Chloracne in 5/7 subjects	185	36-291	5,920[g] 1,047[h]	1480 262	5 10	Schecter et al. 1993
11	Chloracne	604	163-1,935	2,935[i]	646	7	Jansing and Korff 1994
6.5	Immunosuppression	330	43-874	942-1,108[j]	207-244	8.5	Tonn et al. 1996
≥15	No increased risk for peripheral neuropathy	252	2-3,390	2,240[f]	493	NR	Sweeney et al. 1993
<1	Change in sex ratio of children	540-mother 791-father	126-1,650 104-2,340	NA	119[k] 174	NA	Mocarelli et al. 1996
≥15	Increased prevalence of high luteinizing hormone and low testosterone levels	NR	20-3,400	140-30,000[l]	31-6,600	7.1	Egeland et al. 1994
≥1	Increased cancer mortality rate	418	NR	1408-8444[m]	310-1,858	8.5	Fingerhut et al. 1991

Table 2-1. Health Effects Associated with Exposure to 2,3,7,8-TCDD and Body Burdens in Humans (continued)

Exposure duration	Effect	Current serum levels[a] (pg/g lipid)		Estimated levels at the time of exposure termination[b]		Elimination half-life (years)	Reference
		Mean	Range	Serum level (pg/g lipid)	Body burden[c] (ng/kg)		
NR	Increased cancer mortality rate	NR	NR	NR	≥1,000[n]	5.1 or 8.9	Ott and Zober 1996
≥20 years	Increased cancer mortality rate	296	NR	321-4,296[o]	71-945	8.5	Manz et al. 1991

[a] Lipid-adjusted serum levels at the time of examination
[b] Calculated using elimination half-life and the assumption of first order kinetics for the elimination of 2,3,7,8-TCDD from the body.
[c] Unless noted, body burdens were calculated assuming the average worker weighed 70 kg with 22% body fat (DeVito et al. 1995)
[d] Calculated using serum 2,3,7,8-TCDD levels measured shortly after exposure. Body burdens were calculated using body weights of 13 kg for 1-3 year olds, 20 kg for 4-6 year olds, 28 kg for 7-10 year olds, 45 kg for 11 year old males, and 55 kg for 16 year old females (NAS 1989) and body fat percentages of 15% for 0-10 year olds, 15% for 11 year old males, and 20% for 16 year old females (ICRP 1981).
[e] Calculated by the study authors using a half-life of 7.1 years.
[f] Calculated by the study authors using an unspecified half-life.
[g] Calculated by the study authors using a half-life of 5 years; body burdens estimated by using the study authors assumption of reference body weights of 75 kg for males and 65 kg for females and 25% body fat.
[h] Same as footnote g but using a half-life of 10 years.
[i] Calculated by the study authors using a half-life of 7 years.
[j] Calculated using the mean current serum level, a half life of 8.5 years (Michalek et al. 1996), background 2,3,7,8-TCDD concentration of 5 ng/kg lipid, and 13-15 years elapsed time.
[k] Serum 2,3,7,8-TCDD levels were measured shortly after exposure.
[l] Calculated by the study authors using a half-life of 7.1 years and background dioxin levels of 6.08 pg/g lipid.
[m] Calculated using the mean current serum level, a half life of 8.5 years (Michalek et al. 1996), background 2,3,7,8-TCDD concentration of 5 ng/kg lipid, and 15-37 years elapsed time.
[n] Calculated by the study authors using half-lives of 5.1 or 8.9 for people with 20 or 30% body fat, respectively, and the individual's body weight at the time of exposure.
[o] Calculated using the reported mean current adipose tissue 2,3,7,8-TCDD level of 296 ng/kg, half-life of 8.5 years (Michalek et al. 1996), background 2,3,7,8-TCDD concentration of 5 ng/kg lipid, and 1-33 years of elapsed time.

NR Not reported; NA Not applicable, see appropriate footnote for further details.

et al. 1989). In a later study of Ranch Hand personnel, Michalek et al. (1996) calculated a mean serum 2,3,7,8-TCDD half-life of 8.5 years using blood samples collected in 1982, 1987, and 1992 from more than 300 veterans. In the studies of populations exposed several decades prior to measuring 2,3,7,8-TCDD levels in the blood, a difference of 1.4 years in the half-life can yield a large difference in estimated body burdens. For example, in the Manz et al. (1991) study, in which the workers were exposed for 33 years prior to 2,3,7,8-TCDD analysis, a body burden of 945 ng/kg at the time of exposure was calculated using a half-life of 8.5 years; using the 7.1-year half-life, the body burden would be 1606 ng/kg. See Section 2.3.4 for a more complete discussion on estimating human body burdens and the overview to Section 2.5 for information on background exposure.

Occupational exposure to CDDs most likely occurs mainly through inhalation of CDD-contaminated particles or dust and through dermal contact with solutions containing CDDs. However, data indicate that oral exposure to low levels of CDDs from contaminated food (including milk) represents the major route of environmental exposure for the general population and for people living in areas with known dioxin contamination (Connett and Webster 1987; Schecter et al. 1994a; Travis and Hattemer-Frey 1987).

Occupational Exposure. Exposures to 2,3,7,8-TCDD, one of the most potent of the CDD congeners, have occurred occupationally in workers involved in the manufacture and application of trichlorophenols and the chlorophenoxy acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Holmstedt (1980) has reviewed the history of industrial exposures that have occurred between 1949 and 1976, and Kogevinas et al. (1997) summarized recent data on these cohorts. The first reported cases of industrial poisoning were in 1949 at a 2,4,5-T producing factory in Nitro, West Virginia. 2,3,7,8-TCDD formation resulted from uncontrolled conditions in the reactor producing 2,4,5-trichlorophenol (2,4,5-TCP) from tetrachlorobenzene in methanol and sodium hydroxide. Approximately 228 workers (including production workers, laboratory personnel, and medical personnel) were affected. Between 1949 and 1968, 3 other explosive releases were reported: 1 involved 254 workers at the BASF AG facility in Ludwigshafen, Germany, in 1953 (Goldman 1972; Thiess et al. 1982; Zober et al. 1990, 1993); a second similar accident in 1963 involving 106 workers at Philips-Duphar facility in Amsterdam, Netherlands was a problem since the seriousness of the 2,3,7,8-TCDD exposure was not anticipated and cleanup workers were exposed (Holmstedt 1980); and the third was an explosion in a 2,4,5-TCP manufacturing facility in Coalite, England, involving 90 workers (May 1973). Holmstedt (1980) cited papers describing occupational exposure in 24 additional factories producing TCPs or 2,4,5-T during the same period of time. Exposure data on most of these incidents were limited; various numbers of workers were affected, and many of the

published reports are anecdotal. Ott et al. (1994) measured serum 2,3,7,8-TCDD levels in 138 of the 254 exposed workers several decades after the explosion at the BASF facility. More than 35 years after the explosion, serum 2,3,7,8-TCDD levels of <1–553 pg/g lipid were found; these correspond to serum levels of 3.3–12,000 pg/g lipid (calculated using a 7-year half-life) at the time of the accident.

Some of the most comprehensive studies on occupational exposure were conducted by the National Institute for Occupational Safety and Health (NIOSH). They are cross-sectional studies of workers at U.S. chemical facilities involved in the manufacture of 2,3,7,8-TCDD-contaminated products between 1942 and 1984 (Calvert et al. 1991, 1992; Egeland et al. 1994; Fingerhut et al. 1991; Sweeney et al. 1993). Serum 2,3,7,8-TCDD levels were measured in the workers at two of the plants. The mean 2,3,7,8-TCDD serum lipid level in 281 production workers in the Newark, New Jersey, and Verona, Missouri, plants was 220 ppt (range, 2–3,390 ppt) 18–33 years after exposure termination; the referent group of 260 people who had no self-reported occupational exposure and were matched by neighborhood, age, race, and sex had a mean serum 2,3,7,8-TCDD level of 7 ppt (Calvert et al. 1992; Sweeney et al. 1993). Sweeney et al. (1990) estimated current mean lipid-adjusted 2,3,7,8-TCDD levels of 293.4 ppt (range, 2–3,390 ppt) in 103 production workers at the New Jersey facility and 177.3 ppt (range, 3–1,290 ppt) in 32 workers at the Missouri facility; the mean half-life extrapolated levels (using a half-life of 7 years) were 2,664.7 ppt (range, 2–30,900 ppt) and 872.3 ppt (range, 3–6,100 ppt) in the two facilities, respectively. It should be noted that serum 2,3,7,8-TCDD levels were only measured in workers at these two facilities, and it is not known if the levels in these workers are reflective of serum 2,3,7,8-TCDD levels in workers at the other ten facilities.

There are also a number of studies of chlorophenol and phenoxy herbicide applicators. Some of these studies used job histories, questionnaires, and interviews to determine which phenoxy herbicides the workers had used. Many of the studies did not measure exposure levels or internal doses; rather, 2,3,7,8-TCDD exposure was assumed if the worker was exposed to a phenoxy herbicide known to be contaminated with 2,3,7,8-TCDD, such as 2,4,5-T. However, the level of exposure to these 2,3,7,8-TCDD-contaminated products was generally not determined.

Residential/Environmental Exposures. Several incidents in which populations were exposed to potentially high levels of 2,3,7,8-TCDD include: an industrial accident that occurred during the production of 2,4,5-TCP at the ICMESA plant in Seveso, Italy and the spraying of roads and other places with a mixture of waste oil, including chemical waste generated during the manufacture of 2,4,5-TCP, in Missouri.

The most widely studied release of 2,3,7,8-TCDD primarily involving residential exposures occurred in Seveso, Italy in 1976. The ICMESA factory produced trichlorophenol by hydrolysis of 1,2,4,5-tetrachloro-benzene with alkali in ethylene glycol. The reactor overheated and the safety valve ruptured releasing a cloud containing primarily sodium trichlorophenate but also 2,3,7,8-TCDD. It was estimated that more than 1.3 kg of 2,3,7,8-TCDD was released into the atmosphere and that more than 17,000 people in a 2.8-km^2 area adjacent to the facility were exposed. To investigate this accident, the contaminated area was separated into regions A, B, and R based on soil levels of 2,3,7,8-TCDD. The population sizes were 736, 4,737, and 31,800 in areas A, B, and R, respectively. The respective mean (and maximum) surface soil levels of 2,3,7,8-TCDD were 230 (447) $\mu g/m^2$, 3 (43.8) $\mu g/m^2$, and 0.9 (9.7) $\mu g/m^2$ for areas A, B, and R, respectively. Dividing the populations into different zones based on soil levels has been criticized because it does not take into consideration actual-exposure levels and differences in within-zone 2,3,7,8-TCDD exposure (Mastroiacovo et al. 1988). Blood and tissue samples from exposed individuals have been saved and 2,3,7,8-TCDD levels in some of the original samples and in follow-up blood samples have been analyzed. Serum 2,3,7,8-TCDD levels ranged from 828 to 56,000 ppt (lipid adjusted) in 19 residents of zone A (Mocarelli et al. 1991).

Various populations in Missouri were exposed to 2,3,7,8-TCDD in 1971 and 1972 as a result of spraying approximately 29 kg of 2,3,7,8-TCDD-contaminated waste oil on horse arenas, parking lots, and residential roads for dust control (Andrews et al. 1989). The oils originated from an industrial waste residue contaminated with 2,3,7,8-TCDD at levels of 305 ppm (Needham et al. 1991). An exposed group of 51 adults have been the subject of several studies. Adipose tissue levels, as well as paired human serum levels, were measured for 36 of these persons. Sixteen of the individuals were residents of areas where roadways had been sprayed and had mean 2,3,7,8-TCDD adipose tissue levels of 21.1 ppt (range, 1.28–59.1 ppt) in 1985 (Andrews et al. 1989). Eight persons exposed to 2,3,7,8-TCDD at the horse arenas had a mean adipose 2,3,7,8-TCDD concentration of 90.8 ppt (5.0–577 ppt). In a comparison population of 57 people with no known 2,3,7,8-TCDD exposure, 2,3,7,8-TCDD levels in the adipose tissue ranged from 1.4 to 20.2 ppt, with a mean of 7.4 ppt. Although the population of study was not large, they were evaluated in depth for medical effects (Hoffman et al. 1986; Stehr et al. 1986; Webb et al. 1984).

Exposures in Vietnam. During the Vietnam war, a program of aerial spraying of herbicides, code name Ranch Hand, was conducted in 10–20% of the Republic of Vietnam. During the 9 years of the program (1962–70), 19 million gallons of herbicides were dispersed. Six herbicides were used, with Agent Orange being the primary herbicide used (11 million gallons dispersed) (Wolfe et al. 1985). Agent Orange

was a 1:1 mixture of 2,4-D and 2,4,5-T in diesel oil and contained <1–20 ppm 2,3,7,8-TCDD as a contaminant. A number of studies have examined the possible association between Agent Orange exposure and adverse health effects in Vietnam veterans and Vietnamese residents living in the area of spraying. The results of a study comparing blood 2,3,7,8-TCDD levels in Vietnam veterans and the general U.S. population found that on average there was no significant difference between blood 2,3,7,8-TCDD levels between Vietnam veterans and comparison populations (CDC 1987). Thus, "service in Vietnam" or self-reported exposure to Agent Orange is not a reliable index of 2,4,5-T or 2,3,7,8-TCDD exposure. Studies of Air Force personnel participating in Operation Ranch Hand have found increased serum 2,3,7,8-TCDD levels in some of the persons (CDC 1987; USAF 1991). The median level in serum lipids for 888 Ranch Hand personnel was 12.4 ppt (range, 0 to 617.7 ppt) in contrast to 4.2 ppt (0-54.8 ppt) in a comparison group of 856 matched Air Force personnel (Wolfe et al. 1995). The median and high serum 2,3,7,8-TCDD levels would extrapolate to original serum levels of 43 and 3135 ppt, respectively, based on 20 years of elapsed time, and a half-life of 8.5 years. Since the tour of duty in Vietnam for the majority of U.S. veterans was generally less than 1 year, the military exposure was considered to be of intermediate duration if not stated otherwise in the original study.

No studies were located, however, regarding health effects in humans exposed to CDDs by specific routes of exposure (e.g., inhalation, oral, dermal). In this profile, human health effects caused by combined exposure through various exposure routes are discussed separately from effects found in animals that were maintained under controlled experimental conditions (i.e., route, duration, and levels).

2.1.1 Death

None of the studies examining humans acutely exposed to high concentrations of 2,3,7,8-TCDD or other CDD congeners (as contrasted with long-term studies) reported acute instances of death. A number of epidemiology studies have investigated mortality in populations occupationally or environmentally exposed to 2,3,7,8-TCDD or chemicals contaminated with 2,3,7,8-TCDD or other CDD congeners. No significant increases in the number of deaths were observed in workers at phenoxy herbicide or chlorophenol manufacturing facilities (Cook et al. 1986, 1987b; Fingerhut et al. 1991; Ott et al. 1980, 1987; Zack and Suskind 1980) or in workers exposed to 2,3,7,8-TCDD as a result of the accident at the BASF AG facility in Germany (Ott and Zober 1996; Thiess et al. 1982; Zober et al. 1990). Additionally no increases in mortality were observed in the 10-year period after the Seveso accident (Bertazzi et al. 1989b) or in Vietnam veterans involved in Operation Ranch Hand (Wolfe et al. 1985). Although none of these studies found significant

increases in the overall mortality rate, several studies found statistically significant increases in cause-specific mortality. For example, Flesch-Janys et al. (1995) found a significant risk of cardiovascular disease and ischemic heart disease mortality in workers exposed to 2,3,7,8-TCDD and other congeners during the BASF AG accident, and Fingerhut et al. (1991) found a significantly increased risk of cancer mortality in phenoxy herbicide and chlorophenol production workers. More complete descriptions of significant findings in the mortality studies are presented in the appropriate effect portions of Section 2.1.

2.1.2 Systemic Effects

The effects of 2,3,7,8-TCDD exposure in humans exposed in occupational or environmental settings have been described in several studies. Few studies provided precise exposure levels. However, for some cohorts, blood lipid 2,3,7,8-TCDD levels in samples collected shortly after exposure and stored frozen for several years have been analyzed. In other studies, the original blood levels of 2,3,7,8-TCDD were estimated using 2,3,7,8-TCDD levels measured in recent blood samples, the amount of time between exposure and blood sample collection, and a mean serum half-life of 5–12 years. 2,3,7,8-TCDD body burdens calculated from available serum lipid 2,3,7,8-TCDD levels are presented in Table 2-1.

Respiratory Effects. Information regarding respiratory effects of CDDs in humans is limited. Effects of acute massive exposure in workers exposed to 2,3,7,8-TCDD in an industrial accident in Germany included bronchitis and laryngitis a few days after exposure, and hemorrhagic pleuritis 11 months after exposure (Goldman 1973). In an occupationally exposed group, decreased pulmonary function was found in smokers 10 years after the cessation of manufacture of herbicides contaminated with 2,3,7,8-TCDD as compared with nonexposed smokers (Suskind and Hertzberg 1984). In contrast with the results of Suskind and Hertzberg (1984), Calvert et al. (1991) found no significant differences in ventilatory function between a group of 281 workers employed 15 years earlier in the production of NaTCP, 2,4,5-T ester, or hexachlorophene and 260 referents. At the time of the examination, the lipid-adjusted mean serum 2,3,7,8-TCDD concentration was 220 ppt in the exposed workers compared to 7 ppt for the referents. In addition, there was no association between previous occupational exposure to 2,3,7,8-TCDD contamination and elevation in the incidence of chronic bronchitis or in the prevalence of chronic obstructive respiratory disease. Calvert et al. (1991) suggested that the disparity between their results and those of Suskind and Hertzberg (1984) may have been due to the fact that exposed workers in the Suskind and Hertzberg (1984) study were, on average, 10 years older than controls and to the potential exposure to 2,4,5-T acid dust in that study. The 2,4,5-T

acid was finished as a liquid as opposed to a powder in the plant studied by Suskind and Hertzberg (1984), thus limiting inhalation exposure.

No respiratory effects were associated with exposure to 2,3,7,8-TCDD-contaminated herbicides in a group of Vietnam Air Force veterans involved in Operation Ranch Hand examined more than 10 years after the war (Wolfe et al. 1985). In the 1987 follow-up (USAF 1991), no association was found between the initial or current serum level of 2,3,7,8-TCDD and incidences of asthma, bronchitis, pleurisy, pneumonia, or tuberculosis; abnormal spirometric measurements were often associated with CDD blood levels, but according to the authors (USAF 1991), the differences in the mean level between high- and low-exposure subjects were not clinically important. The authors suggested that these findings may have been related to the association between 2,3,7,8-TCDD and body fat because obesity is known to cause a reduction in vital capacity.

A recent follow-up of the cohort involved in the Seveso accident reported a significant increase in deaths (4 deaths) from chronic obstructive pulmonary disease in males from zone A (relative risk [RR]=3.7; 95% confidence internal [CI]=1.4–9.9) and in females from zone B (7 deaths; RR=2.4; 95% CI=1.1–5.1) (Pesatori et al. 1998). The excess found among zone A males was mainly detected in the first 5 years after the accident and mainly affected elderly men. As mentioned below under Cardiovascular Effects among this cohort, Pesatori et al. (1998) stated that stress related to the disaster experience could have precipitated early deaths among people with pre-existing chronic respiratory disease. The investigators also speculated that 2,3,7,8-TCDD, through immunotoxic action, may have impaired protection and defense against episodes of respiratory infection, which play a major role in the natural history of chronic obstructive respiratory disease.

The existing information suggests that acute exposure to high levels of CDDs may cause respiratory effects mainly as a response to upper respiratory tract irritation, but evidence from the numerous cohorts exposed to 2,3,7,8-TCDD that have been studied suggests that the respiratory system is not a target for 2,3,7,8-TCDD toxicity.

Cardiovascular Effects. Most earlier data indicated that exposure to CDDs does not induce cardiovascular effects (Bond et al. 1983; Moses et al. 1984; Suskind and Hertzberg 1984). In a cross-sectional health survey in 1979 of 226 workers who had potential exposure to 2,3,7,8-TCDD from 1948 to 1969 in 2,4,5-T production, 52% had chloracne (present for a mean of 26 years) which was used as a surrogate for heavy exposure. When the chloracne group was compared to workers without chloracne (low

exposure rather than unexposed workers), there was an increased reported incidence of angina and myocardial infarction; when these data were age-adjusted, the prevalence was not statistically increased (Moses et al. 1984). Examination of West Virginia TCP production workers revealed no increases in the prevalence of hypertension or coronary artery disease, abnormal ECG findings, atherosclerotic changes on chest X-ray, or blood pressure elevation (Suskind and Hertzberg 1984).

Cardiovascular examination did not reveal any changes in 17 individuals who were treated for dermal lesions following acute exposure to 2,3,7,8-TCDD in the Seveso industrial accident (Reggiani 1980) or in a group of Missouri residents living in 2,3,7,8-TCDD-contaminated areas for a chronic period of time (mean 2.8 years in one area, 4.9 years in others) (Hoffman et al. 1986). In the 10-year period following the Seveso accident, there was a significant increase in the relative risk (RR) of death from chronic ischemic heart disease in men (RR=1.56; 95% CI=1.2–2.1), which was predominantly due to the increased risk during the first 5-year period (RR=1.76; 95% CI=1.2–2.5) (Bertazzi et al. 1989a). When the residents were divided into contamination zones, the relative risks of death from chronic heart disease in zones A, B, and R were 5.16 (95% CI=1.3–20.9), 1.57 (95% CI=0.6–4.2), and 1.72 (95% CI=1.2–2.5), respectively, for the first 5-year period and 3.28 (95% CI=0.8–13.2), 0.96 (95% CI=0.4–2.6), and 1.61 (95% CI=1.2–2.2), respectively, for the 10-year period (Bertazzi et al. 1989b). In females, there was an increased risk of death from chronic rheumatic heart disease (RR=1.54; 95% CI=0.7–3.2) during the 10-year period (Bertazzi et al. 1989a), which was predominately due to the high relative risk in women living in zone A (RR=27.58; 95% CI=8.5–89.9) (Bertazzi et al. 1989b). Bertazzi et al. (1989b) noted that increased risk of cardiovascular disease deaths may have been due to post-accident stress rather than to 2,3,7,8-TCDD exposure. The results of a 5-year follow-up were recently published (Pesatori et al. 1998). The recent analysis reported five deaths in males from chronic ischemic heart disease (RR=3.0; 95% CI=1.2–7.3); three deaths in females from chronic rheumatic heart disease (RR=15.8; 95% CI=4.9–50.4); and three deaths, also in females, from hypertensive vascular disease (RR=3.6; 95% CI=1.2–11.4), all from zone A, the most severely affected area. Although these observations suggest an association between exposure to 2,3,7,8-TCDD and incidence of cardiovascular effects, they do not necessarily show that the effects were caused by 2,3,7,8-TCDD. As previously suggested by Bertazzi et al. (1989a), Pesatori et al. (1998) also indicates that the disaster experience with its burden of psychosocial stressors may have played a major role in the increased deaths found.

No cardiovascular effects were observed in a group of Air Force veterans exposed to 2,3,7,8-TCDD-contaminated herbicides during the Vietnam war and examined several years post-exposure (Wolfe et al.

1985). However, a follow-up study of the Ranch Hand cohort reported increased mean diastolic blood pressure in those with current serum lipid 2,3,7,8-TCDD levels from 15 to 33.3 ppt, but not in subjects with higher 2,3,7,8-TCDD serum levels (USAF 1991). In addition, the proportion of abnormally low peripheral pulses in all Ranch Hand veterans, regardless of serum levels, was elevated relative to a comparison group. Also, arrhythmias detected on the electrocardiogram were significantly associated with 2,3,7,8-TCDD exposure, but there was no consistent dose-response relationship.

Flesch-Janys et al. (1995) found significant increases in mortality from heart and circulatory diseases in workers exposed to 2,3,7,8-TCDD and other CDD congeners during the accident at BASF AG. Relative risks for cardiovascular disease and ischemic heart disease mortality were 1.96 (95% CI=1.15–3.34) and 2.48 (95% CI=1.32-4.66), respectively, for workers with extrapolated serum lipid 2,3,7,8-TCDD levels of $348 pg/g (ppt) (current 2,3,7,8-TCDD levels were used to estimate 2,3,7,8-TCDD levels at the end of exposure). Additionally, statistically significant dose-response trends for increasing cardiovascular and ischemic heart disease deaths were found. The risk for cardiovascular and ischemic heart disease deaths also increased as the serum lipid CDD and CDF levels increased. However, the results from the Flesch-Janys et al. (1995) study are difficult to interpret since the percentage of chemical workers who died from cardio-vascular disease was 38% compared to 49% for a referent group from a gas supply company with no known special exposure to CDDs/CDFs. An international study comprising 36 cohorts from 12 countries and a total of 21,863 workers exposed to phenoxyacid herbicides and chlorophenols followed from 1939 to 1992 detected an increased risk for death from cardiovascular disease, especially ischemic heart disease (RR=1.67; 95% CI=1.23–2.26) among the exposed workers (Vena et al. 1998). Risks did not differ across latency categories or by year of first exposure, but increased slightly by duration of exposure except for those with 20 or more years of exposure. Vena et al. (1998) indicate, however, that the study was hampered by the reliance on mortality and the crudeness and inaccuracies of death certificate diagnoses. Furthermore, they noted that possible confounding effects from important risk factors for ischemic heart disease such as cigarette smoking, high fat diet, blood pressure, obesity, physical inactivity, and serum lipids cannot be ruled out.

In contrast with the positive findings described in the studies summarized above, a recent study of 281 workers employed 15 years earlier in the manufacturing of 2,4,5-trichlorophenol at two U.S. chemical plants and 260 unexposed referents found no significant association between 2,3,7,8-TCDD exposure and adverse cardiovascular effects (Calvert et al. 1998). The mean serum 2,3,7,8-TCDD concentrations (on a lipid basis) were 220 ppt in the workers and 7 ppt in the referents. Among the workers, the mean 2,3,7,8-TCDD

concentration when occupational exposure ceased was estimated to have been 1,900 ppt using a 7-year estimated half-life for serum 2,3,7,8-TCDD. Cardiovascular outcomes examined included myocardial infarction, angina, cardiac arrhythmias, hypertension, and abnormal, peripheral arterial flow. Calvert et al. (1998) indicated that although the study had sufficient statistical power to detect an elevated risk for cardiac arrhythmias, hypertension, and abnormal peripheral arterial flow, it had low power (approximately 50%) to detect an elevated risk for myocardial infarction and angina and concluded that further examination of the association between exposure to 2,3,7,8-TCDD and cardiovascular diseases is necessary.

In summary, there is suggestive but inconclusive evidence of adverse cardiovascular effects in humans exposed to relatively high concentrations of CDDs. Increased deaths from chronic heart disease were observed among the Seveso cohort, but psychosocial factors could not be ruled out; no clear dose-response relationships were seen among the Ranch Hand cohort; increased deaths from heart and circulatory disease were reported among German workers exposed to CDDs; and no evidence of adverse cardiovascular effects was detected among U.S. workers exposed to CDDs.

Gastrointestinal Effects. Earlier studies of individuals with exposure to substances contaminated with 2,3,7,8-TCDD found significant elevations in self-reported ulcers (Bond et al. 1983; Suskind and Hertzberg 1984), but a study of Vietnam veterans (USAF 1991) failed to find such effects. A more recent study evaluated the gastrointestinal effects of exposure to substances contaminated with 2,3,7,8-TCDD in an occupational cohort (Calvert et al. 1992). More than 15 years earlier, the workers were employed in the manufacture of trichlorophenol and its derivatives at 2 chemical plants. A total of 281 workers participated in the medical study; the control group consisted of 260 unexposed subjects who lived in the same communities as the workers. The participants underwent a comprehensive physical examination of the abdomen and rectum. The mean serum 2,3,7,8-TCDD level (on a lipid basis) for the workers was 220 ppt and was found to be highly correlated with years of exposure to 2,3,7,8-TCDD-contaminated substances; controls had a mean serum 2,3,7,8-TCDD concentration of 7 ppt. At the time of examination, the workers were not found to be at increased risk for any gastrointestinal diseases. Moreover, neither gastrointestinal ulcer disease nor gastritis was found to be significantly associated with any measure of 2,3,7,8-TCDD exposure, as determined by logistic regression analysis. The only significant finding from the physical examination was a statistically significant association between decreased anal sphincter tone and 2,3,7,8-TCDD exposure. This, however, was attributed to examiner bias since all those that exhibited reduced sphincter tone were examined by the same physician (two physicians conducted the blind

examinations). The results of these studies suggest that there is no association between occupational exposure to 2,3,7,8-TCDD and gastrointestinal disease.

Hematological Effects. Human studies regarding exposure to 2,3,7,8-TCDD or 2,3,7,8-TCDD-contaminated chemicals did not find any overt hematological effects after intermediate- (Wolfe et al. 1985) and chronic-duration exposures (Stehr et al. 1986).

Contact with 2,3,7,8-TCDD-contaminated soil in Missouri by physical or recreational activities for 6 months at 100 ppb or for 2 years at 20–100 ppb resulted in a slight but statistically significant increase in total white blood cell (WBC) counts using a prevalence test (5.3% were increased above 10,000 WBC/mm^3 compared to 0.7% for controls, but the increase was slight) (Hoffman et al. 1986). A follow-up study of the same population found no differences in the number of red blood cells, white blood cells, or platelets between exposed and nonexposed individuals (Evans et al. 1988). In a similar cohort, Stehr et al. (1986) found no consistent differences in hematology parameters in a high-risk group (68 persons) compared to a low-risk group (36 persons) except a slightly elevated platelet count. No significant differences in total leukocyte, granulocyte, or lymphocyte levels were observed between workers with high serum lipid CDD and CDF levels and workers with lower serum CDD and CDF levels (Neubert et al. 1993).

A health study of Vietnam veterans involved in Operation Ranch Hand indicated an association between high initial and current serum 2,3,7,8-TCDD levels and increased erythrocyte sedimentation (Wolfe et al. 1995), and an earlier study by Wolfe et al. (1985) indicated an increase in mean corpuscular volume; however, these changes were minor and were not observed in the 1991 follow-up (USAF 1991). Higher serum 2,3,7,8-TCDD levels were also associated with positive dose-response trends for increases in white blood cell and platelet levels.

The existing information suggests that CDDs, at the body burdens seen in the studied populations, do not cause adverse hematological effects.

Musculoskeletal Effects. The only information available comes from two anecdotal reports. In one of them, two individuals exposed to 2,3,7,8-TCDD in a horse arena that was sprayed with waste oil for dust control complained of painful joints (arthralgia) (Kimbrough et al. 1977). In the second case, a chemist exposed to 2,3,7,8-TCDD and 2,3,7,8-tetrabromo-p-dibenzo dioxin (2,3,7,8-TBDD) complained of muscle

pain in the lower extremities and back (Schecter and Ryan 1991). The role that 2,3,7,8-TCDD played in these cases, if any, is unknown. No further information was located.

Hepatic Effects. Two of three laboratory workers synthesizing or working with 2,3,7,8-TCDD in the laboratory developed chloracne 8 weeks after potential acute exposures (Oliver 1975). Blood cholesterol levels (the only biochemical change) were elevated in all three workers and remained elevated for two years. Biochemical examinations were conducted on 55 male workers in Prague who were admitted into a hospital in 1968 and 1969 suffering from chronic 2,3,7,8-TCDD intoxication from exposure in a plant producing 2,4,5-T (Pazderova-Vejlupkova et al. 1981). The first symptoms of intoxication included chloracne (present in the majority of the workers) and neurological symptoms; levels of exposure were never measured. Hypercholesterolemia was seen in 56% of the patients, hyperlipemia in 67%, hyperphospholipidemia in 42%, diabetes mellitus in 8%, a low glucose tolerance level in 19%, increased α and γ globulins in 42% and uroporphyria in 21% (the study did not include a referent group) (Jirasek et al. 1976; Pazderova-Vejlupkova et al. 1981). Liver biopsies revealed mild steatosis, periportal fibrosis, and activated Kupffer cells in those examined. At 10 years postexposure, most of the biochemical changes were not detected; only cholesterol levels remained high (Pazderova-Vejlupkova et al. 1981). Transient alterations of liver function tests were reported in workers exposed to 2,3,7,8-TCDD following an industrial accident in Great Britain (May 1973). Jennings et al. (1988) found nonsignificant increases in serum cholesterol and triglycerides but no effect on gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), or creatinine in a group of 18 workers in England who had been exposed 17 years previously, when comparisons were made with a group of 15 carefully matched controls.

Mocarelli et al. (1986) conducted a 6-year study on clinical laboratory parameters of children exposed to 2,3,7,8-TCDD following the Seveso accident. ALT, aspartate aminotransferase (AST), GGT, alkaline phosphatase, cholesterol, and triglycerides in plasma and delta amino levulinic acid in urine were monitored yearly in exposed and control groups beginning in June, 1977, approximately 1 year after the incident. The children were 6–10 years old at the time of the accident; 69, 528, and 874 resided in the A, B, and R zones, respectively. Chloracne was seen in 19, 0.7, and 4.6%, of the children in areas A, B, and R, respectively. Blood samples were drawn from 69, 83, and 221 children in areas A, B, and R, respectively. A slight increase in GGT and ALT occurred in the highest exposure group (based on zone of residences) compared to controls, but the values were not considered abnormal and returned to baseline levels within 3 years of the initial exposure. 2,3,7,8-TCDD blood levels have been more recently analyzed in about 30 of the subjects (Mocarelli et al. 1991). Similarly altered biochemical values (mainly increased serum transaminases and

GGT) were reported much earlier in individuals residing in an area of Seveso with average soil 2,3,7,8-TCDD concentrations of 580.4 μg/m^2 (Pocchiari et al. 1979). A study of clinical chemistry parameters and urinary porphyrins in Missouri residents living in a 2,3,7,8-TCDD-contaminated area did not indicate any definitive changes that were of clinical importance in 154 persons (high-risk, based on soil levels of dioxins) exposed for up to 11 years (Webb et al. 1989). Multivariate regression analysis with number of years of residence as a surrogate of dose gave a positive trend for GGT and alkaline phosphatase.

A medical survey of workers employed more than 15 years earlier in the manufacture of sodium trichlorophenol and its derivatives at two chemical plants found no evidence of an elevated risk for clinical hepatic disease at the time of examination (Calvert et al. 1992). The cohort consisted of 282 workers and 260 unexposed matched controls. Exposure was assessed by measuring lipid-adjusted serum 2,3,7,8-TCDD levels. The mean serum 2,3,7,8-TCDD level in the workers was 220 ppt, compared with 7 ppt in the control group. The results from abdominal and rectal examination were unremarkable. Similarly, the results from blood and urine tests measuring liver function showed no statistically significant differences between exposed workers and controls, with the exception of a statistically significantly higher mean GGT level in workers. Also, workers were found to have a statistically significant elevated risk for an out-of-range GGT level compared with referents. However, multivariate analysis with logistic regression showed a statistically significant interaction between 2,3,7,8-TCDD exposure and lifetime alcohol consumption, indicating that the elevated risk for an out-of-range GGT was confined to those workers with a history of alcohol consumption and that the risk among the alcohol-consuming workers for an out-of-range GGT increased with increasing 2,3,7,8-TCDD level.

In a follow-up study, Calvert et al. (1996) examined the association between exposure to 2,3,7,8-TCDD and serum lipids. In the follow-up the authors chose not to adjust the 2,3,7,8-TCDD serum concentrations for total lipids to avoid the problems of interpretation that would arise when adjusting a covariate by the dependent variable. Consequently, the results obtained in this study cannot be compared directly with those from the Operation Ranch Hand study (see below). The median serum 2,3,7,8-TCDD concentration among the workers was 406.6 femtograms/g serum (fg/g) compared with 36.9 fg/g among the referents. The results of logistic regression analyses revealed an association between serum 2,3,7,8-TCDD and risk for an abnormally decreased HDL cholesterol concentration that approached statistical significance ($p=0.09$) after controlling for body weight index, use of beta-blocker medication, age, diabetes, and employment at the two plants. The concentration of 2,3,7,8-TCDD was not associated with having either an abnormal total cholesterol concentration or an abnormal total cholesterol/HDL cholesterol ratio. When the workers were

stratified into quartiles according to their serum 2,3,7,8-TCDD concentration, and after controlling for important confounders, those with the highest 2,3,7,8-TCDD concentrations (1,516–19,717 fg/g) had an elevated risk for an abnormal HDL cholesterol concentration (odds ratio [OR]=2.2; 95% CI=1.1–4.7). A small but statistically significant association between triglyceride concentration and serum 2,3,7,8-TCDD was also found (p=0.05) after controlling for gender, plant location, body weight index, cumulative cigarette consumption, use of beta-blocker medication, race, and diabetes. However, in the logistic analyses, abnormal triglyceride concentration was not associated with serum 2,3,7,8-TCDD concentration (p=0.21). Analysis by quartiles showed that workers with the highest 2,3,7,8-TCDD serum concentration had a statistically significant elevation in mean triglyceride concentration compared with the referent group. However, no significant trend was observed in the quartile analyses that evaluated risk for an abnormal triglyceride level. Calvert et al. (1996) concluded that the associations of serum 2,3,7,8-TCDD concentration with triglycerides and HDL (high density lipoprotein) cholesterol were small when compared with the influence of many other factors.

A health study in Vietnam veterans involved in Operation Ranch Hand found no liver diseases linked to 2,3,7,8-TCDD exposure, but biochemical examinations revealed a pattern suggestive of a subclinical effect on lipid metabolism (USAF 1991). Blood triglycerides showed a strong positive association with both the initial levels of 2,3,7,8-TCDD and the current serum levels; the authors indicated that this variable is highly sensitive to body fat, which was increased in the more highly exposed individuals. Cholesterol, HDL, and the cholesterol-HDL ratio also showed significant associations with 2,3,7,8-TCDD.

In conclusion, hepatotoxic effects, such as elevated GGT levels and small alterations in lipid profile, have sometimes been observed in humans following exposure to high 2,3,7,8-TCDD levels. In general, the effects are mild and in some cases appear to have been transient.

Information regarding hepatic effects observed in infants exposed perinatally to CDDs and structurally related compounds is presented in Section 2.5 under Developmental Effects.

Renal Effects. A child who played in a sand box contaminated with waste oils containing 2,3,7,8-TCDD developed hemorrhagic cystitis and focal pyelonephritis (Kimbrough et al. 1977). Since chloracne was not seen and levels of 2,3,7,8-TCDD in the sand were not provided, the effects cannot be definitely attributed to 2,3,7,8-TCDD exposure. No renal effects were reported in other individuals exposed at the same location. An early study in Missouri residents chronically exposed to a 2,3,7,8-TCDD-contaminated environment

found increased incidence of self-reported urinary problems, leukocyturia, and microscopical hematuria (Webb et al. 1984). However, the results of urinalysis on this group did not indicate any kidney effects (Hoffman et al. 1986; Stehr et al. 1986). No renal effects were found in a group of Vietnam veterans exposed to 2,3,7,8-TCDD in Agent Orange based on case histories and evaluation of five laboratory variables comparing Ranch Hand veterans and the various comparison groups (USAF 1991; Wolfe et al. 1985, 1990). Kidney lesions have not been reported in any of the several studies on occupational exposure or for exposed cohorts in Seveso, Italy. These studies suggest that the kidney is not a target organ of 2,3,7,8-TCDD toxicity in humans.

Endocrine Effects. Jennings et al. (1988) examined thyroid function in a group of 18 workers exposed to 2,3,7,8-TCDD as a result of an industrial accident during the manufacture of 2,4,5-T. At the time of the study, 17 years after the accident, all the workers appeared healthy. No measure of exposure was provided. An unexposed group of 15 subjects served as controls. The end points monitored were serum thyroxine (T4), triiodothyronine (T3), and thyroid stimulating hormone (TSH). Without providing further details, the authors indicated that none of the subjects studied had biochemical evidence of thyroid dysfunction. A 35-year follow-up study of workers exposed to 2,3,7,8-TCDD during the BASF accident found a significant increase in the incidence of thyroid disease, as compared to an age-matched referent group (Zober et al. 1994). The workers were divided into two groups based on back-calculated (using a 7-year half-life) serum lipid 2,3,7,8-TCDD levels of $1,000 ppt and <1,000 ppt. For both groups of 2,3,7,8-TCDD-exposed workers, the incidence of thyroid disease was significantly higher than for the referent group, but did not differ between the two groups of workers.

Endocrine function was assessed in Vietnam veterans involved in Operation Ranch Hand. A strong positive association was found between glucose intolerance or increased risk of diabetes and 2,3,7,8-TCDD serum levels (USAF 1991). The diabetes finding remained significant even after adjusting for body fat. Furthermore, subclinical effects in thyroid function (significant decrease in mean T3 uptake and increases in mean TSH) were reported for Operation Ranch Hand veterans with high current 2,3,7,8-TCDD serum levels of $33.3 ppt (USAF 1991). However, the magnitude of the differences was not considered physiologically significant. A follow-up study of Operation Ranch Hand veterans provides further information on the association between serum 2,3,7,8-TCDD levels and the incidence of diabetes mellitus and glucose and insulin levels (Henriksen et al. 1997). The cohort consisted of 989 exposed subjects and 1,276 comparison individuals who served in Southeast Asia (54) during the same period but who were not involved with spraying herbicides. Initial dioxin levels were computed using a first-order pharmacokinetic model with a

constant half-life of 8.7 years. Four exposure categories were defined: 1) comparisons, with current dioxin levels of #10 ppt; 2) background Operation Ranch Hand veterans, with current dioxin levels of #10 ppt; 3) low category, with initial dioxin levels exceeding 10 ppt but #94.2 ppt; and 4) high category, with initial dioxin levels >92.4 ppt. Adjustments were made for age, race, and military occupation. The current median dioxin levels in the low and high categories were 15.0 and 46.2 ppt, respectively. The results of the analysis showed an increase in glucose abnormalities (RR=1.4; 95% CI 1.1, 1.8), diabetes prevalence (RR=1.5; CI 1.2, 2.0), and use of oral medications to control diabetes (RR=2.3; CI 1.3, 3.9) and a decrease in the time-to-diabetes onset with dioxin exposure. Serum insulin abnormalities increased in nondiabetics. Henriksen et al. (1997) pointed out that although some unknown confounder may not have been adjusted for, the strengths of the study included high participation and low attrition rates and accurate serum dioxin measurement and that, taken together, the results indicated a possible relation between dioxin exposure and diabetes mellitus, glucose metabolism, and insulin production. A follow-up evaluation of a cohort from the Seveso accident population found a significant increase in deaths from diabetes among women from zone B (RR=1.9; 95% CI=1.1–3.2) (Pesatori et al. 1998). Thirteen deaths were reported and 9 out of the 13 occurred in the second decade after the accident (RR=3.1; 95% CI=1.6–6.1). Pesatori et al. (1998) indicated that the fact that only women were affected might be explained by the systematically higher 2,3,7,8-TCDD concentrations in females than in males.

In summary, the evidence available from epidemiological studies suggests that exposure to high concentrations of CDDs may induce long-term alterations in glucose metabolism and subtle alterations in thyroid function.

Information regarding endocrine effects observed in infants exposed perinatally to CDDs and structurally related compounds is presented in Section 2.5 under Developmental Effects.

Dermal Effects. The most commonly observed effect of 2,3,7,8-TCDD exposure in humans is chloracne (Jirasek et al. 1976; Kimbrough et al. 1977; May 1973; Oliver 1975; Reggiani 1980). Chloracne is characterized by follicular hyperkeratosis (comedones) occurring with or without cysts and pustules (Crow 1978). Unlike adolescent acne, chloracne may involve almost every follicle in an involved area and may be more disfiguring than adolescent acne (Worobec and DiBeneditto 1984). Chloracne usually occurs on the face and neck, but may extend to the upper arms, back, chest, abdomen, outer thighs, and genitalia. In mild cases, the lesions may clear several months after exposure ceases, but in severe cases they may still be present 30 years after initial onset (Crow 1978; Moses and Prioleau 1985). In some cases lesions may

resolve temporarily and reappear later. Scarring may result from the healing process. Other chlorinated organic chemicals can also cause chloracne.

Acute exposure to 2,3,7,8-TCDD in a chemical laboratory induced the development of chloracne in two of three individuals within 8 weeks of the exposure (Oliver 1975). Chloracne occurred in workers occupationally exposed to 2,3,7,8-TCDD during the manufacture of herbicides (Bond et al. 1989b; Moses and Prioleau 1985; Poland et al. 1971) and after industrial accidents in several locations throughout the world (Goldman 1973; May 1973; Moses et al. 1984; Pocchiari et al. 1979; Suskind and Hertzberg 1984).

Accidental exposure to 2,3,7,8-TCDD in a 1949 explosion in a trichlorophenol plant in Nitro, West Virginia, resulted in an outbreak of severe chloracne. Moses et al. (1984) conducted a cross-sectional survey of workers in this plant in 1979. In reviewing the impact of the accident, the authors indicated that 117 workers had severe chloracne as a result of the explosion; however, 111 additional workers were found to have had chloracne prior to the explosion. A cross-sectional study of 226 workers in 1979 indicated that 52% had chloracne which persisted for 26 years, and in 29 subjects it was still present after 30 years. Blood levels were not measured, but the air dust in the plant was suspected to have contained 2,4,5-T contaminated with 6 ppm 2,3,7,8-TCDD compared to 0.1 ppm in later years. Similarly, high incidences of chloracne were also found in other facilities (Jirasek et al. 1976; May 1973; Poland et al. 1971; Vos et al. 1978). Appearance of chloracne after accidental occupational exposure may be immediate or delayed; since workers may not always be removed from the work environment, the duration of exposure and total exposure is difficult to assess.

Skin lesions from environmental exposures to 2,3,7,8-TCDD have been most thoroughly studied in the population exposed in Seveso, Italy. Reggiani (1980) described dermal lesions for 17 persons (primarily children) hospitalized shortly after the accidental release in Seveso. Acute lesions probably due to alkali and burns were observed immediately and had a duration of up to 2 months; chloracne in children occurred within 2 weeks (earliest occurrence was 3 days) and usually persisted for 8–26 months. Irritative lesions (characterized by erythema and edema of exposed areas, vesiculobollus and necrotic lesions, and papulonodular lesions) were observed in 447 people in Seveso 20–40 days after the accident, and 34 of these individuals later developed chloracne (Caputo et al. 1988). In 1976 and 1978, there were 193 childhood cases of chloracne and 17 of the most severe were in zone A where soil levels were the highest. Bisanti et al. (1980) reported that in zone A, 46 early cases (3–6 months) and 15 late cases (7–10 months) of chloracne were seen, and in zone B, 9 delayed cases were observed. In all zones, 50 early- and 143 late-appearing

cases of chloracne were reported (Caputo et al. 1988). In the 193 people with chloracne, the comedones and cysts progressively decreased in the 2 years following the accident (Caputo et al. 1988). In the most severe cases, regression of the lesions began at the end of 1978. All affected children were clear of lesions by 1982. Histological examination of the lesions from the limbs of severe chloracne patients revealed orthokeratotic hyperkeratosis with loss of adhesiveness, particularly near the follicular ostia; dilated follicular ostia filled with cornified lamellae; acanthosis; horny metaplasia with possible acrosyringeal cyst formation in the dermal and intradermal eccrine duct; and foreign body granulomas around the detached wall of the excretory ducts of some eccrine sweat glands (Caputo et al. 1988). Thirty of the 30,000 samples of serum collected and frozen in 1976 (10 zone A residents with the most severe cases of chloracne types 3 and 4 [chloracne was rated as type 1 for the mildest form to type 4 for the most severe cases], 10 former zone A residents who did not develop chloracne, and 10 controls from non-contaminated zones) were analyzed by Mocarelli et al. (1991). 2,3,7,8-TCDD blood levels (lipid adjusted) of 12,100–56,000 ppt were observed in 6 children with type 4 chloracne and levels of 828, 1,690, 7,420 ppt were found in 3 children with type 3 chloracne. In adults, levels of 1,770–10,400 ppt were associated with no chloracne. No chloracne was observed in Missouri residents who had adipose 2,3,7,8-TCDD levels of 5.2–59.1 ppt 16 years after exposure (using a half-life of 8.5 years, peak tissue levels of 6–204 ppt can be estimated) or in Operation Ranch Hand veterans. While there is a higher incidence of this disorder in those with higher serum 2,3,7,8-TCDD levels, interindividual variability makes it difficult to specify a dose that will result in chloracne (Needham et al. 1991).

The results of a further examination of Operation Ranch Hand veterans was recently published (Burton et al. 1998). The cohort consisted of 930 exposed subjects and 1,200 comparison individuals who served in SEA during the same period but who were not involved with spraying herbicides. The authors examined the associations between serum dioxin levels and a) chloracne, b) occurrence of acne relative to the tour of duty in SEA, and c) anatomical location of acne after service in SEA. Initial dioxin levels were computed using a first-order pharmacokinetic model with a constant half-life of 8.7 years. Four exposure categories were defined: 1) comparisons, with current dioxin levels of #10 ppt; 2) background Operation Ranch Hand veterans, with current dioxin levels of #10 ppt; 3) low category, with current dioxin levels exceeding 10 ppt but #94.2 ppt; and 4) high category, with dioxin levels >92.4 ppt. Adjustments were made for age, race, and military occupation. The range of initial dioxin levels in the low and high categories was 27.7–94.1 ppt and 94.2–3,290 ppt, respectively. Because physicians did not find any cases of chloracne among Operation Ranch Hand veterans at any physical examination and no cases were found via medical record review, the analysis was restricted to cases of acne. The results showed that among Operation Ranch Hand veterans

who had acne only after their service in SEA, the prevalence of acne at any location was increased in the high-exposure category, but the adjusted odds ratio relating acne in the eye-ear-temple location and dioxin category was increased for all three Operation Ranch Hand exposure categories. The increase was greatest in the background exposure category (OR=1.3; 95% CI=0.8–2.2). According to Burton et al. (1998), the results suggest that the Operation Ranch Hand exposure to dioxin, which was much lower than the Seveso exposure, was insufficient for the production of chloracne or that the exposure may have caused chloracne that resolved and was currently undetectable.

The incidence of chloracne was examined in a group of 3 men and 4 women who were among 231 workers exposed to dioxins at a chemical factory in Ufa, Russia, approximately 25 years prior to blood collection in 1991 and 1992 (Schecter et al. 1993). Five of the seven (three males and two females) were diagnosed with chloracne after working in the manufacture of 2,4,5-T contaminated with 2,3,7,8-TCDD between 1965 and 1967. Blood analysis showed 2,3,7,8-TCDD levels (on a lipid basis) ranging from 36 to 291 ppt (mean 185 ppt) in 1991 and 1992 compared with a mean of 4.4 ppt from a sample of 68 subjects from the general Russian population. Polychlorinated dibenzofurans and "dioxin-like" polychlorinated biphenyls (PCBs) were also detected, but it was estimated that in the workers, 2,3,7,8-TCDD contributed over 60% of the total dioxin equivalents (2,3,7,8-TCDD plus "dioxin-like" CDDs and PCBs). One of the workers diagnosed with chloracne had the lowest 2,3,7,8-TCDD blood concentration of the group, whereas two workers with higher levels did not display chloracne. This suggested that the presence of chloracne indicates exposure to dioxin (or similar chlorinated chemical), but its absence does not preclude such exposure, as noted by others (Mocarelli et al. 1991). Schecter et al. (1993) estimated that in the workers, the dioxin toxic equivalents (TEQ) in 1967 ranged from 226 to 1,707 ppt, assuming a 10-year half-life, and from 1,173 to 9,366 ppt assuming a 5-year half-life (see Section 2.5 for a detailed explanation on TEQs and toxicity equivalent factors [TEFs]). They also estimated the total 2,3,7,8-TCDD body burden for the workers to have been between 22 and 172 µg using a 5-year half-life and 4–30 µg using a 10-year half-life (mean present body burden was 3.2 µg versus 0.072 µg for general population). According to Schecter et al. (1993), this is the first reported incidence of chloracne in females with elevated dioxin blood levels from occupational exposure.

A group of 8 individuals who had contracted chloracne between 1973 and 1976 while working in the manufacture of TCP or in the maintenance of a TCP plant were examined 15 years after the exposure (Jansing and Korff 1994). Slight residual chloracne was diagnosed in two subjects, but otherwise the workers were healthy. 2,3,7,8-TCDD levels in blood ranged from 163 to 1,935 ppt (lipid basis), and by assuming a half-life of 7 years, the authors estimated that the blood concentration during the exposure had

ranged from 545 to 9,894 ppt. It was found that the concentration of 2,3,7,8-TCDD in blood correlated well (r=0.93) with duration of chloracne if 2 subjects with a disposition to hypersensitive skin reactions were not included in the analysis.

Other effects manifested as dermal changes have also been noted to accompany chloracne. In addition to chloracne, hyperpigmentation and hirsutism (also known as hypertrichosis or abnormal distribution of hair) were also reported in 2,3,7,8-TCDD-exposed workers (Jirasek et al. 1976; Oliver 1975; Poland and Smith 1971; Suskind and Hetzberg 1984). In the cohort examined by Suskind and Hetzberg (1984), hypertrichosis was observed 25 years after exposure, particularly among workers with persistent chloracne upon clinical examination. In contrast, Moses et al. (1984) found no evidence of hypertrichosis, even though 31% of the exposed workers had evidence of residual chloracne. Webb et al. (1989) observed three cases of hypertrichosis, but not hyperpigmentation, among Missouri residents, one with serum levels of <20 pg/g and two with levels between 20 and 60 pg/g. However, neither condition was noted on examination among residents of the Quail Run Mobile Home Park (Hoffman et al. 1986). Actinic or solar elastosis was also observed among a group of workers diagnosed with active chloracne at the time of their examinations in 1979 (Suskind and Hertzberg 1984).

In conclusion, dermal effects, particularly chloracne, are the most commonly reported effects of 2,3,7,8-TCDD exposure in humans because they are easy to identify. Additional information is needed to determine the level and frequency of 2,3,7,8-TCDD exposure needed to cause chloracne and whether individual susceptibility plays a role in the etiology. Also, chloracne in humans indicates CDD exposure, but lack of chloracne does not indicate that exposure has not occurred. Other dermal conditions reported include hypertrichosis, hyperpigmentation, and solar elastosis.

Ocular Effects. Eye irritation, which correlated with severity of chloracne, was reported by Poland et al. (1971) among workers employed in a 2,4,5-T factory; however, the role of 2,3,7,8-TCDD, if any, cannot be determined.

Body Weight Effects. Limited information was located regarding body weight effects in humans following exposure to CDDs. A transient weight loss was reported in a laboratory worker following an acute exposure to 2,3,7,8-TCDD (Oliver 1975). Weight loss associated with severe cases of chloracne was mentioned in a study among herbicide-manufacturing workers (Jirasek et al. 1976), but further information regarding weight loss was not provided.

2.1.3 Immunological Effects

A limited number of studies have examined the immunotoxicity of 2,3,7,8-TCDD in humans. Most of these studies have found potential alterations in lymphocyte populations (e.g., T cells, B cells), cell surface markers (e.g., $CD4RO^+$, $CD8^+$), or lymphoproliferative responses. The interpretation of these studies is limited by the lack of data correlating changes in immune function measurements and changes in host resistance to disease challenges (Kerkvliet 1995). Based on medical insurance records, Zober et al. (1994) found a significant increase in the incidence of infectious and parasitic disease in 2,3,7,8-TCDD-exposed workers in the 35-year period after the BASF accident. When the workers were divided into groups based on the severity of chloracne or back-calculated serum lipid 2,3,7,8-TCDD levels (assuming a half-life of 7 years), the increase in infectious disease was significant only in the group with severe chloracne and the group with 2,3,7,8-TCDD levels of $1,000 ppt. Among the workers with severe chloracne, one disease subcategory, intestinal infections, accounted for the increased incidence of infectious diseases. A two-fold increase in the incidence of upper respiratory tract infections was also observed in the cohort. Dividing the workers into various groups did not result in evidence of increased respiratory infections in a particular group. Zober et al. (1994) also found a significantly higher incidence of appendicitis in 2,3,7,8-TCDD workers; it is not known if this effect was the result of immunotoxicity or a direct effect on the appendix. Although the results of this study suggest a relationship between 2,3,7,8-TCDD exposure and an increased risk of infection, the authors note that the difference may reflect differences in medical care use between the workers and the referent group. Jennings et al. (1988) examined immunological parameters in a group of 18 workers 17 years after an industrial accident during the manufacture of 2,4,5-T in Coalite, England. At the time of the study all members of the cohort were apparently healthy. An unexposed group of 15 subjects served as controls. No measure of exposure was provided. The exposed group had a significantly increased number of natural killer (NK) cells, and concentration of antinuclear antibodies and immune complexes. Total lymphocytes, B cells, T cells, T-helper cells, T-suppressor cells, the lymphoproliferative response to phytohemagglutinin, and serum levels of immunoglobulins were similar between exposed and control groups. An immunological assessment of 41 subjects exposed to 2,3,7,8-TCDD-contaminated soil in Times Beach, Missouri was conducted by Webb et al. (1989). Sixteen participants had 2,3,7,8-TCDD adipose tissue levels <20 ppt, 13 had levels between 20 and 60 ppt, and 12 had levels >60 ppt. The highest level was 750 ppt. Results from multiple regression analysis showed that increased 2,3,7,8-TCDD levels correlated with an increased percentage and total number of T lymphocytes. The increase was due to $CD8^+$ and $T11^+$ T cells; $CD4^+$ T cells were not altered in percentage or number. The lymphoproliferative responses to T cell mitogens or tetanus toxoid were not altered, and neither was the cytotoxic T cell response. Serum

immunoglobulin A (IgA) was increased, but IgG was not. The study subjects did not exhibit any clinical disease associated with the 2,3,7,8-TCDD levels. In contrast with Webb's findings, depressed cell-mediated immunity was found in residents from the Quail Run Mobile Home Park in Missouri (Hoffman et al. 1986); however, repeated examination of the group failed to confirm the observation (Evans et al. 1988). The levels of 2,3,7,8-TCDD in adipose tissue from this group were unknown.

No indications of immune disease were found in a group of 8 subjects who had worked in a TCP manufacturing plant 15 years earlier and had elevated blood levels of 2,3,7,8-TCDD (163–1,935 ppt) at the time of the examination (Jansing and Korff 1994). The only significant observation was that individuals with the greater exposure (judged by 2,3,7,8-TCDD blood levels and duration of chloracne) showed a tendency of lower gamma-globulin levels. Neubert et al. (1993) examined surface receptors on lymphocyte subpopulations of workers with moderately increased body burden of 2,3,7,8-TCDD and of other CDDs and CDFs. The group consisted of 89 volunteers involved in decontamination work at a chemical plant in Hamburg, Germany. The volunteers were grouped according to their body burden, as defined by the CDD concentration in blood (on a lipid basis). Four groups were formed: a low-, medium-, and higher-level reference group, and the exposed group. Their respective median 2,3,7,8-TCDD blood concentrations were 2, 5, 11, and 41.5 ppt. 2,3,7,8-TCDD was a minor contributor to the total dioxin equivalents. Regression analysis of the data showed some slight trends for some of the biomarkers, such as $CD45R0^{+}$. Except for one, all the trends were increases. The slight increase in the percentage of $CD4^{+}CD45R0^{+}$ cells remained significant even after accounting for age-related changes. The authors concluded that altogether, the data did not provide any evidence for a decrease in cellular components of the human immune system in subjects with moderately increased CDD/CDF body burden. They also pointed out that adult humans appear to be less susceptible to this action of CDDs than adolescent marmoset monkeys. In a follow-up study, Neubert et al. (1995) examined lymphocyte proliferation responses (measured as ^{3}H-thymidine incorporation) in the same volunteers. They found no decrease in the capacity of ^{3}H-thymidine incorporation with any of the proliferation stimulators in the group with the increased 2,3,7,8-TCDD body burden, compared with the other groups. A recent study examined the long-term effects of 2,3,7,8-TCDD on immune function in 11 industrial workers in Germany who had been exposed for several years to high doses of 2,3,7,8-TCDD 20 years earlier (Tonn et al. 1996). Current 2,3,7,8-TCDD blood concentrations (lipid basis) ranged from 43 to 874 ppt, compared with about 4 ppt as the average for the German population. Ten matched unexposed subjects served as controls. End points monitored included determination of lymphocyte subsets, and immunocompetence of T- and B-lymphocytes by mitogen-induced lymphoproliferation assays and by assays using sensitive mixed-lymphocyte cultures. At the time of the study, the workers were generally

healthy, although five persons still exhibited chloracne. Analysis of lymphocyte subsets showed no differences between the control and the 2,3,7,8-TCDD-exposed group. Moreover, there was no statistically significant difference in the response to mitogen stimulation between the two groups, and no correlation was found between individual 2,3,7,8-TCDD levels in the blood or the age of the person and the respective proliferative capacity of their lymphocytes. However, exposed subjects showed a reduced response to human lymphocyte antigen-allogeneic lymphocytes and interleukin-2-boosted proliferation. According to Tonn et al. (1996), this suppression is indicative of a reduced T-helper cell response, although the actual number of T-helper cells was not altered by 2,3,7,8-TCDD. The authors concluded that 2,3,7,8-TCDD immunosuppression is more likely mediated by a reduced functionality of individual cells rather than by a reduction in numbers of cells circulating in the blood. Tonn et al. (1996) further noted that the changes in immunocompetence observed did not correlate with obvious diseases related to severe immunodeficiency such as certain cancers and infections.

In a study of 192 persons exposed to 2,3,7,8-TCDD (and CDFs) in a pesticide-producing factory in Germany, there was also no correlation between the levels of 2,3,7,8-TCDD in blood from exposed workers and the frequency of infectious diseases (Jung et al. 1998). The investigators also conducted a number of assays such as immunoglobulins, serum electrophoresis, monoclonal bands, surface markers, autoantibodies, lymphocyte proliferation, the rise of tetanus antibody concentrations after vaccination, and the *in vitro* resistance of lymphocytes to chromate to evaluate the morphologic and functional state of the immune system. A subgroup of 29 most highly exposed workers was compared to a control group of 28 subjects not exposed to above background levels of 2,3,7,8-TCDD. The median concentration of 2,3,7,8-TCDD in the workers was 217 pg/g blood lipid (range, 33.6–2,252) compared to 3.9 pg/g in the controls (range, 2.9–6.0). There was no significant correlation between the current 2,3,7,8-TCDD concentrations and alterations in any of the immune parameters among the entire exposed group. In addition, the results of the tetanus vaccination and the chromate resistance test were not correlated with exposure to 2,3,7,8-TCDD. The only significant finding was that the chromate resistance of lymphocytes stimulated with phytohemagglutinin of highly exposed persons was significantly lower than that for the control group. This, according to Jung et al. (1998), suggested that the function of lymphocytes can be stressed and possibly impaired by high exposure to 2,3,7,8-TCDD. A separate report on the same group of workers found no significant exposure-related alterations in the phenotype and function of peripheral blood mononuclear cells (PBMC) as judged by the proportions of CD3, CD4, or $CD8^+$ T-lymphocytes; of $CD16^+$ natural killer cells; and of $CD19^+$ B-lymphocytes (Ernst et al. 1998). However, in the 2,3,7,8-TCDD exposed workers, the proportion of $CD8^+$ memory T-cells ($CD45RO^+$) was significantly higher, and that of lymphocytes with naive phenotype

($CD45RA^+$) was significantly lower than in PBMC of the control group. Also, *in vitro* tests of T-cell activation showed a significantly reduced interferon γ release in diluted whole blood cultures but not in isolated PBMC cultures of the TCDD-exposed cohort when T-cells were stimulated with tetanus toxoid. Based on these results, Ernst et al. (1998) suggested that exposure to high concentrations of 2,3,7,8-TCDD can partially impair in the blood milieu those T-cell/monocyte interactions that are essential for antigen-specific T-cell responses, whereas isolated PBMC in the same donor appeared functionally less affected.

The immune status of children exposed to 2,3,7,8-TCDD in the Seveso incident was also examined (Mocarelli et al. 1986). The group consisted of 44 children, 20 of whom had chloracne. The results of the testing showed no abnormalities in serum immunoglobulin concentrations, levels of circulating complement, or lymphoproliferative responses to T- and B-cell mitogens. However, a different cohort of 2,3,7,8-TCDD-exposed children examined 6 years after the explosion showed a significant increase in complement protein levels, which correlated with the incidence of chloracne (Tognoni and Bonaccorsi 1982). The children also had increased numbers of peripheral blood lymphocytes and increased lymphoproliferative responses. No specific health problems were correlated with exposure to 2,3,7,8-TCDD in these children. The findings from the Tognoni and Bonaccorsi (1982) study suggest that chloracne is a more sensitive toxicological endpoint than immunological effects because alteration in complement levels is a subclinical effect and correlated with the incidence of chloracne.

A health study of Vietnam veterans involved in operation Ranch Hand did not find any correlations between clinically significant immunological alterations and serum 2,3,7,8-TCDD levels (USAF 1991). The only significant positive association with exposure to 2,3,7,8-TCDD was an increase in serum IgA levels. The authors suggested that this alteration was indicative of a subtle inflammatory process, but there was no other evidence for an inflammatory response.

Parameters of immunocompetence were assessed in a group of 23 men with high fish consumption from the Baltic Sea (Svensson et al. 1994). Twenty men with almost no fish consumption served as controls. The parameters examined included WBC, lymphocyte levels, serum immunoglobulin levels, and lymphocyte subsets. The mean dioxin equivalent concentration (TEQ, includes CDDS, CDFs, and dioxin-like PCBs) in blood (lipid basis) from fish eaters (n=7) was 64 pg TEQ/g (range, 18–88 pg/g) compared to 21 pg TEQ/g (18–33 pg/g) for controls (n=4). CDDs and CDFs were the major contributors to the total dioxin equivalents in blood. Of all the parameters examined, only the level of NK cells was reduced in fish eaters, but the difference between groups was not statistically significant. No correlation was found between blood levels of

2,3,7,8-TCDD and the reduction in NK levels, but weak correlations existed between the latter and some non-ortho-PCB congeners and p,pN-DDT.

In conclusion, while some studies are suggestive, no consistent exposure-related immunological effects have been observed in human populations exposed to levels of CDDs several orders of magnitude higher than background exposure. This may in part be due to the lack of functional assays of immune competence in humans.

Information regarding immunological effects observed in infants exposed perinatally to CDDs and structurally related compounds is presented in Section 2.5 under Developmental Effects.

2,3,7,8-TCDD human body burdens calculated from available serum lipid 2,3,7,8-TCDD levels are presented in Table 2-1.

2.1.4 Neurological Effects

Symptoms of intoxication including lassitude, weakness of the lower limbs, muscular pains, sleepiness or sleeplessness, increased perspiration, loss of appetite, headaches, and mental and sexual disorders were reported in several of the 117 workers with severe chloracne who had been exposed to 2,3,7,8-TCDD in an occupational setting (Moses et al. 1984; Suskind 1985). Neurological symptoms persisted in these individuals for up to 10 years based on an increased incidence of sensory findings. Similar symptoms of intoxication were observed in a trichlorophenol factory in Czechoslovakia (Jirasek et al. 1976) for which a 10-year follow-up of 55 of the 80 affected workers was conducted (Pazderova-Vejlupkova et al. 1981). At autopsy, damage to peripheral neuron Schwann cells was confirmed in a worker who died (Jirasek et al. 1976). Polyneuropathy and encephalopathy were found in 23 and 7% of the surviving workers, respectively (study did not include a referent group). Most patients suffered from peripheral neuronal lesions of the lower extremities (confirmed by electromyography). Encephalopathy developed in older individuals (aged 50 and above) and was accompanied by an organic psychosyndrome due to atherosclerosis of cerebral arteries. The most-affected patient developed paroxysms of temporal epilepsies and external and internal hydrocephalus. Patients with polyneuropathy did not improve during 10 years postexposure (Pazderova-Vejlupkova et al. 1981). Similarly, neurological effects that included peripheral neuropathy, sensory impairment, tendency to orthostatic collapse, and reading difficulties were reported in workers exposed to 2,3,7,8-TCDD in an industrial accident in Germany (Goldman 1973).

Several studies have investigated the neurological effects of 2,3,7,8-TCDD in Seveso residents. Residents in zone A and zones B and R participated in a neurological screening test in 1977 (Pocchiari et al. 1979). Of the 446 residents in zone A, 6.7% and 3.1% had evidence of "idiopathic clinical neurologic damage" or "idiopathic subclinical neurologic damage" respectively, as compared to 1.2% and 1.2%, respectively, in the 255 residents in zones B or R. No definitions of clinical or subclinical damage were provided. The authors did note that the most frequently observed effects occurred in the peripheral nervous system (particularly reduced nerve conduction velocity, which was considered a subclinical effect). In 1978, 205 residents in zone A were re-tested. At this time, 11.7% had clinical damage and 4.9% had subclinical damage. No relationship between chloracne and neurological symptoms was found (Pocchiari et al. 1979). In another study of zone A residents, 22 cases of peripheral neuropathy were observed in the 470 residents examined in 1977 (prevalence rate of 8.9%, 95% confidence interval [CI] of 6.2 to 11.6) (Filippini et al. 1981). Peripheral neuropathy was diagnosed based on the occurrence of neurological symptoms (paresthesia, hypesthesia, pain, and hyposthenia), clinical signs (superficial and deep sensory impairment, muscular weakness, and tendon hypo- or areflexia), and/or electrophysiological alterations. During a re-evaluation in 1978, 26 of the 308 subjects had neurological symptoms, clinical signs, and/or altered electrophysiological readings and 16/308 had two or more electrophysiological abnormalities (at least one being altered nerve conduction velocity). The 42 subjects with evidence of peripheral neuropathy were divided into three groups: 1) residents with existing predisposing factors (e.g., excess alcohol consumption, diabetes, nutritional diseases), 2) residents with potential high exposure to 2,3,7,8-TCDD (assessed via increased serum levels of several hepatic enzymes [GGT, AST, ALT] and/or chloracne), and 3) remaining residents. The prevalence rate ratio was significantly higher in the first two groups (2.6, 95% CI=1.2–5.6 for predisposing factors group and 2.8, 95% CI=1.2–6.5 for high 2,3,7,8-TCDD exposure group) as compared to the third group. In a 6-year follow-up of 152 subjects with chloracne in Seveso, Barbieri et al. (1988) found no clear-cut peripheral neuropathy but an increase in clinical and electrophysiological signs of peripheral nervous system involvement when compared to 123 age- and sex-matched controls. In 1985, 141 of these subjects were re-examined (Assennato et al. 1989). No statistically significant alterations in motor nerve conduction velocity of the median or peroneal nerves or sensory nerve conduction velocity of the sural nerve, as compared to 167 matched controls, were observed.

No significant increases in neurological effects (based on self-reported neurological effects and neurological examination) were observed in 68 Missouri residents with potential high risk exposure to 2,3,7,8-TCDD as compared to 36 residents with low-risk exposure (Stehr et al. 1986). Abnormal neurological symptoms were observed in a group of 41 Missouri residents with measured 2,3,7,8-TCDD serum lipid levels (Webb et al.

1989). The symptoms included abnormal pain sensation in lower extremities, abnormal vibratory sensation, and abnormal reflexes. However, the distribution of these effects among residents with serum lipid 2,3,7,8-TCDD levels of <20 ppt, 2–60 ppt, or >60 ppt was not dose-related.

Nerve conduction velocities were measured in 55 employees of a 2,4,5-T and 2,4-D manufacturing facility in Jacksonville, Arkansas (Singer et al. 1982). Statistically significant decreases in median motor nerve and sural nerve conduction velocities were observed, as compared to a control group of workers not exposed to phenoxy herbicides. No effect on median sensory nerve conduction velocity was found. Sural nerve conduction velocity was significantly inversely correlated with duration of employment.

Psychological effects have been associated with 2,3,7,8-TCDD exposure in some human studies. Personality changes were reported following acute exposure (Oliver 1975). Depression (Levy 1988; Wolfe et al. 1985), hypochondria, hysteria, and schizophrenia (Wolfe et al. 1985) were found more often in Vietnam veterans exposed to 2,3,7,8-TCDD-contaminated herbicides than in the control group of veterans. A battery of tests used for the psychological evaluation included Minnesota Multiphasic Personality Inventory, Cornell, Wechsler Memory Scale I, Wechsler Adult Intelligence Scale, Wide Range Achievement Test, and Halstead-Reitan Neuropsychological Battery. Peper et al. (1993) reported that the results of neuropsychological testing of 19 persons living in an area with high concentration of dioxins in soil in Germany were within the range of values expected from standardized age samples. However, increased levels of dioxins in blood (but not substantially different from a national sample) were associated with a reduction of cognitive performance in verbal conceptualization, mnemonic organization of verbal and visual stimuli, psychomotor slowing, and a variety of subjective complaints. The concentration of CDDs (including CDFs) in blood (lipid basis) ranged from 16.1 to 80.4 ppt (TEQ), with a mean of 31 ppt. The authors recognized, however, that given the small number of subjects and the relatively low amount of exposure (monitored by blood levels) the results must be interpreted with caution.

A health study in Vietnam veterans involved in Operation Ranch Hand reported that elevated serum 2,3,7,8-TCDD levels were not associated with any neurological disease and were not related to verified psychological or sleep disorders (USAF 1991). In a more recent study, Sweeney et al. (1993) compared the prevalence of chronic peripheral neuropathy in a group of workers employed 15 years earlier in the manufacture of sodium trichlorophenol and its derivatives at two chemical plants. The cohort consisted of 265 exposed workers and an unexposed matched comparison group of 244 subjects. Exposure was assessed by measuring lipid-adjusted serum 2,3,7,8-TCDD levels. The neurologic status was evaluated through a

standardized neurological examination and electrophysiologic measurements and quantitative sensory tests of thermal and vibratory sensitivity. The results showed that the workers had a significantly higher mean serum 2,3,7,8-TCDD level (220 ppt) compared to controls (7 ppt). In both worker and referent groups, 32% met the case definition for peripheral neuropathy; however, logistic regression analyses revealed that serum 2,3,7,8-TCDD level was not related to peripheral neuropathy. The results suggested that despite continued elevated serum 2,3,7,8-TCDD levels, peripheral neuropathy is not a long-term sequela of exposure to TCDD-contaminated chemicals. Nevertheless, the authors (Sweeney et al. 1993) indicated that the study could not preclude the occurrence and subsequent resolution of acute effects caused by high exposure, as observed in Seveso (Fillippini et al. 1981; Pocchiari et al. 1979) and possibly in early case reports (Goldman 1973; Jirasek et al. 1976; Oliver 1975).

The overall evidence from case reports and epidemiological studies showed that exposure to CDDs is associated with signs and symptoms of both central and peripheral nervous system shortly after exposure. In some cases, the effects lasted several years. However, evaluation of individuals 5 to 37 years after the last exposure has not revealed any long-lasting abnormalities.

Information regarding neurological effects observed in infants exposed perinatally to CDDs and structurally related compounds is presented in Section 2.5 under Developmental Effects.

2.1.5 Reproductive Effects

A number of studies have investigated the possible association between 2,3,7,8-TCDD exposure and reproductive toxicity in humans. A common limitation of many of these studies, particularly those conducted prior to the development of assays to quantify serum and adipose levels of 2,3,7,8-TCDD, is the lack of adequate exposure data (Sweeney 1994).

The effects of 2,3,7,8-TCDD exposure on gonadal function (production of germ cells and secretion of sex hormones) has not been extensively investigated. A health study in Vietnam veterans involved in Operation Ranch Hand found a significant association between decreased testicular size and serum 2,3,7,8-TCDD levels, but no association was found for low serum testosterone levels (USAF 1991). When the Operation Ranch Hand cohort was re-examined in 1992 using ultrasound methodology, no significant alterations in testes size were found (Henriksen et al. 1996). No alterations in sperm count or the percentage of abnormal sperm were observed in Vietnam veterans involved in Operation Ranch Hand (Wolfe et al. 1985). No

consistent alterations in testosterone levels, follicle-stimulating hormone (FSH) levels, luteinizing hormone (LH) levels, testicular abnormalities, sperm abnormalities, and sperm counts were found in a follow-up study of the Operation Ranch Hand cohort; reproductive parameters were assessed in 1982, 1987, and 1992 (Henriksen et al. 1996). In workers at two 2,4,5-trichlorophenol manufacturing facilities, serum 2,3,7,8-TCDD levels were positively correlated with follicle stimulating hormone and luteinizing hormone levels and inversely correlated with total testosterone levels (Egeland et al. 1994). However, the magnitude of the change in hormone levels per unit of increase in serum 2,3,7,8-TCDD levels was small. The prevalence of high luteinizing hormone levels, high follicle stimulating hormone levels, and low testosterone levels was significantly increased in workers with half-life extrapolated serum lipid 2,3,7,8-TCDD levels of $140 pg/g, $1,860 pg/g, and $140 pg/g, respectively (based on 2,3,7,8-TCDD levels extrapolated at the time occupational exposure ceased and assuming a 7.1-year half-life). The study authors note that both low testosterone and high LH levels were not observed in the same individuals. Although the number of workers with elevated or depressed hormone levels was significantly higher than in the referent group, the adjusted mean hormone levels (adjusted for age, body mass index, alcohol consumption, smoking, and diabetes mellitus) were within 20% of referent values.

A number of studies have examined pregnancy outcomes following paternal exposure or paternal and maternal exposure to 2,3,7,8-TCDD. No significant alterations in the incidence of spontaneous abortions were found in several studies of Vietnam veterans. In a case-control study conducted by Aschengrau and Monson (1989), no association was observed between paternal military service in Vietnam and the risk of spontaneous abortion (odds ratio [OR] of 0.88, 95% confidence interval [CI] of 0.42–1.86). A limitation of this study is that service in Vietnam is not an adequate exposure surrogate for 2,3,7,8-TCDD exposure; CDC (1988) found that 2,3,7,8-TCDD body burdens in Vietnam veterans were not significantly different than background levels. In a study of Air Force personnel involved in Operation Ranch Hand, no relationship between paternal 2,3,7,8-TCDD exposure (as measured by serum 2,3,7,8-TCDD levels) and the occurrence of spontaneous abortions (relative risk [RR] of 1, 95% CI=0.7–1.3 for veterans with half-life adjusted serum lipid 2,3,7,8-TCDD levels of >110 ppt) or stillbirths (RR of 1.8, 95% CI=0.7–4.7 for veterans with half-life adjusted serum 2,3,7,8-TCDD levels of <110 ppt, current levels >10 ppt; and RR of 0.3, 95% CI=0–2.3 for veterans with half-life adjusted serum 2,3,7,8-TCDD levels of >110 ppt) were observed (Wolfe et al. 1995). No significant alterations in the relative risk of stillbirths (3 stillbirths observed in 2,3,7,8-TCDD-exposed group compared to 0 in control group) or miscarriages (RR of 1.19, 90% CI=0.58–2.45) were observed in the wives of New Zealand 2,4,5-T applicators (Smith et al. 1982). It should be noted that many of the wives were occasionally exposed while helping with spray activities and

while washing contaminated clothing. An increased incidence of spontaneous abortions was reported in women living close to a herbicide manufacturing factory in Sweden (Forsberg and Nordstrom 1985). The residents were exposed to phenoxy acids, chlorophenols, 2,3,7,8-TCDD, and dibenzofurans which were released into the soil and groundwater. The small number of cases in the exposed cohort and concomitant exposure to several other chemicals limits the conclusions which can be drawn from this study.

Several studies have reported alterations in the sex ratio of children of men and women exposed to high levels of CDDs. Mocarelli et al. (1996) observed decreases in the sex ratio of children born to parents living in area A at the time of the accident in Seveso, Italy. More females than males (48 females versus 26 males; normal ratio is 100 females to 106 males) were born between April 1977 (9 months after the accident) and December 1984. Between 1985 and 1994, there was no significant alteration in sex ratio (64 females, 60 males). In nine families in which both parents lived in area A at the time of the accident, serum lipid 2,3,7,8-TCDD levels (blood samples collected at the time of the accident) ranged from 126 to 1,650 ppt and 104 to 2,340 ppt for the mothers and fathers, respectively. Basharova (1996) reported an alteration in sex ratio (more females than males) in children of workers exposed to 2,3,7,8-TCDD-contaminated 2,4,5-T at a production facility in Ufa, Russia. No additional information on the percentage of male and female children or statistical analysis of data was provided. Similarly, more females than males (51.4% versus 48.6%; 19,675 births) were born to 9,512 male workers exposed to chlorophenate wood preservatives contaminated with CDDs (Dimich-Ward et al. 1996). James (1997) statistically analyzed the results of this study and found that the sex ratio was statistically significant, as compared to the expected Caucasian live birth sex ratio of 0.514. Stockbauer et al. (1988) did not find an alteration in the sex ratio (53.% of children were males versus 51.2% in nonexposed controls) among the children of mothers potentially exposed to CDDs in the Times Beach incident. This retrospective cohort study did not monitor where the mothers or fathers lived at the time of conception. Although three studies have found alterations in the sex ratio among the offspring of exposed individuals, a causal relationship between CDD exposure and alterations in the sex ratio cannot be inferred at this time. Further investigation of this positive association is clearly warranted.

In an inadequately reported study, Phuong et al. (1989a) reported a statistically significant increase in the incidence of hydatidiform mole in families living in southern Vietnam potentially exposed to 2,3,7,8-TCDD contaminated herbicides as compared to a group of Ho Chi Minh City residents presumably never exposed to 2,3,7,8-TCDD-contaminated herbicides. In contrast, a case-control study by Ha et al. (1996) did not find a

significant association between exposure to 2,3,7,8-TCDD-contaminated herbicides and the occurrence of complete hydatidiform mole or choriocarcinoma among women living in southern Vietnam.

The results of the human reproductive toxicity studies are inconclusive. In studies which measured 2,3,7,8-TCDD levels (Egeland et al. 1994; Henriksen et al. 1996), mixed results were found for alterations in hormone levels. The Egeland et al. (1994) study of the NIOSH cohort found significant alterations in testosterone and gonadotropins, but the alterations were small, frequently not found in the same individuals, and it is not known if they would adversely affect reproductive performance. In contrast, the Henriksen et al. (1996) study of the Operation Ranch Hand cohort did not find any alterations, but the members of this cohort were exposed to lower concentrations of 2,3,7,8-TCDD and for shorter durations than the NIOSH cohort. Studies which examined pregnancy outcomes following paternal exposure (Aschengrau and Monson 1989; Smith et al. 1982) did not find increases in the incidence of spontaneous abortions and/or stillbirths. Forsberg and Nordstrom (1985) found an increased incidence of spontaneous abortions in residents likely exposed to 2,3,7,8-TCDD. However, the Aschengrau and Monson (1989), Smith et al. (1982), and Forsberg and Nordstrom (1985) studies did not measure 2,3,7,8-TCDD levels, and it is difficult to determine the level of 2,3,7,8-TCDD exposure from the data reported. Three studies found altered sex ratios among offspring of exposed individuals (Basharova 1996; Dimich-Ward et al. 1996; Mocarelli et al. 1996), but the role of CDDs could not be determined with any certainty. Without exposure information, relationships between 2,3,7,8-TCDD exposure and the risk of adverse pregnancy outcomes cannot be established.

2,3,7,8-TCDD body burdens calculated from available serum lipid 2,3,7,8-TCDD levels are presented in Table 2-1.

2.1.6 Developmental Effects

The potential for 2,3,7,8-TCDD to induce developmental effects has been examined in several populations: residents exposed to 2,3,7,8-TCDD during aerial spraying of 2,4,5-T or from accidental releases of 2,3,7,8-TCDD or 2,3,7,8-TCDD-contaminated chemicals, workers involved in manufacturing or application of phenoxy herbicides and/or chlorophenols, and Vietnam veterans. In most of the human studies, exposure was poorly characterized.

In residents of Seveso, Italy, a significant rise in the incidence of birth defects, as compared to pre-accident levels, was observed the year after the accident (Bisanti et al. 1980). A variety of birth defects were

observed, but the incidence for any particular defect was not elevated. The authors suggest that the rise in birth defects may not be related to 2,3,7,8-TCDD exposure. Prior to 1976, birth defects in Italy were usually under reported; the authors note that the reported incidences of birth defects after the accident (23 per 1,000 births) were similar to incidences reported in other western countries. Thus, the increased incidence may be reflective of the increased reporting rather than an increased number of birth defects. In a study which assessed the risk of birth defects for the 6-year period after the Seveso accident, no increases were observed for the risk of total defects (RR of 1.2, 90% CI of 0.88–1.64 for zones A and B and RR of 0.97, 90% CI=0.83–1.13 for zones A, B, and R), major defects RR of 1.02, 90% CI=0.64–1.61 for zones A and B and RR of 0.83, 90% CI=0.67–1.04 for zones A, B and R), and minor defects RR of 1.44 90% CI=0.92–2.24 for zones A and B and RR of 1.14, 90% CI=0.92–1.42 for zones A, B and R) (Mastroiacovo et al. 1988). The small number of observed birth defects limits the statistical power of this study to detect significant increases in a specific defect.

In a study of residents of Northland, New Zealand exposed to 2,4,5-T during aerial spraying, no significant alterations in the total number of birth defects were observed in children born between 1973 and 1976, as compared to the incidence in children born between 1959 and 1960 (before the aerial 2,4,5-T spraying began) (Hanify et al. 1981). Stockbauer et al. (1988) studied the Missouri cohort and found no statistically significant excess risk of birth defects among infants from exposed mothers (n=410) compared to an unexposed referent group (n=820). However, a significant increase in the incidence of talipes (incidence ratio of 1.66, 90% CI=1.2–2.29) was observed in children born after the spraying program began. The relationship between 2,4,5-T usage and the incidence of facial clefts was investigated in residents of Arkansas exposed during the spraying of rice acreage (Nelson et al. 1979). The population was divided into areas of high, medium, and low potential exposure based on herbicide application rates. Increasing trends over time in facial clefts for both the high- and low-exposure groups were observed. The authors attributed this to better case-ascertainment rather than 2,4,5-T exposure.

In the offspring of male workers at a chlorophenol manufacturing facility, no significant increases in the incidence of infant deaths, health defects, or congenital malformations were observed (Townsend et al. 1982). The adjusted odds ratio (95% CI) for infant deaths, health defects, and congenital malformations were 0.63 (0.27–1.39), 0.85 (0.6–1.21), and 0.85 (0.53–1.35), respectively, for workers exposed to any dioxin and 0.82 (0.3–2.09), 0.93 (0.6–1.43), and 1.08 (0.63–7.83) for workers exposed to 2,3,7,8-TCDD only.

Several studies investigated the outcome of pregnancies fathered by Vietnam veterans potentially exposed to 2,3,7,8-TCDD-contaminated herbicides. Two case-control studies (Aschengrau and Monson 1990; Erickson et al. 1984) have examined the risk of Vietnam veterans having a child with birth defects. The Erickson et al. (1984) study used a cohort of 7,133 infants with birth defects registered by the Metropolitan Atlanta Congenital Defects Program and 4,246 control infants; information on military service and possible exposure to Agent Orange was obtained during interviews with the mother and father. The overall risk of having a child with birth defects was not significantly increased in the Vietnam veterans (OR of 0.97, 95% CI=0.83–1.14). However, Vietnam veterans fathered a higher proportion of the children with some birth defects (spina bifida, cleft lips, and congenital tumors including dermoid cysts, teratomas, hepatoblastomas, central nervous system tumors, and Wilm's tumors) (Erickson 1984). The case group (857 infants with congenital anomalies, 61 stillbirths, and 48 neonatal deaths) and control group (998 infants) for the Aschengrau and Monson (1990) study consisted of infants delivered between August 1977 and March 1980. No significant increase in the risk of fathering a child with birth defects was observed for the Vietnam veterans (OR of 1.3, 95% CI=0.7–2.4). Among the children with birth defects, an increased risk of having one or more major systemic malformation (OR of 1.8; 95% CI=1–3.1) was reported in infants fathered by Vietnam veterans. The largest increases were reported for malformations of the nervous system, cardiovascular system, genital organs, and urinary tract. No pattern of multiple malformations was found; the only pattern of multiple malformations observed in more than one infant was ventricular septal defect and talipes. The results of these two case-control studies (Aschengrau and Monson 1990; Erickson et al. 1984) should be interpreted cautiously because there is no documentation of 2,3,7,8-TCDD exposure. CDC (1988) found that in Vietnam veterans self-reporting exposure to Agent Orange, the levels of serum 2,3,7,8-TCDD were not significantly different than levels found in a control population.

In a study of Vietnam veterans participating in Operation Ranch Hand (Wolfe et al. 1995), an increase in nervous system defects with increasing paternal serum lipid 2,3,7,8-TCDD levels was observed (statistical analysis was not performed due to the small number of defects: 3/981 in comparison group, 0/283 in Ranch Hand veterans with current 2,3,7,8-TCDD levels of #10 ppt, 2/241 in veterans with current 2,3,7,8-TCDD levels of >10 ppt and initial levels of #110 ppt, and 3/268 in veterans with current 2,3,7,8-TCDD levels of >10 ppt and initial levels of >110 ppt). However, the authors caution that this relationship is based on a limited amount of data. No relationships between paternal 2,3,7,8-TCDD exposure (based on serum 2,3,7,8-TCDD levels) and the prevalence of other birth defects were observed. In an earlier study by Wolfe et al. (1985) of Air Force personnel involved in Operation Ranch Hand, a significant increase in the number of reported neonatal deaths (no additional details provided), as compared to a comparison group of Air Force

military employees not stationed in Vietnam, was observed. The incidence of major defects, prematurity, learning disabilities, or infant deaths was not increased in the Ranch Hand personnel. A significant increase in the incidence of minor health effects such as birth marks, rashes, and neonatal jaundice was reported by the Ranch Hand veterans. It should be noted that the pregnancy outcomes were self-reported, and this finding was not corroborated by the follow-up study (Wolfe et al. 1995) which used birth certificates, medical records, and death certificates to assess possible relationships between paternal exposure to 2,3,7,8-TCDD and developmental effects in offspring. Michalek et al. (1998) examined birth records of children born between 1959 and 1992 to Operation Ranch Hand veterans. A slight increase in the incidence of preterm births (not statistically significant) was observed in the low (current CDD level of #10 ppt) and high (extrapolated initial CDD level of >79 ppt) exposure groups but not in the medium (extrapolated initial CDD level of #79 ppt) exposure group. An increase in the relative risk of infant deaths was observed in all three groups, as compared to the referent group of veterans in SEA not exposed to Agent Orange); the relative risks in the low, medium, and high groups were 3.2 (95% CI=1.0–10.3), 1.5 (95% CI=0.3–7.5), and 4.5 (95% CI=1.5–14.0). Short gestation and low birth weight were the most common causes of infant deaths. No adverse effect on intrauterine growth was observed. Michalek et al. (1998) concluded that the increased infant mortality may not be due to paternal 2,3,7,8-TCDD exposure because the risk was increased in Operation Ranch Hand cohort members with essentially background current 2,3,7,8-TCDD levels (low exposure group) and in the highest exposure group. In Vietnamese families potentially exposed to 2,3,7,8-TCDD-contaminated herbicides during the Vietnam War, a statistically significant increase in the incidence of unspecified congenital anomalies was observed as compared with a nonexposed population (Phuong et al. 1989a). Serum lipid 2,3,7,8-TCDD levels were not measured and the extent of exposure was based on subject recall of how many times they were exposed to herbicides during the Vietnam war.

The results of the available developmental studies in humans were inconclusive. The lack of exposure data, small sample sizes, and the lack of reliable data for birth defect rates prior to 2,3,7,8-TCDD exposure limit the power of the human studies to determine if an association between 2,3,7,8-TCDD exposure and developmental toxicity exists.

2.1.7 Genotoxic Effects

Data regarding genotoxic effects in humans exposed to CDDs are inconclusive. A statistically significant increase in the incidence of cells with chromosomal aberrations and a greater number of aberrations were found in fetal tissues from induced abortions in women possibly exposed to 2,3,7,8-TCDD after the Seveso

accident (Tenchini et al. 1983). The results from cytogenetic analysis of maternal tissues were comparable to those of the control group. Furthermore, no increase in the frequency of chromosomal aberrations was found in 17 individuals who were treated for chloracne following the Seveso accident (Reggiani 1980). An increased incidence of chromosomal aberrations was found in a group of 10 Vietnam veterans (Kaye et al. 1985); however, in another study, no increases in chromosomal aberrations or sister chromatid exchanges were reported in 15 Vietnam veterans (Mulcahy et al. 1980). None of these studies included 2,3,7,8-TCDD dosimetry and all were limited by using exposed groups that were relatively small (less than 20 individuals) to have the statistical power to reliably assess the cytogenetic damage. A more recent study examined the incidence of chromosomal aberrations and of sister chromatid exchanges in human lymphocytes in 27 workers whose current 2,3,7,8-TCDD concentrations in blood were above 40 ppt, and in 28 age-comparable referents (Zober et al. 1993). The results showed no statistically significant differences between the two groups in the percentages of gaps, chromatid or chromosome exchanges, chromatid or chromosome breaks/fragments/deletions, multiple aberrations, or the overall percentage of aberrations including or excluding gaps. In the exposed group there was an increased rate of sister chromatid exchanges per cell and a higher percentage of cells with more than 10 sister chromatid exchanges. However, these associations were no longer significant when smoking status was included as covariate. Moreover, neither current nor back-calculated 2,3,7,8-TCDD concentration was a significant predictor of these parameters. Zober et al. (1993) indicated that some limitations such as the small number of individuals studied, a possible selection effect, and the possibility that some effects were transient should be considered in the interpretation of the results. The human data on the genotoxicity of 2,3,7,8-TCDD is inconsistent and inconclusive. The lack of exposure data, small sample sizes, and the inconsistent results precludes drawing conclusions from these studies.

2.1.8 Cancer

The carcinogenicity of 2,3,7,8-TCDD in humans has been assessed in numerous case-control and mortality cohort studies of chemical manufacturing and processing workers and phenoxy herbicide and chlorophenols applicators, Vietnam veterans exposed to Agent Orange, and residents of Seveso, Italy. A major weakness in many of these studies is the lack of adequate exposure data. Exposure levels or 2,3,7,8-TCDD body burdens were not measured, rather surrogates of exposure such as exposure to chemicals contaminated with 2,3,7,8-TCDD or chloracne were used to identify subjects likely exposed to 2,3,7,8-TCDD. Another major weakness of most of the human cancer data is concomitant exposure to other compounds. The focus of this discussion on the carcinogenic potential of 2,3,7,8-TCDD and other CDDs will be on studies that have documented exposure by measuring blood levels or in which exposure can be reasonably presumed. The

section is divided into four parts: 1) the effect of CDD exposure on overall cancer risk in workers involved in the manufacture or application of phenoxy herbicides or chlorophenols followed by a discussion of specific types of cancer in this group, 2) cancer risks in Vietnam veterans, 3) cancer risks in Seveso residents, and 4) conclusive statement.

Increases in the overall cancer risk were observed in a number of large cohort mortality studies of chemical manufacturing workers and phenoxy herbicide applicators (Becher et al. 1996; Fingerhut et al. 1991; Hooiveld et al. 1998; Kogevinas et al. 1993, 1997; Manz et al. 1991; Ott and Zober 1996; Zober et al. 1990). Most of the subjects in these studies were males working in chlorophenoxy herbicide or trichlorophenol manufacturing facilities. In one of the few studies assessing the carcinogenicity of 2,3,7,8-TCDD in women, Kogevinas et al. (1993) found a significantly elevated risk for cancer in women probably exposed to 2,3,7,8-TCDD during the production or application of chlorophenoxy herbicides and/or chlorophenols. The Zober et al. (1990), Fingerhut et al. (1991), Manz et al. (1991), Ott and Zober (1996) and Hooiveld et al. (1998) studies used current serum 2,3,7,8-TCDD levels in surviving workers to estimate exposure. The results of these studies, as well as two large multinational cohort mortality studies (Kogevinas et al. 1997; Saracci et al. 1991), are described below.

Saracci et al. (1991) examined cancer mortality in workers on the International Register of Workers Exposed to Phenoxy Herbicides and Their Contaminants. The registry consists of 18,390 workers (16,863 males and 1,527 females) distributed among 20 cohorts from 10 countries. Based on information obtained from questionnaires, factory or spraying records, and job histories, the workers were classified as exposed (13,482 workers), probably exposed (416), exposure unknown (541), or non-exposed (3,951). The exposed workers were workers who sprayed chlorophenoxy herbicides or worked in factories producing chlorophenoxy herbicides or chlorinated phenols. The probably exposed workers worked at facilities producing pentachlorophenol (145 workers) or 2,4-D, 2,4-(dichlorophenoxy)butanoic acid, (4-chloro-2-methyl-phenoxy)acetic acid, and (4-chloro-2-methyl)propanoic acid (275 workers). For 4 of the 20 cohorts, a minimum employment duration of 1 to 12 months was required for inclusion in the registry, for the remaining cohorts, the criterion for inclusion was “ever employed in production or spraying of phenoxy herbicides.” The average follow-up period for the entire cohort was 17 years. Cancer mortalities were not significantly increased in exposed and probably exposed workers (SMR=101; 95% CI=93–110). Duration of exposure or time since first exposure did not appear to influence the number of cancer deaths. The lack of both a clear definition of exposure and uniformity of exposure classification between and within cohorts makes the results difficult to interpret and lessens the confidence in the results.

After publication of the Saracci et al. (1991) study, the IARC cohort was expanded to include 12 manufacturing facilities in the United States (also examined by Fingerhut et al. 1991) and 4 plants in Germany (also examined by Becher et al. 1996). This expanded cohort, studied by Kogevinas et al. (1997), which included almost all workers world-wide who ever produced phenoxy herbicides, comprised 21,863 workers (20,851 males and 1,012 females) in 12 countries. 2,3,7,8-TCDD levels were measured in 573 workers at 10 facilities (approximately 50% of these workers were part of the NIOSH cohort examined by Fingerhut et al. 1991). The levels were 2-34 pg/g blood lipid (measured in 1990, mean not reported), 98–659 (1990, mean of 389 pg/g), 1.9–194 (1993, 53 pg/g), 3.0–131 (1988, 53.3 pg/g), 9–37 (1992, 17 pg/g), 1.3–6.49 (1996, estimated mean of 3.2 pg/g), 3–2,252 (1985–1994, estimated mean of 141 pg/g), 23–1,935 (1989–1992, estimated mean of 401.7 pg/g), and 2–3,400 (1987–1988, mean of 233 pg/g). Mortality rates for the cohort were compared to national mortality rates calculated using data from the WHO mortality data bank. There was a significant increase in the SMRs for all cancers for male workers (1,083 deaths; SMR=1.07; 95% CI=1.01–1.13) but not among female workers (44 deaths; SMR=0.93; 95% CI=0.68–1.25). When mortality rates were calculated for the 13,831 workers (males and females) exposed to phenoxy herbicides contaminated with 2,3,7,8-TCDD or higher chlorinated dioxins, the all-cancer SMR increased to 1.12 (710 deaths; 95% CI=1.04–1.21). The SMR for all cancer deaths was not elevated in workers not exposed to 2,3,7,8-TCDD or higher chlorinated dioxins. 2,3,7,8-TCDD-exposed workers were divided into groups based on years since first exposure, duration of exposure, and year of first exposure. The mortality rate appeared to be related to years since exposure and the related variable of year of first exposure. Significantly increased SMRs were observed in workers with a $20 year latency period (394 deaths; SMR=1.20; 95% CI=1.09–1.33), workers employed before 1955 (335 deaths; SMR=1.12; 95% CI=1.00–1.25), or workers employed between 1955 and 1964 (242 deaths; SMR=1.17; 95% CI=1.03–1.25), but these differences were overall small.

The cancer mortality experience of 247 male workers who were exposed to 2,3,7,8-TCDD during an accidental uncontrolled decomposition reaction and subsequent clean-up activities in a German 2,4,5-TCP production plant (BASF AG facility) in 1953 and followed for 34 years was studied by Zober et al. (1990). Three subcohorts were defined based on qualitative exposure information: Subcohorts 1 (n=69) included workers known to be exposed to 2,3,7,8-TCDD during the accident, and Subcohort 2 (n=84) and 3 (n=94) included workers considered exposed to amounts of 2,3,7,8-TCDD less than in Subcohort 1. Chloracne (114 cases) or erythema (13 cases) developed in 127 (51%) of the main cohort. Subcohorts 1, 2 and 3 contained 69 cases (21 severe, 27 extensive, 21 moderate), 17 cases (1 severe, 3 extensive, 13 moderate) and 28 cases (0 severe, 3 extensive, 25 moderate) of chloracne, respectively, and erythema affected 4 subjects in

Subcohort 2 and 9 subjects in Subcohort 3. Blood 2,3,7,8-TCDD levels were measured in 28 subjects in Subcohorts 1 (median 24.5 ppt, n=11), 2 (median 9.5 ppt, n=7), and 3 (median 8.4 ppt, n=10). Subjects with chloracne/erythema had higher average serum 2,3,7,8-TCDD levels (15 ppt, n=16) than those without (5.8 ppt, n=12). There was no clear increase in all malignant neoplasms or site-specific cancer in the entire cohort or either subcohort based on comparison with national mortality rates in the Federal Republic of Germany. When the 127 workers with chloracne/erythema were examined separately, the standardized mortality ratio (SMR) for all malignant neoplasms was not significantly elevated overall (SMR=139; 95% CI=87–211), although the SMR for all malignancies was significantly increased when analysis was restricted to workers whose first exposure was $20 years earlier (SMR=201; 95% CI=122–315, $p<0.05$).

In a follow-up to the Zober et al. (1990) study, the cohort of workers exposed at the BASF AG facility in Germany, was followed through 1992 (Ott and Zober 1996). The workers were divided into 3 subcohorts based on half-life extrapolated 2,3,7,8-TCDD body burdens of <0.01 µg/kg body weight, 0.1–0.99 µg/kg, and $1 µg/kg. 2,3,7,8-TCDD half-lives were estimated from repeated blood samples from 29 people with initial serum lipid 2,3,7,8-TCDD levels of 29–553 pg/g. The mean half-life was 5.8 years, but the half-life increased with higher percentages of body fat. Half-life estimates of 5.1 and 8.9 years were used for workers with 20 and 30% body fat, respectively. There was suggestive evidence that 2,3,7,8-TCDD exposure affected the occurrence of deaths from cancer (all sites combined) and deaths from respiratory or digestive cancer. The number of cancer deaths increased with increasing body burdens, SMRs of 0.8 (8 deaths; 95% CI=0.4–1.6), 1.2 (8 deaths; 95% CI=0.5–2.3), and 1.6 (15 deaths; 95% CI=0.9–2.6) in the cohorts with body burdens of <0.1, 0.1–0.99, and $1 µg/kg, respectively. No increases in cancer deaths were observed among nonsmokers; among current smokers, there were increases in cancer risks for workers with 2,3,7,8-TCDD body burdens of 1.0–1.99 µg/kg (6 deaths; SMR=3.0; 95% CI=1.1–6.5) and $2.00 µg/kg (6 deaths; SMR=4.0; 95% CI=1.5–8.6) but not for workers with lower 2,3,7,8-TCDD levels. Ott and Zober (1996) concluded that past body burdens of $1.0 µg/kg 2,3,7,8-TCDD were consistent with a 2,3,7,8-TCDD-induced carcinogenic effect. At the same time, they stated that with such a small cohort, the risk estimates are not very stable and could be affected by selection and confounding.

Becher et al. (1996) examined a cohort of 2,479 men employed at 4 German facilities involved in the production of phenoxy acid herbicides and chlorophenols. The workers were divided into 4 subcohorts: 1,144 male workers at facility 1 with a mean duration of employment of 7.7 years, 135 male workers at facility 2 with a mean duration of employment of 21.5 years, 520 male workers at facility 3 with a mean duration of employment of 9.1 years, and 680 workers at facility 4 with a mean duration of employment of

18.5 years. 2,3,7,8-TCDD blood levels in groups of workers in subcohorts 1 (112 males and 18 females) and 2 (8 workers, all with a history of chloracne) were 3–2,252 and 163–1,935 pg/g blood lipid, respectively. The study authors noted that 2,3,7,8-TCDD exposure was probably lower in subcohorts 3 and 4 because 2,3,7,8-TCDD-contaminated products only made up a small percentage of the total products manufactured at these facilities. Messerer et al. (1998) measured serum CDD and CDF levels in 19 of the current employees at facility 4. The mean CDD and CDF levels were slightly higher than background levels, even in the most exposed workers (involved in synthesis); the mean 2,3,7,8-TCDD and TEQ levels in the 7 synthesis workers were 3.8 and 35.4 ppt, respectively, as compared to 3.2 and 25.0 ppt in controls. The SMR for all cancer mortalities was significantly increased in the entire cohort (138 deaths; SMR=119; 95% CI=100–141); subcohort 1 was the only subcohort with a significant increase in all cancer mortalities (97 deaths; SMR=134; 95% CI=109–164). When the entire cohort was divided into groups based on time since first exposure, there were no increases in SMRs for all cancer deaths during the three time periods (0 to <10 years, 10 to <20 years, and $20 years).

In the Fingerhut et al. (1991) study, cancer mortality was evaluated in 5,172 male workers involved in the production of 2,3,7,8-TCDD-contaminated chemicals in 12 U.S. plants, as well as in subcohorts with low (<1 year, n=1,516) or high ($1 year, n=1,520) exposures and $20 years latency. Exposure was documented by reviewing job descriptions and records of 2,3,7,8-TCDD levels in industrial hygiene samples, and measuring lipid-adjusted serum 2,3,7,8-TCDD levels in 253 of the workers from two of the facilities; it was assumed that a similar relationship would exist at the other 10 facilities. Duration of exposure was used as a surrogate for cumulative 2,3,7,8-TCDD exposure on the basis of a high correlation ($r=0.72$, $p<0.001$) between log serum 2,3,7,8-TCDD level and log number of years of exposure. Mean lipid-adjusted serum 2,3,7,8-TCDD levels were 233 ppt (range 2–3,400) for all 253 workers, 418 ppt for 119 workers with $1 year exposure and 7 ppt in a comparison group of 79 unexposed persons. When compared to the U.S. population, mortality from all cancers was significantly increased ($p<0.05$) in the overall cohort (SMR=115; 95% CI=102–130) and the high exposure subcohort (SMR=146; 95% CI=121–176). Cancer mortality increased with increasing latency. The number of deaths were too small to allow meaningful analysis according to duration of exposure.

A cancer mortality follow-up study of 1,583 workers (1,184 men, 399 women) who were employed in a German chemical plant that produced trichlorophenol, 2,4,5-T, and other herbicides known to be contaminated with 2,3,7,8-TCDD and other polychlorinated dioxins and furans (this appears to be the same facility as subcohort 1 in the Becher et al. [1996] study) was reported by Manz et al. (1991). Production of

these chemicals was discontinued during 1954–57 after an outbreak of chloracne. Cohort members worked for at least 3 months during 1952–1984 and were followed through 1989. SMRs were calculated using national mortality rates for West Germany and deaths in a cohort of male gas supply company workers. Exposures of cohort members were classified as high (n=496), medium (n=901), or low (n=186) based on an analysis of production processes in the plants where they had worked. Some validation for these categories was provided by measurement of adipose 2,3,7,8-TCDD levels in 48 males in 1985 (mean concentrations were 296 ng/kg lipid in 37 subjects in the high-exposure group compared with 83 ng/kg for 11 subjects in the medium- and low-exposure groups combined). When compared with national rates, mortality from all cancers combined was increased in the entire cohort (93 deaths, SMR=1.24; 95% CI=1.00–1.52), especially among men in the high exposure subgroup with $20 years employment (8 deaths, SMR=2.54; 95% CI=1.00–5.00) or who began employment before 1955 (18 deaths, SMR=2.11; 95% CI=1.25–3.34). The greatest increase in risk was found in high exposure men with time of entry before 1955 and $20 years employment (8 deaths, SMR=3.5; 95% CI=1.51–6.9). The aforementioned findings were corroborated when the gas workers were used as the comparison group. In a follow-up study (Flesch-Janys et al. 1998) in which the cohort was followed through 1992, the SMR for all cancers was 1.4 (124 deaths; 95% CI=1.17–1.68). When the workers were divided into four exposure groups, a significant increase in SMR (36 deaths; SMR=1.73; 95% CI=1.21–2.40) was only found in the highest exposure group (2,3,7,8-TCDD levels of $2,503 ng/kg blood lipid × years, a measure of cumulative lifetime exposure); the trend toward increasing SMR with increasing dose was also statistically significant.

The cohort in the Manz et al. (1991) study served as the basis for additional analysis by Flesch-Janys et al. (1995, 1998), who investigated the relation between mortality and quantitative measure of PCDD/F exposure. The male chemical workers (n=1,177) were followed for an additional 3 years. Blood levels of each PCDD/F congener at the end of exposure were estimated for all members of the cohort based on work histories (durations of exposure in particular departments) and tissue levels from a subgroup of male workers, and assuming one-compartment first-order elimination kinetics. A total TEQ level was also estimated for all measured CDDs and CDFs combined as the weighted sum of PCDD/F congeners using toxicity equivalent factor (TEF) values (see Section 2.5 for a detailed explanation on TEFs and dioxin equivalents). In the Flesch-Janys et al. (1995) study, risk ratios for the cohort were estimated with year-of-birth stratified Cox regression using seven exposure levels (the reference cohort, the first four quintiles and the ninth and tenth deciles of the estimated 2,3,7,8-TCDD levels and total TEQ); an external cohort of gas supply workers (n=2,158) served as an unexposed control group. The estimated mean 2,3,7,8-TCDD level for the entire cohort at the end of employment in the plant was 141.4 ng/kg blood fat (median of 38.2 ng/kg).

The estimated mean total dioxin equivalents, calculated as the weighted sum of combined PCDD/F congeners, was 296.5 ng/kg (median of 118.3 ng/kg). There was a dose-dependent increase in cancer mortality with increasing levels of 2,3,7,8-TCDD (p=0.01 for trend), predominantly due to increased risk ratio (3.30; 95% CI=2.05–5.31) in the highest-dose group (344.7–3,890.2 ng/kg). Similar findings were obtained when total TEQs were used as the exposure parameter, or when the two lowest-dose groups in the chemical-worker cohort were combined and used for reference. In the Flesch-Janys et al. (1998) study, the mean blood 2,3,7,8-TCDD level in the subgroup (blood or adipose tissue samples collected from 236 males) was 108.3 ng/kg blood lipid (range, 2.0–2252 ng/kg), the mean TEQ for CDD/F congeners was 247.5 ng/kg (11.7–2,985.8), and the mean TEQ without 2,3,7,8-TCDD was 184.0 ng/kg (9.7–1,263.4 ng/kg). When the workers were divided into groups based on cumulative TEQ exposure, statistically significant SMRs were found in the second (TEQ between 360.9 and 1,614.4 ng/kg × years) and fourth (TEQ greater than 5,217.7 ng/kg × years) quartiles; SMRs of 1.64 (34 deaths; 95% CI=1.13–2.29) and 1.64 (34 deaths; 95% CI=1.13–2.29), respectively. A significant relationship between cumulative TEQ level and SMR for all cancer was not found. Potential sources of error and bias in this study include lack of random sampling in the subgroup with PCDD/F assays and the assumption of first-order elimination kinetics. Flesch-Janys et al. (1998) compared age of employment and years spent in each product department for workers with measured 2,3,7,8-TCDD blood levels and workers without blood level data and found no major differences between the two groups.

Workers at two Dutch phenoxy herbicide and chlorophenols production facilities comprised the cohort for the Bueno de Mesquita et al. (1993) study of cancer mortality. The cohorts consisted of any worker employed between 1955 and 1985 (facility A) or between 1965 and 1986 (facility B). An industrial accident at facility A resulted in a release of CDDs, including 2,3,7,8-TCDD. Mortality rates in the workers were compared to national rates. In workers at facility A, there was a slight increase in deaths from all cancers; however, the increase was not statistically significant (26 deaths; SMR=118; 95% CI=77–173); the SMR for all cancers was not increased at facility B or in the combined entire cohort. Likewise, dividing the workers at each facility into groups based on time since first exposure or duration of exposure did not result in any significant increases in SMRs. Hooiveld et al. (1998) followed the workers at facility A for another 6 years. Additionally, serum levels of CDDs, CDFs, and PCBs were measured in a sample of 47 surviving workers who were employed for at least 1 year and whose date of first employment was prior to 1975; 14 of these workers were exposed during the accident, 17 were workers not involved in the accident, and 16 were not exposed to phenoxy herbicides or chlorophenols. The average 2,3,7,8-TCDD serum levels were 105.2, 42.9, 16.6, and 7.6 ppt (lipid adjusted) in the accident-exposed workers with a history of chloracne (12 workers),

accident-exposed workers without chloracne (2 workers), nonaccident-exposed workers, and nonexposed workers, respectively; the mean serum 2,3,7,8-TCDD levels at the time of maximum exposure (extrapolated levels) were estimated at 2,014.4, 806.6, and 244.1 ppt in the accident-exposed workers with a history of chloracne, accident-exposed workers without chloracne, and nonaccident-exposed workers, respectively. There was an increase in deaths from all cancers among all exposed workers (51 deaths; SMR=1.5; 95% CI=1.1–1.9), as compared to national rates; a slightly higher mortality rate (20 deaths; SMR=1.7; 95% CI=1.1–2.7) was observed in the subcohort of accident-exposed workers. When nonexposed workers were used as a comparison group, the relative risk of all cancer deaths was 4.1 (51 deaths; 95% CI=1.8–9.0), the relative risk was adjusted for age, calendar year at end of follow-up, and time since first exposure/ employment. When the workers were divided into three groups based on model-predicted 2,3,7,8-TCDD levels, the relative risk of all cancer deaths was elevated in workers with medium or high exposure, as compared to workers with low exposure (adjusted RR=4.8; 95% CI=2.0–11.3 for the medium exposure group and adjusted RR=4.4; 95% CI=1.9–10.4 for the high exposure group).

Case-control studies have been designed to determine if 2,3,7,8-TCDD exposure results in increased risks for site-specific cancers. Case-control studies have found significant increases in the risk of soft-tissue sarcomas in Swedish agricultural, forestry, and horticultural workers (Eriksson et al. 1981, 1990; Hardell and Eriksson 1988; Hardell and Sandstrom 1979), workers involved in manufacturing and application of phenoxy herbicides (Kogevinas et al. 1995), and New Zealand farmers (Smith et al. 1984a). In the Eriksson et al. (1990) study, the risk ratio of soft-tissue sarcoma was 1.80 (95% CI=1.02–3.18) in subjects exposed to phenoxyacetic acid herbicides and/or chlorophenols. In subjects exposed to phenoxyacetic acid herbicides only or chlorophenols only, the risk ratios were 1.34 (95% CI=0.7–2.56) and 5.25 (95% CI=1.69–16.34), respectively. When the phenoxyacetic acid herbicide-only subjects were divided into two groups of subjects predominantly exposed to 2,4,5-T and those exposed to phenoxyacetic acid herbicides other than 2,4,5-T, risk ratios of 1.81 (95% CI=0.85–3.87) and 0.60 (95% CI=0.18–2.06), respectively, were calculated. Hardell et al. (1995) conducted a meta-analysis of their four Swedish case-control studies (Eriksson et al. 1981, 1990; Hardell and Eriksson 1988; Hardell and Sandstrom 1979). The odds ratios for workers exposed to phenoxyacetic acid herbicides or chlorophenols, phenoxy herbicides only, or chlorophenols only were 2.8 (90 cases; 95% CI=2.1–4.4), 2.7 (59 cases; 95% CI=1.9–4.7), and 3.3 (34 cases; 95% CI=1.8–6.1), respectively. The data from this study suggest that the increased possible risks of soft-tissue sarcomas observed in phenoxy herbicide and chlorophenols applicators may be due to exposure to the 2,3,7,8-TCDD (or other CDDs) contamination of the mixture. However, the results of the Kogevinas et al. (1995) study suggest that the possible risk of soft-tissue sarcoma may not be specific to 2,3,7,8-TCDD-contaminated

phenoxy herbicides. An increase in the possible risk of soft-tissue sarcoma (OR of 10.3; 95% CI=1.2–90.6) was found in multinational workers involved in the production and spraying of phenoxy herbicides (Kogevinas et al. 1995). Exposure to chlorophenols was not associated with an increase in the possible risk of soft-tissue sarcomas (OR=1.29; 95% CI=0.24–6.91). Using job histories, the workers were divided into groups based on the type of phenoxy herbicide used. Excess possible risks of soft-tissue sarcoma were observed in workers predominantly exposed to 2,4-dichlorophenoxyacetic acid (2,4-D, OR=5.7; 95% CI=1.1–29), 2,4,5-T (OR=4.3, 90% CI=0.7–26), 4-chloro-2-methylphenoxyacetic acid (MCPA, OR=11.3; 95% CI=1.3–98), exposure to any CDDs or CDFs (OR=5.6; 95% CI=1.1–28), and exposure to 2,3,7,8-TCDD (OR=5.2, CI=0.9–32). Although this study suggests that exposure to 2,3,7,8-TCDD increases the possible risk of soft-tissue sarcoma, it also suggests that exposure to phenoxy herbicides on their own may also increase the possible risk of soft-tissue sarcoma.

Cohort mortality studies have also found increases in the incidences of soft-tissue sarcomas. Fingerhut et al. (1991) found significant increase in deaths from soft-tissue sarcomas (SMR=922; 95% CI=190–2,695) in the high-exposure cohort, although this was only based on 3 deaths. In the Saracci et al. (1991) multinational cohort, an increase in deaths from soft-tissue sarcoma was observed in phenoxy herbicide sprayers (3 deaths, SMR=297; 95% CI=61–868) and in workers dying 10–19 years after first exposure (4 deaths, SMR=606; 95% CI=165–1,552). Similarly, the Kogevinas et al. (1997) study of the IARC cohort found 3 cases of soft-tissue sarcoma in workers exposed for 10–19 years (SMR=6.52; 95% CI=1.35–19.06); shorter exposure durations did not result in significantly elevated SMRs. Additionally, when workers were divided into latency groups, the SMRs were not elevated. Duration of probable exposure to 2,3,7,8-TCDD did not appear to influence soft tissue sarcoma cancer risks.

Case-control studies and/or cohort mortality studies have also found significant increase in the possible risk of malignant lymphoma (RR=4.8; 95% CI=2.9–8.1) in Swedish agricultural, forestry, and horticultural workers Hardell et al. 1981) and German phenoxy herbicide and chlorophenols manufacturer workers (SMR=239; 95% CI=119–427) (Becher et al. 1996); non-Hodgkin's lymphoma in Wisconsin farmers (OR=1.22; 95% CI=0.98–1.51) (Cantor 1982), multinational phenoxy herbicide manufacturers and applicators (OR of 1.25; CI=0.54–2.90) (Kogevinas et al. 1995), and German workers at a phenoxy herbicide and chlorophenols facility (SMR=375; 95% CI=101–957) (Becher et al. 1996); and stomach cancer in railroad workers (rate ratio of 6.1) (Axelson et al. 1980) and workers at a German trichlorophenol facility (observed/expected ratio of 3/0.52) (Thiess et al. 1982).

Cohort mortality studies by Fingerhut et al. (1991), Zober et al. (1990), Manz et al. (1991) (including the Flesch-Janys et al. [1998] follow-up data), and Kogevinas et al. (1997) found significant increases in risk of respiratory tract cancer. In the Manz et al. (1991) study, a slightly increased risk of lung cancer was observed in the entire cohort compared to the risk in the West German population (30 deaths, SMR=1.41; 95% CI=0.95–2.01) or the reference gas workers (26 deaths, SMR=1.67; 95% CI=1.09–2.44); the increase in lung cancer risk was statistically significant when the gas workers were used as the referent group (Manz et al. 1991). Smoking does not appear to be a major confounder because available data (partial cohort) suggest that the percentage of smokers in the study cohort and gas-worker control group were similar. In the Flesch-Janys et al. (1998) follow-up of this cohort, the SMRs for lung (38 deaths) and respiratory (44 deaths) cancer were 1.51 (95% CI=1.07–2.08) and 1.71 (95% CI=1.24–2.29), respectively. When the cohort was divided into four cumulative exposure categories, the SMRs were not significantly elevated for any exposure group. In this cohort, blood 2,3,7,8-TCDD levels were not correlated with smoking status (Flesch-Janys et al. 1995). The study authors additionally noted that when lung cancer deaths were removed from total cancer deaths, there was a stronger relationship between 2,3,7,8-TCDD levels and cancer rates, suggesting that smoking was not a strong confounder. Death from cancers of the respiratory tract (SMR=142; 95% CI=103–192) were significantly increased in the high-exposure subcohort of the Fingerhut et al. (1991) study. The expected number of lung cancers was adjusted for smoking using smoking-prevalence data for a small subset of workers; the adjusted SMR for the high-exposure subcohort was 137 (95% CI=98–187). The authors concluded that the increased lung cancer risk was probably not due to smoking because the incidences of smoking-related diseases were not higher than expected in the subcohort and mortality from non-malignant respiratory disease was lower than expected. However, when smoking was included as a confounding factor, the increased risk for lung cancer was no longer statistically significant. Zober et al. (1990) found a borderline increase in mortality from cancer of the trachea bronchus/ lung (SMR=252; 95% CI=99–530) in workers with chloracne/erythema and $20 years latency. In the follow-up to the Zober et al. (1990) study, Ott and Zober (1996) found an increased number of deaths from respiratory cancer in the subcohort with 2,3,7,8-TCDD body burdens of $1 µg/kg (SMR=2.4; 95% CI=1.0–5.0). However, in 10 of the 11 cases, the worker smoked, which makes it difficult to determine if the cancer deaths were due to 2,3,7,8-TCDD exposure. The Kogevinas et al. (1997) examination of the expanded IARC cohort did not find a significant elevation in deaths from lung cancer (225 deaths; SMR=1.12; 95% CI=0.98–1.28) among workers exposed to phenoxy herbicides contaminated with 2,3,7,8-TCDD or higher chlorinated dioxins, but it did find a rise in deaths from other respiratory organ cancers (9 deaths; SMR=3.20; 95% CI=1.46–6.08).

Using regression analysis based on Cox's proportional hazards model, Ott and Zober (1996) found evidence of association between 2,3,7,8-TCDD exposure and digestive cancer (conditional risk ratio of 1.46; 95% CI=1.13–1.89); the primary tumor sites were the liver, stomach, and pancreas.

A number of studies have looked at cancer incidences among Vietnam veterans to determine if exposure to Agent Orange with its 2,3,7,8-TCDD contamination resulted in a higher cancer risk. Many of these studies compared cancer incidences in Vietnam veterans to Vietnam-era veterans stationed outside of Vietnam. A limitation of this study design is that not all veterans in Vietnam were exposed to Agent Orange and exposure was lower than that of occupational workers. CDC (1988) found that the levels of 2,3,7,8-TCDD in Vietnam veterans were usually similar to a comparison group. Thus, studies which examined cancer incidences in "Vietnam veterans" may not be adequate to assess the carcinogenicity of 2,3,7,8-TCDD. Wolfe et al. (1985) focused on Air Force personnel involved in Operation Ranch Hand. 2,3,7,8-TCDD levels in 888 Ranch Hand personnel was 12.4 ppt as compared to 4.2 ppt in a referent group of Air Force personnel (CDC 1987; USAF 1991). No significant alterations in the incidence of systemic malignancies were observed in the Ranch Hand personnel, as compared to a group of veterans flying cargo in Southeast Asia during the Vietnam war. A significant increase in non-melanomic skin cancer (predominantly basal cell carcinoma) was found in the Ranch Hand personnel; however, cancer incidences were not adjusted for sun exposure. No significant alterations in systemic malignancy indices were found. In a similar study of Air Force veterans involved in Operation Ranch Hand, a significant increase in benign systemic neoplasms was observed (USAF 1991). No alterations in the risk of malignant neoplasm were observed. No increases in the risk of Hodgkin's disease, non-Hodgkin's lymphoma or soft-tissue sarcoma were observed; however, the statistical power of the study to detect significant risk ratios for site-specific cancers was limited by the small number of cancers and the small sample size. The incidence of benign neoplasms (primarily lipomas) was highest in veterans with the highest blood dioxin levels. The incidence of basal cell skin neoplasms was not positively associated with serum dioxin levels except among enlisted flyers with basal cell carcinomas at sites other than the ear, face, head, or neck.

Increases in the risk of several types of cancer have been observed in residents of Seveso, Italy. In the residents with the highest exposure (zone A), no increases in the risk ratio of all malignancies were observed (Bertazzi et al. 1993). However, the small number of zone A residents (724) limits the statistical power of the analysis. Among residents living in zone B (4,824 people), significant increases were observed for the risk of hepatobiliary cancer (risk ratio of 3.3; 95% CI=1.3–8.1) and multiple myeloma (risk ratio of 5.3; 95% CI=1.2–22.6) in women and lymphoreticulosarcoma in men (RR of 5.7; 95% CI=1.7–19). In zone R

(31,647 residents), the risk ratio of soft-tissue sarcomas in men (2.8; 95% CI=1–7.3) was significantly increased. In both zone B and R, the risk ratios of all malignancies was not significantly altered. Estrogen-dependent cancers (breast cancer and corpus uteri cancer) were consistently decreased in the women living in zone A, B, and R. It should also be noted that the latency period of 10 years may be too short to find increases in other types of cancer. A study of children (aged 0–19 years at the time of the accident) exposed to 2,3,7,8-TCDD during the accident in Seveso found increased risks of Hodgkin's lymphoma [RR =2; 95% CI=0.5–7.6), myeloid leukemia (RR=2.7; 95% CI=0.7–11.4), and thyroid cancer (RR=4.6; 95% CI=0.6–32.7) (Pesatori et al. 1993). However, the differences in RRs for these cancer types between the Seveso residents and the control population did not reach statistical significance. The small number of detected cancers and the relatively short latency period (10 years) limits the interpretation of the results of this study. Similar results were found in the 15-year follow-up study (Bertazzi et al. 1997). No significant increases in cancer mortality were found in zone A residents (805 residents, 70 deaths). In zone B, death from all cancers was not significantly elevated in males (104 deaths; RR=1.1; 95% CI=0.9–1.3) or females (48 deaths; RR=0.9; 95% CI=0.7–1.2). However, significant increases in site-specific cancers were observed, including rectal cancer in males (7 deaths; RR=2.9; 95% CI=1.2–5.9), pleural cancer in males (31 deaths; RR=5.3; 95% CI=1.1–5.5), lymphohemopoietic cancer in males (12 deaths; RR=2.4; 95% CI=1.2–4.1), leukemia in males (7 deaths; RR=3.1; 95% CI=1.3–6.4), and myeloma in females (4 deaths; RR=6.6; 95% CI=1.8–16.8). The cancer with an elevated incidence among zone R residents was bone cancer in females (7 deaths; RR=2.4; 95% CI=1.0–4.9).

The available epidemiology data suggest that 2,3,7,8-TCDD may be a human carcinogen. Statistically significant increases in risks for all cancers were found in highly exposed workers with longer latency periods. Although the estimated SMRs are low, they are consistent across studies with the highest exposures. The evidence for site-specific cancers is weaker, with some data suggesting a possible relationship between soft-tissue sarcoma, non-Hodgkin's lymphoma, or respiratory cancer with 2,3,7,8-TCDD exposure. It should be emphasized that some of the human studies do not provide adequate exposure data and were confounded by concomitant exposure to other chemicals.

2,3,7,8-TCDD body burdens calculated from available serum lipid 2,3,7,8-TCDD levels are presented in Table 2-1.

2.2. ANIMAL STUDIES

This section contains descriptions and evaluations of studies and presents levels of significant exposure for CDDs based on toxicological studies.

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, developmental, reproductive, 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. These distinctions are intended to help the users of the document identify the levels of exposure at which adverse health effects start to appear. They should also help to determine 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 tables and figures may differ depending on the user's perspective. For example, physicians concerned with the interpretation of clinical findings in exposed persons may be interested in levels of exposure associated with "serious" effects. Public health officials and project managers 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 (NOAEL) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels, MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of CDDs are indicated in Tables 2-2, 2-3, and 2-4 and Figures 2-1 and 2-2.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made (see Section 2.5), where data were believed reliable, for the most sensitive noncancer effect for each exposure duration. MRLs include adjustments to reflect human variability and extrapolation of data from laboratory animals to humans.

Although methods have been established to derive these levels (Barnes et al. 1988; EPA 1989c), 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.

2.2.1 Inhalation Exposure

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

2.2.1.1 Death

2.2.1.2 Systemic Effects

2.2.1.3 Immunological Effects

2.2.1.4 Neurological Effects

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

No studies were located regarding cancer in animals after inhalation exposure to CDDs.

2.2.2 Oral Exposure

Information regarding adverse health effects in animals exposed to CDDs via the oral route was located for the following congeners: 2-monochlorodibenzo-*p*-dioxin (2-MCDD), 2,3-dichlorodibenzo-*p*-dioxin (2,3-DCDD), 2,7-dichlorodibenzo-*p*-dioxin (2,7-DCDD), 1,2,3-trichlorodibenzo-*p*-dioxin (1,2,3-TrCDD), 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TCDD), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD), 1,2,3,4,7,8-hexachloro-dibenzo-*p*-dioxin (1,2,3,4,7,8-HxCDD), 1,2,4,7,8-pentachlorodibenzo-*p*-dioxin (1,2,4,7,8-PeCDD),

1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (1,2,3,6,7,8-HxCDD), 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin (1,2,3,7,8,9-HxCDD), 1,2,3,4,6,7,8,-heptachlorodibenzo-*p*-dioxin (1,2,3,4,6,7,8,-HpCDD), and 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD). Some of the animal studies used a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD. Of all the CDD congeners, 2,3,7,8-TCDD has been the one most extensively studied.

2.2.2.1 Death

Numerous studies provided doses associated with death following exposure to CDDs in animals. LD_{50} (lethal dose, kill for 50% of dosed animals during a certain time interval) values for each congener varied not only among species, but also among different strains.

LD_{50} values following a single oral dose of 2,3,7,8-TCDD were calculated as 22 µg/kg (males) and 45 µg/kg (females) in Sherman rats (Schwetz et al. 1973); and 164 µg/kg, 297 µg/kg, 303 µg/kg, and 340 µg/kg in Fischer 344 rats from Charles River Breeding Laboratories, Charles River CD, Frederick Cancer Research Center, and Harlan Industries, respectively (Walden and Schiller 1985); 165 µg/kg (males) and 125 µg/kg (females) in Osborne Mendel rats (NTP 1982b); 43 µg/kg in male Sprague-Dawley rats (Stahl et al. 1992), and 60 and 100 µg/kg in female and male Long Evans rats, respectively (Fan and Rozman 1995). A single gavage dose of 100 µg/kg caused death in 95% of exposed male Fischer 344 rats (Kelling et al. 1985), and a dose of 25 µg/kg led to the death of 25% of exposed male Sprague-Dawley rats (Seefeld et al. 1984a). Furthermore, the reported LD_{50} values were 4.2 µg/kg in minks (Hochstein et al. 1988), 115 µg/kg in New Zealand albino rabbits (Schwetz et al. 1973b), 1.75 µg/kg in male Hartley guinea pigs (McConnell et al. 1984), 0.6 µg/kg (males) and 2.1 µg/kg (females) in Hartley guinea pigs (Schwetz et al. 1973b), and 1,157 µg/kg (Olson et al. 1980a) or 5,051 µg/kg (Henck et al. 1981) in Syrian hamsters. A 42-day LD_{50} of 2.5 µg/kg was calculated for female Hartley guinea pigs when 2,3,7,8-TCDD was administered in corn oil and 19 µg/kg when administered in methyl cellulose (Silkworth et al. 1982). No effect on survival was observed after a single oral dose of 200 µg/kg in B6C3F_1 mice (NTP 1982b), but 69% of C57BL/6 mice died following exposure to 360 µg/kg (Kelling et al. 1985), and an LD_{50} was calculated as 146 µg/kg 2,3,7,8-TCDD in male C57BL mice (Smith et al. 1981). An acute LD_{50} in excess of 3,000 µg/kg was reported for male DBA/2J mice (Weber et al. 1995). Increased lethality was observed in Hartley guinea pigs exposed to 0.03 µg/kg/day 2,3,7,8-TCDD in the feed for 11 days (DeCaprio et al. 1986) and in pregnant rabbits following 10 daily doses of 1 µg/kg during gestation (Giavini et al. 1982). Beagle dogs survived a single dose of 300 µg/kg but not 3,000 µg/kg (Schwetz et al. 1973). In addition, 3 of 12 pregnant rhesus

monkeys died following a single dose of 1 µg/kg (McNulty 1984). It is evident from the above results that guinea pigs were the most sensitive species, while hamsters were the most resistant (up to 5,000 times greater lethal doses). In all studies cited above, the animals died following a latency period of several days (mean values varied from 9 to 43). In almost all laboratory animals, a pronounced wasting syndrome appears to be a major contributor to lethality.

In the intermediate-duration experiments, increased lethality was observed in Osborne Mendel rats exposed to 2,3,7,8-TCDD by gavage in oil vehicle at 0.56 µg/kg/day for up to 13 weeks (NTP 1982b). Mortality of 5% (no deaths in controls) was observed in Sprague-Dawley rats administered 2,3,7,8-TCDD by gavage at a rate of approximately 0.8 µg 2,3,7,8-TCDD/kg/day for 13 weeks (Viluksela et al. 1994); the first death occurred on day 57. Four of 7 male Sprague-Dawley rats dosed by gavage with approximately 1.6 µg 2,3,7,8-TCDD/kg/day died in a 10-week study (Li and Rozman 1995); the mean time to death was 53.5 days. Increased mortality was reported in Hartley guinea pigs exposed daily for up to 60 days to diets that provided 0.03 µg/kg/day (DeCaprio et al. 1986); 4 of 10 males died by day 42 and 4 of 10 females by day 59. In a dietary study, all male Sprague-Dawley rats that received the diet that provided the highest doses (3.4 µg/kg/day or more) died within 4 weeks (Van Miller et al. 1977). C57BL/6 mice had decreased survival following exposure by gavage to 3 µg/kg/day of 2,3,7,8-TCDD 3 days a week for 25 weeks (Umbreit et al. 1987). Two monkeys were exposed intermittently by gavage to 0.6 µg/kg/day of 2,3,7,8-TCDD for 3 weeks and both died (McNulty 1984); 5 of 8 monkeys died within 2 months following exposure to diets that provided 0.02 µg/kg/day (Hong et al. 1989); also, 5 of 8 monkeys died within 9 months of dietary exposure to 0.011 µg/kg/day (Allen et al. 1977). In all species, severe weight loss and body fat depletion were experienced prior to death, but usually no other overt toxic signs were observed. Pancytopenia, a secondary effect, was the cause of death in monkeys.

Decreased survival was reported after chronic exposure to CDDs. Chronic dietary exposure to 2,3,7,8-TCDD increased the mortality over controls in Sprague-Dawley rats at 0.1 µg/kg/day (Kociba et al. 1978a). Increased mortality also occurred in Swiss mice given 2,3,7,8-TCDD by gavage at 1.0 µg/kg/day (Toth et al. 1979) and in $B6C3F_1$ mice at 0.36 µg/kg/day (Della Porta et al. 1987). In both studies, the mice were dosed once a week for 1 year and followed for the rest of their lives or until 110 weeks of age. No treatment-related effects on survival were observed in Osborne-Mendel rats or in $B6C3F_1$ mice administered up to 0.25 µg 2,3,7,8-TCDD/kg/day, 2 days a week by gavage for 104 weeks(0.071 µg/kg/day for rats and male mice; 0.3 µg/kg/day for female mice) (NTP 1982b).

Increased mortality occurred after acute exposure to other congeners. After a single oral dose of a mixture of 1,2,3,7,8,9-HxCDD and 1,2,3,6,7,8-HxCDD, LD_{50} values were calculated as 1,800 µg/kg and 800 µg/kg in male and female Osborne-Mendel rats, respectively, and 750 µg/kg and 500 µg/kg in male and female $B6C3F_1$ mice, respectively (NCI/NTP 1980). In addition, LD_{50} values were calculated for several congeners in guinea pigs (29,444 µg/kg for 1,2,3-TrCDD, 1,125 µg/kg for 1,2,4,7,8-PeCDD, 3.1 µg/kg for 1,2,3,7,8-PCDD, 70–100 µg/kg for 1,2,3,6,7,8-HxCDD, 60–100 µg/kg for 1,2,3,7,8,9-HxCDD, and 72.5 µg/kg for 1,2,3,4,7,8-HxCDD) and in mice (825 µg/kg for 1,2,3,4,7,8-HxCDD and 337.5 µg/kg for 1,2,3,7,8-PCDD) following a single oral exposure by gavage in oil vehicle (McConnell et al. 1978b). In male Sprague-Dawley rats, the oral LD_{50} for 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD administered in corn oil/acetone (95/5) was 206, 887, and 6,325 µg/kg, respectively (Stahl et al. 1992). Other CDD congeners have a much lower order of toxicity, as evidenced by data showing no effects on mortality at much higher doses than those of 2,3,7,8-TCDD, TrCDD, HxCDD, or PCDD that cause death. No deaths were observed after a single oral dose of 1×10^6 and 2×10^6 µg/kg 2,7-DCDD in Sprague-Dawley rats and Swiss Webster mice, respectively (Schwetz et al. 1973). In addition, rats and mice survived acute oral doses of 1×10^6 and 4×10^6 µg/kg OCDD, respectively (Schwetz et al. 1973). The relative species differences in sensitivity for 2,3,7,8-TCDD also applied for other congeners.

Mortality rates of 15 and 50% were reported in groups of male Sprague-Dawley rats administered 73 and 110 µg 1,2,3,4,6,7,8-HpCDD/kg/day by gavage for 13 weeks, respectively (Viluksela et al. 1994). At the highest dose, the first death occurred on day 31; at the 73 µg/kg/day dose, on day 41. Fifteen out of 20 female Sprague-Dawley rats died during a 13-week treatment period with daily doses of approximately 2.6 µg 1,2,3,7,8-PeCDD/kg (total dose was 233 µg/kg) (Viluksela et al. 1998a). The first death occurred on day 16. The same mortality rate was observed in males treated with approximately 3.8 µg/kg/day (total dose was 350 µg/kg). In the same study, administration of approximately 10.3 µg 1,2,3,4,7,8-HxCDD resulted in a 25% death rate (5/20, first death on day 61) in female rats; the same death rate was seen among male rats treated with approximately 15.4 µg/kg/day (first death on day 24). The main causes of death were wasting syndrome, hemorrhage, and anemia (Viluksela et al. 1998a). No effects on survival were observed following chronic dietary exposure of Osborne-Mendel rats and $B6C3F_1$ mice to 5×10^5 µg/kg/day of 2,7-DCDD and to 1.3×10^6 µg/kg/day of 2,7-DCDD, respectively (NCI/NTP 1979a), or following chronic gavage dosing with a mixture of 1,2,3,7,8,9-HxCDD and 1,2,3,6,7,8-HxCDD at 0.34 µg/kg/day and 0.7 µg/kg/day, respectively (NCI/NTP 1980).

In conclusion, 2,3,7,8-TCDD was the most toxic of all congeners tested, and doses on the order of several µg/kg body weight have led to death in all species tested, except hamsters and dogs, in acute-exposure experiments. In contrast, of the congeners tested, 2,7-DCDD and OCDD were the least toxic as tested animals survived very high doses (g/kg body weight). The wasting syndrome was the major toxic effect of acute- and intermediate-duration exposure to CDDs in most species. It was characterized by body weight loss, adipose tissue depletion, and eventual death. In most of the chronic duration studies the cause of death was not determined.

The LD_{50} values and all reliable representative LOAEL values for death in each species and duration category for each congener tested are recorded in Tables 2-2 and 2-3 and plotted in Figures 2-1 and 2-2.

2.2.2.2 Systemic Effects

The highest NOAEL values and all reliable representative LOAEL values for each systemic effect in each species and duration category for each congener tested are recorded in Tables 2-2 and 2-3 and plotted in Figures 2-1 and 2-2.

Respiratory Effects. Few studies have examined the respiratory system in animals following oral exposure to CDDs. However, serious respiratory effects have been observed in monkeys that died from 2,3,7,8-TCDD exposure.

Bleeding from the nose was reported in rhesus monkeys exposed via gavage to 0.1 µg/kg/day, 3 days a week for 3 weeks (McNulty 1984). Hemorrhage, hyperplasia, and metaplasia of the bronchial epithelium (as well as at other organ sites that had mucous-secreting cells) developed in monkeys exposed to diets providing 0.011 µg/kg/day for 9 months (Allen et al. 1977); 5 of 8 monkeys died with this dose level. Focal alveolar hyperplasia and squamous metaplasia and carcinoma were reported in Sprague-Dawley rats chronically exposed to 0.1 µg/kg/day 2,3,7,8-TCDD in the feed (Kociba et al. 1978a). Since powdered feed containing 2,3,7,8-TCDD was given to the rats, there is a distinct possibility that the respiratory effects were attributable to inhalation exposure rather than oral systemic absorption. In contrast, no respiratory effects were observed in rats or mice chronically exposed by gavage to 2,3,7,8-TCDD at approximately 0.071 µg/kg/day or 0.3 µg/kg/day, respectively (NTP 1982b).

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
	ACUTE EXPOSURE						
	Death						
1	Monkey (Rhesus)	once (GO)				70 (1/3 died)	McConnell et al. 1978
2	Monkey (Rhesus)	once (GO)				1.0 (3/12 dams died)	McNulty 1984
3	Rat (Long- Evans)	once (GO)				60 F (LD_{50})	Fan and Rozman 1995
4	Rat (Fischer- 344)	once (GO)				100 M (95% died)	Kelling et al. 1985
5	Rat (Osborne-Mendel)	once (GO)				165 M (LD_{50})	NTP 1982b
						125 F (LD_{50})	
6	Rat (Sherman)	once (GO)				22 M (LD_{50})	Schwetz et al. 1973
						45 F (LD_{50})	
7	Rat (Sprague-Dawley)	once (GO)				25 M (25% died)	Seefeld et al. 1984
8	Rat (Sprague-Dawley)	once (GO)				43 (LD_{50})	Stahl et al. 1992

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL		Reference
					Less Serious (ug/kg/day)	Serious (ug/kg/day)	
9	Rat (Fischer- 344)	once (GO)				164 M (LD_{50})	Walden and Schiller 1985
10	Mouse (C57BL/6)	once (GO)				360 M (69% died)	Kelling et al. 1985
11	Mouse (C57BL)	once (GO)				146 M (LD_{50})	Smith et al. 1981
12	Mouse (C57BL/6N)	once (GO)				100 M (LD_{50})	Weber et al. 1995
13	Mouse (DBA/2)	once (GO)				>3000 M (LD_{50})	Weber et al. 1995
14	Gn pig (Hartley)	11 d (F)				0.03 (10% died)	Decaprio et al. 1986
15	Gn pig (Hartley)	once (GO)				1.75 M (LD_{50})	McConnell et al. 1984
16	Gn pig (Hartley)	once (GO)				0.6 M (LD_{50}) 2.1 F (LD_{50})	Schwetz et al. 1973
17	Hamster (Syrian)	once (GO)				5051 M (LD_{50})	Henck et al. 1981
18	Hamster (Golden Syrian)	once (GO)				1157 (LD_{50})	Olson et al. 1980a

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
19	Dog (Beagle)	once (C)				3000 M (2/2 died)	Schwetz et al. 1973
20	Rabbit (New Zealand)	Gd 6-15 1 x/d (GO)				1 F (4/10 dams died)	Giavini et al. 1982
21	Rabbit (New Zealand)	once (GO)				115 (LD_{50})	Schwetz et al. 1973
22	Mink	once (GO)				4.2 M (LD_{50})	Hochstein et al. 1988
	Systemic						
23	Monkey (Rhesus)	once (GO)	Cardio		70 F (reduced heart weight)		McConnell et al. 1978
			Gastro		70 F (epithelial hyperplasia of the stomach)		
			Hemato		70 F (mild anemia)		
			Hepatic		70 F (increased liver weight)		
			Renal		70 F (epithelial hyperplasia in the renal pelvis)		
			Dermal		70 F (blepharitis, acne, facial alopecia)		
			Bd Wt			70 F (28% weight loss)	

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
24	Rat (Sprague-Dawley)	once (GO)	Endocr		25 (decreased corticosterone levels on days 14 and 21 after dosing)		Balk and Piper 1984
25	Rat (Sprague-Dawley)	once (GO)	Gastro		100 M (malabsorption of glucose)		Ball and Chabra 1981
			Bd Wt	5 M		100 M (25% decrease weight)	
26	Rat (Sprague-Dawley)	once (GO)	Endocr		50 M (increased serum ACTH; increased serum corticosterone on days 1 and 5 but decrease on days 10 and 14)		Bestervelt et al. 1993
27	Rat (Sprague-Dawley)	once (GO)	Cardio	75 M			Christian et al. 1986a
			Gastro	75 M			
			Hepatic		25 M (enlarged hepatocytes)		
			Renal		25 M (dilated convoluted tubules)		
			Bd Wt			25 M (36-48% body weight loss)	

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
28	Rat (Wistar)	1 x/d 10 d (GO)	Bd Wt	25 M	50 M (significant but unspecified reduction in body weight gain)		De Heer et al. 1994a
29	Rat (Wistar)	once (GO)	Bd Wt	25 M			De Heer et al. 1994b
30	Rat (Long- Evans)	once (GO)	Endocr	5.3 F	12 F (44% decrease in serum total T4 four days after dosing)		Fan and Rozman 1995
31	Rat (CRCD)	2 wk 7 d/wk 1 x/d (GO)	Bd Wt	0.125		0.5 (28% reduced weight gain in dams)	Giavini et al. 1983
32	Rat (Sprague-Dawley)	once (GO)	Hepatic		6.25 (altered vitamin A storage)		Hakansson et al. 1989c
33	Rat (Sprague-Dawley)	once (GO)	Hepatic		100 (ED_{50} for liver enlargement)		Hanberg et al. 1989
			Bd Wt		89 (ED_{50} for reduced body weight gain)		

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL				Reference
					Less Serious (ug/kg/day)		Serious (ug/kg/day)		
34	Rat (Sprague-Dawley)	3 d 1 x/d (GO)	Cardio				40 F	(27% decrease heart rate, 20% decrease mean blood pressure)	Hermansky et al. 1988
			Hepatic				40 F	(hepatocyte enlargement, focal sinusoidal dilation, pericentral acinar necrosis, nuclear vacuolation, portal inflammation)	
			Endocr		40 F	(decreased thyroxine levels)			
35	Rat (Fischer- 344)	once (GO)	Hepatic		100 M	(degenerative changes)			Kelling et al 1985
			Bd Wt				100 M	(40% weight loss)	
36	Rat (Sprague-Dawley)	once (GO)	Cardio		6.25 M	(increased basal tension of the left atria)			Kelling et al. 1987
37	Rat (Sprague-Dawley)	once (GO)	Endocr		50 M	(decreased adrenal 21-hydroxylase activity)			Mebus and Piper 1986
38	Rat (Sprague-Dawley)	once (GO)	Bd Wt		6.25 M	(10% weight loss)	100 M	(23% weight loss)	Moore et al. 1985

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
39	Rat (Sprague-Dawley)	once (GO)	Renal	5 M			Pegg et al. 1976
40	Rat (Sprague-Dawley)	once (GO)	Endocr	0.032 M	0.32 M (decreased serum T4 and T3 levels)		Roth et al. 1988
			Bd Wt	3.2 M		10.6 M (30% decrease body weight gain)	
41	Rat (Sprague-Dawley)	once (GO)	Bd Wt			15 M (100% decreased body weight gain)	Seefeld and Peterson 1984
42	Rat (Sprague-Dawley)	once (GO)	Bd Wt		5 M (transiently decreased body weight gain)	50 M (50% body weight loss)	Seefeld et al. 1984a
43	Rat (Sprague-Dawley)	once (GO)	Bd Wt			15 M (60% decreased weight gain)	Seefeld et al. 1984b
44	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)	Bd Wt	0.125 F	0.5 F (decreased body weight gain in dams)		Sparschu et al. 1971
45	Rat (Sprague-Dawley)	once (GO)	Gastro		19 M (increased weight of antral mucosa)		Theobald et al. 1991

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL		Reference
					Less Serious (ug/kg/day)	Serious (ug/kg/day)	
46	Rat (Fischer- 344)	once (GO)	Hepatic		45 M (hyperlipidemia, hypoglycemia)		Walden and Schiller 1985
			Bd Wt			164 M (38.9% body weight loss at lethal dose)	
47	Rat (CD)	10-14 d 1 x/d (GO)	Hemato		10 (increase in packed cell volume, erythrocytes, neutrophils; decrease in mean corpuscular hemoglobin and platelet count)		Weissberg and Zinkl 1973
48	Mouse (CD-1)	10 d Gd 7-16 1 x/d	Hepatic		25 F (increased liver weight in dams)		Courtney 1976
			Bd Wt	50 F		100 F (22% decrease weight gain in dams)	
49	Mouse (CD-1)	14 d 1 x/d (GO)	Bd Wt	10 F			Courtney 1976
50	Mouse (B6C3F1)	once (GO)	Hepatic	1 F	10 F (23% increase in liver weight; significant induction of CYP1A1 and 1A2 activities)		Diliberto et al. 1995
			Bd Wt	10 F			

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
51	Mouse (A2G-hr/+)	once (GO)	Hepatic			75 (3-fold increase in serum ALT, 100-fold increase in hepatic porphyrins, paracentral necrotic foci, fatty changes in midzonal region)	Greig 1984, 1987
			Dermal		75 (skin thickening)		
52	Mouse (C57BL/6)	once (GO)	Hepatic		1000 (ED_{50} for liver enlargement)		Hanberg et al. 1989
			Bd Wt		890 (ED_{50} for reduced body weight gain)		
53	Mouse (B6C3F1)	14 d 1 x/d (GO)	Resp	1 F			Holsapple et al. 1986
			Hemato	1 F			
			Hepatic		1 F (hydropic degeneration, increased liver weight induced microsomal enzymes)		
			Renal	1 F			

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
54	Mouse (C57BL/6N)	once (G)	Hepatic	0.5 M	15 M (35% increase in relative liver weight in young mice; significant induction of hepatic EROD and ACOH activities)		Pegram et al. 1995
			Bd Wt	15 M			
55	Mouse (C57BL/6J)	10 d Gd 6-15 1 x/d (GO)	Hepatic		0.5 F (38% increase in relative liver weight)		Silkworth et al. 1989b
			Bd Wt	8 F			
56	Mouse (CF-1)	10 d Gd 6-15 1 x/d (GO)	Hepatic	1.0 F	3.0 F (increased liver weight in dams)		Smith et al. 1976
			Bd Wt	3.0 F			
57	Mouse (C57BL/10)	once (GO)	Hepatic	15		50 (50-fold increase in hepatic porphyrins)	Smith et al. 1981

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
58	Mouse (C57BL/6N)	once (GO)	Hepatic	1 M	3 M (increased relative liver weight and EROD activity; decreased PEPCK activity)		Weber et al. 1995
			Renal	235 M			
			Endocr	0.03 M	0.1 M (significant decrease in serum T3 and T4)		
			Bd Wt	235 M			
59	Mouse (DBA/2)	once (GO)	Hepatic	10 M	97.5 M (increased liver weight and EROD activity; decreased PEPCK activity)		Weber et al. 1995
			Renal	1500 M	1950 M (decreased kidney weight)		
			Endocr	10 M	97.5 M (significant decrease in serum T3 and T4)		
			Bd Wt	375 M	1500 M (17% decrease in weight gain)		
60	Mouse (CD-1)	once (GO)	Hemato		1 F (reversible decreases in leuko- and lymphocyte counts)		Zinkl et al. 1973

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
61	Gn pig (Hartley)	once (GO)	Bd Wt		1.8 (ED_{60} for reduced body weight gain)		Hanberg et al. 1989
62	Gn pig (Hartley)	once (G)	Hepatic		0.1 (focal necrosis)		Turner and Collins 1983
63	Hamster (Golden Syrian)	once (GO)	Hepatic		14 (ED_{50} for liver enlargement)		Hanberg et al. 1989
			Bd Wt		1000 (ED_{60} for reduced body weight gain)		
64	Hamster (Syrian)	once (GO)	Gastro	6000 M			Henck et al. 1981
			Dermal	600 M	1000 M (rough hair)		
			Bd Wt	600 M	1000 M (decreased body weight gain)		
65	Rabbit (New Zealand)	Gd 6-15 1 x/d (GO)	Bd Wt	0.1 F		0.25 F (44% decreased weight gain in dams)	Giavini et al. 1982

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
66	mink	once (GO)	Cardio	2.5 M	5 M (increased heart relative weight)		Hochstein et al. 1988
			Gastro	2.5 M		5 M (gastrointestinal ulcerations)	
			Hemato	7.5 M			
			Hepatic	2.5 M	5 M (pale liver)		
			Renal	2.5 M	5 M (increased kidney relative weight)		
			Endocr		2.5 M (increased adrenal absolute weight)		
			Bd Wt		2.5 M (11% weight loss)	5 M (27% weight loss)	
	Immunological/Lymphoreticular						
67	Monkey (Rhesus)	once (GO)				70 F (severe atrophy of the thymus)	McConnell et al 1978a
68	Rat (Sprague-Dawley)	once (GO)			25 M (reduced germinal centers and increased hemosiderin deposits in the spleen)		Christian et al. 1986a
69	Rat (Wistar)	1 x/d 10 d (GO)		1 M	5 M (reduced relative thymus weight)		De Heer et al. 1994a

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
70	Rat (Wistar)	once (GO)			25 M (reversible thymic atrophy starting on day 13)		De Heer et al. 1994b
71	Rat (Wistar)	1 x/d 4 d (GO)			1 M (reduced number of immature CD4CD8 double positive thymocytes; decreased thymus weight)		De Heer et al. 1994b
72	Rat (Wistar)	once (GO)			50 M (decreased absolute thymus weight)		DeWall et al. 1992
73	Rat (Sprague-Dawley)	once (GO)			10 M (altered cell-mediated immunity judged by an increased delayed-type hypersensitivity reaction)		Fan et al. 1996
74	Rat (Sprague-Dawley)	once (GO)				26 (ED_{50} for thymic atrophy)	Hanberg et al. 1989
75	Rat (Fischer- 344)	1 x/d 14 d (GO)			0.72 F (supression in virus-augmented NK cell activity)		Yang et al. 1994

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
76	Mouse (B6C3F1)	once (GO)			5 (reduced polymorphonuclear activity)		Ackermann et al. 1989
77	Mouse (C57BL/6)	9 d Gd 6-14 1 x/d (GO)				1.5 F (inhibition of thymocyte maturation fetal thymocytes)	Blaylock et al. 1992
78	Mouse (B6C3F1)	once (GO)		0.005[b] F	0.01 F (decreased influenza virus host resistance)		Burleson et al. 1996
79	Mouse (C57BL/6N)	once (GO)		2.5 M	5 M (decreased cytotoxic T lymphocyte activity)		De Krey and Kerkvliet 1995
80	Mouse (B6C3F1)	once (GO)		1 F	10 F (28% decrease in thymus weight 14 days after dosing)		Diliberto et al. 1995
81	Mouse (C57BL/6)	once (GO)				280 (ED_{50} for thymic atrophy)	Hanberg et al. 1989
82	Mouse (B6C3F1)	once (GO)		0.5 F	1.0 F (suppressed antibody response)		Holsapple et al. 1986
83	Mouse (B6C3F1)	once (GO)			20 F (18% reduction in serum Complement 3)		Lin and White 1993

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
84	Mouse (C57BL/6J)	10 d Gd 6-15 1 x/d (GO)		0.5 F	1 F (decreased relative thymus weight)		Silkworth et al. 1989b
85	Mouse (B6C3F1)	once (GO)			14 F (suppressed serum complement activity)		White et al. 1986
86	Mouse (B6C3F1)	14 d 1 x/d (GO)			0.01 F (suppressed serum complement activity)		White et al. 1986
87	Gn pig (Hartley)	once (GO)				0.8 (ED_{50} for thymic atrophy)	Hanberg et al. 1989
88	Gn pig (NS)	once (GO)				6 (thymic atrophy)	Umbreit et al. 1985
89	Hamster (Golden Syrian)	once (GO)				48 (ED_{50} for thymic atrophy)	Hanberg et al. 1989
	Neurological						
90	Rat (CRCD)	2 wk 7 d/wk 1 x/d (GO)		0.5	2 (decreased activity in dams)		Giavini et al. 1983
91	Rat (Sprague-Dawley)	once (GO)			5 M (decreased motor activity)		Seefeld et al. 1984a

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
92	Mink	once (GO)		2.5 M	5 M (increased brain relative weight)		Hochstein et al. 1988
	Reproductive						
93	Monkey (Rhesus)	once (GO)				1.0 (abortions in 10/12)	McNulty 1984
94	Rat (Sprague-Dawley)	once (GO)				10 M (ED_{50} altered regulation of leutinizing hormone secretion)	Bookstaff et al. 1990a
95	Rat (CRCD)	2 wk 7 d/wk 1 x/d (GO)		0.125		0.5 F (increased pre- and post-implantation loss)	Giavini et al. 1983
96	Rat (Wistar)	7 d 1x/d (GO)			4 (epididymal inflammation)		Khera and Ruddick 1973
97	Rat (Sprague-Dawley)	once 1 x/d (GO)		3 F		10 F (increased luteinizing and follicle stimulating hormone levels, altered ovulation)	Li et al. 1995a
98	Rat (Sprague-Dawley)	once 1 x/d (GO)				10 F (irregular estrous cycle and ovulation)	Li et al. 1995b

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
99	Rat (Sprague-Dawley)	once (GO)			4.5 M (decreased seminal vesicle weight)		Moore et al. 1985
100	Rat (Sprague-Dawley)	once (GO)				12.5 M (decreased plasma testosterone levels)	Moore et al. 1985
101	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)		0.03 F		0.125 F (increased resorptions)	Sparschu et al. 1971
102	Mouse (CF-1)	10 d Gd 6-15 1 x/d (GO)		0.1 F		1.0 F (increased resorptions)	Smith et al. 1976
103	Rabbit (New Zealand)	10 d Gd 6-15 1 x/d (GO)		0.1		0.25 (increased post- implantation loss)	Giavini et al. 1982
	Developmental						
104	Monkey (Rhesus)	Gd 40 1 x/d (GO)				1 (2/3 fetuses died)	McNulty 1984
105	Rat (Holtzman)	Gd 15 1 x/d (GO)				1.0 M (demascularization and feminization of sexual behavior; delayed puberty)	Bjerke and Peterson 1994

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
106	Rat (Holtzman)	Gd 15 1 x/d (GO)				0.7 M (impaired development of reproductive system)	Bjerke et al. 1994a
107	Rat (Holtzman)	Gd 15 1 x/d (GO)				0.7 M (demascularization and feminization of sexual behavior)	Bjerke et al. 1994b
108	Rat (Sprague-Dawley)	25, 27, 29, and 31 days of age 1 x/d (GO)			2.5 F (decreased mammary gland size)		Brown and Lamartiniere 1995
109	Rat (Sprague-Dawley)	once Gd 15 (GO)				1 F (alteration in mammary gland differentiation in 50-day old pups; increased number of chemically-induced mammary adenocarcinomas in pups)	Brown et al. 1998
110	Rat (Holtzman)	once (GO)			1 F (decreased serum estrogen levels in female offspring)		Chaffin et al. 1996
111	Rat (Holtzman)	once (GO)		1 F			Chaffin et al. 1997

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL				Reference
					Less Serious (ug/kg/day)		Serious (ug/kg/day)		
112	Rat (F344)	Ld 0, 7, 14 1 x/d (GO)					5	(reversible suppression of cell-mediated immunity in offspring)	Faith and Moore 1977
113	Rat (Fischer- 344)	once (GO)			1	(alterations in lymphocyte phenotypes)			Gehrs et al. 1979a
114	Rat (Fischer- 344)	once (GO)			3	(alterations in lymphocyte phenotypes)			Gehrs et al. 1979b
115	Rat (Fischer- 344)	once (GO)			1	(alterations in lymphocyte phenotypes and decreased DTH response)			Gehrs et al. 1979b
116	Rat (CRCD)	2 wk 7 d/wk 1 x/d (GO)		0.5			2	(reduced number of live fetuses, fetal malformation)	Giavini et al. 1983
117	Rat (Long- Evans)	Gd 15 1 x/d (GO)			1 M	(decreased core body temperature)			Gordon et al. 1995

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
118	Rat (Long- Evans)	Gd 8 1 x/d (GO)				1 F (malformations of external genitalia, decreased fertility, shortened reproductive lifespan)	Gray and Ostby 1995
119	Rat (Long- Evans)	Gd 15 1 x/d (GO)				1 F (malformations of external genitalia, decreased fertility)	Gray and Ostby 1995
120	Rat (Holtzman)	Gd 15 1 x/d (GO)				1 F (malformations of external genitalia, decreased fertility)	Gray and Ostby 1995
121	Rat (Long- Evans)	Gd 8 or 15 1 x/d (GO)				1 M (impaired development of reproductive system)	Gray et al. 1995
122	Rat (Long- Evans)	once (GO)		0.05 F	0.20 F (urogenital morphological alterations, presence of vaginal thread, and cleft phallus)		Gray et al. 1997a

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

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL				Reference
					Less Serious (ug/kg/day)		Serious (ug/kg/day)		
123	Rat (Long- Evans)	once (GO)			1 F	(urogenital morphological alterations, presence of vaginal thread, and cleft phallus)			Gray et al. 1997a
124	Rat (Long- Evans)	once (GO)			0.05 M	(reduction in ejaculated sperm count)			Gray et al. 1997a
125	Rat (Sprague-Dawley)	Ld 1 (GO)					10	(thymic atrophy, liver enlargement, and decreased weight gain in pups)	Hakansson et al. 1987
126	Rat (Holtzman)	once (GO)			1 F	(decreased number of antral and preantral ovarian follicles)			Heimler et al. 1998
127	Rat (Long- Evans)	Gd 8 1 x/d (GO)		1			5	(cleft palate, thymic atrophy, decreased number of live fetuses)	Huuskonen et al. 1994

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
128	Rat (Han/Wistar)	Gd 8 1 x/d (GO)		1		10 (hydronephrosis, thymic atrophy, gastrointestinal hemorrhage, decreased number of live fetuses)	Huuskonen et al. 1994
129	Rat (Han/Wistar)	Gd 12 1 x/d (GO)		1		10 (hydronephrosis, gastrointestinal hemorrhages, decreased number of live fetuses)	Huuskonen et al. 1994
130	Rat (Wistar)	10 d Gd 6-15 1 x/d (GO)		0.125		0.25 (subcutaneous edema, hemorrhages in GI tract and brain)	Khera and Ruddick 1973
131	Rat (Holtzman)	Gd 15 (GO)			0.064 (decreased testosterone)	0.16 M (delayed testis descent) 0.40 M (decreased growth during lactation--male pups)	Mably et al. 1992a
132	Rat	Gd 15 (GO)				0.064 (decreased masculine sexual behavior in male offspring)	Mably et al. 1992b
133	Rat	Gd 15 (GO)				0.064 M (reduced sperm production in offspring at all ages)	Mably et al. 1992c

[a]

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
134	Rat (Sprague-Dawley)	Gd10 (GW)				1.5 (fetal mortality, thymic dysfunction)	Olson and McGarrigle 1992
						3.6 (cleft palate)	
135	Rat (Sprague-Dawley)	Gd 10-16 1x/d (GO)		0.025 F	0.1 F (decreased thyroxine levels)		Seo et al. 1995
136	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)		0.03		0.125 (intestinal hemorrhage in fetuses)	Sparschu et al. 1971
137	Mouse (C57BL/6N)	Gd 10 or 12 1 x/d (GO)				6 (cleft palates)	Abbott and Birnbaum 1989
138	Mouse (C57BL/6N)	Gd 10 (GO)				12 (fetal hydronephrosis, hydroureter)	Abbott et al. 1987a
139	Mouse (C57BL/6N)	Gd 10-13 1 x/d (GO)		3			Abbott et al. 1992
140	Mouse (C57B1/6)	Gd 6-14 1 x/d (GO)				.1.5 M (thymic atrophy and delayed thymocyte maturation)	Blaylock et al. 1992

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
141	Mouse (C57BL/6J)	Gd 9 1 x/d (GO)				15 (cleft palate)	Dasenbrock et al. 1992
142	Mouse (DBA2J)	Gd 9 1 x/d (GO)				150 (cleft palate)	Dasenbrock et al. 1992
143	Mouse (B6C3F1)	9 d Gd 6-14 (GO)				1.5 (immunosupression in pups, thymic atrophy, abnormal fetal thymoctye-maturation)	Holladay et al. 1991
144	Mouse (B6C3F1)	Gd 14 Ld 1, 7, 14 1 x/d (GO)			1 (increased neutrophils in pups)	5 (bone marrow toxicity)	Luster et al. 1980
145	Mouse (C57B1/6)	Gd 10 1 x/d (GO)				1 (hydronephrosis)	Moore et al. 1973
146	Mouse (NMR1)	4 d Gd 14-17 1 x/d (GO)				12.5 (75% pup mortality)	Nau et al. 1986
147	Mouse (NMRI)	10 d Gd 6-15 1 x/d (GO)		0.3		3.0 (cleft palates)	Neubert and Dillmann 1972

[a]

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)		Reference
148	Mouse (C57BL/6J, DBA/2J)	10 d Gd 6-15 1 x/d (GO)				0.5	(hydronephrosis)	Silkworth et al. 1989b
149	Mouse (CF-1)	10 d Gd 6-15 1 x/d (GO)		0.1		1.0	(cleft palate)	Smith et al. 1976
150	Gn pig (Hartley)	Gd 14 (GO)		0.15		1.5	(fetal mortality, increase resorption, decreased spleen and thymus weight)	Olson and McGarrigle 1992
151	Hamster (Golden Syrian)	Gd 11 1 x/d (GO)				2 M	(impaired development of reproductive system)	Gray et al. 1995
						2 M	(nephrosis)	
152	Hamster (Golden Syrian)	Gd 7 or 9 (GO)				1.5	(hydronephrosis, thymic dysfunction)	Olson and McGarrigle 1992
						18	(fetal mortality)	
153	Rabbit (New Zealand)	10 d Gd 6-15 1 x/d (GO)				0.1	(skeletal anomalies hydronephrosis)	Giavini et al. 1982

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
INTERMEDIATE EXPOSURE							
	Death						
154	Monkey (Rhesus)	9 mo 7 d/wk (F)				0.011 F (5/8 died)	Allen et al. 1977
155	Monkey (Rhesus)	2-33 mo 7 d/wk (F)				0.02 (5/8 died by 2 months)	Hong et al. 1989
156	Monkey (Rhesus)	3 wk 3 d/wk 1 x/d (GO)				0.6 (2/2 died)	McNulty 1984
157	Rat (Sprague-Dawley)	10 wk 1 x/ wk (GO)				1.6 M (4/7 deaths; mean time to death was 54 days)	Li and Rozman 1995
158	Rat (Sprague-Dawley)	48 wk 7 d/wk (F)				3.4 M (100% mortality by 3rd week)	Van Miller et al. 1977
159	Mouse (C57B/6)	25 wk 3 d/wk 1 x/d (GO)				3 F (70% died)	Umbreit et al. 1987
160	Gn pig (Hartley)	35 d 7 d/wk (F)				0.8 (LD_{50} for 27 days)	DeCaprio et al. 1986

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL		Reference
					Less Serious (ug/kg/day)	Serious (ug/kg/day)	
	Systemic						
161	Monkey (Rhesus)	9 mo 7 d/wk (F)	Resp			0.011 F (lung hemorrhage)	Allen et al. 1977
			Cardio			0.011 F (hemorrhage in epicardium, myocardium, and endocardium)	
			Gastro			0.011 F (gastric ulcers)	
			Hemato			0.011 F (pancytopenia, bone marrow atrophy)	
			Musc/skel			0.011 F (hemorrhage in muscles from the extremities)	
			Hepatic		0.011 F (epithelial biliary hyperplasia)		
			Renal		0.011 F (tubular epithelial hyperplasia)		
			Dermal			0.011 F (periorbital edema, facial alopecia, squamous metaplasia, hyperkeratoses; subcutaneous edema)	
			Bd Wt		0.011 F (12% weight loss)		

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL		Reference
					Less Serious (ug/kg/day)	Serious (ug/kg/day)	
162	Monkey (Rhesus)	3 wk 3 d/wk 1 x/d (GO)	Resp	0.02 F	0.1 F (epistaxis)		McNulty 1984
			Gastro	0.02 F	0.1 F (metaplasia of gastric mucosa)		
			Hemato	0.02 F		0.1 F (anemia, bone marrow hypoplasia)	
			Hepatic		0.1 F (biliary hyperplasia)		
			Dermal	0.02 F		0.1 F (hair loss, periorbital edema, hyperkeratosis, squamous metaplasia of sebaceaus glands)	
			Bd Wt	0.02 F		0.1 F (weight loss)	
163	Rat (Sprague-Dawley)	16 wk 1 x/wk (GO)	Hepatic	0.01 F	0.14 F (hepatic porphyria)		Goldstein et al. 1982
			Bd Wt	0.01 F	0.14 F (16% reduction in body weight gain)		
164	Rat (Sprague-Dawley)	10 wk 1 x/ wk (GO)	Endocr	0.003 M	0.03 M (almost 50% reduction in total serum T4)		Li and Rozman 1995
			Bd Wt	0.03 M		0.2 M (38% decrease in body weight gain)	

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL		Reference
					Less Serious (ug/kg/day)	Serious (ug/kg/day)	
165	Rat (Osborne-Mendel)	13 wk 2 d/wk 1 x/d (GO)	Hepatic		0.07 F (unspecified lesions in female)		NTP 1982b
			Bd Wt		0.07 (unspecified decrease in weight gain)		
166	Rat (Sprague-Dawley)	30 wk 1 x/2 wk (GO)	Endocr	0.011 F	0.036 F (reduction in serum T4)		Sewall et al. 1995
167	Rat (Sprague-Dawley)	13 wk (F)	Hepatic		0.014 F (reduction in hepatic retinol)		Van Birgelen et al. 1995
			Renal	0.026 F	0.047 F (increased relative kidney weight)		
			Endocr	0.026 F	0.047 F (reduction in total serum T4)		
			Bd Wt	0.026 F	0.047 F (10% reduction in body weight gain)	1.02 F (72% reduction in body weight gain)	

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL		Reference
					Less Serious (ug/kg/day)	Serious (ug/kg/day)	
168	Rat (Sprague-Dawley)	13 wk 1 x/2 wk (GO)	Hemato			0.8 M (decrease in platelet count; increase in prothrombin time in some rats)	Viluksela et al. 1994
			Hepatic		0.8 M (increased relative liver weight and liver EROD activity; decreased liver PEPCK activity)		
			Endocr		0.8 M (decrease in total serum T4)		
			Bd Wt			0.8 M (30% decrease in body weight gain)	
169	Rat (CD)	6 wk 1 d/wk 1 x/d (GO)	Hemato	0.71 F			Vos et al. 1973
			Bd Wt	0.14 F	0.71 F (14% decreased body weight gain)		
170	Rat (CD)	30 d 1 x/d (GO)	Hemato		0.1 F (trombocytopenia)		Zinkl et al. 1973
			Hepatic	0.1 F	1 F (increased serum transaminase)		

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
171	Mouse (B6C3F1)	13 wk 5 d/wk (F)	Hepatic	0.150 F			DeVito et al. 1994
			Bd Wt	0.150 F			
172	Mouse (B6C3F1)	13 wk 2 d/wk 1 x/d (GO)	Hepatic	0.28 F	0.7 F (unspecified lesions in females)		NTP 1982b
173	Mouse (C57BL/6Jfh)	4 wk 1 d/wk 1 x/d (GO)	Hepatic	5 M	10 M (hepatocellular cytomegaly)		Thigpen et al. 1975
			Bd Wt	10 M		20 M (36% decreased weight gain)	
174	Mouse (Swiss-Webster)	10 wk 7 d/wk (F)	Dermal	0.65 F	1.3 F (alopecia, edema in dams)		Thomas and Hinsdill 1979
175	Mouse	4 wk 1 d/wk 1 x/d (GO)	Bd Wt	0.71 M	3.6 M (17% reduced weight gain)		Vos et al. 1973

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)		LOAEL Serious (ug/kg/day)		Reference
176	Gn pig (Hartley)	90 d 7 d/wk (F)	Hemato	0.005					DeCaprio et al. 1986
			Hepatic	.0007	0.005	(hepatocellular inclusions, hypertriglyceridemia)			
			Bd Wt	.0007	0.005	(15-20% reduced weight gain)			
177	Gn pig (Hartley)	8 wk 1 d/wk 1 x/d (GO)	Hemato		0.001 F	(decreased lymphocytes)			Vos et al. 1973
			Bd Wt	0.006	0.03 F	(14% decreased body weight gain)			
	Immunological/Lymphoreticular								
178	Monkey (Rhesus)	9 mo 7 d/wk (F)					0.011	(lymph nodes atrophy)	Allen et al. 1977
179	Rat (Sprague-Dawley)	90 d (F)		0.001	0.01	(decreased thymus weight in F3)			Murray et al 1979

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
180	Rat (Sprague-Dawley)	13 wk (F)			0.014 F (21% reduction in final thymus weight)	1.02 F (80% reduction in final thymus weight)	Van Birgelen et al. 1995
181	Rat (Sprague-Dawley)	13 wk 1 x/2 wk (GO)			0.8 M (significant reduction in absolute and relative thymus weight)		Viluksela et al. 1994
182	Rat (CD)	6 wk 1 d/wk 1 x/d (GO)		0.14 F	0.71 F (decreased thymus weight slight cortical atrophy)		Vos et al. 1973
183	Mouse (B6C3F1)	13 wk 5 d/wk (F)		0.150 F			DeVito et al. 1994
184	Mouse (C57BL/6Jfh)	4 wk 1 d/wk 1 x/d (GO)		0.5 M		1 M (increased mortality after infection)	Thigpen et al. 1975
185	Mouse (C57BL/6, DBA/2)	5-8 wk 1 d/wk 1 x/d (GO)				0.5 M (suppoessed humoral activity in C57BL/6 strain)	Vecchi et al. 1983a

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
186	Mouse	4 wk 1 d/wk 1 x/d (GO)		0.14 M		0.71 M (decreased cell-mediated immunity)	Vos et al. 1973
187	Gn pig (Hartley)	90 d 7 d/wk (F)		0.0007[c]	0.005 (37% decrease in absolute thymus weight; 24% decrease in relative thymus weight)	0.028 (atrophy of thymus cortex in 1/4 males and 2/4 females)	DeCaprio et al. 1986
188	Gn pig (Hartley)	8 wk 1 d/wk 1 x/d (GO)			0.001 F (decreased lymphocytes)	0.03 F (decreased humoral immunity, thymic atrophy)	Vos et al. 1973
	Reproductive						
189	Monkey (Rhesus)	3 wk 3 d/wk 1 x/d (GO)		0.02		0.1 (abortions in 3/4 monkeys)	McNulty 1984
190	Rat (Sprague-Dawley)	90 d (F)		0.001		0.01 (decreased fertility in F1 and F2)	Murray et al. 1979
191	Rat (Sprague-Dawley)	4 wk 7 d/wk (F)		0.286 M		3.4 M (reduced spermatogenesis)	Van Miller et al. 1977a

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
192	Mouse (B6C3F1)	13 wk 5 d/wk (GO)		0.150 F			DeVito et al. 1994
193	Mouse (Swiss-Webster)	10 wk 7 d/wk (F)		0.65		1.3 (increased mortality in offspring)	Thomas and Hinsdill 1979
194	Mouse (C57B/6)	25 wk 3 d/wk 1 x/d (GO)				3 F (estrus blocked)	Umbreit et al. 1987
195	Mouse (C57B/6)	30 wk 1 d/wk 1 x/d (GO)		3 M			Umbreit et al. 1988
	Developmental						
196	Rat (Sprague-Dawley)	90 d (F)		0.001		0.01 (decreased neonatal survival and neonatal growth in F1 and F2)	Murray et al. 1979
197	Mouse (Swiss-Webster)	10 wk 7 d/wk (F)				0.35 (thymus atrophy)	Thomas and Hinsdill 1979
198	Mouse (C57B/6)	30 wk 1 d/wk 1 x/d (GO)		3			Umbreit et al. 1988

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)		LOAEL Serious (ug/kg/day)		Reference
	CHRONIC EXPOSURE								
	Death								
199	Rat (Sprague-Dawley)	2 yr 7 d/wk (F)					0.1	(increased mortality)	Kociba et al. 1978
200	Mouse (B6C3)	52 wk 1 d/wk 1 x/d (GO)					0.36	(increased mortality)	Della Porta et al. 1987
201	Mouse (Swiss)	1 yr 1 d/wk (GO)					1.0 M	(decreased survival)	Toth et al. 1979
	Systemic								
202	Rat (Sprague-Dawley)	2 yr 7 d/wk (F)	Resp	0.01	0.1	(focal alveolar hyperplasia)			Kociba et al. 1978a
			Cardio	0.01			0.1	(myocardial degeneration, periarteritis)	
			Gastro	0.1					
			Hemato	0.01	0.1	(decreased erythocytes)			
			Musc/skel	0.1					
			Hepatic		0.001 F	(hepatocellular alterations in females)	0.01	(severe and extensive hepatic necrosis)	
			Renal	0.1					
			Bd Wt		0.1	(unspecified decrease in body weight gain)			

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
203	Rat (Osborne-Mendel)	104 wk 2 d/wk (GO)	Resp	0.071			NTP 1982b
			Cardio	0.071			
			Gastro	0.071			
			Hemato	0.071			
			Musc/skel	0.071			
			Hepatic	0.0071		0.071 (toxic hepatitis)	
			Renal	0.071			
			Endocr	0.071			
			Dermal	0.071			
			Ocular	0.071			
			Bd Wt			0.0014 (21% decrease in body weight gain)	
204	Rat (Sprague-Dawley)	78 wk 7 d/wk (F)	Gastro	0.286			Van Miller et al. 1977
			Hemato	0.286			
			Hepatic	0.286			
			Bd Wt	0.057		0.286 M (26% decreased weight gain)	

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)		LOAEL Serious (ug/kg/day)		Reference
205	Mouse (B6C3)	52 wk 1 x/wk (GO)	Dermal		0.36	(dermatitis)			Della Porta et al. 1987
			Bd Wt				0.36	(33% decreased weight gain)	
206	Mouse (B6C3F1)	104 wk 2 d/wk (GO)	Resp	0.3					NTP 1982b
			Cardio	0.3					
			Gastro	0.3					
			Hemato	0.3					
			Musc/skel	0.3					
			Hepatic	0.0071			0.071	(toxic hepatitis)	
			Renal	0.0071	0.071	(lymphocytic inflamatory infiltration in kidneys)			
			Endocr	0.3					
			Dermal	0.3					
			Ocular	0.3					
			Bd Wt	0.3					
207	Mouse (C57BL/6N)	14.5 mo 1 x/wk (GO)	Hemato	0.03 F					Oughton et al. 1995
			Bd Wt	0.03 F					

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
208	Mouse (Swiss)	1 yr 1 d/wk (GO)	Dermal		0.001 M (skin lesions and generalized amyloidosis in 5/44 mice; 0/38 in controls)		Toth et al. 1979
	Immunological/Lymphoreticular						
209	Monkey (Rhesus)	2-33 mo 7 d/wk (F)				0.002 (bone marrow and lymphoid tissue degeneration)	Hong et al. 1989
210	Monkey (Rhesus)	4 yr 12 mo/yr 7 d/wk (F)		0.001			Hong et al. 1989
211	Rat (Sprague-Dawley)	2 yr 7 d/wk (F)		0.01		0.1 (thymic atrophy)	Kociba et al. 1978
212	Rat (Osborne-Mendel)	104 wk 2 d/wk (GO)		0.071			NTP 1982b
213	Rat (Sprague-Dawley)	78 wk 7 d/wk (F)		0.286 M			Van Miller et al. 1977
214	Mouse (B6C3F1)	104 wk 2 d/wk (GO)		0.3			NTP 1982b

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference
215	Mouse (C57BL/6N)	14.5 mo 1 x/wk (GO)			0.03 F (decrease in the effector and memory T cell phenotypes)		Oughton et al. 1995
	Neurological						
216	Rat (Sprague-Dawley)	2 yr 7 d/wk (F)		0.01		0.1 (hemorrhage in brain)	Kociba et al. 1978
217	Rat (Osborne-Mendel)	104 wk 2 d/wk (GO)		0.071			NTP 1982b
218	Mouse (B6C3F1)	104 wk 2 d/wk (GO)		0.3			NTP 1982b
	Reproductive						
219	Monkey (Rhesus)	4 yr 12 mo/yr 7 d/wk (F)		0.00012 F		0.00064 F (50% increased abortions)	Bowman et al. 1989b; Hong et al. 1989
220	Monkey (Rhesus)	3.5-4 yr (F)		0.00012 F		0.00064 F (reduced reproduction)	Bowman et al. 1989b; Hong et al. 1989
221	Monkey (Rhesus)	3.5-4 years daily (F)			0.00012 F (moderate endometriosis)	0.00064 F (severe endometriosis)	Rier et al. 1993

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)		LOAEL Serious (ug/kg/day)		Reference
222	Rat (Sprague-Dawley)	2 yr 7 d/wk (F)		0.1					Kociba et al. 1978
223	Rat (Osborne-Mendel)	104 wk 2 d/wk (GO)		0.071					NTP 1982b
224	Mouse (B6C3F1)	104 wk 2 d/wk (GO)		0.3					NTP 1982b
	Developmental								
225	Monkey (Rhesus)	4 yr 7 d/wk (F)		.00012			0.00064	(decreased off-spring survival)	Bowman et al. 1989b
226	Monkey (Rhesus)	16 mo 7 d/wk (F)			0.00012[d]	(altered social behavior)			Schantz et al. 1992
227	Monkey (Rhesus)	3.5-4 yrs (F)					0.00064	(learning impairment)	Schantz et al. 1992; Bowman et al. 1989a
	Cancer								
228	Rat (Sprague-Dawley)	2 yr 7 d/wk (F)					0.1	(CEL: hepatocellular carcinoma, squamous cell carcinoma in lung and hard palate)	Kociba et al. 1978

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL			Reference
					Less Serious (ug/kg/day)	Serious (ug/kg/day)		
229	Rat (Osborne-Mendel)	104 wk 2 d/wk (GO)				0.0071 M	(CEL: increased incidence of thyroid follicular cell adenoma or carcinoma)	NTP 1982b
						0.071 F	(CEL: increased incidence of neoplastic nodule in liver or hepatocellular carcinoma)	
230	Mouse (B6C3)	52 wk 1 x/wk (GO)				0.36	(CEL: hepatocellular adenoma or carcinoma)	Della Porta et al. 1987
231	Mouse (B6C3F1)	104 wk 2 d/wk (GO)				0.071 M	(CEL: increased incidence of hepatocellular adenoma or carcinoma)	NTP 1982b
						0.3 F	(CEL: increased inceidence of thyroid follicular cell adenoma and histiocytic lymphomas)	

Table 2-2. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL			Reference
					Less Serious (ug/kg/day)	Serious (ug/kg/day)		
232	Mouse (Swiss)	1 yr 1 d/wk (GO)				0.1	(CEL: hepatocellular carcinoma)	Toth et al. 1979

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

[b] Used to derive an acute-duration oral minimal risk level (MRL) of $2x10^{-4}$ µg/kg/day; dose divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability) and by a modifying factor of 0.7 to adjust for the difference in higher bioavailability of 2,3,7,8-TCDD from oil gavage vehicle than from food.

[c] Used to derive an intermediate-duration oral MRL of $2x10^{-5}$ µg/kg/day; dose divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability).

[d] Used to derive a chronic-duration oral MRLof $1x10^{-6}$ µg/kg/day; dose divided by an uncertainty factor of 90 (3 for use of a minimal LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

ACOH = acetanilide-4-hydroxylase; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); ED_{50} = dose eliciting a 50% decrease or increase relative to controls; Endocr = endocrine; EROD = ethoxyresorufin-O-deethylase; F = female; (F) = food; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; hr = hour(s); LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; LH = luteinizing hormone; M = male; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; min = minute(s); mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; PEPCK = phosphoenolpyruvate carboxykinase; RBC = red blood cell; Resp = respiratory; WBC = white blood cell; wk = week(s); yr = year(s): x = times.

Figure 2-1. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral

Acute (≤14 days)

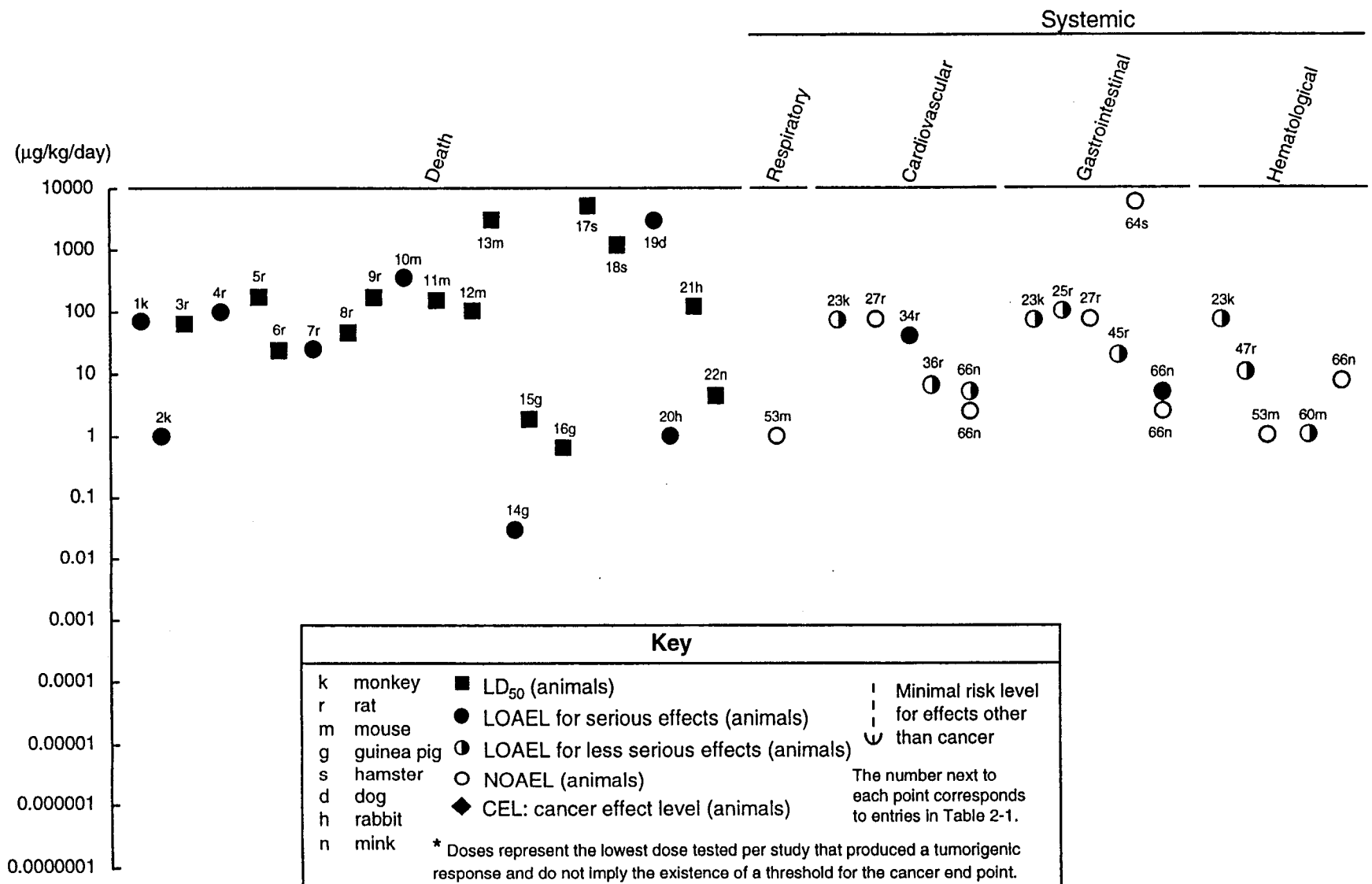
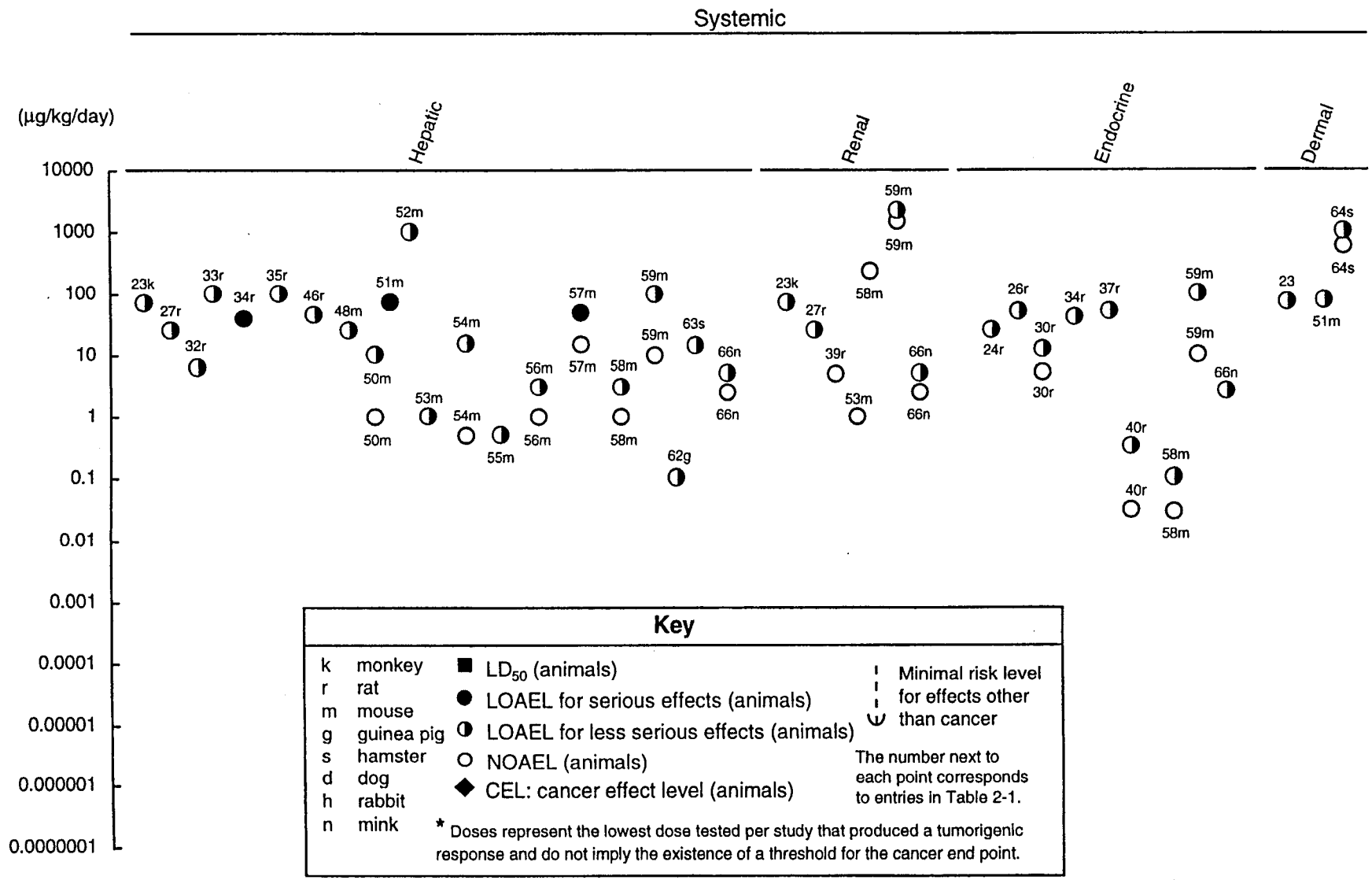


Figure 2-1. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (cont.)

Acute (≤14 days)



Key

k	monkey
r	rat
m	mouse
g	guinea pig
s	hamster
d	dog
h	rabbit
n	mink

■ LD_{50} (animals)
● LOAEL for serious effects (animals)
◑ LOAEL for less serious effects (animals)
○ NOAEL (animals)
◆ CEL: cancer effect level (animals)

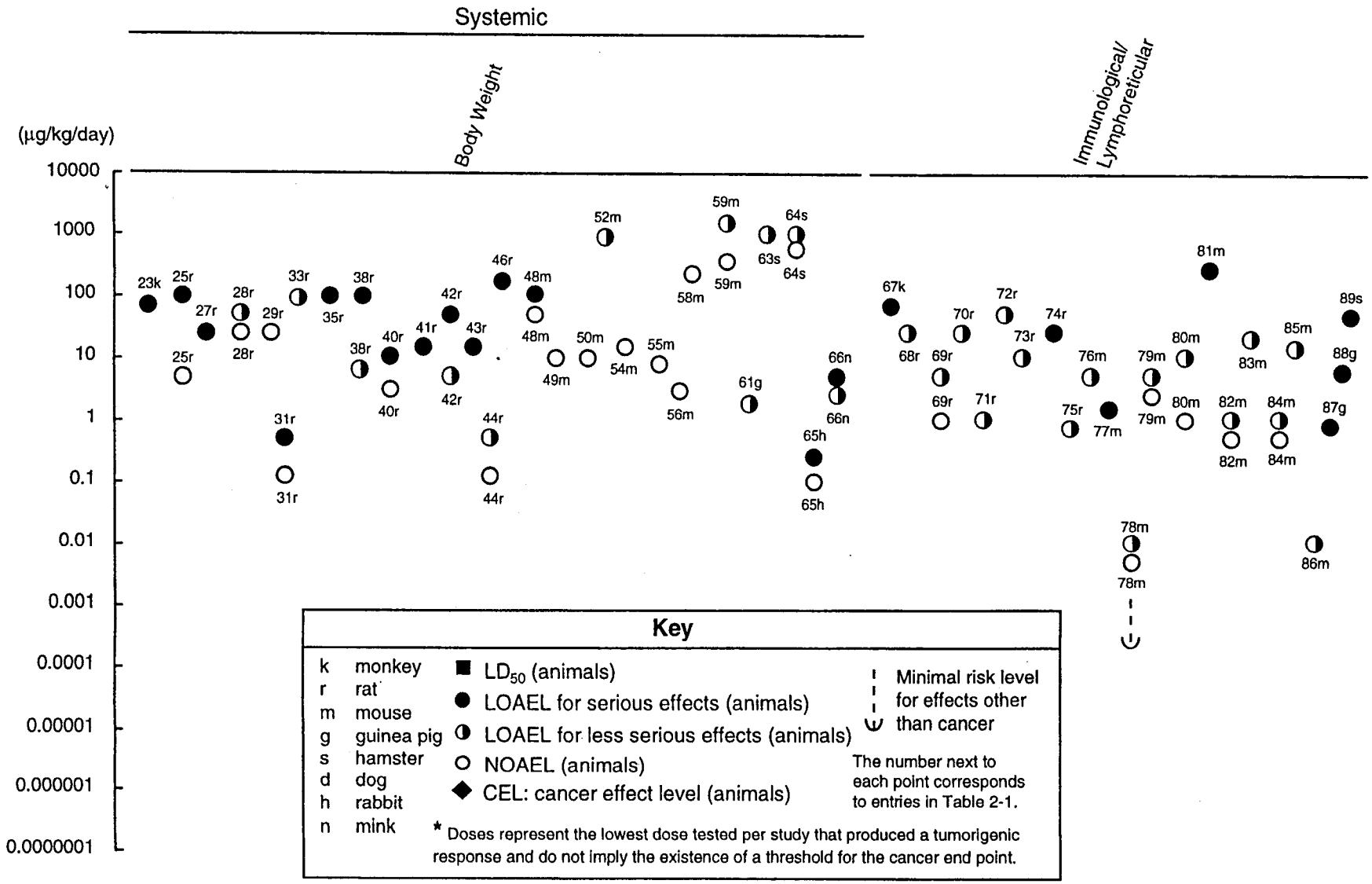
Minimal risk level for effects other than cancer

The number next to each point corresponds to entries in Table 2-1.

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

Figure 2-1. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (cont.)

Acute (≤14 days)



Key

k	monkey	■ LD_{50} (animals)	Minimal risk level for effects other than cancer
r	rat	● LOAEL for serious effects (animals)	
m	mouse	◑ LOAEL for less serious effects (animals)	The number next to each point corresponds to entries in Table 2-1.
g	guinea pig	○ NOAEL (animals)	
s	hamster	◆ CEL: cancer effect level (animals)	
d	dog		
h	rabbit		
n	mink		

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

Figure 2-1. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (cont.)

Acute (≤14 days)

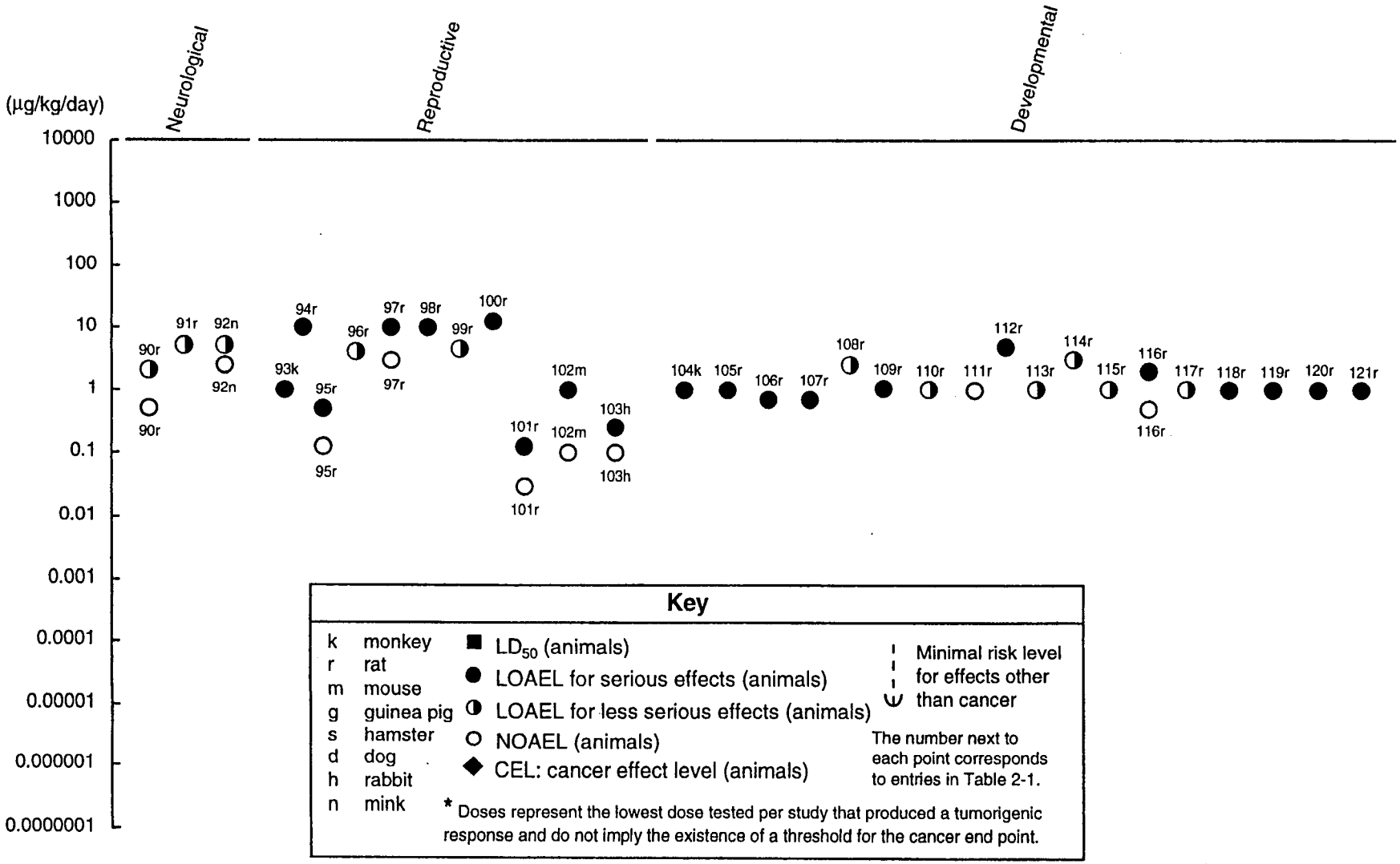


Figure 2-1. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (cont.)

Acute (≤14 days)

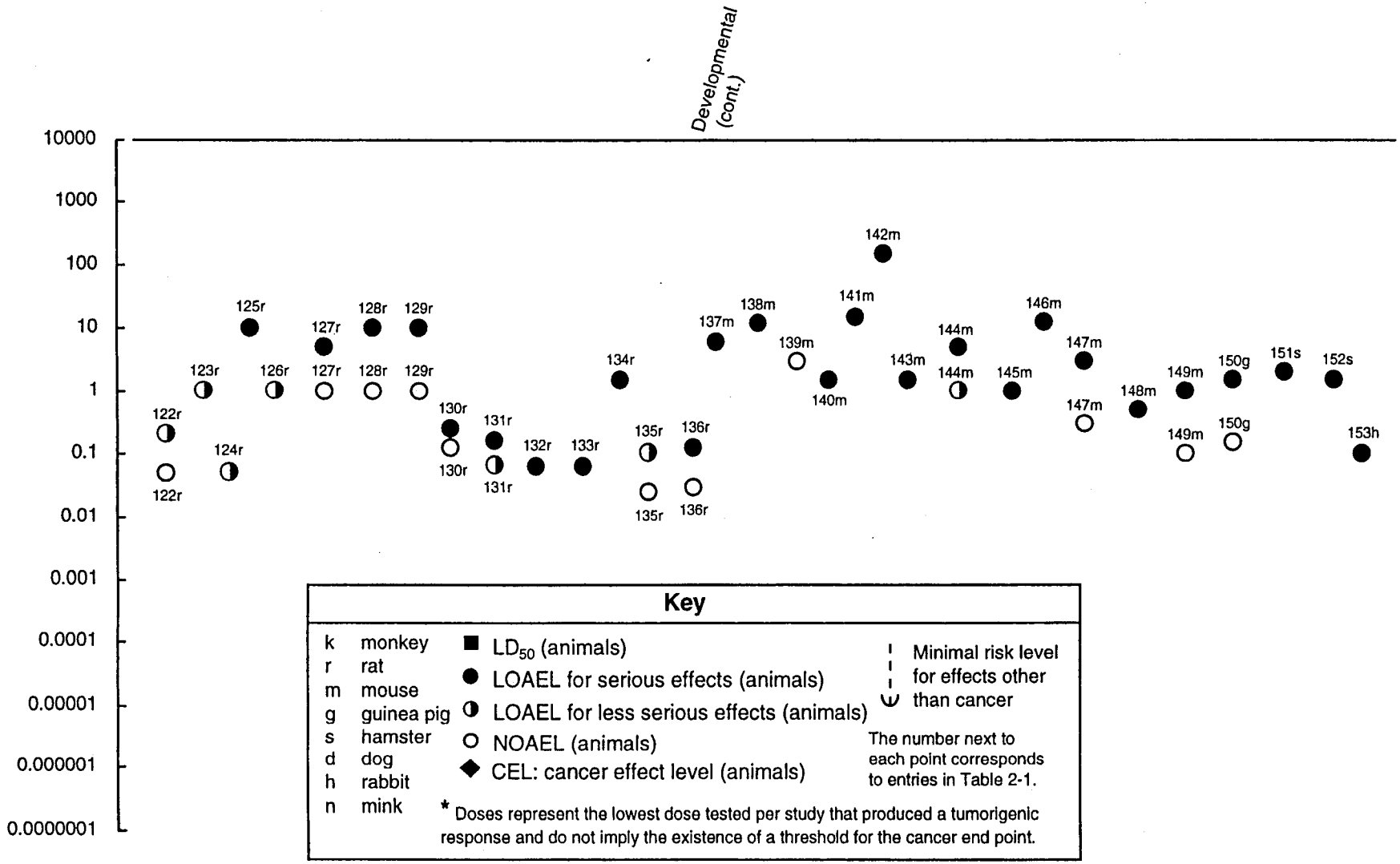


Figure 2-1. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (cont.)

Intermediate (15-364 days)

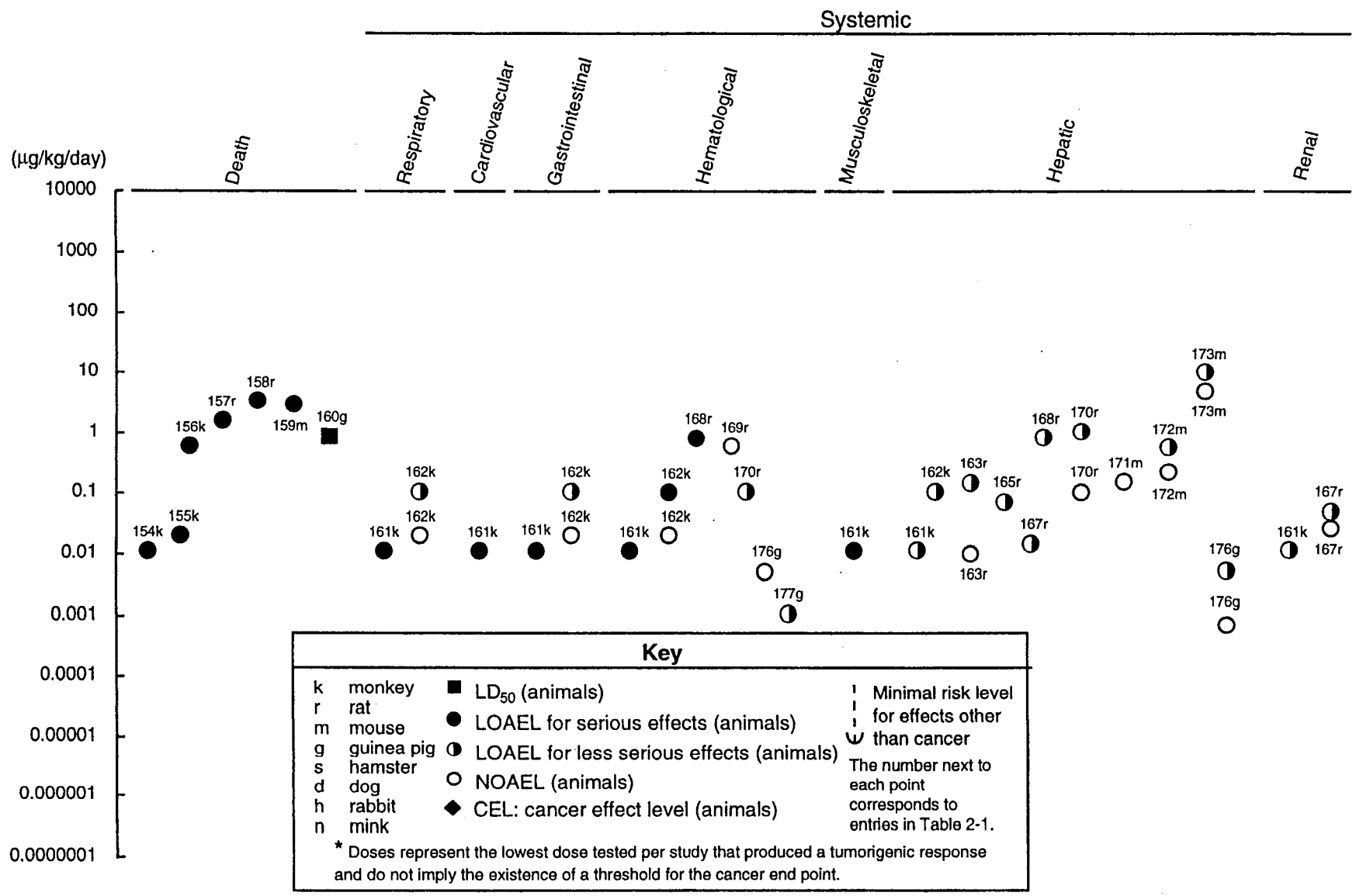


Figure 2-1. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (cont.)

Intermediate (15-364 days)

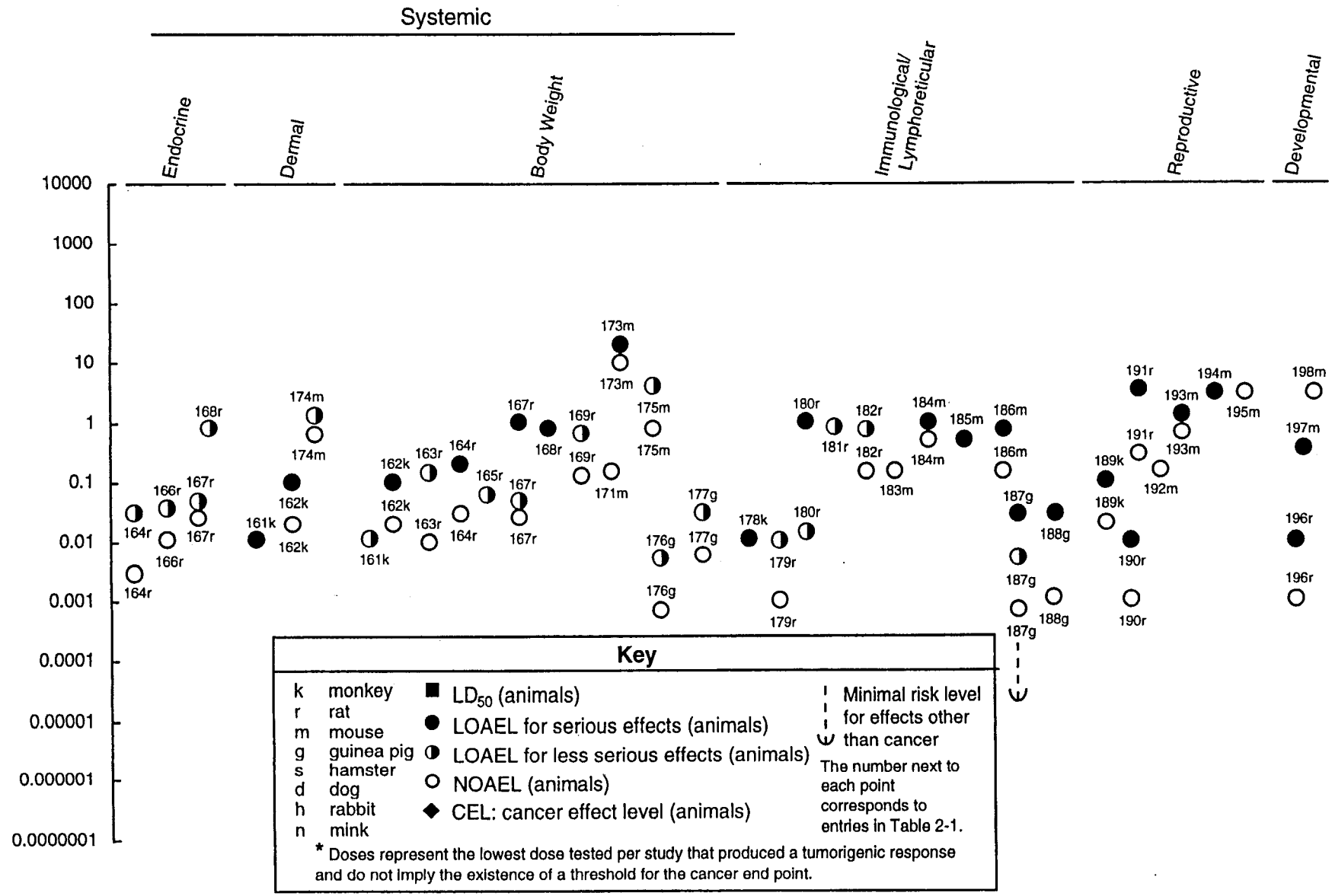


Figure 2-1. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (cont.)

Chronic (≥365 days)

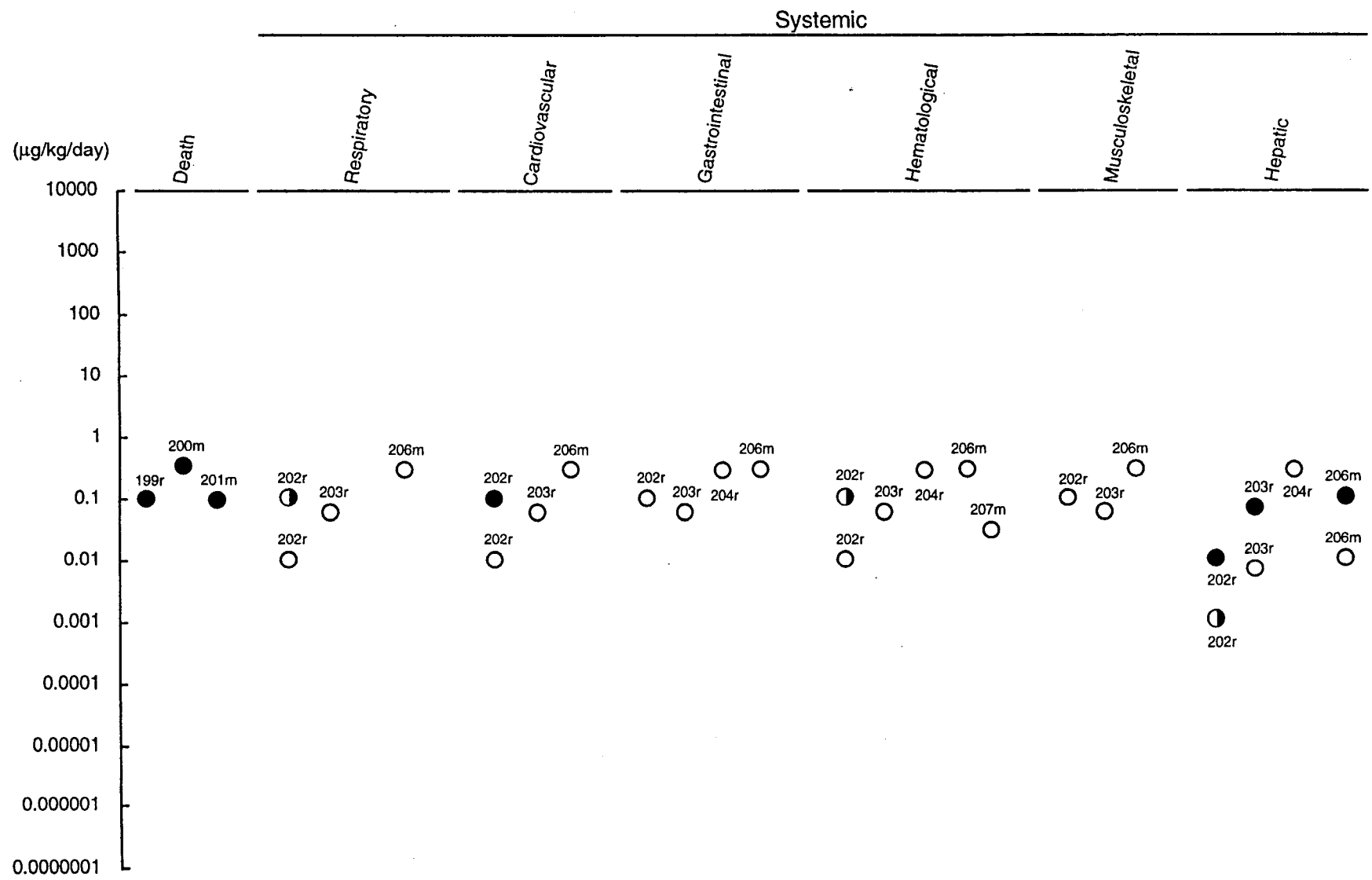


Figure 2-1. Levels of Significant Exposure to 2,3,7,8-TCDD - Oral (cont.)

Chronic (≥365 days)

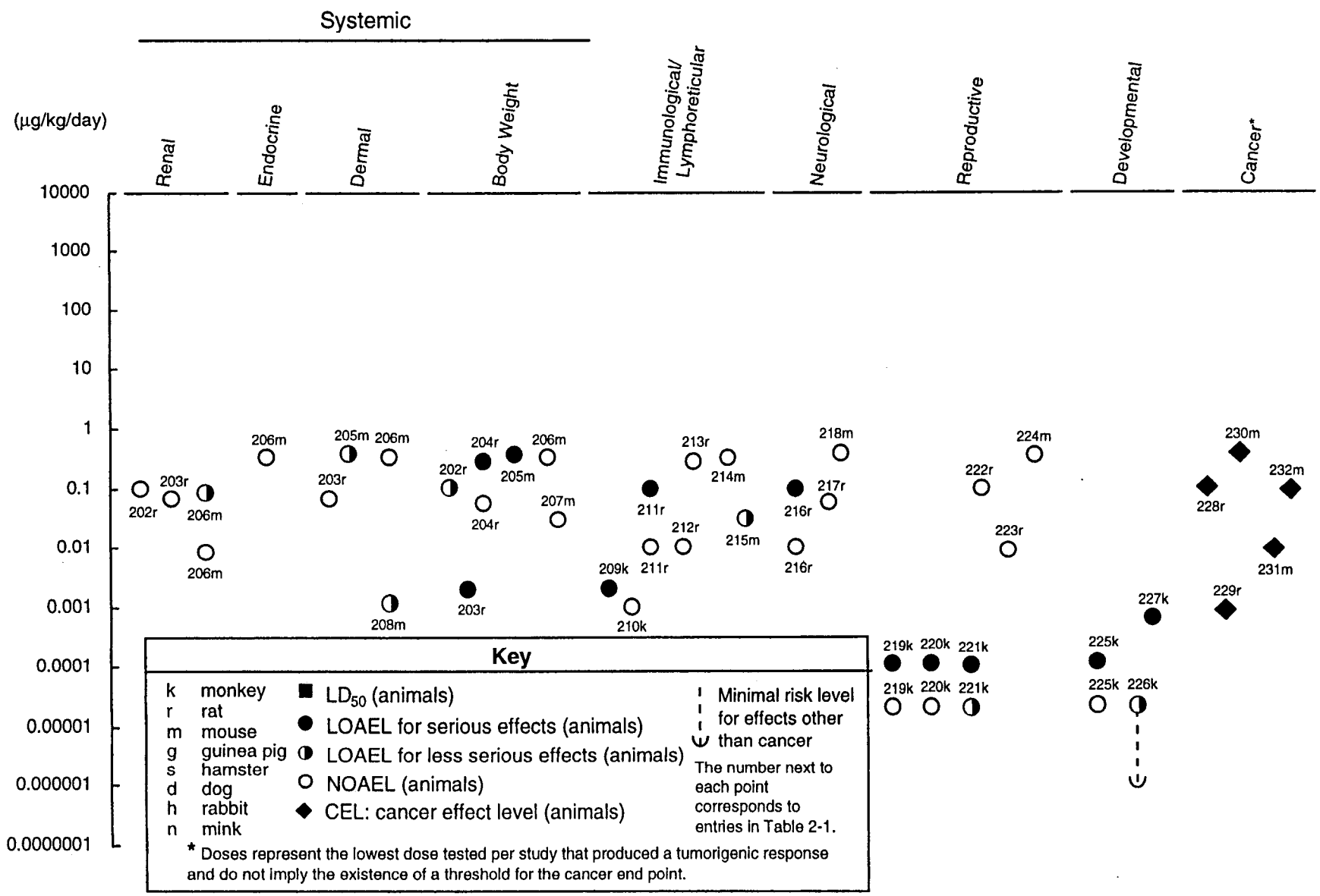


Table 2-3 Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (ug/kg/day)	Serious (ug/kg/day)	
	ACUTE EXPOSURE						
	Death						
1	Rat (Osborne-Mendel)	once (GO)				1800 M (LD_{50})	NCI/NTP 1980a HCDD1
						800 F (LD_{50})	
2	Rat (Sprague-Dawley)	1 d 4 x/d (GO)				6325 (LD_{50})	Stahl et al. 1992 HpCDD
3	Rat (Sprague-Dawley)	once (GO)				206 M (LD_{50})	Stahl et al. 1992 PCDD1
4	Rat (Sprague-Dawley)	1 d 2 x/d (GO)				887 (LD_{50})	Stahl et al. 1992 HCDD2
5	Mouse (C57BL/6N)	once (GO)				825 M (LD_{50})	McConnell et al. 1978 HCDD2
6	Mouse (C57BL/6N)	once (GO)				337.5 M (LD_{50})	McConnell et al. 1978 PCDD1
7	Mouse (B6C3F1)	once (GO)				750 M (LD_{50})	NCI/NTP 1980 HCDD1
						500 F (LD_{50})	

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (ug/kg/day)	Serious (ug/kg/day)	
8	Gn pig (Hartley)	once (GO)				1125 M (LD_{50})	McConnell et al. 1978 PCDD2
9	Gn pig (Hartley)	once (GO)				60 M (LD_{50})	McConnell et al. 1978 HCDD3
10	Gn pig (Hartley)	once (GO)				600 M (LD_{50})	McConnell et al. 1978b HpCDD
11	Gn pig (Hartley)	once (GO)				72.5 M (LD_{50})	McConnell et al. 1978b HCDD2
12	Gn pig (Hartley)	once (GO)				3.1 M (LD_{50})	McConnell et al. 1978b PCDD1
13	Gn pig (Hartley)	once (GO)				29444 M (LD_{50})	McConnell et al. 1978b TrCDD
14	Gn pig (Hartley)	once (GO)				70 M (LD_{50})	McConnell et al. 1978b HCDD4
	Systemic						
15	Rat (Fischer- 344)	2 wk 5 d/wk 1 x/d (GO)	Hemato	50 M			Couture et al. 1988 OCDD
			Hepatic	50 M			

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference Chemical Form
16	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)	Hepatic	10 F	100 F (pale livers)		Schwetz et al. 1973 HCDD5
			Bd Wt	1 F		10 F (39% decreased maternal weight gain)	
17	Mouse (CD-1)	14 d 1 x/d (GO)	Bd Wt	1000 F			Courtney 1976 OCDD
18	Mouse (CD-1)	14 d 1 x/d (GO)	Bd Wt	1000 F			Courtney 1976 DCDD1
19	Mouse (CD-1)	10 d Gd 7-16 1 x/d	Hepatic	1000 F			Courtney 1976 TCDD, 1234
			Other	1000 F			
20	Mouse (CD-1)	10 d Gd 7-16 1 x/d (GO)	Hepatic	20			Courtney 1976 OCDD
21	Mouse	14 d 1 x/d (GO)	Hepatic	10 F			Holsapple et al. 1986 DCDD1
	Immunological/Lymphoreticular						
22	Mouse (B6C3F1)	14 d 1 x/d (GO)				0.1 F (suppressed antibody response)	Holsapple et al. 1986 DCDD1

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference Chemical Form
23	Mouse (B6C3F1)	14 d 1 x/d (GO)		10 F			Holsapple et al. 1986 OCDD
24	Mouse (C57B1/6)	once (GO)				33 (decreased splenic antibody response)	Kerkvliet and Brauner 1987 HpCDD
25	Mouse (B6C3F1)	14 d 1 x/d (GO)		0.1 F	1.0 F (suppressed serum complement activity)		White et al. 1986 HCDD4
	Reproductive						
26	Rat (Wistar)	10 d Gd 6-15 1 x/d (GO)		2000 F			Khera and Ruddick 1973 MCDD
27	Rat (Wistar)	10 d Gd 6-15 1 x/d (GO)		2000 F			Khera and Ruddick 1973 DCDD1
28	Rat (Wistar)	10d Gd 6-15 1 x/d (GO)		2000 F			Khera and Ruddick 1973 DCDD1
29	Rat (Wistar)	110 d Gd 6-15 1 x/d (GO)		800 F			Khera and Ruddick 1973 TCDD

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)		Reference Chemical Form
30	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)		5000000 F				Schwetz et al. 1973 OCDD
31	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)		100000 F				Schwetz et al. 1973 DCDD1
32	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)		1		10 F	(25% increased resorption)	Schwetz et al. 1973 HCDD5
	Developmental							
33	Rat (Wistar)	10 d Gd 6-15 1 x/d (GO)		800				Khera and Ruddick 1973 TCDD
34	Rat (Wistar)	10 d Gd 6-15 1 x/d (GO)		1000		2000	(edematous separation of cardiac myofibrils in fetuses)	Khera and Ruddick 1973 DCDD1
35	Rat (Wistar)	10 d Gd 6-15 1 x/d (GO)		2000				Khera and Ruddick 1973 MCDD

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (ug/kg/day)		Serious (ug/kg/day)		
36	Rat (Wistar)	10 d Gd 6-15 1 x/d (GO)		2000					Khera and Ruddick 1973 DCDD2
37	Rat (Wistar)	Gd 16 (G)			0.5	(induced microsomal enzyme activity, decreased thymus weight)			Madsen and Larsen 1989 PCDD1
38	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)		0.1	1	(subcutaneous edema)	10	(growth retardation, dilated renal pelvis, delayed ossification)	Schwetz et al. 1973 HCDD5
39	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)		100000					Schwetz et al. 1973 DCDD1
40	Rat (Sprague-Dawley)	10 d Gd 6-15 1 x/d (GO)		100000	500000	(subcutaneous edema)			Schwetz et al. 1973 OCDD
41	Mouse (CD-1)	10 d Gd 7-16 1 x/d (GO)		20					Courtney 1976 OCDD
42	Mouse (CD-1)	10 d Gd 7-16 1 x/d		1000					Courtney 1976 TCDD

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Death						
43	Rat (Sprague-Dawley)	13 wk 1 x/2 wk (GO)				73 M (3/20 died; first death on day 41)	Viluksela et al. 1994 HpCDD
44	Rat (Sprague-Dawley)	13 wk (GO)				2.6 F (15/20 died during treatment period; first death on day 16)	Viluksela et al. 1998a,1998b PCDD1
45	Rat (Sprague-Dawley)	13 wk (GO)				10.3 F (5/20 died during treatment period; first death on day 61)	Viluksela et al. 1998a,1998b HCDD2
	Systemic						
46	Rat (Fischer- 344)	13 wk 5 d/wk 1 x/d (GO)	Hemato		50 (mild anemia)		Birnbaum et al. 1989 OCDD
			Hepatic		50 (liver hypertrophy, enzyme induction)		
47	Rat (Fischer- 344)	4-13 wk 5 d/wk 1 x/d (GO)	Hemato		50 M (increased lymphocytes, decreased MCH, MCV, HGB)		Couture et al. 1988 OCDD
			Hepatic		50 M (cytoplasmic vacuolization)		

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference Chemical Form
48	Rat	13 wk 1 d/wk 1 x/d (GO)	Hemato	1.4	7.1 (splenic hyperplasia)		NCI/NTP 1980 HCDD1
			Hepatic	0.36	0.71 (unspecified moderate hepatoxicity)		
			Bd Wt	0.36	0.71 (13-18% decreased weight gain)		
49	Rat (Sprague-Dawley)	13 wk 1 x/2 wk (GO)	Hemato	24 M	73 M (decrease in platelet count)		Viluksela et al. 1994 HpCDD
			Hepatic	0.3 M	4 M (increased relative liver weight and EROD activity)		
			Endocr	4 M	24 M (decrease in serum total T4)		
			Bd Wt	24 M	73 M (13% decrease in body weight gain)	110 M (48% decrease in body weight gain)	

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (ug/kg/day)	Serious (ug/kg/day)	
50	Rat (Sprague-Dawley)	13 wk (GO)	Hemato		2.6 F (decreased hematocrit; reduced platelet count)		Viluksela et al. 1998a,1998b PCDD1
			Hepatic		2.6 F (decreased liver PEPCK and increased EROD activities)		
			Endocr		3.8 M (69% decrease in serum T4)		
			Dermal		2.6 F (occasional hair loss; sores in ears, nose, neck, tail, and feet)		
			Bd Wt		2.6 F (body weight reduced 18% relative controls at the end of dosing period)	3.8 M (body weight reduced by 27% relative to controls at the end of dosing period)	

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference Chemical Form
51	Rat (Sprague-Dawley)	13 wk (GO)	Hemato		10.3 F (decreased hematocrit; reduced platelet count)		Viluksela et al. 1998a,1998b HCDD2
			Hepatic		10.3 F (decreased liver PEPCK and TdO activities and increased EROD activity)		
			Endocr		15.4 M (69% decrease in serum T4)		
			Dermal		10.3 F (occasional hair loss; sores in ears, nose, neck, tail, and feet)		
			Bd Wt	10.3 F		15.4 M (body weight reduced by 24% relative to controls at the end of dosing period)	
52	Mouse	13 wk 1 d/wk 1 x/d (GO)	Hepatic	0.71	1.4 (mild hepatoxicity)		NCI/NTP 1980a HCDD1
			Bd Wt		0.18 (13-17% decreased weight gain)		
	Immunological/Lymphoreticular						
53	Rat	13 wk 1 d/wk 1 x/d (GO)		1.4	7.1 (splenic hyperplasia)		NCI/NTP 1980 HCDD1

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference Chemical Form
54	Rat (Sprague-Dawley)	13 wk 1 x/2 wk (GO)		0.3 M	4 M (decrease in absolute and relative thymus weight)		Viluksela et al. 1994 HpCDD
	CHRONIC EXPOSURE						
	Systemic						
55	Rat (Osborne-Mendel)	110 wk 7 d/wk (F)	Resp	500000			NCI/NTP 1979a DCDD1
			Cardio	500000			
			Gastro	500000			
			Hemato	500000			
			Musc/skel	500000			
			Hepatic		250000	(fatty changes)	
			Renal	500000			
			Dermal	500000			
			Bd Wt		250000	(17% decreased body weight gain)	

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (ug/kg/day)		Serious (ug/kg/day)		
56	Rat (Osborne-Mendel)	104 wk 2 d/wk (GO)	Resp		0.18	(adenomatous hyperplasia of the lungs)			NCI/NTP 1980a HCDD1
			Cardio	0.7					
			Gastro	0.7					
			Hemato	0.7					
			Musc/skel	0.7					
			Hepatic				0.18	(toxic hepatitis)	
			Renal	0.7					
			Dermal	0.7					
			Bd Wt				0.18	(38% decreased weight gain)	

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL Less Serious (ug/kg/day)	LOAEL Serious (ug/kg/day)	Reference Chemical Form
57	Mouse (B6C3F1)	90 wk 7 d/wk (F)	Resp	1300000			NCI/NTP 1979a DCDD1
			Cardio	1300000			
			Gastro	1300000			
			Hemato	1300000			
			Musc/skel	1300000			
			Hepatic			650000 (toxic hepatitis)	
			Renal	1300000			
			Dermal	1300000			
			Bd Wt		650000 (16% decreased body weight gain)		
58	Mouse (B6C3F1)	104 wk 2 d/wk (GO)	Resp	1.4			NCI/NTP 1980a HCDD1
			Cardio	1.4			
			Gastro	1.4			
			Hemato	1.4			
			Musc/skel	1.4			
			Hepatic			0.7 (toxic hepatitis)	
			Renal	1.4			
			Dermal	1.4			
			Bd Wt	1.4			

Table 2-3. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ug/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (ug/kg/day)	Serious (ug/kg/day)		
	Cancer							
59	Rat (Osborne-Mendel)	104 wk 2 d/wk (GO)				0.34	(CEL: hepatocellular carcinoma or liver neoplastic nodules)	NCI/NTP 1980a HCDD1
60	Mouse (B6C3F1)	90 wk 7 d/wk (F)				650000 M	(CEL: hepatocellular carcinoma or adenoma, lymphoma, leukemia, hemangiosarcomas)	NCI/NTP 1979a DCDD1
61	Mouse (B6C3F1)	104 wk 2 d/wk (GO)				0.7 M	(CEL: hepatocellular adenoma or carcinoma)	NCI/NTP 1980 HCDD

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

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); DCDD1 = 2,7-dichlorodibenzo-p-dioxin; DCDD2 = 2,3-dichlorodibenzo-p-dioxin; Endocr = endocrine; EROD = ethoxyresorufin-O-deethylase; F = female; (F) = food; Gastro = gastrointestinal; Gd = gestational day; Gn Pig = guinea pig; (GO) = gavage in oil; HCDD1 = mixture of 1,2,3,7,8,9- and 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin; HCDD2 = 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin; HCDD3 = 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin; HCDD4 = 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin; HCDD5 = unspecified mixture of hexachlorodibenzo-p-dioxins; Hemato = hematological; HGB = hemoglobin; HpCDD = 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin; hr = hour(s); LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; M = male; MCDD = 2-monochlorodibenzo-p-dioxin; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; NS = not specified; OCDD = octachlorodibenzo-p-dioxin; PCDD1 = 1,2,3,7,8-pentachlorodibenzo-p-dioxin; PCDD2 = 1,2,4,7,8-pentachlorodibenzo-p-dioxin; RBC = red blood cell; Resp = respiratory; TCDD = 1,2,3,4-tetrachlorodibenzo-p-dioxin; TrCDD = 2,3,7-trichlorodibenzo-p-dioxin; wk = week(s); x = times

Figure 2-2. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral

Acute (≤14 days)

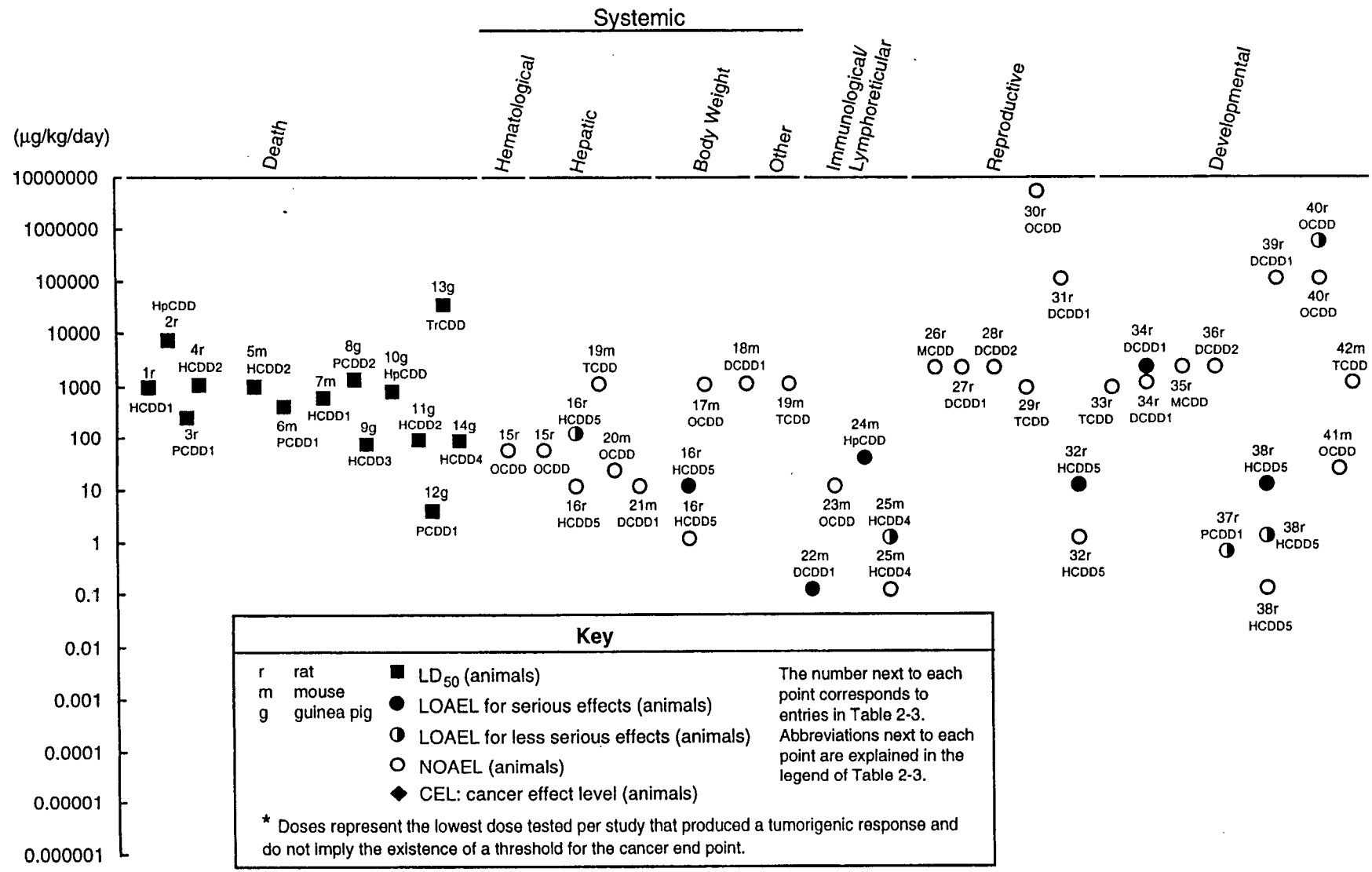


Figure 2-2. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (cont.)

Intermediate (15-364 days)

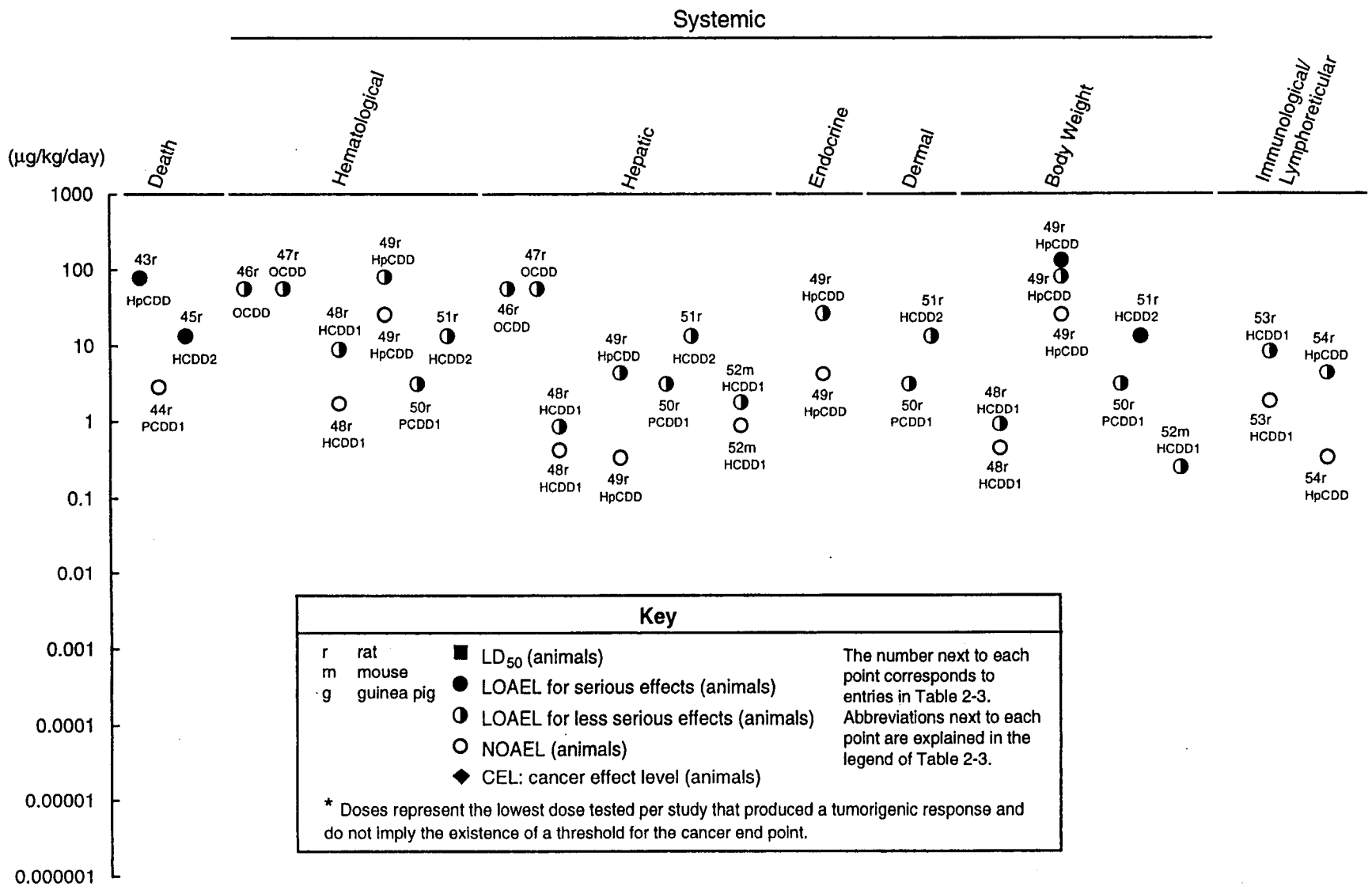
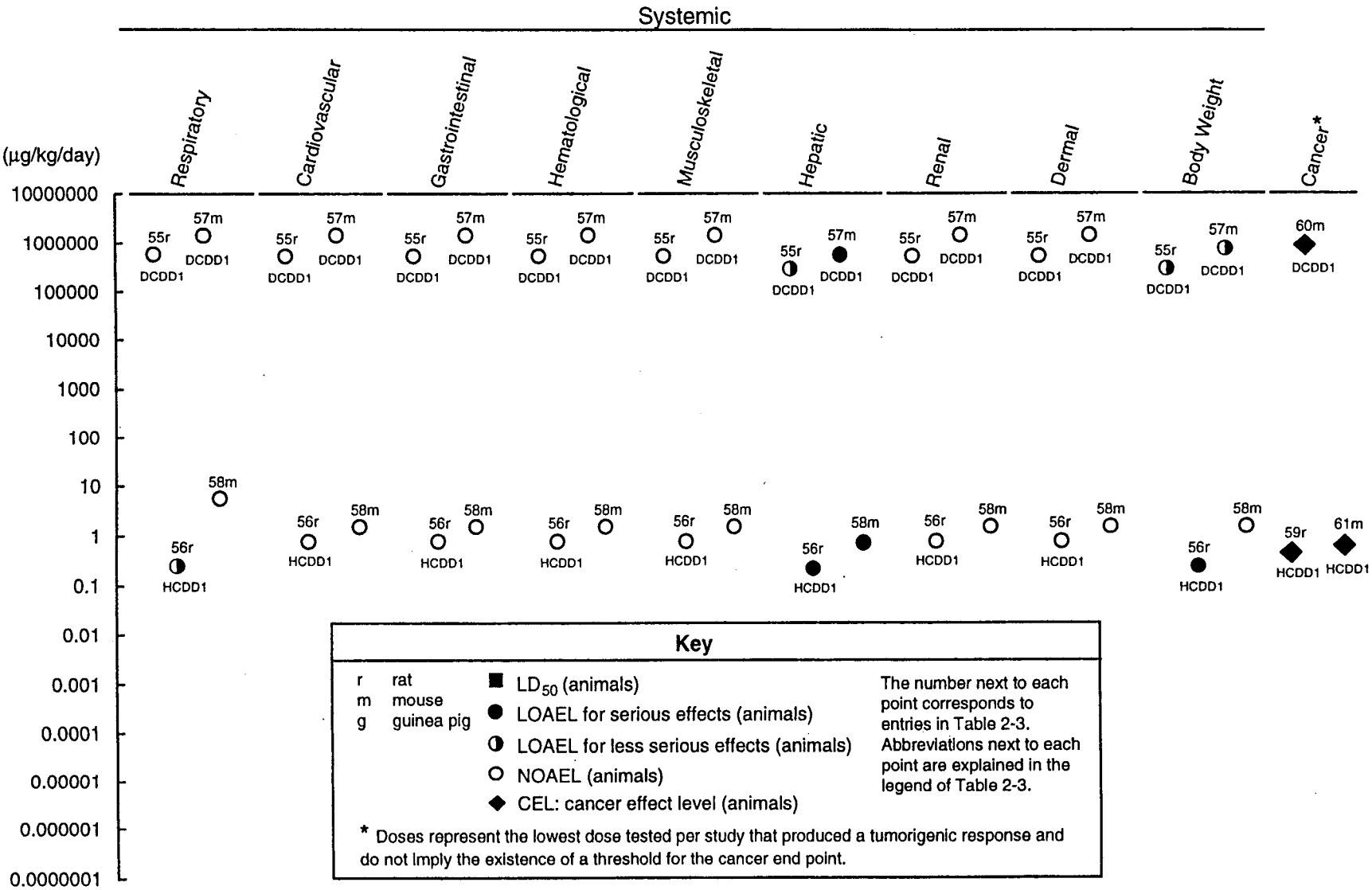


Figure 2-2. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Oral (cont.)

Chronic (≥365 days)



Similarly, no respiratory effects were found in rats and mice chronically exposed by diet to 5×10^5 and 1.3×10^6 µg/kg/day of 2,7-DCDD, respectively (NCI/NTP 1979a). In contrast, rats exposed chronically by gavage to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD at 0.18, 0.34, and 0.7 µg/kg/day had a dose-related increased incidence of adenomatous hyperplastic lesions in terminal bronchioles and adjacent alveoli of both males and females; no such effects were found in mice exposed chronically to 0.7 µg/kg/day of that same mixture (NCI/NTP 1980). The existing information suggests that in animals, the respiratory system is not a sensitive target for CDDs toxicity via oral exposure.

Cardiovascular Effects. Cardiovascular effects have been detected in animals following acute-, intermediate-, and chronic-duration oral exposure to 2,3,7,8-TCDD. These included changes in heart weight, pathophysiological effects, and degenerative changes. However, exposures at or near a lethal dose were required to elicit these effects.

Decreased absolute heart weight was reported in minks 28 days after a single oral dose of 5 µg/kg, but not at 2.5 µg/kg (Hochstein et al. 1988). A reduction of absolute heart weight which is attributed to weight loss was also found in monkeys at 70 µg/kg (relative heart weight was increased) (McConnell et al. 1978a). Histological examinations of the heart were normal in the monkeys. This examination was not performed in minks. Doses in both species were near the lethal dose.

Kelling et al. (1987) assessed the effects of 2,3,7,8-TCDD on cardiac function tests in male Sprague-Dawley rats 7 days after single oral doses of 6.25, 25, or 100 µg/kg. At 100 µg/kg (near-lethal dose), an increased sensitivity to the inotropic (left atrium) and chronotropic (right atrium) effects of isoproterenol were observed. Three daily oral doses of 40 µg/kg caused decreased heart rate, depressed blood pressure, and increased myocardial peroxidase activity in rats (Hermansky et al. 1988). All of these effects may have been secondary to the modulation of adenylate cyclase activity at β-adrenergenic receptors as a result of hypothyroidism (Hermansky et al. 1987).

In intermediate-duration experiments, monkeys that died after exposure to diets providing 0.011 µg/kg/day of 2,3,7,8-TCDD (lethal dose) had hemorrhages in the epicardium, myocardium, and endocardium (Allen et al. 1977). Myocardial degenerative changes and periarteritis were reported in Sprague-Dawley rats chronically exposed to a diet providing a lethal dose of 0.1 µg/kg/day of 2,3,7,8-TCDD, but not in those receiving 0.01 µg/kg/day (Kociba et al. 1978a). In contrast, no histopathological lesions were observed in

the hearts of rats and mice chronically exposed by gavage to approximately 0.071 and 0.3 µg/kg/day of 2,3,7,8-TCDD, respectively (NTP 1982b).

No histopathological lesions were observed in the hearts of rats and mice chronically exposed in the diet to 5×10^5 and 1.3×10^6 µg/kg/day of 2,7-DCDD, respectively (NCI/NTP 1979a); or exposed for 104 weeks by gavage to approximately 0.34 and 0.7 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, respectively (NCI/NTP 1980).

Gastrointestinal Effects. One of the major 2,3,7,8-TCDD-induced effects in various animal species is the wasting syndrome and hypophagia which occur after a single near-lethal dose or after repeated dosing (discussed under Body Weight Effects). Studies of effects on the gastrointestinal system have been carried out to investigate the mechanism of this starvation-like syndrome. Ulceration of the gastrointestinal tract and bloody stools were observed in minks after a single oral exposure to 5 µg 2,3,7,8-TCDD/kg (3 of 4 mink died) but not at a dose of 2.5 µg/kg/day. The response of the antral mucosa of the rat stomach to 2,3,7,8-TCDD has been studied by Theobald et al. (1991). In Sprague-Dawley rats, a single oral dose of 100 µg 2,3,7,8-TCDD/kg caused a 7–10-fold increase in serum gastrin (secreted by G-cells in the antrum) that was not detected until 14 days after dosing, whereas control rats fed a restricted diet had atrophic changes in the antral mucosa and no increase in gastrin (Theobald et al. 1991). The number of G-cells in the antral mucosa was not affected by treatment with 2,3,7,8-TCDD or paired-feed restriction, indicating that hypergastrinemia in treated rats is not due to reduced feed intake or antral G-cell hyperplasia. In 2,3,7,8-TCDD-treated rats, both gastrin and somatostatin (which inhibits gastrin release) levels in the antral mucosa were significantly decreased, and these changes were observed a week earlier than the hypergastrinemia. Moreover, the ED_{50} values (half maximum effect level of 2,3,7,8-TCDD) for the decrease in antral mucose content and concentration of gastrin (29 and 22 µg/kg, respectively) and somatostatin (24 and 19 µg/kg, respectively) was less than that for hypergastrinemia (46 µg/kg). This suggested that hypergastrinemia in 2,3,7,8-TCDD-treated rats is not a consequence of reduced antral levels of gastrin or somatostatin. Epithelial hyperplasia of the stomach occurred in rhesus monkeys following a single oral dose of 70 µg/kg; this response is unique to monkeys and cows and is not seen in rats, mice, or guinea pigs (McConnell et al. 1978b). Monkeys undergo a similar wasting syndrome as rodents after a single oral lethal dose. Moderate to severe ileitis, characterized by hyperplasia of the mucosal epithelium with hemorrhaging and necrosis, and peritonitis were observed in hamsters that died after oral administration of $1000 µg/kg 2,3,7,8-TCDD (Olson et al. 1980a).

Repeated dosing of rats at 3.4 µg/kg/day or higher caused gastrointestinal hemorrhaging in rats that died in a chronic oral-dosing study (Van Miller et al. 1977). Metaplasia of the gastric mucosa was found in rhesus monkeys exposed to 0.1 µg/kg/day of 2,3,7,8-TCDD for 3 weeks (McNulty 1984), and gastric ulcers developed after exposure to 0.011 µg/kg/day for 9 months in the feed (Allen et al. 1977). No gastrointestinal effects were observed in rats and mice chronically exposed by gavage to approximately 0.071and 0.3 µg/kg/day of 2,3,7,8-TCDD, respectively (NTP 1982b) or in rats on diets that provided 0.1 µg/kg/day (Kociba et al. 1978a).

Gastrointestinal lesions were not observed following exposure of rats and mice to 5×10^5 and 1.3×10^6 µg/kg/day of 2,7-DCDD, respectively, in the diet (NCI/NTP 1979a) or to 0.34 and 0.7 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, respectively, by gavage for 104 weeks (NCI/NTP 1980).

The above studies demonstrated that monkeys are more sensitive to gastrointestinal effects of 2,3,7,8-TCDD than rodents.

Hematological Effects. Hematological effects were reported in some animals following exposure to lethal or near-lethal doses of 2,3,7,8-TCDD. Increases in erythrocyte counts, hemoglobin, and hematocrit were observed 10–14 days after CD rats received a single oral dose of 10 µg/kg 2,3,7,8-TCDD. Increases in total leukocyte count and neutrophil counts, and a decrease in platelet counts were also observed, but bleeding time and megakaryocytes were not altered (Weissberg and Zinkl 1973). Reduction of germinal centers and increased hemosiderin deposits were seen histologically in the spleen of Sprague-Dawley rats after a single oral dose of 25 µg/kg (Christian et al. 1986a). Mild anemia developed in rhesus monkeys after a single oral dose of 70 µg/kg (McConnell et al. 1978a). No effects were found in minks exposed acutely to a lethal dose of 2,3,7,8-TCDD (7.5 µg/kg) (Hochstein et al. 1988) or in $B6C3F_1$ mice exposed to 1 µg/kg/day for 14 days (Holsapple et al. 1986a). Reversible changes (suppression of progenitor cells, decreases in leukocyte and lymphocyte counts) were reported in CD-1 mice at doses between 1 and 10 µg/kg 2,3,7,8-TCDD (Zinkl et al. 1973).

Hematological effects have also been reported following intermediate-duration exposures to 2,3,7,8-TCDD. Decreased white blood cell counts were reported in guinea pigs exposed by gavage to 0.008 µg/kg/day 2,3,7,8-TCDD for 8 weeks (Vos et al. 1973), but no hematological changes were observed following dietary exposure to 0.005 µg/kg/day 13 weeks (DeCaprio et al. 1986). Exposure to higher doses

(3.4 µg/kg/day or more) caused splenic atrophy in Sprague-Dawley rats that died during the first 4 weeks of exposure in a chronic-duration dietary study (Van Miller et al. 1977). In contrast, no hematological changes were found in rats exposed to 0.71 µg/kg/day of 2,3,7,8-TCDD for 6 weeks (Vos et al. 1973). One month of intermittent exposure to 0.1 µg/kg/day 2,3,7,8-TCDD induced thrombocytopenia in CD rats (Zinkl et al. 1973); exposure to 1 µg/kg/day caused increased erythrocyte counts and hemoglobin levels. Administration of 2,3,7,8-TCDD by gavage for 13 weeks to male Sprague-Dawley rats at doses equivalent to 0.8 µg/kg/day (only dose level tested) produced a significant decrease in platelet counts, and in some animals, increased prothrombin times (Viluksela et al. 1994). Anemia and bone marrow hypoplasia were observed in rhesus monkeys exposed to 0.1 µg/kg/day of 2,3,7,8-TCDD by gavage 3 days a week for 3 weeks (McNulty 1984). The changes were more severe with longer exposure; pancytopenia and bone marrow atrophy developed in monkeys exposed to 0.011 µg/kg/day (a lethal dose) in the feed for 9 months (Allen et al. 1977).

In chronic-duration studies, reduced erythrocyte counts were found in Sprague-Dawley rats at dietary doses of 0.1 µg/kg/day of 2,3,7,8-TCDD but not at 0.01 µg/kg/day (Kociba et al. 1978a). No hematological effects were observed in Osborne-Mendel rats or B6C3F_1 mice chronically exposed by gavage to approximately 0.071or 0.3 µg/kg/day of 2,3,7,8-TCDD, respectively (NTP 1982b). Results from a more recent study showed that 2,3,7,8-TCDD administered by gavage to female C57BL/6 mice in gavage doses equivalent to approximately 0.03 µg/kg/day (0.2 µg/kg once/week) for 14–15 months produced no significant effects on the total number of circulating red or white blood cells or in white blood cell differentials (Oughton et al. 1995).

Hematological effects have been reported in some animals following exposure to other CDDs. No hematological effects were observed in rats after 2 weeks of intermittent exposure to 50 µg/kg/day OCDD (Couture et al. 1988), but increased neutrophils, decreased mean cell volume, and hemoglobin (Couture et al. 1988), and mild anemia was observed at the same exposure level after 13 weeks of intermittent exposure (Birnbaum et al. 1989a). A dose-dependent decrease in platelet counts was observed in male Sprague-Dawley rats following administration by gavage of doses equivalent to 73 or 110 µg 1,2,3,4,6,7,8-HpCDD/kg/day for 13 weeks (Viluksela et al. 1994); no such effect was observed with doses #24 µg/kg/day. Some rats administered the highest dose also showed increased prothrombin times. Administration of doses equivalent to 2.6 µg 1,2,3,7,8-PCDD/kg/day or 10.3 µg 1,2,3,47,8-HxCDD/kg/day for 13 weeks resulted in decreased hematocrit and reduced platelet count in female Sprague-Dawley rats (Viluksela et al. 1998a); these doses also caused mortality.

Splenic hyperplasia was observed in rats exposed by gavage to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD at 7.1 µg/kg/day, but not at 1.4 µg/kg/day for 13 weeks (NCI/NTP 1980). No hematological effects were observed in Osborne-Mendel rats or $B6C3F_1$ mice chronically exposed to 5×10^5 and 1.3×10^6 µg/kg/day of 2,7-DCDD, respectively, in feed (NCI/NTP 1979a) or exposed to 0.34 and 0.7 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, respectively, 2 days a week for 104 weeks by gavage (NCI/NTP 1980).

The above results demonstrated hematological effects in animals following CDD exposure; however, the observed changes in the red and white blood cell counts were nonspecific and were probably due to the broad systemic toxicity of 2,3,7,8-TCDD rather than to a direct effect on the hematological system.

Musculoskeletal Effects. The musculoskeletal system does not appear to be a major target of toxicity in animals exposed to CDDs. Only one study reported hemorrhages in the musculoskeletal system of severely debilitated monkeys following dietary exposure to 0.011 µg/kg/day of 2,3,7,8-TCDD for an intermediate duration (Allen et al. 1977).

No musculoskeletal effects were observed in Sprague-Dawley rats exposed to 0.1 µg/kg/day in the diet for 2 years (Kociba et al. 1978a) or in Osborne-Mendel rats and $B6C3F_1$ mice chronically exposed 2 days a week by gavage to 0.071and 0.3 µg/kg/day of 2,3,7,8-TCDD, respectively (NTP 1982b).

Chronic experiments with other congeners showed no musculoskeletal effects in Osborne-Mendel rats and $B6C3F_1$ mice exposed in the diet to 5×10^5 and 1.3×10^6 µg/kg/day of 2,7-DCDD, respectively (NCI/NTP 1979a) or by gavage to approximately 0.34 and 0.7 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, respectively (NCI/NTP 1980).

Hepatic Effects. Effects on the liver are seen after acute oral exposure or after intermediate and chronic exposure to CDDs. Alterations in metabolism, biochemical changes, and increases in liver weights (without histologic changes) are sensitive markers of effects, but they are not clearly overt toxic effects; they may predict a toxic or histopathologic effect that will occur at higher doses or after longer exposure. Likewise induction of mixed-function oxidases (MFO) and cytochrome P-450s are generally considered adaptive effects; they may be associated with increased liver weight, but are not necessarily associated with histopathologic changes. However, alterations in cytochrome P-450 (e.g., CYP1A1) may lead to altered metabolism and/or toxicity of other xenobiotics and endogenous compounds. Increases in liver enzymes

such as AST and ALT in serum are an indication of cell death or necrosis. Effects on the liver that occur at near-lethal doses or when animals are debilitated and approaching moribundity are secondary effects and are not specific to the action of CDDs on the liver. The liver should not be implicated as a target organ in these cases. The types of histological changes caused by CDDs and their severity vary widely between species and strains of laboratory animals and the doses administered.

Histological changes in the liver were observed after acute-, intermediate-, and chronic-duration exposures. Enlarged hepatocytes with finely vacuolated cytoplasm in the centrilobular region were observed 20 days after administration of a single oral lethal dose of 75 µg/kg 2,3,7,8-TCDD to adult Sprague-Dawley rats (Christian et al. 1986a); cellular degeneration in the centrilobular region was observed in Fischer 344 rats 20 days after a single oral dose of 1000 µg/kg (Kelling et al. 1985). Three daily doses of 40 µg/kg caused centriacinar necrosis and enlarged hepatocytes in Sprague-Dawley rats (Hermansky et al. 1988). In all the above citations, the changes were secondary to the wasting syndrome and occurred after severe weight loss. Focal areas of mild hydropic degeneration of the liver associated with increased liver weight was seen in $B6C3F_1$ mice receiving 1 µg/kg/day 2,3,7,8-TCDD for 14 days (non-lethal) (Holsapple et al. 1986a). Swelling of hepatocytes, disruption of cell membranes, and dilation of sinusoids in the central vein area of the liver accompanied by cellular necrosis were observed after a single lethal dose of 360 µg/kg in C57B46 mice (Kelling et al. 1985); central necrosis in the livers of hairless A2G hr/+ mice dosed once at 75 µg/kg was reported (Greig 1984; Greig et al. 1987). Guinea pigs only showed minimal focal necrosis at 42 days after a single oral dose of 0.1 µg/kg 2,3,7,8-TCDD (Turner and Collins 1983). Hypertrophy, steatosis, cytoplasmic degeneration, and hyaline-like cytoplasmic inclusion bodies were observed at non-lethal and lethal doses (0.5 to 20 µg/kg); no between group qualitative differences in this histological alteration were found (Turner and Collins 1983). No degenerative changes were observed by Kelling et al. (1985) prior to death in guinea pigs given a single oral lethal dose of 2 µg/kg. In minks, a sensitive species, pale and mottled livers were observed at gross necropsy 28 days after a single lethal oral dose of 5 µg/kg (Hochstein et al. 1988).

Mild-to-moderate hepatic effects were also seen after intermediate-duration exposure to 2,3,7,8-TCDD. Unspecified histopathological hepatic lesions were reported following intermittent exposure to 2,3,7,8-TCDD in female Osborne-Mendel rats at 0.07 µg/kg/day and in female $B6C3F_1$ mice at 0.7 µg/kg/day for 13 weeks (NTP 1982b). Cytomegaly with focal necrosis was observed in C57BL/6J mice exposed to 10 µg/kg/day by gavage 1 day/week for 4 weeks (Thigpen et al. 1975). Increase relative liver weight and hepatocellular inclusions were found in guinea pigs exposed to 0.005 µg/kg/day in the feed

for 90 days (DeCaprio et al. 1986); this dose level also significantly reduced serum ALT activity in females and increased triglycerides in males. Biliary epithelial hyperplasia has been reported in monkeys following exposure to lethal levels (0.011 µg/kg/day) in feed for 9 months (Allen et al. 1977) and after intermittent exposure at 0.1 µg/kg/day by oral gavage for 3 weeks (McNulty 1984).

Liver necrosis occurred in Sprague-Dawley rats that died during the first 4 weeks of dietary exposure to 2,3,7,8-TCDD at 3.4 µg/kg/day in a chronic-exposure experiment, while no non-cancerous liver effects were found in rats chronically exposed to 0.286 µg/kg/day (Van Miller et al. 1977). Toxic hepatitis characterized by lipidosis and hydropic degeneration of hepatocytes with proliferation of bile ductules and by mild fibrosis was observed in 14/50 male and 32/49 female Osborne-Mendel rats following exposure by gavage to approximately 0.071 µg/kg/day of 2,3,7,8-TCDD administered for 104 weeks and in 44/50 male $B6C3F_1$ mice receiving 0.071µg/kg/day or 34/47 female mice receiving 0.3 µg/kg/day (NTP 1982b). In addition, cytoplasmic vacuolation, hyperplasia, hepatocellular degeneration, and liver necrosis occurred in Sprague-Dawley rats chronically exposed to diets providing doses of 0.001 (females) and 0.01 (both sexes) µg/kg/day 2,3,7,8-TCDD, respectively (Kociba et al. 1978a).

Biochemical changes indicating liver effects following acute oral exposure to 2,3,7,8-TCDD included hypoglycemia and increased serum triglycerides and cholesterol 10 days after a single sublethal oral dose of 45 µg/kg in Fischer 344 rats (Walden and Schiller 1985); earlier, Albro (1978) found increased triglycerides and decreased sterol esters after a non-lethal dose of 2,3,7,8-TCDD and increased cholesterol and free fatty acids after a lethal dose. Reduced retinol storage in the liver was found in Sprague-Dawley rats exposed to a single dose of 1 µg/kg 2,3,7,8-TCDD (Thunberg 1984). Reduction of hepatic retinol by 2,3,7,8-TCDD was greater (87%) in younger rats with lower initial weights (Thunberg et al. 1984) than in more mature rats (60%) (Thunberg et al. 1979, 1980). Significant and maximum induction of hepatic ethoxyresorufin-O-deethylase (EROD, marker for CYP1A1 activity) activity and dose-related decrease in liver phosphoenolpyruvate carboxykinase (PEPCK, a key enzyme of gluconeogenesis) was observed in female Long Evans rats 4 days after a single gavage dose of 5.3–60 µg 2,3,7,8-TCDD/kg (Fan and Rozman 1995). Hepatic activity of tryptophan 2,3-dioxygenase (TdO, a key enzyme of tryptophan metabolism) was elevated by 2,3,7,8-TCDD-treatment (significantly at 5.3, 12, and 18 µg/kg but not 60 µg/kg), whereas serum tryptophan levels were not altered. EROD activity had diminished considerably 90 days after dosing, although it was still 10 times the control values, and PEPCK and TdO activities had returned to control values. The authors concluded that gluconeogenesis is inhibited by 2,3,7,8-TCDD in Long Evans rats by reducing liver PEPCK activity (Fan and Rozman 1995).

Hepatic porphyria has been found after acute oral dosage of mice with 2,3,7,8-TCDD. A single oral dose of 150 µg/kg or 4 weekly doses of 25 µg/kg caused an accumulation of porphyrins and induction of delta aminolevulinic acid synthetase in $B6C3F_1$ mice (Goldstein et al. 1973). These were lethal doses and caused severe histologic liver damage. Single oral doses as high as 30 µg/kg did not produce porphyria acutely or within 16 weeks (Goldstein 1982). Elevation of serum levels of ALT and sorbitol dehydrogenase, which are indicative of subclinical toxic effects on the liver, have been found in mice after a single oral non-lethal dose (Greig 1984; Rosenthal et al. 1989; Smith et al. 1981). Smith et al. (1981) compared the sensitivity of C57BL/10 mice and DBA/2 mice, and found that the DBA/2 mouse was 20 times less sensitive than the C57 strain to 2,3,7,8-TCDD-induced porphyria. Recent results from Weber et al. (1995) suggested that acute toxicity of 2,3,7,8-TCDD occurs between 37.5 and 235 µg/kg in male C57BL/6J mice and between 375 and 3,295 µg/kg in male DBA/2J mice, as judged by decreases in liver PEPCK and glucose-6-phosphatase (G-6-Pase, also a key enzyme of gluconeogenesis) activities, reduction in blood glucose, and changes in relative liver weight 8 days after a single gavage dose of 2,3,7,8-TCDD. The ED_{50} for induction of hepatic EROD activity in male C57BL/6J mice was estimated at 1.1 µg/kg compared with 16 µg/kg in DBA/2J mice. Also there was no evidence of a reduction of liver TdO activity or of elevation of serum tryptophan levels over the dose range tested (0.03–235 µg/kg in C57BL/6J mice and 1–3,295 µg/kg in DBA/2J mice). Dose-dependent induction of EROD has also been observed in the liver from female $B6C3F_1$ mice after single (Diliberto et al. 1995) and repeated (DeVito et al. 1994) oral 2,3,7,8-TCDD doses. In the repeated-dosing study (DeVito et al. 1994), both EROD and acetanilide-4-hydroxylase (marker for CYP1A2) activities were induced with doses as low as 1.5 ng 2,3,7,8-TCDD/kg/day. Pegram et al. (1995) found no differences in the dose-response curves for hepatic EROD induction between young and old male C57BL/6N mice 8 days after a single dose of 0.015–15 µg 2,3,7,8-TCDD/kg. However, induction of acetanilide-4-hydroxylase was significantly greater in old than in young mice. Also a trend of greater relative liver weight with increasing dioxin dose was observed in young mice, whereas liver weight was not altered in old mice (Pegram et al. 1995).

Increased liver weights were reported in pregnant mice that received 25 µg/kg/day (Courtney 1976), 3 µg/kg/day (Smith et al. 1976), or 0.5 µg/kg/day (Silkworth et al. 1989b) for 10 days during gestation, and in monkeys receiving a single oral dose of 70 µg/kg (McConnell et al. 1978a). The ED_{50} values for liver enlargement following a single oral dose of 2,3,7,8-TCDD were calculated as 100 µg/kg 2,3,7,8-TCDD in Sprague-Dawley rats, 1,000 µg/kg in C57BL/6 mice, and 14 µg/kg in Syrian hamsters (Hanberg et al. 1989). These ED_{50} values approach and exceed the LD_{50} values for rats and mice (see Section 2.2.2.1).

In a 1-week dietary study in female Sprague-Dawley rats, a dose of 1 µg 2,3,7,8-TCDD/kg/day induced a significant increase in absolute liver weight and a lower dose of 0.32 µg/kg/day significantly increased relative liver weight (Van Birgelen et al. 1995). Other hepatic effects observed in this study included significantly dose-related increased hepatic microsomal activities of EROD and acetanilide-4-hydroxylase, beginning at the lowest dose tested (0.014 µg/kg/day), and dose-related decrease in hepatic retinol at $0.014 µg/kg/day. In agreement with the results of Van Birgelen et al. (1995), Viluksela et al. (1994) also reported increases in absolute and relative liver weights in male Sprague-Dawley rats administered 2,3,7,8-TCDD by gavage for 13 weeks at doses that supplied approximately 0.8 µg 2,3,7,8-TCDD/kg/day (the only dose level tested). An increase in liver EROD activity and decrease in liver PEPCK activity were also reported in the study. Liver TdO activity and total serum tryptophan were not significantly altered in surviving rats, but TdO activity was significantly decreased in moribund animals with signs of wasting syndrome, whereas serum tryptophan levels were doubled in these animals. Li and Rozman (1995) examined the reversibility of 2,3,7,8-TCDD-induced changes in some liver enzymes in male Sprague-Dawley rats treated by gavage with doses equivalent to 0.003–1.6 µg/kg/day for 10 weeks and allowed to recover for an additional 6-week period. As reported by others, there was a dose-dependent decrease in TdO activity with a concurrent increase in serum tryptophan levels (both significant at the highest-dose level) and a decrease in PEPCK activity (significant at $1 µg/kg/day). These dose responses were very similar to the dose response for body weight reduction. EROD was induced even at the lowest dose and maximum induction was attained at $35 µg/kg/day. After the 6-week recovery period, PEPCK and TdO activities, as well as serum tryptophan levels, returned to near-control levels; however, EROD still remained induced. The authors (Li and Rozman 1995) indicated that the results supported the hypothesis that subchronic toxicity of 2,3,7,8-TCDD is similar to its acute toxicity when the dose is corrected for pharmacokinetics. In other words, toxicity is determined by the body burden represented by the cumulative dose minus the portion of the dose already eliminated.

Hepatic effects have also been reported in animals following exposure to other CDDs. Pale, friable livers were observed in 3/20 Sprague-Dawley rat dams exposed to 100 µg/kg/day of mixed HxCDD during gestation (incidence in control group was not reported), but not in those exposed to 10 µg/kg/day (Schwetz et al. 1973). No effects on the liver were observed at 10 µg/kg/day of 2,7-DCDD in B6C3F_1 mice (Holsapple et al. 1986b), at 20 µg/kg/day of OCDD in pregnant CD-1 mice (Courtney 1976), or at 1,000 µg/kg/day of 1,2,3,4-TCDD in pregnant CD-1 mice (Courtney 1976).

Data for hepatic effects after intermediate-duration exposure to the other congeners were available for 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, a mixture of 1,2,3,7,8,9-HxCDDs and 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD. Mild hepatotoxicity (not otherwise specified) was recorded in rats exposed to 0.71 µg/kg/day of a mixture of 1,2,3,7,8,9-HxCDD and 1,2,3,6,7,8-HxCDD, and in mice exposed to the same mixture by gavage for 13 weeks to 1.4 µg/kg/day (NCI/NTP 1980). No effects were seen at 0.36 µg/kg/day and at 0.71 µg/kg/day in rats and mice, respectively. Absolute liver weight was significantly increased in male Sprague-Dawley rats administered $24 µg 1,2,3,4,6,7,8-HpCDD/kg/day by gavage for 13 weeks (Viluksela et al. 1994). Relative liver weight was increased at $4 µg/kg/day. Liver activity of PEPCK was significantly decreased with the 2 highest-dose levels tested, 73 and 110 µg/kg/day, whereas hepatic EROD activity was dose-dependently induced over the dose range tested, 0.3 to 110 µg/kg/day. Liver TdO activity and serum total tryptophan were not significantly altered at #24 µg/kg/day; however, TdO was decreased and serum tryptophan was increased in rats that died at the two highest dose levels. Similar results were reported in rats treated with 1,2,3,7,8-PCDD (2–4 µg/kg/day) or 1,2,3,4,6,7-HxCDD (10–15 µg/kg/day) (Viluksela et al. 1998b). Cytoplasmic vacuolization of hepatocytes (Couture et al. 1988) and liver hypertrophy with induced hepatic enzymes (Birnbaum et al. 1989a) were reported in rats gavaged with 50 µg/kg/day OCDD 5 days a week for up to 13 weeks.

Toxic hepatitis was reported in Osborne-Mendel rats and in B6C3F_1 mice following gavage exposure to 0.18 µg/kg/day and to 0.34 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, respectively, for 104 weeks (NCI/NTP 1980). The corresponding NOAEL values are recorded in Table 2-3. Furthermore, fatty changes in the liver were found in rats chronically exposed to 2,7-DCDD at a dose of 2.5×10^5 µg/kg/day in the feed (NCI/NTP 1979a). In contrast, no liver effects were observed in mice following chronic exposure to 1.3×10^6 µg/kg/day of 2,7-DCDD in the feed (NCI/NTP 1979a).

In conclusion, the above studies demonstrated that the liver is a primary target of CDD toxicity. 2,3,7,8-TCDD was the most toxic congener, but other congeners were also capable of inducing hepatic effects. The induced effects were dose-related and species- and strain-related. It also appeared that for some hepatic end points, and after repeated dosing, toxicity is determined by the body burden represented by the cumulative dose minus the portion of the dose eliminated.

Renal Effects. Mild-to-moderate renal effects have been reported in some mature animals exposed to lethal or near-lethal levels of 2,3,7,8-TCDD. Acute exposure to 2,3,7,8-TCDD caused dilation of

convoluted tubules and Bowman's spaces at 25 µg/kg in Sprague-Dawley rats (Christian et al. 1986) and epithelial hyperplasia in the renal pelvis at 70 µg/kg in rhesus monkeys (McConnell et al. 1978a). Similar findings were reported in monkeys exposed to 0.011 µg/kg/day of 2,3,7,8-TCDD for 9 months (Allen et al. 1977). Kidney weights were not affected in male C57BL/6J and DBA/2J mice treated with a single 0.03–235 µg/kg or 1–3,295 µg/kg 2,3,7,8-TCDD dose, respectively (Weber et al. 1995). Rats administered doses of $0.047 µg/kg/day for 13 weeks exhibited an increase in relative kidney weight; a dose of 0.026 µg/kg/day was without effect (Van Birgelen et al. 1995). Chronic exposure of $B6C3F_1$ mice by gavage to approximately 0.071 µg/kg/day of 2,3,7,8-TCDD induced renal inflammatory changes; no effects were found at 0.0071 µg/kg/day (NTP 1982b). In contrast, no renal effects were found in Osborne-Mendel rats exposed to 0.071 µg/kg/day of 2,3,7,8-TCDD for 104 weeks (NTP 1982b) or in Sprague-Dawley rats exposed to 0.1 µg/kg/day of 2,3,7,8-TCDD in the feed for 2 years (Kociba et al. 1978a).

Studies with other congeners reported no renal effects following chronic exposure to 0.34 µg/kg/day and 0.7 µg/kg/day of a mixture of 1,2,3,7,8,9-HxCDD and 1,2,3,6,7,8-HxCDD by gavage in rats and mice, respectively (NCI/NTP 1980) or 5×10^5 and 1.3×10^6 µg/kg/day of 2,7-DCDD in the feed in rats and mice, respectively (NCI/NTP 1979a).

The above data suggest that the observed renal effects in mature animals may be secondary to the general response to 2,3,7,8-TCDD toxicity with the exception of the epithelial hyperplasia reported in monkeys. However, developmental studies clearly show that the ureteral epithelium is altered by *in utero* exposure to CDDs as manifested by hyperplasia of ureteral lining epithelial cells leading to hydronephrosis (see Section 2.2.2.6).

Endocrine Effects. Blood corticosterone levels were decreased to 29 and 26% of control values in male Sprague-Dawley rats at 14 and 21 days after a single oral dose of 25 µg/kg 2,3,7,8-TCDD, respectively (Balk and Piper 1984). Since 11-β-hydroxyprogesterone levels were elevated, the authors suggested that 2,3,7,8-TCDD produced a block at the 21-hydroxylase step in the synthesis of corticosterone. This was directly demonstrated in a follow-up study in which the authors observed a 35% decrease in 21-hydroxylase activity 7 days after a single oral dose of 50 µg/kg 2,3,7,8-TCDD (Mebus and Piper 1986). Corticosterone serum levels from samples taken late in the light phase decreased up to 40% in male Sprague-Dawley rats administered a single 50 µg 2,3,7,8-TCDD/kg dose (DiBartolomeis et al. 1987). The effect was attributed to 2,3,7,8-TCDD-induced inhibition of cholesterol side-chain cleavage. In samples taken early in the light cycle, corticosterone levels increased 4-fold relative to controls; however,

this increase was shown to result from nutritional deprivation rather than from a direct effect of 2,3,7,8-TCDD. The possibility that altered levels of corticosterone result from a 2,3,7,8-TCDD-induced effect on adrenocorticotropin (ACTH) was examined by Bestervelt et al. (1993). ACTH serum levels were significantly increased in male Sprague-Dawley rats over a 14-day period following administration of a single dose of 50 µg 2,3,7,8-TCDD/kg; maximum increases were observed on days 3 and 14. Plasma corticosterone levels were significantly increased on days 1 and 5, but were reduced below control levels on days 10 and 14. Treatment with 2,3,7,8-TCDD did not affect the activity of the rate-limiting enzyme for adrenal steroidogenesis, mitochondrial cytochrome P-450 cholesterol side chain cleavage. Basal corticosterone concentration in adrenal glands from 2,3,7,8-TCDD-treated rats was significantly lower than in controls on days 5, 7, and 14 after dosing; however, secretion of corticosterone induced by stimulation with exogenous ACTH was not altered by treatment with 2,3,7,8-TCDD. Based on these results, the authors concluded that 2,3,7,8-TCDD may interfere with secretion or synthesis of appropriate, bioactive ACTH from the anterior pituitary gland, which could compromise adrenal steroidogenesis.

The effects of 2,3,7,8-TCDD on thyroid function has been extensively studied. For example, a single gavage dose of 25 µg 2,3,7,8-TCDD/kg significantly decreased serum levels of T4 and increased serum levels of triiodothyronine (T3) in male hooded rats 9 days after dosing (Bastomsky 1977). The decrease in T4 appeared to be the result of an increased biliary excretion of T4-glucuronide, and this was attributed to induction of UDP-glucuronyltransferase (UDPGT) by 2,3,7,8-TCDD. UDPGT catalyzes glucuronidation of T4 and clearance. The increase in T3 was consistent with increased thyroid secretion from thyrotropin (TSH) stimulation. Administration of a single dose of 6.25–100 µg 2,3,7,8-TCDD/kg by gavage to adult male Sprague-Dawley rats produced a significant dose-related decrease in serum T4 levels (50% of control with the lowest dose) 7 days after dosing (Potter et al. 1986). Serum levels of T3 were elevated in a dose-related manner, whereas levels of TSH achieved a maximum increase with the lowest dose. Potter et al. (1986) also observed a small 2,3,7,8-TCDD-related increase in thyroid weight, but no consistent pattern of histological alterations. Hermansky et al. (1988) reported a 65% decrease in serum T4 levels in female Sprague-Dawley rats 6 days after administration of 3 doses of 40 µg 2,3,7,8-TCDD/kg; however, in this study the authors observed a 9% increase in serum T3. A dose-related decrease in serum total T4 was observed in female Long Evans rats 4 days after a single dose of 5.3–60 µg 2,3,7,8-TCDD/kg; statistical significance was achieved with a 12 µg/kg dose (Fan and Rozman 1995). Total serum T3 was not significantly altered. However, 90 days after single doses of 27–60 µg 2,3,7,8-TCDD/kg total serum T4 and T3 were elevated, which led the authors to suggest that 2,3,7,8-TCDD triggers adaptive responses which persist after most of the chemical has cleared the organism (Fan and Rozman 1995). In male mink,

relative thyroid gland weight was increased over a 4-week period after administration of a single dose of 7.5 µg 2,3,7,8-TCDD/kg, but a dose of 5 µg/kg was without effect (Hochstein et al. 1988). Hochstein et al. (1988) also reported a significant dose-related increase in relative adrenal gland weight over a 2.5–7.5 µg 2,3,7,8-TCDD/kg dose range. However, when the weights were expressed as a percentage of brain weight, only the increase in the adrenal gland at 7.5 µg/kg was significant. The concentrations of plasma cortisol and free and bound T3 and T4 were slightly reduced as a result of 2,3,7,8-TCDD treatment (2.5 µg/kg, only dose level tested), but the differences relative to controls were not significant.

Acute effects of 2,3,7,8-TCDD on thyroid function have been also reported in mice. In contrast with observations in rats, in which 2,3,7,8-TCDD appears to have independent effects on T4 and T3 levels, serum T4 and T3 levels were decreased in a dose-dependent fashion in male C57BL/6J mice 8 days after a single gavage dose of 0.03–235 µg 2,3,7,8-TCDD/kg (Weber et al. 1995). A similar effect was observed in male DBA/2J mice treated with a single dose of 1–3,295 µg/kg. In C57BL/6J mice, maximum depression of thyroid hormones (35% of controls) was achieved with a dose of 133 µg/kg. In male DBA/2J mice, maximum reductions in T3 and T4 levels (40 and 20% of controls, respectively) were attained with the highest dose level (Weber et al. 1995). It should be noted that the Weber et al. (1995) study did not include statistical analysis of the results.

A significant decrease in serum total T4 was observed in male Sprague-Dawley rats administered 2,3,7,8-TCDD by gavage for 13 weeks at doses equivalent to 0.8 µg/kg/day, the only dose level tested (Viluksela et al. 1994). Serum total T3 was not significantly altered and neither was the relative or absolute weight of the pituitary. Similar results on thyroid function were reported in female Sprague-Dawley rats administered 2,3,7,8-TCDD by gavage for 30 weeks at doses equivalent to 0.0001–0.125 µg/kg/day (Sewall et al. 1995). The dose-related decrease in serum T4 was statistically significant beginning at the 0.035 µg/kg/day dose level. Serum levels of T3 were not significantly altered by treatment. Sewall et al. (1995) also reported that serum levels of TSH were increased about 3-fold in the highest-dose group. Treatment with 2,3,7,8-TCDD also induced UDP-glucuronosyltransferase-1. Administration of 2,3,7,8-TCDD in the diet also affected thyroid function as demonstrated by Van Birgelen et al. (1995) who found a significant dose-related decrease in plasma total T4 in female Sprague-Dawley rats at dietary doses of $0.047 µg/kg/day for 13 weeks; plasma total T3 was not altered with 2,3,7,8-TCDD doses of up to 1 µg/kg/day. Li and Rozman (1995) examined the reversibility of the 2,3,7,8-TCDD-induced decrease in serum T4 in male Sprague-Dawley rats. The rats were gavaged once a week for 10 weeks with doses equivalent to approximately 0.003–1.6 µg 2,3,7,8-TCDD/kg and this was

followed by a 6-week recovery period. Serum T4 levels were significantly depressed with 2,3,7,8-TCDD doses of $0.03 µg/kg in a dose-dependent fashion and remained low during the recovery period. Based on these results, the authors suggested that the ED_{50} for this dose-response is close to a total cumulative dose of 1 µg/kg.

No significant non-neoplastic lesions were observed in the thyroid, parathyroid, adrenal, and pituitary gland from male and female Sprague-Dawley rats maintained for 2 years on a diet that supplied 0.001, 0.01, or 0.1 µg 2,3,7,8-TCDD/kg/day (Kociba et al. 1978a). Similar results were obtained in male and female Osborne-Mendel rats and in male $B6C3F_1$ mice administered up to approximately 0.071 µg 2,3,7,8-TCDD/kg/day by gavage for 104 weeks, and in female $B6C3F_1$ mice given up to 0.3 µg 2,3,7,8-TCDD/kg/day (NTP 1982b).

Information regarding other CDD congeners is limited. Administration of 1,2,3,4,6,7,8-HpCDD by gavage for 13 weeks to male Sprague-Dawley rats in doses equivalent to 24–110 µg/kg/day produced a dose-related decrease in serum total T4 (Viluksela et al. 1994). Doses of #4 µg/kg/day were without significant effect. Serum levels of total T3 were not significantly affected by treatment. A more recent study reported a 69% decrease in serum T4 levels in male Sprague-Dawley rats administered doses equivalent to 3.8 µg 1,2,3,7,8-PeCDD/kg/day or 15.4 µg 1,2,3,4,7,8-HxCDD/kg/day for 13 weeks (Viluksela et al. 1998b). After an additional 13-week CDD-free period, T4 levels returned to near control levels. In females administered doses of 2.6 µg/kg/day of the penta-CDD or 10.3 µg/kg/day of the hexa-CDD, T4 serum levels were 40% below control levels at the end of the dosing period and 62% below controls at the end of the additional 13-week period. Serum T3 levels were not significantly affected by treatment with either congener (Viluksela et al. 1998b).

In summary, CDDs were shown to alter endocrine parameters mostly in rodent studies. One of the better characterized effects was a decrease in serum T4, caused apparently by CDD-induced T4 metabolism and excretion. Alterations in T3 levels were less consistent. Results from additional studies suggested that 2,3,7,8-TCDD may interfere with secretion and synthesis of ACTH in the pituitary.

Dermal Effects. A number of changes in the skin have been observed in rodents and monkeys. In monkeys, skin lesions seen after a single oral dose or repeated dosing resemble the chloracne observed in humans. Distinctive changes in rhesus monkeys included swelling and inflamed eyelids, nail loss, and facial hair loss with acneform lesions following acute exposure to a single dose of 70 µg/kg (McConnell et

al. 1978a). Monkeys had hair loss due to squamous metaplasia and keratinization of the sebaceous glands and hair follicles, and periorbital edema following intermediate-duration exposure to 0.011 µg/kg/day of 2,3,7,8-TCDD in the diet or exposure to 0.1 µg/day, 3 days a week for 3 weeks, but not in those exposed to 0.02 µg/kg/day (Allen et al. 1977; McNulty 1984). Rough hair coats were described in Syrian hamsters exposed to a single dose of 1,000 µg/kg 2,3,7,8-TCDD, but not in those exposed to 600 µg/kg (Henck et al. 1981). Skin thickening was observed in A2G-hr/+ mice exposed to 75 µg/kg 2,3,7,8-TCDD (Greig 1984). Chronic exposure by gavage to 2,3,7,8-TCDD induced dermatitis in $B6C3F_1$ mice at 0.36 µg/kg/day (Della Porta et al. 1987) and amyloidosis in Swiss mice at 0.001 µg/kg/day (Toth et al. 1979). In the $B6C3F_1$ mice, dermatitis regressed after discontinuation of treatment (Della Porta et al. 1987). In contrast, no dermal effects were observed in Osborne-Mendel rats and in $B6C3F_1$ mice following chronic exposure to 0.71 µg/kg/day and 0.3 µg/kg/day of 2,3,7,8-TCDD, respectively, by gavage for 104 weeks (NTP 1982b).

No dermal effects were found in Osborne-Mendel rats and $B6C3F_1$ mice gavaged with approximately 0.34 µg/kg/day and 0.7 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, respectively, for 104 weeks (NCI/NTP 1980). However, male and female Sprague-Dawley rats treated with doses equivalent to 2.6–3.8 µg 1,2,3,7,8-PeCDD/kg/day or 10.3–15.4 µg 1,2,3,4,7,8-HxCDD/kg/day for 13 weeks exhibited occasional hair loss and sores in the ears, nose, neck, tail, and feet (Viluksela et al. 1998a). No effects were observed following chronic exposure of Osborne-Mendel rats and $B6C3F_1$ mice to 5×10^5 µg/kg/day and 1.3×10^6 µg/kg/day of 2,7-DCDD, respectively, in the feed (NCI/NTP 1979a).

Ocular Effects. No ocular effects were observed in Osborne-Mendel rats and in $B6C3F_1$ mice following chronic exposure to 0.071 µg/kg/day and 0.3 µg/kg/day of 2,3,7,8-TCDD, respectively, by gavage for 104 weeks (NTP 1982b). Also no ocular effects were found in Osborne-Mendel rats and $B6C3F_1$ mice gavaged with approximately 0.34 µg/kg/day and 0.7 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, respectively, for 104 weeks (NCI/NTP 1980). Similarly, no effects were observed following chronic exposure of Osborne-Mendel rats and $B6C3F_1$ mice to 5×10^5 µg/kg/day and 1.3×10^6 µg/kg/day of 2,7-DCDD, respectively, in the feed (NCI/NTP 1979a).

Body Weight Effects. A characteristic effect of exposure to 2,3,7,8-TCDD in animals is the wasting syndrome. This is observed following exposure in all duration categories.

Weight loss or decreased weight gain were recorded in Sprague-Dawley rats following a single dose of 6.25 µg/kg (Moore et al. 1985), 10.6 µg/kg (Roth et al. 1988), 15 µg/kg (Seefeld and Peterson 1984), and 25 µg/kg 2,3,7,8-TCDD (Christian et al. 1986a), and in Fischer 344 rats following a single oral dose of 100 µg/kg (Kelling et al. 1985). Furthermore, about 40% weight loss was recorded in the range of LD_{50} values (164–340 µg/kg) for Fischer 344 rats from different breeding stations (Walden and Schiller 1985). None of the studies provided a NOAEL value. Acute exposure (10–14 days) to lower doses of 2,3,7,8-TCDD caused reduced weight gain in rats at 0.5 µg/kg/day, but not at 0.125 µg/kg/day (Giavini et al. 1983; Sparschu et al. 1971b).

Decreased body weight gain was observed in guinea pigs after a single dose of 6 µg/kg 2,3,7,8-TCDD in oil vehicle but not after 12 µg/kg in soil (Umbreit et al. 1985). A decreased weight gain was recorded in pregnant rabbits exposed during gestation to 0.25 µg/kg/day (Giavini et al. 1982) and in hamsters exposed to 1,000 µg/kg (Henck et al. 1981). ED_{50} values (doses causing a 50% decrease in a measurable parameter relative to the control value) for reduced body weight gain were calculated for 2,3,7,8-TCDD as 1.8 µg/kg for Hartley guinea pigs, 89 µg/kg for Sprague-Dawley rats, 890 µg/kg for C57BL/6 mice, and 1,000 µg/kg for Syrian hamsters (Hanberg et al. 1989). Single doses of $75 µg 2,3,7,8-TCDD/kg produced a slight reduction in body weight in male C57BL/6J mice 8 days after dosing (Weber et al. 1995); feed intake was not affected during this period. In the same study, it was found that body weights of male DBA/2J mice dosed with 1–3,295 µg 2,3,7,8-TCDD/kg were significantly reduced at $1,500 µg 2,3,7,8-TCDD/kg. It should be noted, however, that in mice, decreases in body weight resulting from 2,3,7,8-TCDD exposure do not become evident until 5–7 days after dosing (Shen et al. 1991), and that in C57BL/6J mice reduction of feed intake is insignificant during the first week after dosing (Kelling et al. 1985). Weight loss (28%) was also found in monkeys after a single dose of 70 µg/kg 2,3,7,8-TCDD (McConnell et al. 1978a).

Decreases in body weight gain or body weight loss have been consistently reported in animals following intermediate-duration exposures to 2,3,7,8-TCDD. A decreased weight gain was observed in Osborne-Mendel rats exposed intermittently for 13 weeks by gavage to 0.07 µg/kg/day (NTP 1982b) or in Sprague-Dawley rats treated with $0.2 µg/kg/day for 10–13 weeks (Li and Rozman 1995; Viluksela et al. 1994), in guinea pigs exposed to 0.005 µg/kg/day in the feed (DeCaprio et al. 1986), and in mice intermittently exposed to 20 µg/kg/day by gavage (Thigpen et al. 1975). A weight loss was recorded in rhesus monkeys after 0.1 µg/kg/day of 2,3,7,8-TCDD for 3 weeks of intermittent exposure (McNulty 1984). However, weight loss occurred with longer exposure of 9 months at 0.011 µg/kg/day (Allen et al. 1977). A recent study reported a 10% decrease in body weight gain in female Sprague-Dawley rats fed a diet that supplied

a daily dose of 0.047 µg 2,3,7,8-TCDD/kg for 13 weeks (Van Birgelen et al. 1995). At the highest exposure level (1 µg/kg/day) terminal body weights were reduced to 72% of controls; this group consumed 32% less food than controls.

In chronic-duration experiments with 2,3,7,8-TCDD, decreased body weight gain was reported in Sprague-Dawley rats exposed to 0.1 µg/kg/day (Kociba et al. 1978a) and 0.286 µg/kg/day (Van Miller et al. 1977) in the feed; Osborne Mendel rats exposed to approximately 0.0014 µg/kg/day by gavage for 104 weeks (NTP 1982b); and in $B6C3F_1$ mice exposed to 0.36 µg/kg/day by gavage for 52 weeks (Della Porta et al. 1987), but not in C57BL/6 mice gavaged once per week for 14–15 months with 0.03 µg 2,3,7,8-TCDD (Oughton et al. 1995).

Experiments with other congeners showed milder effects. Acute exposure during gestation caused a decreased maternal weight gain in Sprague-Dawley rats exposed to 10 µg/kg/day mixed HxCDD (Schwetz et al. 1973). Decreased weight gains were observed in rats and mice gavaged for intermediate-duration with 0.71 µg/kg/day and 0.18 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD, respectively (NCI/NTP 1980). Chronic-duration exposure induced decreased weight gain in Osborne Mendel rats exposed to 0.18 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD by gavage for 104 weeks (NCI/NTP 1980). In contrast, no effects on body weight were observed in mice exposed to 0.7 µg/kg/day of a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD for 104 weeks (NCI/NTP 1980). Male Sprague-Dawley rats administered 1,2,3,4,6,7,8-HpCDD by gavage at dose levels equivalent to 73 and 110 µg/kg/day for 13 weeks exhibited a 5.1% and 19.3% reduction in body weight gain, respectively, at the end of the study period (Viluksela et al. 1994). No significant effect was observed with doses #24 µg/kg/day. Relative to controls, the body weight of male Sprague-Dawley rats administered doses equivalent to 3.8 µg 1,2,3,7,8-PeCDD/kg/day or 15.4 µg 1,2,3,4,7,8-HxCDD/kg/day for 13 weeks was reduced by 27% and 24%, respectively, at the end of the dosing period (Viluksela et al. 1998a). In females, doses equivalent to 2.6 µg 1,2,3,7,8-PeCDD/kg/day for 13 weeks resulted in an 18% reduction in body weight relative to controls, whereas doses of approximately 10.3 µg 1,2,3,4,7,8-HxCDD/kg/day for 13 weeks were without significant effect (Viluksela et al. 1998a).

No effect on the body weight of CD-1 mice was observed after 14 daily doses of OCDD at 1 µg/kg/day or 2,7-DCDD at 1,000 µg/kg/day (Courtney 1976). Chronic-duration exposure induced decreased weight gain in Osborne Mendel rats and in $B6C3F_1$ mice exposed to 2.5×10^5 µg/kg/day and 6.5×10^5 µg/kg/day of 2,7-DCDD, respectively, in the feed (NCI/NTP 1979a).

As summarized above, body weight effects were consistently observed in all species exposed to CDDs. Effects occurred after intermittent exposure by gavage and after exposure in a diet. In acute- and intermediate-duration exposure experiments, the wasting syndrome seemed to be the primary cause of death.

2.2.2.3 Immunological Effects

An effect of sublethal exposures (acute, intermediate-term, or chronic) to 2,3,7,8-TCDD common to all species studied is thymic atrophy. Depletion of lymphocytes results in suppression of T-cell immunity. The T-cell responses studied have included delayed hypersensitivity responses, rejection of skin allografts, and *in vitro* mutagen responses of lymphoid cells. T-cell immunotoxicity is probably the most sensitive end point. Effects on T-cells can occur at levels of exposure three orders of magnitude lower than the effects on thymus cellularity. B-lymphocytes are also affected by 2,3,7,8-TCDD, but higher exposure levels are necessary for suppression of humoral immunity. CDDs suppress resistance to different infectious agents by various mechanisms (see Section 2.4 for more detailed information).

Acute ED_{50} values for thymic atrophy following a single dose of 2,3,7,8-TCDD were calculated as 26 µg/kg in Sprague-Dawley rats, 0.8 µg/kg in Hartley guinea pigs, 280 µg/kg in C57BL/6 mice, and 48 µg/kg in Syrian hamsters (Hanberg et al. 1989). A significant dose-related reduction in absolute thymus weight was reported in young male Wistar rats administered single doses of $1 µg/kg 2,3,7,8-TCDD; this effect was paralleled by a significant decrease in thymic cellularity (De Heer et al. 1994b). Thymic atrophy was shown to be initiated in the thymus cortex on day 4 after a single dose of 25 µg/kg 2,3,7,8-TCDD (De Heer et al. 1994a). The initial lymphodepletion in the cortex was followed by a secondary depletion of medullary thymocytes on day 6, and on day 10, a preferential depletion of cortical thymocytes was no longer observed. Decreased thymus weight was reported in pregnant C57BL/6J mice exposed to 0.5 µg/kg/day 2,3,7,8-TCDD for 10 days (Silkworth et al. 1989b). Offspring of C57BL/6J mice similarly exposed to 1.5 µg/kg/day had severe thymic atrophy, cellular depletion and altered thymocyte antigen expression, and immune function (Holladay et al. 1991). In contrast, similar changes were observed in DBA/2J mice only after exposure to higher doses of 8 µg/kg/day. Furthermore, thymic atrophy was observed in rhesus monkeys after a single dose of 70 µg/kg (McConnell et al. 1978a) and in guinea pigs after a dose of 6 µg/kg (Umbreit et al. 1985).

Treatment of rats with daily doses of 0.72 µg 2,3,7,8-TCDD/kg/day by gavage for 14 days did not alter spontaneous NK-cell activity in the lung, but significantly suppressed influenza virus-augmented NK activity (Yang et al. 1994). A significantly higher virus titer was observed on days 2, 3, and 4 in whole lung homogenate from rats treated with a single dose of 10 µg/kg (Yang et al. 1994). Decreased resistance to infection, as evidenced by increased mortality, was observed in $B6C3F_1$ mice infected with *Streptococcus pneumoniae* and administered 1 µg/kg/day 2,3,7,8-TCDD for 14 days (White et al. 1986), and in $B6C3F_1$ mice infected with influenza A virus and administered a single gavage dose of 0.01, 0.05, or 0.1 µg/kg 2,3,7,8-TCDD (Burleson et al. 1996). The Burleson et al. (1996) study identified a NOAEL of 0.005 µg/kg for this effect. Acute exposure to 2,3,7,8-TCDD reduced polymorphonuclear activity in $B6C3F_1$ mice at 5 µg/kg (no effect was seen in DBA/2N mice) (Ackermann et al. 1989). Suppressed antibody response to sheep erythrocytes (SRBC) was reported in $B6C3F_1$ mice that were given a single gavage dose of 1 µg/kg; no such effect was found after a single dose of 0.5 µg/kg (Holsapple et al. 1986a). However, suppression of the antibody response occurred after 14 daily doses of 0.1 µg/kg/day. In rats, a single dose of 20 µg 2,3,7,8-TCDD/kg administered 5 days before immunization significantly enhanced the primary antibody response to SRBC as judged by a significant increase in serum IgG levels 7 days after immunization (Fan et al. 1996). However, serum IgM levels were not significantly affected by doses of 2,3,7,8-TCDD of up to 40 µg/kg. Fan et al. (1996) also observed that cell-mediated immunity, tested with a delayed-type hypersensitivity (DTH) assay, exhibited a U-shaped response to treatment with 2,3,7,8-TCDD, as doses of 1–20 µg/kg increased the DTH response, whereas doses of 30–90 µg/kg decreased it, even below control levels.

Suppressed total serum complement activity was observed in female $B6C3F_1$ mice exposed to a single gavage dose of 14 µg/kg or 14 daily doses of 0.01 µg/kg/day (White et al. 1986). Serum levels of complement component C3 were also suppressed at doses of $0.5 µg/kg 2,3,7,8-TCDD (White et al. 1986). Subsequent studies by the same group showed that the 2,3,7,8-TCDD-induced reduction in serum C3 is not the result of a decrease in C3 production by hepatocytes but, at least in part, may be due to increased catabolism (Lin and White 1993). Single gavage doses of $2.5 µg 2,3,7,8-TCDD/kg suppressed cytotoxic T-lymphocyte (CTL) activity in mice challenged with a tumor allograft by a mechanism that did not involve elevation in plasma glucocorticoid levels (De Krey and Kerkvliet 1995). This was directly correlated with reduced numbers of splenic CTL effector cells (Kerkvliet et al. 1996). In these same animals, a suppression of the alloantibody response was correlated with a decreased expansion of the B-cell splenocyte population. This dose of 2,3,7,8-TCDD also initially induced interferon-γ, interleukin-2, and tumor necrosis factor production, but the normal increase of these in response to the tumor allograft was

not observed. Based on these and additional studies, the authors concluded that these effects are due to TCDD initially interfering with the activation of $CD4^+$ T cells and possibly T helper-B cell interactions. A recent study from the same group of investigators presented evidence that immune 2,3,7,8-TCDD-induced suppression in C57BL/6 mice is not caused by direct alterations in the production of immunomodulatory metabolites of arachidonic acid (Lawrence and Kerkvliet 1997). The above results indicate that immunological effects occur after moderate-to-low single doses or after repeated low doses that accumulate in the body, suggesting that the total dose of 2,3,7,8-TCDD is important. As shown in Figure 2-1, immunotoxicity was a very sensitive end point; the lowest LOAEL for immune effects is 0.01 µg/kg/day (Burleson et al. 1996; White et al. 1986). In the Burleson et al. (1996) study, decreased resistance to infection was observed in mice receiving a single gavage dose of 0.01 µg/kg, and no effects were observed at 0.005 µg/kg. Reduced serum complement levels were observed in mice exposed to 0.01 µg/kg/day for 14 days (White et al. 1986); no NOAEL was identified in this study. The NOAEL of 0.005 µg/kg/day identified in the Burleson et al. (1996) study was used to derive an acute oral MRL for 2,3,7,8-TCDD of 2×10^{-4} µg/kg/day as described in the footnote to Table 2-2, Section 2-5, and in Appendix A.

Several immunological effects were observed following intermediate-duration exposure to 2,3,7,8-TCDD. Decreased thymus weight after 2,3,7,8-TCDD exposure was observed in rats dosed by gavage with 0.71 µg/kg/day for 6 weeks (Vos et al. 1973), in the F_3 generation of rats receiving 0.01 µg/kg/day (Murray et al. 1979), and in guinea pigs receiving 0.005 µg/kg/day or 0.03 µg/kg/day (thymic atrophy) in the feed for 90 days (DeCaprio et al. 1986). A significant reduction in absolute and relative thymus weight was observed in male Sprague-Dawley rats administered 2,3,7,8-TCDD by gavage at doses equivalent to 0.8 µg/kg/day (only dose level tested) for 13 weeks (Viluksela et al. 1994). Spleen weight was not significantly altered. Similar results were reported in female Sprague-Dawley rats fed for 13 weeks a diet that supplied doses of $0.014 µg 2,3,7,8-TCDD/kg/day (Van Birgelen et al. 1995). Relative spleen weight was increased at $0.047 µg 2,3,7,8-TCDD/kg/day. Decreased cell-mediated immunity was found in mice and guinea pigs exposed by gavage to 0.71 µg/kg/day for 4 weeks and 0.03 µg/kg/day for 8 weeks, respectively (Vos et al. 1973). Guinea pigs seem to be especially sensitive to 2,3,7,8-TCDD toxicity; an intermediate-duration exposure to 0.001 µg/kg/day reduced the lymphocyte counts, and exposure to 0.03 µg/kg/day caused decreased humoral immunity and thymic atrophy (Vos et al. 1973). A recent study examined the effect of low-level dietary exposure to 2,3,7,8-TCDD to young adult male Leeds strain rats (Badesha et al. 1995). A 30-day exposure to approximately 0.1 µg/kg/day (or a total dose of approximately 3 µg/kg) resulted in an exposure duration-dependent reduction of *in vitro* lipopoly-saccharide-induced production of interleukin-1 in cultures of their splenic macrophages. A 180-day

exposure to approximately 0.017 µg/kg/day suppressed the production of interleukin-2 by either concanavalin A or phorbol ester/calcium ionophore stimulation, and reduced the lectin-induced proliferation of splenic T cells. The authors concluded that exposure to a low dietary dose of 2,3,7,8-TCDD suppresses the functions of several T-cell subsets. The highest NOAEL value for immunological effects (decreased thymus weight) was 0.0007 µg/kg/day 2,3,7,8-TCDD given to the most sensitive species, guinea pigs, in the diet (DeCaprio et al. 1986). The NOAEL value of 0.0007 µg/kg/day was used to derive an intermediate-duration oral MRL for 2,3,7,8-TCDD of 2×10^{-5} µg/kg/day as described in the footnote to Table 2-2, Section 2.5, and in Appendix A.

Increased mortality that was indicative of altered immunity was also observed in C57BL/6Jfh mice challenged with *Salmonella bern* following exposure to $1 µg/kg/day of 2,3,7,8-TCDD by gavage once a week for 4 weeks (Thigpen et al. 1975); no significant effects were observed at 0.5 µg/kg/day. In the same study, using the same experimental design, doses of up to 20 µg/kg/day of 2,3,7,8-TCDD had no significant effect on mortality in mice infected with *Herpesvirus suis* (Thigpen et al. 1975). Exposure to 0.5 µg/kg/day 2,3,7,8-TCDD once a week for 5–8 weeks caused suppression of humoral activity in C57BL/6 mice (Vecchi et al. 1983a). In addition, lymph node atrophy was reported in monkeys exposed to a lethal dose of 0.011 µg/kg/day in the feed for 9 months (Allen et al. 1971).

Administration of 2,3,7,8-TCDD at approximately 0.071 µg/kg/day to Osborne-Mendel rats or at about 0.3 µg/kg/day to B6C3F1 mice by gavage for 104 weeks produced no histological alterations in the spleen or thymus (NTP 1982b). Chronic exposure to 2,3,7,8-TCDD in food induced thymic atrophy in Sprague-Dawley rats at 0.1 µg/kg/day in a 2-year study (Kociba et al. 1978a) with the highest NOAEL of 0.01 µg/kg/day. Furthermore, rhesus monkeys exposed chronically to 0.002 µg/kg/day 2,3,7,8-TCDD in the feed exhibited degeneration of the bone marrow and lymphoid tissues (Hong et al. 1989). A recent study examined the effect of long-term exposure to 2,3,7,8-TCDD on various immune cell phenotypes of female C57 BL/6 mice (Oughton et al. 1995). The mice were administered 0.2 µg 2,3,7,8-TCDD/kg once per week for 14–15 months; this resulted in a cumulative dose of 12–13 µg/kg (approximately 0.03 µg/kg/day) and a concentration of 2,3,7,8-TCDD in adipose tissue of 1.27 ng/g abdominal fat. There were no significant 2,3,7,8-TCDD-related effects on thymus and spleen weight or in the cellularity of these tissues. Exposure to 2,3,7,8-TCDD induced subtle changes in thymic phenotypes which, according to the authors, were of questionable biological relevance given the age-related decrease in thymic cellularity observed. 2,3,7,8-TCDD did not alter the frequencies of the major leukocyte subpopulations, but significantly altered functionally discrete subpopulations within the T-cell compartment. The most notable

change was a decrease in the frequency of memory T helper cells, with a concomitant increase in the proportion of naive T helper cells. Oughton et al. (1995) also presented preliminary data suggesting that phenotypic changes in spleen cells correlated with similar changes in blood cells.

Other CDD congeners also appear to affect the immune system. Significant dose-related decreases in absolute and relative thymus weight were observed in male Sprague-Dawley rats administered doses equivalent to 4–110 µg/kg/day 1,2,3,4,6,7,8-HpCDD for 13 weeks by gavage (Viluksela et al. 1994). A dose level of 0.3 µg/kg/day was without significant effect. Treatment with 1,2,3,4,6,7,8-HpCDD had no significant effect on spleen weight. Suppressed antibody response was reported in $B6C3F_1$ mice after 2 weeks of exposure to 0.1 µg/kg/day of 2,7-DCDD, but not after exposure to 10 µg/kg/day of OCDD (Holsapple et al. 1986b). Depressed antibody response was found in C57BL/6 mice exposed to a single dose of 33 µg/kg/day 1,2,3,4,6,7,8-HpCDD (Kerkvliet and Brauner 1987). Suppressed serum complement activity was found in $B6C3F_1$ mice following 2 weeks of exposure to 1 µg/kg/day 1,2,3,6,7,8-HxCDD (White et al. 1986). Splenic hyperplasia was observed in Osborne-Mendel rats after exposure to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD at 7.1 µg/kg/day, 1 day/week for 13 weeks (NCI/NTP 1980).

In conclusion, the immunological system was a sensitive target of CDD toxicity under experimental conditions in animals. Effects on all types of mediated immunity were seen at doses of 2,3,7,8-TCDD as low as 0.01 µg/kg. Doses of 2,3,7,8-TCDD that were well below the lethal dose affect humoral immunity. Thymic atrophy occurs as single or multiple doses approach those that may increase lethality. Neonates and young animals are much more sensitive than adults to most of the immunological responses.

The highest NOAEL values and all reliable LOAEL values for immunological effects in each species and duration category for each congener are recorded in Table 2-2 and 2-3 and plotted in Figures 2-1 and 2-2.

2.2.2.4 Neurological Effects

Limited information was obtained regarding neurological effects in animals. Decreased motor activity was observed in Sprague-Dawley rats after a single dose of 5 µg/kg of 2,3,7,8-TCDD that was not associated with mortality (Seefeld et al. 1984a) and after 14 daily doses of 2 µg/kg/day to pregnant females that were sacrificed on day 21 of gestation for developmental effects evaluation (Giavini et al. 1983). The NOAEL value was 0.01 µg/kg/day. Administration of 2,3,7,8-TCDD by gavage to male and female Osborne-

Mendel rats and male B6C3F1 mice at doses of up to 0.071 µg/kg/day for 104 weeks did not result in significant histological alterations in the brain, spinal cord, or sciatic nerve (NTP 1982b). The same was found for female B6C3F1 dosed with up to 0.3 µg/kg/day for the same time period (NTP 1982b).

Although motor effects have been described in rats dosed with 2,3,7,8-TCDD, in most studies, the neurological system was not specifically examined; therefore the issue of whether CDDs have a direct effect on the nervous system of animals has not been conclusively resolved.

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species and duration category are recorded in Tables 2-2 and 2-3 and plotted in Figures 2-1 and 2-2.

2.2.2.5 Reproductive Effects

A number of reproductive effects have been observed in animals orally exposed to 2,3,7,8-TCDD, including reduced fertility, pre- and post-implantation losses, decreases in gonad weights, decreased androgen levels, and altered estrus cycle and ovulation. Increased pre- and postimplantation losses were observed in CRCD rats exposed to 0.5 µg/kg/day for 2 weeks before mating (Giavini et al. 1983). Increased resorptions were found in Sprague-Dawley rats exposed to 0.125 µg/kg/day (Sparschu et al. 1971a), in CF-1 mice exposed to 1.0 µg/kg/day (Smith et al. 1976), and in NMRI mice exposed to 9 µg/kg/day (Neubert and Dillmann 1972) on gestation days (Gd) 6–15. In rabbits, increased postimplantation losses were recorded in a group exposed to 0.25 µg/kg/day, but not in those exposed to 0.1 µg/kg/day on Gd 6–15 (Giavini et al. 1982). Furthermore, increased abortions (10 of 12) were observed in monkeys after a single gavage dose of 1 µg/kg (McNulty 1984).

Reproductive toxicity has also been observed in non-pregnant female rats. Significant decreases in ovarian weight, ovulation rate, and the number of ova released have been observed in female Sprague-Dawley rats receiving a single gavage dose of $10 µg/kg (Li et al. 1995a, 1995b). Effects on hormone levels were also observed in the rats. Within 24 hours after dosing, significant increases in LH and follicle stimulating hormone levels were observed; prolactin levels were not altered (Li et al. 1995a). Following the administration of 17β-estradiol, LH and follicle stimulating hormone levels dropped below control levels.

2,3,7,8-TCDD is also a reproductive toxicant in males. Decreased seminal vesicle weight was reported in Sprague-Dawley rats after a single dose of 4.5 µg/kg, with decreased androgen levels detected only on day

6 postexposure (Moore et al. 1985). Inflammation of the epididymis with sperm granuloma formation was reported in Wistar rats exposed to 4 µg/kg/day for 7 days, and decreases in the weight of male reproductive organs together with reduced levels of serum testosterone and dihydrotestosterone (compared with the pair-fed controls) were seen in Sprague-Dawley rats after a single gavage dose of 12.5 µg/kg 2,3,7,8-TCDD (Khera and Ruddick 1973). An ED_{50} for altered regulation of LH levels was calculated as 10 µg/kg 2,3,7,8-TCDD in Sprague-Dawley male rats (Bookstaff et al. 1990a). No dominant lethality was reported when male Wistar rats were given 12 µg/kg/day 2,3,7,8-TCDD for 7 days before mating (Khera and Ruddick 1973).

In intermediate-duration studies with 2,3,7,8-TCDD, increased mortality was found in the offspring of Swiss Webster mice that were kept on a diet providing 0.35 µg/kg/day 2,3,7,8-TCDD for 4 weeks before mating, during gestation, and for 3 weeks of lactation (Thomas and Hinsdill 1979). Blocked estrous cycle was observed in female C57BL/6 mice exposed by gavage to 3 µg/kg/day, 3 days a week for 25 weeks (Umbreit et al. 1987), but no reproductive effects were seen in male mice exposed 1 day/week for 30 weeks to the same dose (Umbreit et al. 1988). However, reduced spermatogenesis was found in Sprague-Dawley rats exposed for 4 weeks to 3.4 µg/kg/day in the feed, but not in those similarly exposed to 0.286 µg/kg/day (Van Miller et al. 1977). Exposure of female rhesus monkeys to 0.1 µg/kg/day 3 days a week by gavage for 3 weeks caused abortions in 3 of the 4 monkeys; 1 of the 4 monkeys administered 0.02 µg/kg/day aborted (McNulty 1984). In a 3-generation study with 2,3,7,8-TCDD, significantly reduced fertility was observed among F_1- and F_2-generation rats exposed before mating to 0.01 µg/kg/day in the feed for 90 days, but not in those exposed to 0.001 µg/kg/day (Murray et al. 1979).

In chronic-duration studies, increased abortions and reduced reproduction rates were reported in monkeys exposed to 0.00064 µg/kg/day of 2,3,7,8-TCDD in the feed (Bowman et al. 1989b; Hong et al. 1989; Schantz et al. 1992). No reproductive effects were found at 0.00012 µg/kg/day. No changes were observed in the reproductive organs of Sprague-Dawley rats chronically exposed to 0.1 µg/kg/day in the feed (Kociba et al. 1978a), or Osborne-Mendel rats and $B6C3F_1$ mice exposed by gavage to approximately 0.0.71µg/kg/day and 0.3 µg/kg/day of 2,3,7,8-TCDD, respectively (NTP 1982b).

Rier et al. (1993) found a dose-related increase in the incidence and severity of endometriosis in monkeys chronically exposed to 0.00012 or 0.00064 µg/kg/day of 2,3,7,8-TCDD in the diet. Surgical-induced endometriosis was enhanced by 2,3,7,8-TCDD exposure in rats and mice. In a surgically induced endometriosis model, significant increases in the diameter of the endometriotic site and an acceleration of

growth were observed in rats (Cummings et al. 1996) and mice (Cummings et al. 1996; Johnson et al. 1997), respectively. In this model, the animals received a gavage dose of 2,3,7,8-TCDD every 3 weeks (first dose was administered 3 weeks prior to surgical induction of endometriosis) for a total of five doses. Mice appear to be more sensitive than rats in terms of the magnitude of the effect on endometrial site diameter and adverse effect levels (endometriosis promotion was observed at 1, 3, and 10 µg/kg in mice [Cummings et al. 1996; Johnson et al. 1997] and at 10 µg/kg [Cummings et al. 1996]; no effects were observed in rats at 3 µg/kg). In contrast to these results, Foster et al. (1997) found that 2,3,7,8-TCDD exposure suppressed endometrial growth in mice. In their model, the mice were not pre-exposed to 2,3,7,8-TCDD prior to the induction of endometriosis. Foster et al. (1997) notes that pre-exposure to 2,3,7,8-TCDD results in endometriosis development due to immune suppression rather than an estrogen-responsive disease.

Acute-duration studies examining reproductive effects have been conducted with other congeners. Increased resorptions were found in Sprague-Dawley rats exposed to 10 µg/kg/day mixed HxCDD during gestation but not in those exposed to 1 µg/kg/day (Schwetz et al. 1973). No reproductive effects were found in rats exposed to 1×10^5 µg/kg/day 2,7-DCDD or 5×10^5 µg/kg/day OCDD during gestation. Similarly, no reproductive effects were found in rats exposed to 800 µg/kg/day 1,2,3,4-TCDD or 2,000 µg/kg/day 2-MCDD, 2,3-DCDD, or 2,7-DCDD on Gd 6–15 (Khera and Ruddick 1973).

The above data demonstrate that exposure to CDDs caused reproductive effects in animals. 2,3,7,8-TCDD was the most potent congener. The effects included increased pre- and postimplantation losses in females, morphological and functional changes in male and female reproductive organs, and hormonal imbalance in both sexes.

The highest NOAEL values and all reliable representative LOAEL values for reproductive effects in each species and duration category for each congener are recorded in Tables 2-2 and 2-3 and plotted in Figures 2-1 and 2-2.

2.2.2.6 Developmental Effects

A number of developmental effects have been observed in animals acutely exposed to 2,3,7,8-TCDD by the oral route. The types of effects observed in the offspring of animals exposed to 2,3,7,8-TCDD include structural malformations-cleft palate and kidney anomalies, functional alterations-damage to the immune

system and impaired development of the reproductive system, decreased growth, and fetal/newborn mortality.

Cleft palate and other skeletal anomalies have been observed in the offspring of CRCD rats exposed to 2 µg/kg/day for 2 weeks prior to conception (Giavini et al. 1983), in Long Evans rats exposed to 5 µg/kg on Gd 8 (Huuskonen et al. 1994), in rabbits exposed to 0.1 µg/kg/day during Gd 6–15 (Giavini et al. 1982), in C57BL/6N mice exposed to 12 µg/kg once on Gd 10 (Weber et al. 1985), in C57BL/6 mice exposed to 6 µg/kg once on Gd 10 or 12 (Abbott and Birnbaum 1989a), in C57BL/6J mice exposed once to 15 µg/kg on Gd 9 (Dasenbrock et al. 1992), in CD-1 mice exposed to 25 µg/kg/day during Gd 7–16 (Courtney 1976), in CF-1 mice exposed to 1 µg/kg/day on Gd 6–15 (Smith et al. 1976), in DBA2J mice exposed to 150 µg/kg on Gd 9 (Dasenbrock et al. 1992), and in NMRI mice exposed to 3 µg/kg/day during Gd 6–15 (Neubert and Dillmann 1972). The incidence of cleft palate was not significantly altered in Han/Wistar rats exposed to 1 or 10 µg/kg on Gd 8 (Huuskonen et al. 1994), in Long Evans rats exposed to 1 µg/kg on Gd 8 (Huuskonen et al. 1994), or in C57BL/6N mice exposed to 3 µg/kg/day on Gd 10–13 (Abbott et al. 1992).

Kidney anomalies, mainly hydronephrosis, were found in the offspring of CRCD rats exposed to 2 µg/kg/day for 2 weeks prior to conception (Giavini et al. 1983), in Han/Wistar rats exposed to 10 µg/kg on Gd 8 (Huuskonen et al. 1994), in C57BL/6N mice exposed to 12 µg/kg on Gd 10 (Abbott et al. 1987a, 1987b), in C57BL/6N mice exposed to 1 µg/kg on Gd 10 (Moore et al. 1973), in CD-1 mice exposed during Gd 7–16 to 25 µg/kg/day (Courtney 1976), in C57BL/6J and DBA/2J mice exposed to 0.5 µg/kg/day 2,3,7,8-TCDD on Gd 6–15 (Silkworth et al. 1989b), in C57BL/6N mice exposed postnatally through contaminated mothers' milk (Couture-Haws et al. 1991b), and in Golden Syrian hamsters exposed to 1.5 µg/g on Gd 7 or 9 (Olson and McGarrigle 1992). An increase in the severity of nephrosis was observed in 4–5-month-old Syrian hamsters receiving a single dose of 2 µg/kg *in utero* on Gd 11(Gray et al. 1995). No significant increases in the incidence of hydronephrosis or dilatation of renal pelvis were observed in Long Evans rats exposed to 1 or 5 µg/kg on Gd 8 (Huuskonen et al. 1994) or Han/Wistar rats exposed to 1 µg/kg on Gd 8 (Huuskonen et al. 1994).

The immune system effects include thymic atrophy and immunosuppression. Thymic atrophy was found in pups of Sprague-Dawley rats exposed to a single dose of 10 µg/kg 2,3,7,8-TCDD on lactation day 1 (Håkansson et al. 1987) and in Long Evans and Han/Wistar rats exposed to 5 or 10 µg/kg, respectively, on Gd 8 (Huuskonen et al. 1994). At lower doses, the thymic atrophy may be transitory; thymic atrophy was

observed on Gd 19 in the offspring of F344 rats exposed to 3.0 µg/kg on Gd 14 but not on Gd 22 (Gehrs et al. 1997a). Similarly, transient thymus atrophy was observed in offspring of BALB/cGa mice exposed to 10 µg/kg on Gd 14 (Fine et al. 1989). A dose-related decrease in relative thymus weights was seen in offspring of rats dosed at levels of 0.005–0.35 µg/kg 2,3,7,8-TCDD on Gd 16 (Madsen and Larsen 1989). Severe thymic atrophy and cellular depletion occurred in offspring of C57BL/6N mice exposed to 1.5 or 3 µg/kg/day on Gd 6–14 (Blaylock et al. 1992; Holladay et al. 1991). Thymus size was not affected in the offspring of Long Evans or Han/Wistar rats exposed to 1 µg/kg on Gd 8 (Huuskonen et al. 1994). Reversible suppression of cell-mediated immunity was reported in pups of Fischer 344 rats exposed to 2,3,7,8-TCDD through the dosing of dams on lactation day 0, 7, and 14 with 5 µg/kg/day (Faith and Moore 1977). Increased neutrophils were found in pups of $B6C3F_1$ mice exposed to 1 µg/kg/day 2,3,7,8-TCDD on Gd 14 and lactation days 1, 7, and 14 (Luster et al. 1980). Furthermore, increased lymphocytes and decreased erythrocytes and hematocrit were recorded in groups exposed to 5 µg/kg/day. Alterations in thymocyte phenotypes have also been observed following *in utero* and/or lactational exposure. A decrease in the percentage of $CD3^-/CD4^-CD8^-$, $CD3^+/CD4^-CD8^-$, and $CD3^+/CD4^+CD8^+$ thymocytes and an increase in $CD3^+/CD4^-CD8^+$ thymocytes were observed in the offspring of F344 rats exposed to 1.0 or 3.0 µg/kg on Gd 14 (Gehrs et al. 1997a). A decrease in $CD4^-/CD8^-$ thymocytes was observed following *in utero*, lactation only, or *in utero* and lactational exposure to 1.0 µg/kg (administered on Gd 14) (Gehrs et al. 1997b). *In utero* and lactational exposure also resulted in an increase in the percentage of $CD4^-/CD8^+$ lymphocytes; this was not observed in the *in utero* only or lactation only groups. Gehrs et al. (1997b) also found a suppression of the delayed hypersensitivity response to BSA in 5-month-old male offsprings receiving *in utero* and lactational exposure.

A number of studies have found impaired development of the reproductive system in male and female animals exposed to 2,3,7,8-TCDD during gestation. 2,3,7,8-TCDD affects androgen levels, secondary sex organs, spermatogenesis, fertility, and sexual behaviors. Effects have been observed in male and female offspring, although most of the studies have focused on males. Malformations of external genitalia (clefting, hypospadias, and vaginal thread), delayed vaginal opening (only significant in rats exposed on Gd 15; Gray and Ostby 1995), decreased number of ovarian follicles (only tested in Gd 15-exposed rats), and decreased fertility have been observed in female offspring of Holtzman and Long Evans rats exposed to a single dose of 1 µg/kg on Gd 8 or 15 (Gray and Ostby 1995; Flaws et al. 1997; Heimler et al. 1998). Gd 8 exposure also resulted in accelerated onset of constant estrus, shortened reproductive lifespan, and increased incidences of cystic hyperplasia of the endometrium. Malformations of the external genitalia were also observed in the offspring of Long Evans rats exposed to 0.20 or 0.80 µg/kg on Gd 15 but not at

0.05 µg/kg (Gray et al. 1997a). The fertility rate was not adversely affected in the offspring, but there was an increase in time to pregnancy in the 0.80 µg/kg group. In a cross-fostering experiment (Gray et al. 1997a), similar morphological reproductive alterations were observed following *in utero* exposure to 1.0 µg/kg (Gd 15) but not after lactation-only exposure. Chaffin et al. (1996) found significant decreases in serum estrogen levels in the female offspring of Holtzman rats receiving a single dose of 1 µg/kg on Gd 15. This study also found an increase in estrogen receptor mRNA in the hypothalamus, uterus, and ovary and a decrease in the pituitary; an increase and a decrease in estrogen receptor binding DNA were found in the uterus and hypothalamus, respectively. 2,3,7,8-TCDD exposure did not alter gonadotropin secretion; no alterations in serum FSH, LH, or androstenedione levels were observed on postnatal day 21 (Chaffin et al. 1997).

In male Holtzman rats exposed to 1 µg/kg on Gd 15, significant decreases in plasma testosterone were observed on Gd 18–21 (Mably et al. 1992a). Additionally, the normal surge in testosterone levels that occurs 2 hours after birth was delayed until 4 hours after birth and the amplitude of the surge was lower in 2,3,7,8-TCDD-exposed male offspring. Bjerke and Peterson (1994) observed significant decreases in plasma testosterone levels in Holtzman rats at age 63 days following exposure to 1 µg/kg on Gd 15. But studies by Mably et al. (1992a) and Gray et al. (1995) did not find significant alterations in plasma testosterone levels in pre- and post-pubescent rats; although Mably et al. (1992a) did report a tendency toward dose-related decreases in plasma testosterone and 5α-dihydrotestosterone levels in Holtzman rats at age 32, 49, 63, and 120 days following perinatal exposure to 0.064–1 µg/kg on Gd 15. Roman et al. (1995) found similar decreases at age 32 and 49 days, but at day 63 the testosterone levels were similar to controls. Most studies found large inter-animal variations in plasma testosterone levels; this coupled with small sample sizes may have been a contributing factor in the conflicting results that were found. Luteinizing hormone levels (primary hormone stimulating testosterone production) were decreased in the 32-day-old male offspring of dams receiving a dose of 1 µg/kg on Gd 15; no significant alterations in LH levels were observed in 49-, 63-, or 120-day-old rats (Mably et al. 1992a). Additional effects on androgenic status (androgen concentrations and androgen-dependent structures and functions) have been observed. Significant decreases in anogenital distance (corrected for differences in body size by dividing by crown-rump length) were observed in male Holtzman rats exposed to $0.16 µg/kg on Gd 15 (Mably et al. 1992a); however, Bjerke and Peterson (1994), Bjerke et al. (1994a), and Gray et al. (1995) did not find significant alterations in anogenital distance (also corrected for body size) in Holtzman and Long Evans rats exposed to 0.7 or 1 µg/kg on Gd 15. More consistent results were found for other measures of androgenic status. Significant alterations included a delay in testis descent (Bjerke et al. 1994a), delay in

preputial separation (external indicator of delayed puberty) (Bjerke et al. 1994a; Gray et al. 1995, 1997b), decreased ventral prostate weight (Bjerke and Peterson 1994; Mably et al. 1992a), and decreased seminal vesicle weight (Bjerke and Peterson 1994; Bjerke et al. 1994a; Mably et al. 1992a). The results of the Mably et al. (1992a) study suggest that the decrease in ventral prostate weight may be the most sensitive indicator of 2,3,7,8-TCDD-induced toxicity on androgen status. Decreases were observed in the rats exposed to $0.064 µg/kg on Gd 15; the other effects were observed in rats exposed to $0.16 µg/kg. Decreases in absolute testis weight and cauda epididymis weight were observed in juvenile (Mably et al. 1992c), pubertal (Mably et al. 1992c), postpubertal (Bjerke and Peterson 1994; Mably et al. 1992c), and sexually mature (Gray et al. 1995; Mably et al. 1992c) rats prenatally exposed to 2,3,7,8-TCDD on Gd 15. The lowest LOAEL for these effects was 0.064 µg/day (decreased testes weight), identified in the Mably et al. (1992c) study. Recent studies from the same group of investigators have focused on evaluating the potential role of the Ah receptor (see section 2.4.2 for a detailed discussion on the Ah receptor-mediated mechanism of action of CDDs) on the developmental alterations of the male reproductive tract (Roman and Peterson 1998; Roman et al. 1998a, 1998b). These studies are summarized in Section 2.5.

Significant decreases in daily sperm production (Bjerke and Peterson 1994; Mably et al. 1992c; Sommer et al. 1996), the amount of mature sperm stored in the cauda epididymis (Bjerke and Peterson 1994; Gray et al. 1995; Mably et al. 1992c), and the amount of sperm ejaculated (Gray et al. 1995, 1997b) were observed in gestationally exposed rats. These adverse effects on spermatogenesis occurred at doses of $0.05 µg/kg (Gray et al. 1997b). Sommer et al. (1996) suggest that observed decreases in cauda epididymal sperm number is likely due to a decrease in daily sperm production and an increase in sperm phagocytosis in the excurrent duct system. Sommer et al. (1996) and Wilker et al. (1996) did not find alterations in sperm epididymal transit time. Significant decreases in follicle stimulating hormone levels (necessary for the initiation of spermatogenesis) have been observed in 32-day-old male rats receiving a perinatal dose of 0.064, 0.40, or 1 µg/kg on Gd 15, but not in 49-, 63-, or 120-day-old rats (Mably et al. 1992c). In 70- and 120-day-old males exposed to #1 µg/kg and mated with unexposed females, no significant alterations in reproductive outcomes (fertility index, gestational index, survival index) were observed (Mably et al. 1992c); however, a non-significant decrease in fertility index was observed in the 0.4 and 1 µg/kg males. Gray et al. (1995) found a significantly decreased number of implants when males exposed to 1 µg/kg on Gd 15 were mated with unexposed females. Altered fertility was not observed in the male offspring of rats exposed to 2,3,7,8-TCDD on Gd 8 (Gray et al. 1995). Several studies have found a demasculinization of sexual behavior (prolonged intromission latency, increased number of intromissions prior to ejaculation) (Bjerke et al. 1994b; Mably et al. 1992b) and partial feminization of sexual behavior (increased intensity of

lordosis and lordosis quotient) in male offspring of rats dosed with 2,3,7,8-TCDD on Gd 15 (Bjerke and Peterson 1994; Bjerke et al. 1994b; Mably et al. 1992b). Mably et al. (1992b) identified 0.16 µg/kg on Gd 15 as the lowest LOAEL for demasculinized and feminized behaviors with a NOAEL of 0.064 µg/kg. A demasculinization and feminization of male rats has also been observed in 2,3,7,8-TCDD-exposed males. In perinatally exposed castrated rats, no significant alteration in plasma LH levels were observed following injection of estradiol benzoate. However, the response to progesterone administration in the rats receiving 0.40 or 1 µg/kg on Gd 15 was similar to that seen in unexposed ovariectomized females, and the plasma LH levels were significantly higher than in control males (Mably et al. 1992b). Impaired development of the reproductive system has also been observed in male Syrian hamsters exposed to 2 µg/kg on Gd 11 (Gray et al. 1995). Decreased epididymal sperm reserves, decreased testis and cauda epididymides weight, and delayed puberty were observed.

Alterations in mammary gland differentiation (less differentiation) were observed in 50-day-old offspring from rats treated by gavage with 1 µg 2,3,7,8-TCDD/kg on gestation day 15 (Brown et al. 1998). Specifically, treatment with 2,3,7,8-TCDD resulted in significantly more terminal end buds and fewer lobules II. However, no such effect was seen in 21-day-old rats. Prenatal treatment with 2,3,7,8-TCDD also resulted in an increased number of mammary adenocarcinomas in the offspring in response to dimethylbenz[a]anthracene (DMBA) relative to rats treated with DMBA alone. The authors speculated that the decreased differentiation may have rendered the gland more susceptible to mammary cancer.

Intestinal hemorrhage (Khera and Ruddick 1973; Sparschu et al. 1971a), subcutaneous edema, and hemorrhages in brain (Khera and Ruddick 1973) were observed in the offspring of Wistar rats treated with 0.125 or 0.25 µg/kg/day 2,3,7,8-TCDD during Gd 6–15, and gastrointestinal hemorrhage was observed in Han/Wistar rats exposed to 10 µg/kg on Gd 8 or 12 (Huuskonen et al. 1994). Decreases in mammary gland size due to inhibition of cell proliferation and gland development were observed in female Sprague-Dawley rats dosed with 2.5 µg/kg/day at ages 25, 27, 29, and 31 days (Brown and Lamartiniere 1995). Exposure to 0.10 µg/kg/day 2,3,7,8-TCDD during gestational days 10–16 resulted in significant decreases in T4 levels in female Sprague-Dawley rat pups. Thyroxine levels were not significantly altered in male rats or in females exposed to 0.025 µg/kg/day, and no alterations were observed in triiodothyronine or TSH values in males and females exposed to either dose (Seo et al. 1995). A decrease in core body temperature was observed in the offspring of Long Evans rats exposed to 1 µg/kg on Gd 15; no effect on metabolic rate or evaporative heat loss was observed (Gordon et al. 1995).

Decreases in fetal and newborn body weight were observed in Holtzman rats exposed to 0.7 or 1 µg/kg on Gd 15 (Bjerke and Peterson 1994; Bjerke et al. 1994a). No body weight effects were observed in C57BL/6N mouse fetuses exposed to maternal doses of 3 µg/kg on Gd 10–13 (Abbott et al. 1992). Crown-rump length was also decreased in Holtzman rats exposed to 1 µg/kg on Gd 15 (Bjerke and Peterson 1994).

Several studies have reported increased mortality in the offspring of rats and monkeys exposed to 2,3,7,8-TCDD during gestation. Fetal/newborn deaths have occurred at doses which were either non-toxic or minimally toxic to the mothers. Increased newborn mortality was observed in Holtzman rat pups exposed to 0.7 or 1 µg/kg on Gd 15 (Bjerke and Peterson 1994; Bjerke et al. 1994a); and decreased numbers of live fetuses, caused by increased resorption and fetal deaths, were observed in monkeys after a single exposure to 1 µg/kg on Gd 25, 30, 35, or 40 (McNulty 1984) and in Long Evans or Han/Wistar rats exposed to 5 or 10 µg/kg, respectively, on Gd 8 (Huuskonen et al. 1994). After exposure of mouse dams to 12.5 µg/kg/day 2,3,7,8-TCDD on Gd 14–17, 75% lethality was observed in the pups (Nau et al. 1986).

In intermediate-duration exposure experiments, decreased neonatal survival was found in the F_1 and F_2 generations of Sprague-Dawley rats exposed via the feed to 0.01 µg/kg/day, but not to 0.001 µg/kg/day, of 2,3,7,8-TCDD in a 3-generation study (Murray et al. 1979). Thymic atrophy was found in offspring of Swiss Webster mice that were kept on a diet providing 0.35 µg/kg/day 2,3,7,8-TCDD for 4 weeks before mating, during gestation, and for 3 weeks of lactation (Thomas and Hinsdill 1979). No developmental effects were found in the offspring of C57BL/6 male mice treated with 3 µg/kg/day of 2,3,7,8-TCDD by gavage (in oil or soil vehicle) for 30 weeks (Umbreit et al. 1988). No fetal abnormalities were found in the 3 fetuses of rhesus monkeys administered 0.02 µg/kg/day 2,3,7,8-TCDD 3 days a week for 3 weeks (McNulty 1984). At the higher dosages (0.1 and 0.6 µg/kg/day) only 1 fetus (from the 0.1 µg/kg/day group) was not aborted.

Developmental effects of 2,3,7,8-TCDD after chronic exposure were studied in rhesus monkeys. Decreased offspring survival was found when mothers were exposed continuously during pregnancy to 6.4×10^{-4} µg/kg/day in the feed (Bowman et al. 1989b). In addition, alterations in peer-group behavior (Bowman et al. 1989b; Schantz et al. 1992) and cognitive deficits were observed in the offspring of rhesus monkeys exposed to 1.2×10^{-4} µg/kg/day in the diet for 7 months prior to mating and during mating and lactation (16 months total duration). Significant alterations were observed in play behavior, displacement, and self directed behavior. Exposed monkeys tended to initiate more rough-tumble play bouts and retreated

less from play bouts than controls, were less often displaced from preferred positions in the playroom than the controls, and engaged in more self-directed behavior than controls. Cognitive function was altered as evidenced by impaired-reversal-learning performance in the absence of impaired delayed-spatial-alterations performance (Bowman et al. 1989b; Schantz and Bowman 1989); no NOAEL was identified for these effects. Schantz et al. (1986) also found increased and prolonged maternal care of these infants. The LOAEL of 1.2×10^{-4} µg/kg/day identified for neurobehavioral effects identified in the Schantz et al. (1992) study was used to derive a chronic oral MRL of 1×10^{-6} µg/kg/day, as described in the footnote to Table 2-2, Section 2.5, and in Appendix A.

Other CDD congeners have also been found to induce developmental toxicity. Rat pups exposed *in utero* to 2,000 µg/kg/day 2,7-DCDD had edematous separation of the cardiac myofibrils (Khera and Ruddick 1973). Schwetz et al. (1973) found no developmental effects in fetuses of rats exposed to 100,000 µg/kg/day 2,7-DCDD during gestation, but histological examinations of soft tissues were not performed. Decreased thymic weight was found in the offspring of rats exposed once on Gd 16 to 0.125 µg/kg 1,2,3,7,8-PCDD (Madsen and Larsen 1989). Subcutaneous edema was found in the offspring of Sprague-Dawley rats exposed to 1 µg/kg/day of mixed HxCDD during Gd 6–15 (Schwetz et al. 1973). Furthermore, decreased fetal body weight, reduced crown-rump length, delayed ossification, and dilated renal pelvis were observed at 10 µg/kg/day, and an increased incidence of cleft palate was found at 100 µg/kg/day. The NOAEL for the mixture of HxCDD isomers was 0.1 µg/kg/day. Subcutaneous edema was also reported in fetuses of rats exposed to 5×10^{5} µg/kg/day of OCDD during Gd 6–15; no effects were found in the 1×10^{5} µg/kg/day OCDD-exposure group (Schwetz et al. 1973) or in mice exposed to 20 µg/kg/day of OCDD during Gd 7–16 (Courtney 1976). In contrast to most experiments with 2,3,7,8-TCDD, the 1,2,3,4-TCDD isomer did not induce developmental effects in the offspring of Wistar rats treated on Gd 6–15 with 800 µg/kg/day (Khera and Ruddick 1973) or CD-1 mice exposed to 1,000 µg/kg/day during gestation (Courtney 1976). No developmental effects were seen in the offspring of Wistar rats exposed to 2,000 µg/kg/day 2,3-DCDD or 2-MCDD on Gd 6–15 (Khera and Ruddick 1973).

In conclusion, studies in rodents and monkeys demonstrated that oral exposure to CDDs induced developmental effects with congeners primarily having chlorine atoms at the 2, 3, 7, and 8 positions. Following CDD exposure of dams, the primary effects observed in the offspring included cleft palates, hydronephrosis, impaired development of the reproductive system, immunotoxicity, and death. Effects were observed in offspring from dams exposed before mating and/or during gestation; transfer of CDDs via maternal milk also resulted in adverse developmental effects. Mice appear to be particularly sensitive to

the induction of cleft palate; this alteration also occurred in rats, but at dose levels that were maternally toxic. Alterations to the immune system from offspring (mostly rats and mice) included thymic atrophy, alterations in cell-mediated immunity, and changes in lymphocyte surface cell markers. The development of the reproductive systems of male and female rats was also affected by parental exposure to CDDs. Chronic exposure of monkeys starting before mating and continuing throughout gestation and lactation resulted in neurobehavioral alterations in the infants; this effect was used to derive a chronic oral MRL.

The highest NOAEL values and all reliable representative LOAEL values for developmental effects in each species and duration category for each congener are recorded in Tables 2-2 and 2-3 and plotted in Figures 2-1 and 2-2.

2.2.2.7 Genotoxic Effects

Mostly negative results were obtained in animal studies following oral exposure to 2,3,7,8-TCDD. Cytogenetic analysis of the bone marrow did not reveal any increase in chromosomal aberrations in CD-COBS rats exposed to 1 µg/kg 2,3,7,8-TCDD by gavage once a week for 45 weeks (Loprieno et al. 1982), but an increased incidence was reported in Osborne-Mendel rats exposed to 4 µg/kg twice a week for 13 weeks (Green et al. 1977). However, chromosomal aberrations were not increased in peripheral lymphocytes of monkeys exposed to 0.001 µg/kg/day in the feed for 4 years (Lim et al. 1987). Furthermore, 7 daily doses of 12 µg/kg did not induce dominant lethality in Wistar rats (Khera and Ruddick 1973). In addition, an intermediate-duration exposure to 1 µg/kg/week of 2,3,7,8-TCDD or 1,2,3,7,8-PCDD for up to 6 months did not enhance the formation of deoxyribonucleic acid (DNA) adducts in rat hepatocytes (Randerath et al. 1989). In conclusion, CDDs were not genotoxic in most animal studies. Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

The carcinogenicity of CDDs has been demonstrated in several experiments in animals. Chronic exposure of male Osborne-Mendel rats to approximately 0.0071 µg 2,3,7,8-TCDD/kg/day by gavage significantly increased the incidence of thyroid follicular cell adenoma; in females, doses of approximately 0.071 µg/kg/day increased the incidence of neoplastic nodules in the liver and of hepatocellular carcinoma (NTP 1982b). Exposure to 0.00014 µg/kg/day 2,3,7,8-TCDD in the feed resulted in an increase in the number of tumor bearing male Sprague Dawley rats (5/6 versus 0/4 in control) (Van Miller et al. 1977).

The types of neoplasms found were ear duct carcinoma, lymphocytic leukemia, kidney adenocarcinoma, peritoneal malignant histiocytoma, skin angiosarcoma, and Leydig cell adenoma. Each tumor-bearing rat had different tumor types, and one rat had 2 types of tumors. The high mortality in the control group, inadequately reported results, and small group sizes (10/group) limit the interpretation of these results. Exposure to 0.1 µg/kg/day in the feed induced hepatocellular carcinoma, squamous cell carcinoma of lungs, and hard palate and tongue in Sprague-Dawley rats (Kociba et al. 1978a). A significant increase in hepatocellular hyperplastic nodules was observed in the female rats exposed to 0.01 or 0.1 µg/kg/day. Females were more affected by 2,3,7,8-TCDD exposure than males. 2,3,7,8-TCDD was also carcinogenic in mice exposed chronically by gavage. Hepatomas and hepatocellular carcinomas were induced in Swiss mice exposed to 0.1 µg/kg/day for 1 year (Toth et al. 1979). Increased incidence of hepatocellular carcinomas was observed in male B6C3F1 mice administered 2,3,7,8-TCDD at approximately 0.071 µg/kg/day by gavage for 104 weeks (NTP 1982b); females exhibited an increase in thyroid follicular cell adenomas and in histiocytic lymphoma at a dose of approximately 0.3 µg/kg/day (NTP 1982b). Hepatocellular carcinomas (males and females) and adenomas (females) were found in $B6C3F_1$ mice exposed to 0.36 µg/kg/day given by gavage for 1 year (Della Porta et al. 1987).

Experiments with other congeners showed that chronic exposure to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD by gavage induced hepatocellular carcinoma, adenoma, and neoplastic nodules at approximately 0.34 µg/kg/day in female Osborne-Mendel rats and at 0.71 µg/kg/week in $B6C3F_1$ male mice (NCI/NTP 1980). Therefore, HxCDD is approximately 1/20 as potent a liver carcinogen as 2,3,7,8-TCDD. Furthermore, chronic exposure to 6.5×10^5 µg/kg/day of 2,7-DCDD in the feed caused leukemias, lymphomas, hemangiosarcomas, hemangiomas, and dose-related increased incidences of hepatocellular adenomas and carcinomas in male $B6C3F_1$ mice (NCI/NTP 1979a). In contrast, no cancer effects were observed following chronic exposure of Osborne-Mendel rats to 5×10^5 µg/kg/day of 2,7-DCDD (NCI/NTP 1979a) in the feed.

In conclusion, the tested congeners (2,3,7,8-TCDD, mixed HxCDDs) were carcinogenic in rodents. 2,7-DCDD was carcinogenic in mice, but not in rats that received a lower dose.

The cancer effect levels (CELs) for each species and duration category for each congener are recorded in Tables 2-2 and 2-3 and plotted in Figures 2-1 and 2-2.

2.2.3 Dermal Exposure

2.2.3.1 Death

Information regarding mortality following dermal exposure to CDDs in animals is limited. For 2,3,7,8-TCDD, a dermal LD_{50} value in rabbits was calculated as 275 µg/kg (Schwetz et al. 1973). Deaths occurred within 12–22 days, but the cause of death was not specifically indicated. Decreased survival was observed in Swiss Webster mice exposed 3 days a week to 2,3,7,8-TCDD at 0.05 µg for 13 weeks and 0.001 µg for chronic duration (NTP 1982a). In the subchronic study (NTP 1982a), male mice exhibited a higher mortality rate than females; lethal doses in males caused marked effects in lymphoid and hematopoietic tissues as well as on the liver and lung. The cause of death in the chronic study (NTP 1982a) was not specified. No increase in lethality was reported in HRS/J hairless mice dermally exposed to 0.0025 µg, 2 days a week, for 20 weeks (Hebert et al. 1990).

The LD_{50} value for rabbits and LOAEL values in mice for increased mortality for the intermediate- and chronic-duration categories are recorded in Tables 2-4 and 2-5.

2.2.3.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values for each systemic effect in each species and duration category for each congener are recorded in Table 2-4 and 2-5.

Respiratory Effects. Bronchiolar adenomatoid changes were found in Swiss Webster mice exposed 3 days a week to 0.05 µg 2,3,7,8-TCDD for 13 weeks, but no respiratory effects were observed in mice exposed 3 days per week to 0.01 µg for a chronic exposure period (NTP 1982a).

Cardiovascular Effects. Information regarding cardiovascular effects in animals after dermal exposure to CDDs is limited. Chronic dermal exposure of Swiss Webster mice to 2,3,7,8-TCDD at 0.005 µg, 3 days per week, did not induce any cardiovascular changes observable under histopathological examination (NTP 1982a).

Table 2-4. Levels of Significant Exposure to 2,3,7,8-TCDD - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference
ACUTE EXPOSURE						
Death						
Rabbit (NS)	once				275 µg/kg (LD_{50})	Schwetz et al. 1973b
Systemic						
Mouse (HRS/J)	2 wk 3 d/wk 1x/d	Dermal		0.01 µg F (hyperkeratosis, involution of sebaceaus glands)		Puhvel and Sakamoto 1988
Rabbit (NS)	once	Dermal		2000 µg (transient inflammation of conjunctiva)		Schwetz et al. 1973b
INTERMEDIATE EXPOSURE						
Death						
Mouse (Swiss-Webster)	13 wk 3 d/wk				0.05 µg (10% died in both sexes)	NTP 1982a
Systemic						
Mouse (CD-1)	30 wk 2 d/wk	Dermal		0.1 µg F (acne-like lesion)		Berry et al. 1978; 1979

Table 2-4. Levels of Significant Exposure to 2,3,7,8-TCDD - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL		Reference
				Less Serious	Serious	
Mouse (HRS/J hairless)	20 wk 2 d/wk	Hepatic		0.0025 µg F	(increased relative liver weight)	Hebert et al. 1990
		Bd Wt		0.01 µg F	(16% decreased body weight gain)	
Mouse (Swiss-Webster)	13 wk 3 d/wk	Resp	0.01 µg	0.05 µg	(bronchiolar adenomatoid changes with hyperplasia)	NTP 1982a
		Hepatic		0.005 µg	(fatty degeneration)	
Mouse (HRS/J, Skh:HR-1)	4 wk 3 d/wk 1x/d	Hepatic		0.1 µg F	(increased microsomal enzyme-activity)	Puhvel et al. 1982
		Dermal		0.1 µg F	(hyperkeratosis absence of sebaceous glands)	

Table 2-4. Levels of Significant Exposure to 2,3,7,8-TCDD - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL			Reference
				Less Serious		Serious	
Immunological/Lymphoreticular							
Mouse (HRS/J)	20 wk 2 d/wk		0.005 µg F	0.01 µg F	(decreased thymus/ body weight ratio in non-initiated mice)		Herbert et al. 1990
Cancer							
Mouse (HRS/J hairless)	20 wk 2 d/wk					0.0025 µg F (increased number of skin squamous cell papilloma and hyperproliferative nodules)	Herbert et al. 1990
Mouse (HRS/J hairless)	20 wk 2 d/wk					0.00375 µg (skin papilloma following initiation)	Poland et al. 1982
CHRONIC EXPOSURE							
Death							
Mouse (Swiss-Webster)	99-104 wk 3 d/wk					0.001 µg (decreased survival)	NTP 1982a

Table 2-4. Levels of Significant Exposure to 2,3,7,8-TCDD - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL		Reference
				Less Serious	Serious	
Systemic						
Mouse (Swiss-Webster)	99-104 wk 5 d/wk	Resp	0.005 µg			NTP 1982a
		Cardio	0.005 µg			
		Gastro	0.005 µg			
		Hemato	0.005 µg			
		Hepatic	0.005 µg			
		Renal	0.005 µg			
		Dermal	0.005 µg			
		Bd Wt	0.005 µg			
Reproductive						
Mouse (Swiss-Webster)	99-104 wk 3 d/wk		0.005 µg			NTP 1982a

Table 2-4. Levels of Significant Exposure to 2,3,7,8-TCDD - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL			Reference
				Less Serious	Serious		
Cancer							
Mouse (Swiss-Webster)	99-104 wk 3 d/wk				0.005 µg	(CEL: fibrosarcoma without initiation)	NTP 1982a

Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); F = female; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observable-adverse-effect level; NOAEL = no-observable-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s) x = times.

Table 2-5. Levels of Significant Exposure to Dioxins (non-2,3,7,8-TCDD) - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL (ug)	LOAEL		Reference Chemical Form
				Less Serious (ug)	Serious (ug)	
ACUTE EXPOSURE						
Systemic						
Rabbit (NS)	once	Dermal		2000 (transient inflammation of conjunctiva)		Schwetz et al. HCDD5
Rabbit (NS)	once	Dermal		(transient inflammation of conjunctiva)		Schwetz et al. OCDD
Rabbit (NS)	once	Dermal		(transient inflammation of conjunctiva)		Schwetz et al. 1973b DCDD1

DCDD1 = 2,7-dichlorodibenzo-p-dioxin; HCDD5 = unspecified mixture of hexachlorodibenzo-p-dioxins; LOAEL = lowest-observable-adverse-effect level; NOAEL = no-observable-adverse-effect level; NS = not specified; OCDD = octachlorodibenzo-p-dioxin

Gastrointestinal Effects. Information regarding gastrointestinal effects in animals after dermal exposure to CDDs is limited. No histopathological changes were observed in the gastrointestinal tract of Swiss Webster mice chronically exposed to 0.005 µg 2,3,7,8-TCDD 3 days per week (NTP 1982a).

Hematological Effects. Hematological examination of Swiss Webster mice chronically exposed to 0.005 µg 2,3,7,8-TCDD 3 days per week did not reveal any differences between exposed and control groups (NTP 1982a).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in animals after dermal exposure to CDDs.

Hepatic Effects. Hepatic effects have been observed in animals after dermal exposure to 2,3,7,8-TCDD. Necrosis, peripheral fibrosis, and bile duct proliferation were observed in rabbits acutely exposed to 2,3,7,8-TCDD on the ear surface (Kimbrough et al. 1977). Increased liver/body weight ratio and hepatocellular hypertrophy were seen in HRS/J hairless mice topically exposed to 0.0025 µg 2,3,7,8-TCDD twice a week for 20 weeks (Hebert et al. 1990). Fatty degeneration and hepatocellular necrosis were observed in the livers of Swiss Webster mice exposed to 0.005 µg 2,3,7,8-TCDD 3 days a week for 13 weeks (NTP 1982a). No hepatic effects were found in mice chronically exposed to 0.005 µg/day 2,3,7,8-TCDD (NTP 1982a). The data indicated that 2,3,7,8-TCDD induced hepatotoxic effects similar to those observed after oral exposure.

Renal Effects. Information regarding renal effects in animals after dermal exposure to 2,3,7,8-TCDD is limited. No histopathological changes were found in Swiss Webster mice exposed to 0.005 µg 2,3,7,8-TCDD 3 days per week for 99–104 weeks (NTP 1982a).

Dermal Effects. Dermal effects of several CDD congeners have been studied in animals. Acute dermal exposure to 0.01 µg (newborn) and 0.1 µg 2,3,7,8-TCDD (adult) per animal caused hyperkeratosis and epidermal hyperplasia in hairless HRS/J mice (Puhvel and Sakamoto 1988). An involution of sebaceous glands was found in both (haired and hairless) strains. Similar results were found following intermediate-duration exposure (Puhvel et al. 1982). Furthermore, acne-like lesions in the ears were found in CD-1 mice following exposure to 0.1 µg 2,3,7,8-TCDD applied on the pre-shaved back 2 days a week for 30 weeks (Berry et al. 1978, 1979). In contrast, no dermal effects were observed in Swiss Webster mice exposed to 0.005 µg 2,3,7,8-TCDD/application, 3 days a week for up to 104 weeks (NTP 1982a).

Ocular Effects. A single application of 2,000 µg 2,7-DCDD, 2,3,7,8-TCDD, mixed HxCDD, or OCDD into the conjunctival sac of rabbits caused transient pain and conjunctival inflammation (Schwetz et al. 1973). Delayed conjunctival chemosis was observed with 2,3,7,8-TCDD. None of the CDDs caused corneal injury or iritis.

Body Weight Effects. In animal studies, decreased body weight was observed in HRS/J and Skjh:HR-1 mice following intermediate-duration dermal exposure to 0.1 µg 2,3,7,8-TCDD (Puhvel et al. 1982) and in Swiss Webster mice following chronic exposure to 0.005 µg 2,3,7,8-TCDD 3 days per week (NTP 1982a).

2.2.2.3 Immunological Effects

The only information regarding immunological effects in animals after dermal exposure to CDDs was obtained from an intermediate-duration study in HRS/J mice (Hebert et al. 1990). Mice dermally exposed to 0.01 µg 2,3,7,8-TCDD 2 days per week for 20 weeks had decreased thymus/body weight ratio. No effects were observed at 0.005 µg.

The NOAEL and LOAEL values for immunological effects in mice after intermediate-duration exposure are recorded in Table 2-4.

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in animals after dermal exposure to CDDs.

2.2.3.5 Reproductive Effects

Data regarding reproductive effects following dermal exposure in animals are scarce. No treatment-related changes were observed in the reproductive system of Swiss Webster mice after chronic exposure to 0.005 µg 2,3,7,8-TCDD per application (NTP 1982a).

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in animals after dermal exposure to CDDs.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in animals after dermal exposure to CDDs. Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

Acute- and intermediate-duration studies in animals investigated the interactions of 2,3,7,8-TCDD with known carcinogens. A single dermal pretreatment of CD-1 Charles River mice with 0.01 µg 2,3,7,8-TCDD inhibited the development of skin papillomas otherwise initiated by 1,3-dimethylbenz(*o*)anthracene (DMBA) (Berry et al. 1979). In intermediate-duration experiments, 2,3,7,8-TCDD did not promote skin tumors initiated by DMBA (Berry et al. 1978, 1979). In contrast, the promoting ability of 2,3,7,8-TCDD at 0.0025 µg/day (and higher), 2 days a week, for 20 weeks, was reported in HRS/J hairless mice following the initiation with N-methyl-N-nitro-N-nitrosoguanidine in intermediate-duration experiments (Hebert et al. 1990; Poland et al. 1982). The effect was not observed in mice heterozygous for the hairless trait (Poland et al. 1982). In a chronic study, significantly increased incidence of fibrosarcoma of the integumentary system was found in Swiss Webster female mice following dermal exposure to 2,3,7,8-TCDD at 0.005 µg, 3 days a week for 2 years (NTP 1982a). The cancer effect level (CEL) from this study is shown in Table 2-4.

2.3 TOXICOKINETICS

Data regarding toxicokinetics of CDDs in humans are limited to information derived from exposures that occurred after industrial accidents, exposures of Vietnam veterans, and ingestion of 2,3,7,8-TCDD by a volunteer. Humans can absorb CDDs by the inhalation, oral, and dermal routes of exposure. CDDs, when administered orally, are well absorbed by experimental animals, but they are absorbed less efficiently when administered by the dermal route. Limited data in rats showed that transpulmonary absorption of 2,3,7,8-TCDD may be at least as efficient as oral absorption. In a human volunteer, >86% of the administered single oral dose appeared to have been absorbed. In general, absorption is vehicle-dependent and congener-specific. Passage across the intestinal wall is predominantly limited by molecular size and solubility. These parameters are most significant for hepta- and octachlorinated congeners, which exhibit decreased absorption in mammals. The predominant CDD carriers in human plasma are serum lipids and

lipoproteins, but chlorine substitution plays a role in the distribution in these fractions. For most mammalian species, the liver and adipose tissue are the major storage sites of CDDs; in some species, skin and adrenals also can act as primary deposition sites. 2,3,7,8-Substituted CDDs are the predominant congeners retained in tissues and body fluids. Tissue deposition is congener-specific and depends on the dose, the route of administration, and age. CDDs are very slowly metabolized by the microsomal monooxygenase system to polar metabolites that can undergo conjugation with glucuronic acid and glutathione. The major routes of excretion of CDDs are the bile and the feces; smaller amounts are excreted via the urine. In mammalian species, lactation is an effective way of eliminating CDDs from the liver and other extrahepatic tissues. Physiologically based pharmacokinetic (PBPK) models have been developed to describe disposition of 2,3,7,8-TCDD in humans and animals. Some of these models included parameters to describe complex interactions of 2,3,7,8-TCDD with cellular proteins that lead to specific biological responses.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No quantitative data were located regarding absorption of CDDs in humans following inhalation exposure. However, based on data from studies with structurally related chemicals it is reasonable to assume that CDDs are absorbed by this route. Furthermore, data on levels of CDDs in blood from populations with above-background exposures (occupational, accidental) also suggest that transpulmonary absorption occurs in humans; see Section 2.1 for more information.

Systemic effects (hepatic aryl hydrocarbon hydroxylase [AHH] and cytochrome P-450 induction, hepatic histological alterations) were observed in rats following a single intratracheal instillation of 2,3,7,8-TCDD in a corn oil vehicle or as a laboratory-prepared contaminant of gallium oxide particles (Nessel et al. 1990). In a subsequent study, the same group of investigators (Nessel et al. 1992) using a similar protocol found that the relative pulmonary bioavailability of 2,3,7,8-TCDD on respirable soil particles was 100% as compared to the gallium oxide vehicle. One and 7 days post-treatment, 13.9 and 11.9% of the administered dose were detected in the liver, respectively, and this was similar to the percentage found after instillation of contaminated gallium oxide particles. Twenty-eight days after treatment, 5.2% of the administered dose was detected in the liver from soil-treated rats and 2.9% in liver from gallium oxide-treated rats suggesting that redistribution and retention of 2,3,7,8-TCDD differed in the two treatment groups. Recently, Diliberto

et al. (1996) reported that 3 days after intratracheal application of a single dose of 0.32 µg 2,3,7,8-TCDD/kg to male Fischer 344 rats, 95% of the applied dose was absorbed, suggesting that inhalation can be an effective route of exposure. The extent of inhalation absorption was higher than when the same dose was administered orally (88%) or dermally (40%). The available data suggest that inhaled CDDs will be absorbed. However, the degree of absorption and the rate will depend on the media on which the CDDs are adsorbed and the degree of chlorination.

2.3.1.2 Oral Exposure

The absorption of 2,3,7,8-TCDD was estimated to be >87% in a human volunteer following ingestion of a single radioactively labeled dose of 0.00114 µg 2,3,7,8-TCDD/kg in corn oil (Poiger and Schlatter 1986). Data regarding absorption of CDDs from breast milk in nursing infants are provided in Section 2.3.4.4.

Gastrointestinal absorption of radiolabeled 2,3,7,8-TCDD has been investigated in rodents. About 73.5% of the total dose of 2,3,7,8-TCDD (administered by gavage in corn oil vehicle) was absorbed in Syrian hamsters, the species most resistant to acute 2,3,7,8-TCDD toxicity (Olson et al. 1980b). In Sprague-Dawley rats given a single gavage dose of 50 µg/kg 2,3,7,8-TCDD in corn oil, at least 70% was absorbed (Piper et al. 1973). Rose et al. (1976) found a mean of 84% of a single oral gavage dose of 1 µg/kg absorbed within a day in a similar study and a steady-state body burden was achieved after dosing with 0.01, 0.1, or 1 µg/kg in corn oil, 5 days a week for 7 weeks. When ^{14}C-2,3,7,8-TCDD was fed to Sprague-Dawley rats at 0.35 µg/kg/day or 1 µg/kg/day in the diet for 42 days, about 60% of the consumed dose was absorbed (Fries and Marrow 1975). Intestinal absorption of 2,3,7,8-TCDD did not vary with age of Fischer 344 rats (13 weeks, 13 or 26 months) when *in vivo* absorption was studied with an *in situ* intestinal perfusion technique (Hebert and Birnbaum 1987). When ICR/Ha Swiss mice were given a single dose of radioactively labeled 2,3,7,8-TCDD, 67–76% of the administered dose was excreted in feces and 1–2% in urine within the first 24 hours (Koshakji et al. 1984). The authors (Koshakji et al. 1984) concluded that most of the dose was not absorbed. The more highly chlorinated CDD congeners are absorbed from the gastrointestinal tract to a lesser extent than 2,3,7,8-TCDD.

Gastrointestinal absorption of 2,3,7,8-TCDD may differ depending on the vehicle used. When hepatic concentrations were used as a measure of absorbed dose, the levels observed in rats 24 hours after 2,3,7,8-TCDD administration in 50% ethanol were higher than in an aqueous suspension of soil (Poiger and Schlatter 1980). Use of activated carbon as a vehicle almost completely eliminated 2,3,7,8-TCDD

absorption. It was further demonstrated that the absorption of 2,3,7,8-TCDD from the gastrointestinal tract of rats was . 50% less from contaminated soil than from corn oil (Lucier et al. 1986), which is supported by the finding that 2,3,7,8-TCDD-contaminated soil was less toxic to guinea pigs than an equivalent amount of 2,3,7,8-TCDD in oil (Umbreit et al. 1985). Gastrointestinal absorption of OCDD was <10% of the administered dose in Sprague-Dawley and Fischer 344 rats following single or repeated (3-week) exposures by gavage in oil vehicle (Birnbaum and Couture 1988; Norback et al. 1975). Low doses (50 µg/kg) in a *o*-dichlorobenzene:corn oil (1:1) vehicle were found to give the best oral bioavailability for this extremely insoluble compound (Birnbaum and Couture 1988). The bioavailability of CDDs (2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD) to rats was lower on fly ash (0.4% for 2,3,7,8-TCDD) as compared to extracts of the same fly ash administered in an oily vehicle (45% for 2,3,7,8-TCDD) (Van den Berg et al. 1983, 1987c). The differences in hepatic levels between fly ash- and extract-treated rats were greater for the more highly chlorinated congeners.

2.3.1.3 Dermal Exposure

No quantitative data were located regarding absorption of CDDs in humans following dermal exposure. However, based on data from studies with structurally related chemicals it is reasonable to assume that CDDs are absorbed by this route. Furthermore, data on levels of CDDs in blood from populations with above-background exposures (i.e., occupational, accidental) also suggest that dermal absorption occurs in humans. Due to the relatively low vapor pressure and high lipid solubility, dermal uptake of 2,3,7,8-TCDD in the workplace may be a significant source of occupational exposure (Kerger et al. 1995).

Kerger et al. (1995) examined the potential contribution of dermal exposure to 2,3,7,8-TCDD for three different occupational exposure scenarios: 1) trichlorophenoxy herbicide manufacturing worker (20-year exposure), 2) contract maintenance mechanic exposed by repairing a trichlorophenol reactor after an explosion accident (6-week exposure), and 3) trichlorophenoxy applicator handling only diluted trichlorophenoxy herbicides (seasonal exposure for 20 years). In their evaluation, the authors used a conceptual model of workplace exposure, dermal bioavailability/uptake calculations, and simple pharmacokinetic modeling techniques (details of the model were not provided). The contribution of background uptake of 2,3,7,8-TCDD from dietary sources in the United States was accounted for in the estimates of steady-state adipose concentrations. The results of the modeling showed that considerable occupational uptake can occur following both long-term continuous exposure and short-term high exposure. In the former case, occupational uptake can be distinguished from background exposures when

body burden is measured within a 10-year period following cessation of exposure. In contrast, seasonal exposure to dilute 2,3,7,8,-TCDD residues may result in little or no change in 2,3,7,8-TCDD body burden.

The *in vitro* penetration of ^{3}H-labeled 2,3,7,8-TCDD into human cadaver skin was studied at concentrations of 6.5 and 65 ng 2,3,7,8-TCDD/cm^2 of skin (Weber et al. 1991). Two vehicles were used: acetone to simulate exposure to 2,3,7,8-TCDD as a dry material, and mineral oil to simulate exposure in an oily medium. The experiments were conducted in intact skin and in skin with stripped stratum corneum and penetration was monitored for 30, 100, 300, and 1,000 minutes. The results showed that acetone as a vehicle allowed 2,3,7,8-TCDD to penetrate deeply into the loose surface of the lamellae of the stratum corneum, but there was little further penetration. On the other hand, mineral oil appeared to compete with lipophilic constituents of the stratum corneum for 2,3,7,8-TCDD, thus slowing its penetration even more. Removal of the stratum corneum increased the amount of 2,3,7,8-TCDD absorbed into layers of the skin. Rates of absorption were calculated in two ways: a worst case scenario where 2,3,7,8-TCDD absorbed into any layer of skin including the stratum corneum was used for analysis; and a physiological approach where only the amount of 2,3,7,8-TCDD which had penetrated beyond the epidermis into the region of dermal vascularization was considered absorbed. In the former case, the stratum corneum appeared to mediate dermal absorption of 2,3,7,8-TCDD since the rates decreased when stripped skin was exposed to 2,3,7,8-TCDD. With the physiological approach, the rate of absorption was a function of the amount applied suggesting that the rate of absorption per unit time was a first-order function. The amount of 2,3,7,8-TCDD that penetrated the skin also correlated with exposure duration. The rate of 2,3,7,8-TCDD penetration with acetone as vehicle ranged from 100 to 800 pg 2,3,7,8-TCDD per hour-cm^2 (worst-case scenario), or 6–170 pg per hour-cm^2 with the physiological approach. The corresponding values with mineral oil as a vehicle were 20–220 pg and 1.4–18 pg per hour-cm^2, respectively.

Data regarding dermal absorption of CDDs in animals are limited. When 200 pmol 2,3,7,8-TCDD was applied to the skin of Fischer 344 rats, absorption followed first-order kinetics with an absorption rate constant of 0.005 hour^{-1} (Banks and Birnbaum 1991). Within 120 hours postexposure, about 0.026 µg 2,3,7,8-TCDD was absorbed (less than 50% of the applied dose); at each interval of measurement, about 70% of detected radioactivity on the skin could be removed by swabbing with acetone. About 15% of the dose was detected in the liver of rats 24 hours after dermal exposure to 26 ng of 2,3,7,8-TCDD in 50% methanol (Poiger and Schlatter 1980). It was estimated that the amount absorbed from the dermal exposure represents .40% of the amount absorbed from an equivalent oral dose. Absorption of 2,3,7,8-TCDD was significantly reduced by application in Vaseline or polyethylene glycol and practically

eliminated in soil or activated carbon. Dermal absorption of radioactively labeled 2,3,7,8-TCDD in soil vehicle was reported to be only 1% of the administered dose during a 24-hour contact in rats (Shu et al. 1988). The dermal absorption of 2,3,7,8-TCDD after 4 hours of contact was about 60% of that after 24-hour contact. The uptake was not influenced by the 2,3,7,8-TCDD concentration in soil, nor were there any differences between normal and hairless rats.

Dermal absorption in rats was found to be age-related. Banks et al. (1990) found that in Fischer 344 rats, the percutaneous absorption was decreased in middle-aged (36-week-old) and senescent (120-week-old) rats compared to that in young adults (10-week-old) 72 hours after application of a dose of 40 nmol (approximately 12.9 μg) of ^{3}H-labeled 2,3,7,8-TCDD. The authors suggested a decrease in blood flow through the skin between 3 and 4 months of age as a possible explanation for their findings. In a subsequent and similar study, the same group of investigators examined the dermal absorption of 2,3,7,8-TCDD in 3-, 5-, 8-, 10-, and 36-week-old Fischer 344 rats 72 hours after application of 200 pmol 2,3,7,8-TCDD in acetone (Banks et al. 1993). Dermal absorption was greatest in 3-week-old rats (approximately 64% of the applied dose), decreased to about 40% of the applied dose in 5-, 8-, and 10-week-old rats and to about 22% in 36-week-old rats. In each age group, 70–80% of the radioactivity remaining at the application site 72 hours after dosing could be removed with acetone swabs.

2.3.2 Distribution

As discussed in Section 2.1, occupational or environmental human exposure to CDDs is not readily classifiable as to route of exposure. Human data regarding distribution obtained at autopsy indicated that accumulation in the liver following low levels of exposure is based in part on lipid solubility (Leung et al. 1990a). However, this may not be the case with higher exposure levels that cause hepatic enzyme induction (see Section 2.4.1). When human hepatic and adipose tissues were examined for the presence of 2,3,7,8-TCDD, the concentration detected in the liver was about 1/10 of that in the adipose tissue on a whole-tissue-weight basis. However, on the basis of the total tissue lipid, the concentration in adipose tissue lipid was one-half that in the liver lipid (Thoma et al. 1990). It was further demonstrated that over a wide range of concentrations, the serum 2,3,7,8-TCDD levels highly correlated with adipose tissue 2,3,7,8-TCDD levels when both are expressed on a lipid weight basis (Patterson et al. 1988). Adipose tissue serves as a storage depot for 2,3,7,8-TCDD in the body, and detectable levels (up to 20.2 ppt) were found in the general population with no known risk of high exposure to CDDs (Andrews et al. 1989). An average concentration of 2,3,7,8-TCDD in serum lipid of 5.38 ppt has been estimated for the U.S.

population (Orban et al. 1994). The distribution of highly chlorinated CDDs among tissue lipid fractions is not equal. For example, the distribution of OCDD is 12:1 (Thoma et al. 1990) between liver and adipose tissue lipid factions and 2:1 between serum and adipose tissue lipid fractions (Schecter et al. 1990).

Increased adipose tissue levels of CDDs were reported in populations with known high residential or occupational exposure (Beck et al. 1989c; Fingerhut et al. 1989; Patterson et al. 1989b; Schecter et al. 1994). For example, high levels of 2,3,7,8-TCDD were found in fat (42–750 ppt) and serum lipid (61–1,090 ppt) of Missouri chemical workers (Patterson et al. 1989b). Measurable CDDs and CDFs levels were reported in the liver tissue of human stillborn neonates suggesting that the transplacental intrauterine transfer of these persistent chemicals resulted from environmentally exposed mothers (Schecter et al. 1990). In addition, CDDs are distributed to human milk (i.e., Fürst et al. 1994; Schecter et al. 1987a, 1987b, 1989e) and numerous studies have published concentrations of various congeners in human milk samples (see section 5.5.1). Levels of CDDs in human milk have been found to be significantly and positively associated with of proximity of residence to waste sites and to dietary fat intake per week (Schaud et al. 1995).

2.3.2.1 Inhalation Exposure

The tissue distribution of 2,3,7,8-TCDD-derived radioactivity was recently examined in male Fischer 344 rats 3 days after intratracheal application of a single dose of 0.32 µg 2,3,7,8-TCDD/kg (Diliberto et al. 1996). The liver and adipose tissue were the major tissue depots for 2,3,7,8-TCDD-derived radioactivity with 33 and 15% of the applied dose distributing to these respective tissues. The skin (ear) and muscle followed with 4.3 and 1.3%, respectively. All other tissues had less than 0.5% of the administered dose. The 2/1 liver/adipose ratio was in contrast to the approximately 1/1 ratio found after gavage administration of the same dose.

2.3.2.2 Oral Exposure

Following an ingested dose of ^{3}H-2,3,7,8-TCDD of 0.00114 µg/kg by a volunteer, the concentration of 2,3,7,8-TCDD in the adipose tissue were 3.09 and 2.86 ppt at 13 and 69 days following exposure, respectively (Poiger and Schlatter 1986). The authors estimated that about 90% of the body burden was distributed to the fatty tissue. Increased radioactivity was detected in the blood only during the first 2 days

postexposure; no radioactivity was detected in serum lipid after 5 days, but was in the feces for several months.

Studies in animals have shown that 2,3,7,8-TCDD distributes preferentially to the liver and adipose tissue. In Sprague-Dawley rats, the highest levels of radioactivity (expressed as percentage of dose per gram of tissue) were located in the liver (3.18, 4.49, and 1.33% at days 3, 7, and 21 post-exposure, respectively) and adipose tissues (2.6, 3.22, and 0.43% at days 3, 7, and 21, respectively) following a single oral dose of labeled 2,3,7,8-TCDD at 50 µg/kg (Piper et al. 1973). Much smaller amounts were found in muscles, testes, lungs, stomach, and other organs. In male Fischer 344 rats administered a single gavage dose of 0.32 µg 2,3,7,8-TCDD/kg, 24.4 and 26.2% of the administered dose was found in the liver and adipose tissue, respectively, 3 days after dosing (Diliberto et al. 1996); skin and muscle had 7.3 and 1.8%, respectively. 2,3,7,8-TCDD accumulated mainly in the liver and adipose tissue, with smaller amounts in the brain of pregnant Wistar rats after 10 daily doses of 2 µg/kg (Khera and Ruddick 1973). Similarly, the highest levels of radioactivity were found in the liver, adipose tissue, and the adrenals of Golden Syrian hamsters after a single gavage dose of 650 µg/kg labeled 2,3,7,8-TCDD (Olson et al. 1980b). In addition, about 36% of the total radioactivity administered remained in the adipose tissue by day 45 postexposure in Hartley guinea pigs; only about 7% (each) was found in the liver, muscles, and carcass (Olson 1986). Essentially all of the administered dose was unchanged 2,3,7,8-TCDD. When pregnant NMRI mice were exposed to a single oral, intraperitoneal, or subcutaneous dose of 2,3,7,8-TCDD, hepatic levels were about the same, indicating that there is no major first pass effect after oral 2,3,7,8-TCDD exposure (Nau and Bass 1981). Liver, then adipose tissue and skin, were the major depots of OCDD in Fischer 344 rats treated with single oral doses of this congener (Birnbaum and Couture 1988).

The dose- and time-dependent tissue distribution of 2,3,7,8-TCDD in mice has been recently examined (Diliberto et al. 1995). Female B6C3F_1 mice were administered a single dose of 0.1, 1, or 10 µg [^{3}H]-2,3,7,8-TCDD/kg by gavage in corn oil and the distribution of radioactivity was followed in 18 tissues for up to 35 days after dosing. The results showed dose-dependent distribution of 2,3,7,8-TCDD-derived radioactivity in all tissues. The highest concentrations of radioactivity were found in liver and adipose tissues, and both tissues accounted for 50% of the body burden. Relatively high concentrations of 2,3,7,8-TCDD-derived radioactivity were also found in skin, adrenal glands, thyroid, pancreas, olfactory epithelium, spleen, mesenteric lymph nodes, thymus, lung, and bone marrow. The liver concentration of radioactivity increased disproportionally with increasing doses, whereas relative concentration and percentage dose/total tissue in extrahepatic tissues decreased with increasing dose and over time.

Liver/adipose tissue concentration ratios were shown to be dose- and time-dependent. At the low-, mid- and high-dose, the ratios ranged from 0.6 to 0.2, 2.3–0.5, and 3.1–1.4 over time, respectively. This variation over time was thought to have been due to redistribution of 2,3,7,8-TCDD between the two storage sites and/or hepatic metabolism and subsequent excretion.

The effect of age of the animal on 2,3,7,8-TCDD tissue distribution has also been examined. Pegram et al. (1995) administered a single dose of 0.015, 0.5 or 15 µg [^{3}H]2,3,7,8-TCDD/kg to 10-week- and 28-month-old male C57BL/6N mice and monitored 2,3,7,8-TCDD-derived radioactivity in blood, liver, skin, kidney, and muscle 7 days after dosing. The results showed that in young mice given the low- and high-dose, the concentration of 2,3,7,8-TCDD in blood relative to all other tissues was significantly greater than in older mice. Also, in older mice, the concentration of 2,3,7,8-TCDD in skin and the percentage of the dose in the skin were greater than in the young mice. The same trend was observed in kidney and muscle. The concentration of 2,3,7,8-TCDD in liver, as well as the percentage of the dose in the liver, were greater in young than old animals at both the mid- and high-doses. In both young and old mice the ratios of liver to adipose tissue increased with increasing doses. According to the authors, the higher hepatic concentration of 2,3,7,8-TCDD in young mice could be due to the old mice having a larger fat compartment, such that the hepatic 2,3,7,8-TCDD sequestering action of CYP1A2 or other inducible binding factors may have been less effective in the more obese older mice. In addition, decreased perfusion in the liver and adipose compartments in the old mice may have limited the effectiveness of hepatic 2,3,7,8-TCDD accumulation. The greater accumulation of 2,3,7,8-TCDD in the skin, muscle, and kidney from old mice were attributed to altered perfusion and possibly greater lipid infiltration in these tissues.

The subcellular distribution of 2,3,7,8-TCDD-derived radioactivity in the liver, lungs, and kidneys from female Sprague-Dawley rats and B6C3F1 mice was studied by Santostefano et al. (1996). In the liver of rats given a single oral dose of 0.1, 1, or 10 µg [^{3}H]-2,3,7,8-TCDD/kg, radioactivity accumulated equally in the supernatant (S9, cytosol, and microsomes) and pellet (P9, nucleus, lysosomes, and mitochondria) fractions; within the S9 fraction, accumulation was predominantly in the microsomal fraction. In contrast, in kidneys and lungs radioactivity accumulated preferentially in P9, but radioactivity detected in S9 was mostly in the cytosolic fraction. The pattern of distribution of radioactivity in liver and lungs from mice was similar to that found in rats, but in mice kidneys, 2,3,7,8-TCDD detected in S9 was equally distributed between the microsomal and cytosolic fractions. Accumulation of 2,3,7,8-TCDD in the various fractions in this single-dose study was not dose-dependent. The investigators also conducted a 17-week oral dosing study in B6C3F1 mice given 1.5 or 150 ng/kg that showed that increasing the dose resulted in equal

accumulation between liver S9 and P9 fractions, whereas the kidney P9 had the most radioactivity regardless of the dose. In addition, liver S9 accumulated 2,3,7,8-TCDD in the microsomal fraction, whereas kidney S9 did it predominantly in the cytosol. These results are consistent with the hypothesis that hepatic microsomal sequestration of 2,3,7,8-TCDD is mediated by cytochrome P-4501A2 (CYP1A2), a dioxin-inducible protein. This hypothesis was subsequently confirmed by experiments in transgenic mice lacking expression of CYP1A2 (CYP1A2-/-) (Diliberto et al. 1997). These mice, as judged by 2,3,7,8-TCDD liver/fat concentration ratios, failed to sequester 2,3,7,8-TCDD in the liver after administration of a single dose of 2,3,7,8-TCDD.

Intermediate-duration exposure to 2,3,7,8-TCDD in the feed has been shown to produce higher liver accumulation in male (85%) than in female rats (70%) (Fries and Marrow 1975). The percentage retained was related to intake, and at steady state, the total amount retained was about 10.5 times the average daily intake.

Intermediate-duration studies have also been conducted with radioactively labeled OCDD. OCDD had similar patterns of distribution and similar half-lives as 2,3,7,8-TCDD in Sprague-Dawley (Norback et al. 1975) and Fischer 344 rats (Birnbaum and Couture 1988; Birnbaum et al. 1989a). Most of the absorbed amount (50–97%) was found in the liver and was associated with the microsomal fractions. Skin- and adipose-tissue levels were much lower. Radioactivity was also detected in the kidneys, heart, testes, skeletal muscle, and serum.

2.3.2.3 Dermal Exposure

Male Fischer 344 rats absorbed 40% of a single dermal dose of 0.32 µg of radioactive 2,3,7,8-TCDD/kg over a period of 120 hours after dosing (Banks and Birnbaum 1991). The major depots for 2,3,7,8-TCDD-derived radioactivity were the liver and adipose tissue. Seventy-two hours after dosing, the liver and adipose tissue retained approximately 21 and 8% of the administered dose, respectively. Distribution to the liver increased significantly between 4 and 8 hours and between 12 and 72 hours after dosing. Distribution in fat increased significantly between 12 and 120 hours after dosing. Skin and muscle accumulated considerably less 2,3,7,8-TCDD-derived radioactivity than liver and fat. Within 120 hours of dosing, less than 4% of the administered dose was found in either of these tissues. When 2,3,7,8-TCDD was dermally applied to HRS/J hairless mice for an intermediate duration, about 5–6% of the total administered dose (0.0025–0.01 µg/kg, 2 days a week, for 20 weeks) was detected in the liver (Hebert et al. 1990).

2.3.3 Metabolism

No data were located regarding metabolic pathways of CDDs in humans. However, there is some evidence that 2,3,7,8-TCDD is partially excreted in the feces in the form of metabolites (Wendling et al. 1990).

Studies in animals indicate that 2,3,7,8-TCDD is metabolized slowly in mammals (Koshakji et al. 1984). Metabolic transformation by phase I metabolizing enzymes includes oxidation and reductive dechlorination, as well as oxygen bridge cleavage. This is followed by conjugation reactions catalyzed by phase II type enzymes, which facilitate excretion by adding more polar groups to the molecule. For example, a study in guinea pigs showed that only 28% of the radioactivity in the tissues 45 days following exposure to ^{3}H-2,3,7,8-TCDD was in the form of metabolites (Olson 1986). Results from high performance liquid chromatography (HPLC) suggested the presence of at least five ^{3}H-labeled metabolites of 2,3,7,8-TCDD, but their structure was not established. The results indicated that in the guinea pig, the metabolites of 2,3,7,8-TCDD may not leave the body rapidly. In rats and hamsters, metabolism appears to be required for urinary and biliary excretion (Olson et al. 1980a). Metabolites of 2,3,7,8-TCDD are not generally detected in tissues, suggesting that for most species, 2,3,7,8-TCDD is readily eliminated following metabolism. An *in vitro* study with isolated rat hepatocytes identified 1–hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-TrCDD as metabolites (Sawahata et al. 1982). 2-Hydroxy-1,3,7,8-TCDD was found to be the major metabolite of 2,3,7,8-TCDD in dogs but not in rats (Poiger et al. 1982). The metabolites from dogs administered to rats were eliminated as conjugates in the bile (Weber et al. 1982). Self induction of 2,3,7,8-TCDD metabolism was reported in both species (Poiger and Schlatter 1985; Weber et al. 1982). A single 10 µg/kg dose of unlabeled 2,3,7,8-TCDD 9 days prior to administration of ^{3}H-2,3,7,8-TCDD resulted in a doubling of the amount of radioactivity eliminated in the bile of dogs. When the 2,3,7,8-TCDD metabolites, 2-hydroxy-2,3,7-TrCDD and 2-hydroxy-1,3,7,8-TCDD, were synthesized and injected intraperitoneal into Wistar rats, no toxic effects were observed (Mason and Safe 1986). This supports the observation that the extract from the bile of 2,3,7,8-TCDD-treated dogs is about 100 times less toxic to rats and guinea pigs than pure 2,3,7,8-TCDD (Poiger et al. 1982). The lack of toxicity of the 2,3,7,8-TCDD metabolites suggests that autoinduction of its own metabolism in animals is a detoxification mechanism.

Data regarding other 2,3,7,8-substituted CDDs are limited. Wacker et al. (1986) found at least three phenolic radiolabeled metabolites of ^{14}C-1,2,3,7,8-PeCDD in rat bile after treatment with glucuronidase and methylation, indicating the probability of formation of hydroxymetabolites. Results from studies in

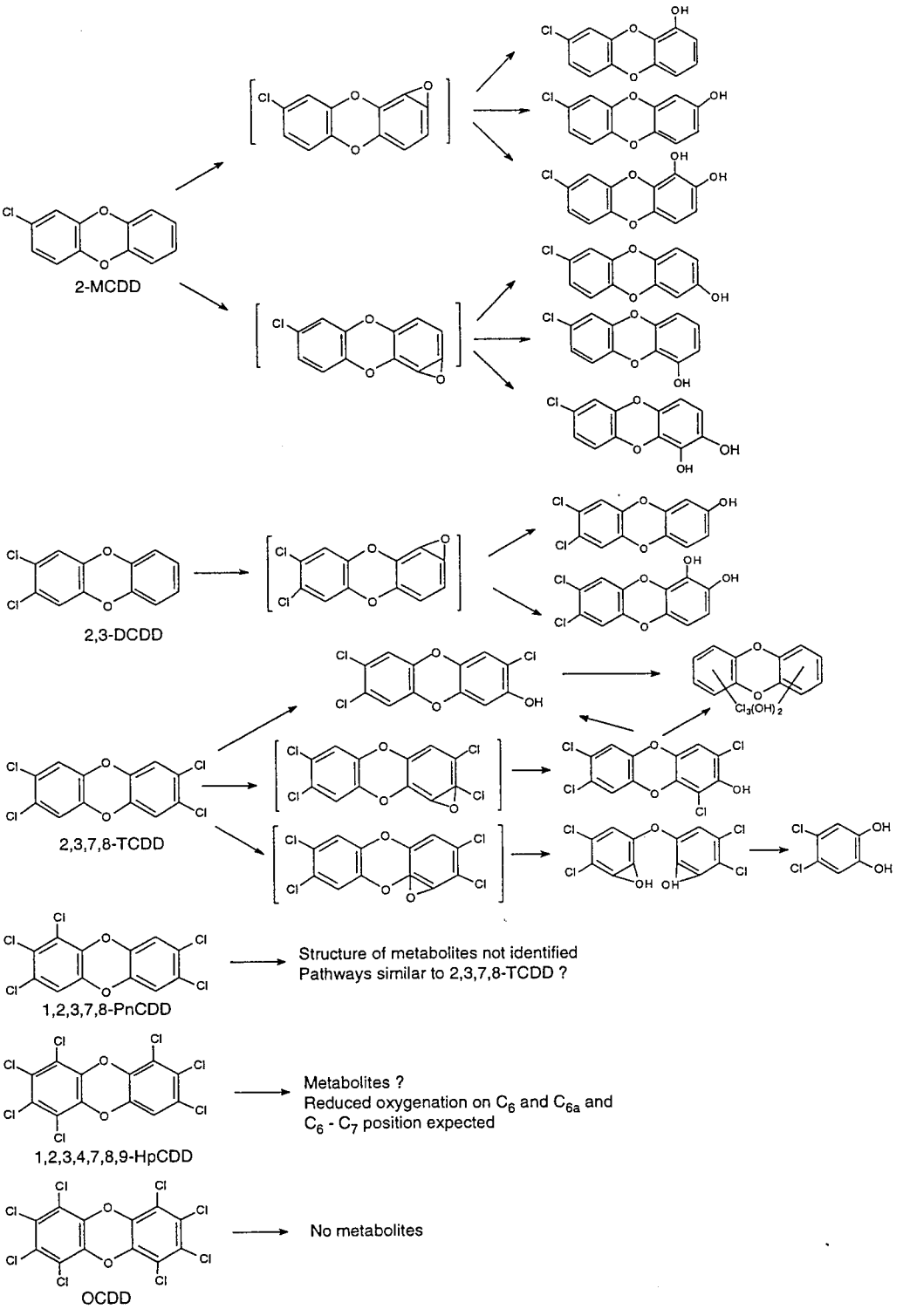
rats revealed no metabolites of OCDD, as expected from the fully chlorinated molecule (Birnbaum and Couture 1988; Tulp and Hutzinger 1978).

CDDs induce both phase I and phase II drug-metabolizing enzymes including AHH, EROD, ACOH, glucuronosyl transferase, glutathione S-transferase, and DT-diaphorase (Van den Berg et al. 1994). These enzymes are responsible for the metabolism of a variety of exogenous and endogenous substances. Pretreatment of C57BL/6J mice with 2,3,7,8-TCDD increased hepatic accumulation of a subsequent radiolabeled dose (total liver burden increased about 50%), whereas distribution to the kidney, fat, heart, lung, and gastrointestinal tract were reciprocally decreased (Curtis et al. 1990). The data indicated that an inducible, saturable system is involved in 2,3,7,8-TCDD toxicokinetics. The pretreatment, however, did not alter the hepatic metabolism of 2,3,7,8-TCDD in exposed mice. Similarly, the rate of metabolism of 2,3,7,8-TCDD in hepatocytes from 2,3,7,8-TCDD-pretreated (induced) guinea pigs and mice was unchanged from that in untreated animals (Olson and Wroblewski 1985; Shen et al. 1989; Wroblewski and Olson 1985). In contrast, the rate of metabolism in hepatocytes from 2,3,7,8-TCDD-pretreated rats was 3.2-fold greater than the rate in hepatocytes from control rats and about 9 times greater than in hepatocytes from 2,3,7,8-TCDD-pretreated guinea pigs. The difference between the 2,3,7,8-TCDD ability to induce its own rate of metabolism in rats and guinea pigs could be a factor in the difference between the susceptibility to 2,3,7,8-TCDD-induced toxicity in these two species, because the parent compound rather than metabolites is the toxic agent (Poland and Glover 1979). A generalized scheme of metabolic pathways for CDDs based on information from *in vivo* mammalian studies was proposed by Van den Berg et al. (1994) and is presented in Figure 2-3.

2.3.4 Elimination and Excretion

A median half-life of 7.1 years was estimated for 2,3,7,8-TCDD in a group of 36 Vietnam veterans (CDC 1987; Pirkle et al. 1989). The calculation was based on the decrease of 2,3,7,8-TCDD serum levels that

Figure 2-3. A Generalized Scheme of Pathways for the Biotransformation of CDDs Based on Information from *In Vivo* Mammalian Studies



Source: adapted from Van der Berg et al. 1994

were measured in these individuals in 1982 and again in 1987. The individual half-life values varied from 2.9 to 26.9 years. In an expanded half-life study of 343 Vietnam veterans participating in Operation Ranch Hand, which included the subjects of the Pirkle et al. (1989) study, a half-life estimate of 8.7 years (95% CI of 8.0–9.5 years) was calculated (Michalek et al. 1996). The half-life estimate was calculated using 2,3,7,8-TCDD levels in blood samples collected in 1982, 1987, and 1992. An earlier study of these subjects (Wolfe et al. 1994), which used data from two blood collection periods (1982 and 1987) estimated a half-life of 11.3 years (95% CI of 10–14.1 years). This half-life of 11.3 years was considered too high because it was based on restricted analysis of veterans with 2,3,7,8-TCDD levels above 10 ppt. By conditioning the data to lie above a line with slope equal to the negative of the decay rate, the analysis yielded a revised half-life of 8.7 years. The Michalek et al. (1996) half-life estimate of 8.7 years supersedes other estimates for this group of veterans because it includes an additional measurement of serum lipid 2,3,7,8-TCDD levels and controls for potential biases.

Several other studies have calculated 2,3,7,8-TCDD half-lives. A mean half-life of 5.8 years was estimated from repeated samples from 29 BASF AG facility workers whose initial 2,3,7,8-TCDD serum lipid concentrations ranged from 29 to 553 ppt (Ott and Zober 1996). In a study of 48 German workers at a pesticide facility who were exposed to a mixture of CDDs/CDFs, a median half-life of 7.2 years was estimated for 2,3,7,8-TCDD (Flesch-Janys et al. 1996). Needham et al. (1994) estimated a half-life of 8.2 years in 27 Seveso residents with initial serum 2,3,7,8-TCDD levels of 130 to 3,830 ppt. Using data from a human subject ingesting a single dose of 1.14 ng/kg 2,3,7,8-TCDD, Poiger and Schlatter (1986) calculated a half-life of 2,120 days (5.8 years). Geyer et al. (1986a) noted that they calculated a half-life of 3.5-6.9 years, but did not describe the basis of this estimation. Overall, there is good agreement between the 2,3,7,8-TCDD half-lives estimated in 4 different populations (Vietnam veterans, BASF AG cohort, German pesticide workers, and Seveso residents); the half-lives ranged from 5.8 to 8.7 years (Flesch-Janys et al. 1996; Michalek et al. 1996; Needham et al. 1994; Ott and Zober 1996). Several studies have found correlations between percentage of body fat and 2,3,7,8-TCDD elimination half-times (Flesch-Janys et al. 1996; Michalek et al. 1996; Ott and Zober 1996; Wolfe et al. 1994). Ott and Zober (1996) estimated half-lives of 5.1 and 8.9 years in subjects with 20 and 30% body fat, respectively.

There are limited data available on the elimination of other CDD congeners in humans. In the Flesch-Janys et al. (1996) study of 48 workers at a German pesticide facility, elimination half times were estimated for several CDD congeners. The estimated half-lives were 15.7 years for 1,2,3,7,8-PeCDD, 8.4 years for 1,2,3,4,7,8-HxCDD, 13.1 years for 1,2,3,6,7,8-HxCDD, 4.9 years for 1,2,3,7,8,9-HxCDD, 3.7 years for

1,2,3,4,6,7,8-HpCDD, and 6.7 years for OCDD. In a study of six German workers with high CDD/CDF body burdens, elimination half-lives corrected for alterations in body weight ranged from 3.5 years for 1,2,3,4,6,7,8-HpCDF to 7.9 years for 2,3,7,8-TCDD and 15 years for 1,2,3,4,7,8-HxCDD (Rohde et al. 1997). In the same study, half-lives for elimination due only to fecal excretion ranged from 10 years for OCDD to 22 years for 2,3,7,8-TCDD and 27 years for 1,2,3,7,8-PeCDD. The half-lives for 2,3,4,7,8-PeCDF in humans exposed to contaminated rice oil in the Yusho incident range from 2 to 30 years, and were inversely dependent on adipose tissue concentrations above approximately 10 ng/kg body weight (i.e., the higher the body burden, the faster the elimination) (Ryan et al. 1993a).

Elimination of CDDs through lactation is discussed in Section 2.3.4.4.

2.3.4.1 Inhalation Exposure

In male Fischer 344 rats administered a single intratracheal dose of 0.32 µg labeled 2,3,7,8-TCDD/kg, feces was the major route of excretion over a 3-day period after dosing (Diliberto et al. 1996). The cumulative excretion of 26.3% of the administered dose was observed over 3 days following exposure. Approximately 4% of the dose was excreted in the feces on day 3. The cumulative urinary excretion was only 1.3% of the administered dose.

2.3.4.2 Oral Exposure

The half-life for elimination of a single oral dose of 0.00114 µg/kg ^{3}H 2,3,7,8-TCDD in a human volunteer was calculated as 5.8 years (Poiger and Schlatter 1986). The excretion in feces was high during the first few days (up to day 6) probably because of elimination of unabsorbed material. During these first few days, about 12% of the administered dose was excreted. However, during days 7–125 only about 3.5% of the administered dose was eliminated. Urinary levels of radioactivity did not exceed the background levels.

Studies in animals indicated that elimination of 2,3,7,8-TCDD is a relatively slow process. However, the results showed a great variability among species. The half-life for 2,3,7,8-TCDD elimination was 14.95 days in Syrian hamsters (Olson et al. 1980b), 12 and 14 days in male and female Sprague-Dawley rats, respectively (Fries and Marrow 1975), 17 days in male Sprague-Dawley rats in another study (Piper et al. 1973), and 94 days in guinea pigs, the most sensitive species to the acute toxicity of 2,3,7,8-TCDD (Olson 1986). In contrast, the elimination half-life was 391 days in monkeys chronically exposed to low

doses of 2,3,7,8-TCDD in the feed (Bowman et al. 1989b). A similar half-life of about 1 year was observed in monkeys after a single-dose exposure (McNulty et al. 1982). In addition, studies of 2,3,7,8-TCDD half-life in highly exposed rats (Abraham et al. 1988), rhesus monkeys (McNulty et al. 1982) and marmoset monkeys showed that rates of excretion decreased with dose.

The clearance of radioactivity after oral exposure to labeled 2,3,7,8-TCDD followed first-order kinetics in most studies. Fecal elimination was the major route, though excretion in the urine, expired air, and milk was also reported.

When Sprague-Dawley rats were given radioactively labeled 2,3,7,8-TCDD, a total of 53% of the administered radioactivity was excreted by feces in the first 21 days (Piper et al. 1973). Elimination of 2,3,7,8-TCDD-derived radioactivity in urine and expired air was 13 and 3% of the administered dose, respectively. Thirty-two percent of a single gavage dose of 0.32 µg of 2,3,7,8-TCDD/kg was eliminated in the feces of male Fischer 344 rats over a 3-day period after dosing (Diliberto et al. 1996). Only 1.4% of the administered dose was excreted in the urine over the same period. About 20–30% of the total oral 2,3,7,8-TCDD dose was eliminated in the bile of cholecystectomized and cannulated dogs (Poiger et al. 1982). In addition, excretion of unchanged 2,3,7,8-TCDD in milk was demonstrated in NMRI mice (Nau et al. 1986) and in monkeys (Bowman et al. 1989b), after oral exposure.

Of the other congeners, several have been studied. An elimination half-life of 29.5 days was estimated for 1,2,3,7,8-PCDD in Sprague-Dawley rats following a single oral exposure (Wacker et al. 1986). OCDD was more persistent in Fischer 344 rats with an estimated elimination half-life of 3–5 months following 10 daily oral doses (Birnbaum and Couture 1988). These congeners were excreted primarily in the feces following biliary elimination as metabolites (1,2,3,7,8-PCDD, at least three phenolic metabolites) or parent compound. A 13-week dosing study in which Sprague-Dawley rats were administered various mixtures of CDDs estimated liver half-lives of 14.5, 29.3, 45.6, and 100 days for 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD, respectively (Viluksela et al. 1998a).

2.3.4.3 Dermal Exposure

Within 120 hours after dermal administration of 0.32 µg/kg 2,3,7,8-TCDD to the clipped back skin of male Fischer 344 rats, 4% of the administered dose was excreted in the feces and <1% was excreted in the urine (Banks and Birnbaum 1991). The rate of 2,3,7,8-TCDD elimination significantly increased over time.

2.3.4.4 Transfer of CDDs Through the Placenta and Breast Milk

CDDs are lipophilic compounds which can concentrate in maternal milk. Therefore, lactation provides an efficient mechanism for decreasing the body burden of these compounds (Schecter and Gasiewicz 1987a). CDD levels in breast milk samples from 193 German women ranged from 2.5 to 47 ng TEQ/kg milk fat (mean of 13 ng/kg) (Fürst et al. 1989b). More than 50% of the total CDDs detected in samples was represented by OCDD, which was detected at a mean concentration of 195 ng/kg milk fat (range, 13–664 ng/kg). The amounts of other congeners in human milk decreased with decreasing chlorination; the mean concentration of 2,3,7,8-TCDD in milk fat was 2.9 ng/kg (range, <1–7.9 ng/kg). A more recent analysis of 526 individual milk samples from the German general population revealed a mean 2,3,7,8-TCDD concentration of 3.2 ng/kg milk fat (Fürst et al. 1994). The analysis also showed the presence of only 2,3,7,8-chlorine-substituted CDD congeners. OCDD was the most concentrated congener with a mean level of 208 ng/kg milk fat. In general, the levels in milk decreased with decreasing degree of chlorination from octa- to tetra-CDD. Schecter and coworkers have published information on levels of dioxins in human breast milk from various countries (Schecter et al. 1989d, 1989e) (see also Section 2.6.1). In general, milk samples from industrial countries had higher CDD levels than those from less developed countries. Representative mean levels of CDDs in samples of human breast milk from various countries are presented in Table 2-6.

Fürst et al. (1989b) also found that the levels of CDDs found in the milk of mothers breast-feeding their second child were about 20–30% lower than in those breast-feeding their first child. It was further noted that the highest excretion of CDDs was during the first few weeks after delivery. The sharpest decline was observed with OCDD; its excretion was reduced by half between the 1st and 5th week of lactation. In contrast, there was no significant decline in total HxCDDs in milk during the first year of lactation. The concentration of 1,2,3,4,6,7,8-HpCDD in milk fat showed a steady decline over the 1-year period, but its levels stayed relatively high. 2,3,7,8-TCDD represented the smallest portion of the total CDDs, and its levels in milk continuously declined over the year of lactation. Levels of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD were measured in a mother of twins prior to nursing and after 2 years of nursing (Schecter et al. 1996a). There was a 49.5% decrease in the total amount of CDDs in the lipid fraction of the breast milk. 2,3,7,8-TCDD had the largest percent decline in CDD levels, a decrease of 83.9%. A 52.4% decrease in maternal serum lipid levels of total CDD was also observed; the largest percent decline was an 86.8% decline in 1,2,3,7,8-PeCDD levels.

Table 2-6. Mean Levels of CDDs in Breast Milk (ng/kg milk fat)

	The Netherlands[a] N=35	Canada[b] N=96	USA[c] N=42	Germany[d] N=526	Siberia[c] N=23	United Kingdom[e] N=57	South Vietnam (1973)[c] N=7	Cambodia[c] N=8
2,3,7,8-TCDD	3.8	2.3	3.3	3.2	2.7	5.6	131.0	0.49
1,2,3,7,8-PeCDD	10.6	4.8	6.7	10.1	3.3	13	ND	1.6
1,2,3,4,7,8-HxCDD	1.3	34.6[f]	4.9	8.4	1.6	62.0[f]	70.0[f]	0.6
1,2,3,6,7,8-HxCDD	49.1		30.5	35.8	5.6			3.4
1,2,3,7,8,9-HxCDD	6.5	6.4	6.2	6.4	1.2	8.3		1.1
1,2,3,4,7,8,9-HpCDD	54.3	40.5	42.0	14.2	8.1	10.5	99.0	11.0
OCDD	297.5	131.7	233.0	207.9	50.2	287	494.0	59.0
Total TEQ for CDDs only[g]	15.6	9.34	11.5	13.9	5.3	20.1	139.5	20.0

[a] Pluim et al. 1994a
[b] Dewailly et al. 1991
[c] Schecter et al. 1991a
[d] Fürst et al. 1994
[e] Duarte-Davidson et al. 1992
[f] For unseparated congeners
[g] See Section 2.5 for additional information

ND = not detected; TEQ = toxicity equivalency

Several studies have shown that CDDs in breast milk are readily absorbed by nursing infants. In a 19-week-old nursing infant, absorption was estimated as the difference between ingestion and the amount of CDDs found in the feces over a period of 12 days (McLachlan 1993). The mother was 32 years old and nursing for the first time. Several CDD congeners were determined in the milk: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, three hexacloro-substituted congeners, 1,2,3,4,6,7,8-HpCDD, and OCDD. The percentage of dose absorbed ranged from 90 to 95% except for the hepta-substituted congeners and OCDD which exhibited absorption rates of 61 and 23%, respectively. The percentage of the dose absorbed increased slightly if corrections were made for background levels in the diapers. Similar results were reported by Pluim et al. (1993b) who measured the amount of CDDs consumed via breast milk and excreted in the feces in 3 infants at the ages of 4, 8, and 12 weeks. Because of the high content of CDDs of the diapers relative to the feces, the percentage of dose absorbed was not determined. However, the results showed that, with the exception of OCDD, the bioavailability from breast milk was greater than 95%. At 4 weeks of age, the average cumulative intake of CDDs from breast milk was 132.1 pg TEQ per kg body weight. Of these, 37.4 corresponded to 2,3,7,8-TCDD, 46.2 to 1,2,3,7,8-PCDD, and 24.4 to 1,2,3,6,7,8-HxCDD. With the inclusion of CDFs, the total TEQ at 4 weeks was approximately 257 pg/kg body weight. Exposure to CDDs and CDFs from lactation decreased at 8 and 12 weeks mainly due to a decrease in their concentration in whole breast milk which resulted from a reduced fat content of the milk (the depletion of body burden of the mother while nursing may have also contributed). Abraham et al. (1994, 1996) and Dahl et al. (1995) also reported almost complete absorption of lower chlorinated CDDs and CDFs in breast-fed infants during the first year of life. It was also noticed that intake of CDDs and CDFs was up to 50 times higher in breast-fed infants compared with a formula-fed infant (Abraham et al. 1996). The latter study further showed that despite much lower intake of CDDs and CDFs after weaning, the concentration of these compounds in stool fat did not decrease substantially, suggesting that concentration in fecal fat more or less reflect that in body fat. Also, at 11 months of age, TEQ concentrations in blood from formula-fed infants were less than 25% of maternal values and about 10 times lower than in infants breast-fed for 6-7 months (Abraham et al. 1996).

Schecter et al. (1996b) recently presented data on the levels of CDDs and CDFs in human fetuses (8–14 weeks gestational age with placenta removed) and in placentas from women from the general population who had normal deliveries. On a lipid basis, the total TEQs (CDDs plus CDFs) in a pool of 14 placentas was 10.1 ng/kg; half this amount (5.3 ng/kg) was measured in a pool of 10 fetuses. In an analysis of 43 samples of human milk, Schecter et al. (1996b) found that the total concentration of CDDs and CDFs was 16.7 ng/kg (expressed as TEQ). The authors also calculated that the TEQ body burden for

the pooled fetal tissue was 0.034 ng/kg body weight; for pooled placentas, they calculated a total TEQ of 0.086 ng/kg wet weight. These results suggest that the transfer of CDDs to the fetus may be somewhat limited.

The influence of maternal transfer (placental and via breast milk) of CDDs/CDFs on the body burden of newborns and infants was further investigated by Kreuzer et al. (1997). These investigators also developed a pharmacokinetic model for 2,3,7,8-TCDD that allowed them to simulate body and tissue burden for the entire human lifetime as a function of 2,3,7,8-TCDD uptake from contaminated nutrition. On a lipid basis, the concentrations of 2,3,7,8-TCDD in adipose tissue and liver of breast-fed infants who died of sudden infant death syndrome were 0.4–4 ppt and 0.5–4 ppt, respectively. The corresponding values in nonbreast-fed infants were 0.2–0.8 ppt and 0.3–0.7 ppt. Similar values were detected in adipose tissue and livers of three stillborns, confirming the placental transfer of these chemicals to the fetus. The model developed by Kreuzer et al. (1997) reflected sex- and age-dependent changes in body weight, volumes of liver, adipose and muscle tissue, food consumption, and excretion of feces and was used to predict the half-life of elimination of 2,3,7,8-TCDD and its concentrations in adipose tissue, blood, liver, and feces at different ages. Also, the influence of breast-feeding on the 2,3,7,8-TCDD burden of the mother, her milk, and her child was simulated. The authors used their own data, as well as those from others, to validate the model. For nonbreast-fed infants, the model predicted a decrease in the concentration of 2,3,7,8,-TCDD in lipids during the first year, and this was supported by the empirical data. For infants exclusively breast-fed, the model predicted an increase in 2,3,7,8-TCDD burden followed by a decrease after weaning, and this was also confirmed by the measured data. Model validation of 2,3,7,8-TCDD concentrations in liver for the 20 infants investigated and in adipose tissue, blood, and feces for data in infants published by others showed good agreement between the simulated and experimental values. Since one of the model's assumption was that the concentration of 2,3,7,8-TCDD in fecal lipids reflected the concentration in lipids of the organism, the good correlation between predicted and empirical data validated the assumption. Under the assumption that the 2,3,7,8-TCDD concentration in lipids of breast milk equals the concentration in the maternal organism, the model predicted a value of 2.23 ng 2,3,7,8-TCDD/kg lipids for the beginning of the nursing period. The model further predicted that the concentration of 2,3,7,8-TCDD in milk decreases with duration of breast-feeding, such that after 6 months of daily nursing the concentration in milk and maternal body lipids is approximately 70% of the value at the time of delivery. These predictions were in good agreement with published values. Lastly, the investigators modeled the concentration of 2,3,7,8-TCDD in lipids or blood of a male subject for a time span of 60 years and compared it with literature values for German subjects. One of two curves constructed was computed assuming breast-feeding for the first

6 months of life followed by formula up to 1 year and the other considering feeding only formula for the same period of time. In both cases further nutrition was simulated to consist of the common diet. The predicted curves differed considerably during the first years of life. For the nonbreast-fed case, 2,3,7,8-TCDD concentrations decreased during the first year and subsequently increased, reaching a maximum at 16 years. For the breast-fed case, the simulation yielded a rapid rise of 2,3,7,8-TCDD in lipids followed by a 3-year decrease after weaning and merging at about 7 years with the concentrations of nonbreast-fed individuals. Subsequently, 2,3,7,8-TCDD concentrations leveled at between 2 and 3 ng 2,3,7,8-TCDD/kg body lipids until the end of life. The latter value was in agreement with average background levels for the German population. The half-life of nonmetabolic elimination (unchanged 2,3,7,8-TCDD) was calculated to be 0.42 years in newborns and 9.5 years in 40-year-old adults. The half-life of the fraction metabolized by the liver ranged from 1.5 years for newborns to approximately 10 years for a 40-year-old individual. The three times greater elimination half-life for the metabolized fraction relative to the nonmetabolized fraction in infants suggests that metabolic elimination does not play a major role in the elimination of 2,3,7,8-TCDD in infants. A key finding from the Kreuzer et al. (1997) study is the model prediction that the increased 2,3,7,8-TCDD burden observed as a result of breast-feeding does not lead to a raised lifetime value.

In rodents, placental transfer of CDDs to the fetus is relatively limited, but transfer during sensitive periods of organogenesis is biologically important as evidenced by effects on fetuses or offspring exposed *in utero*. Excretion into milk represents a major pathway for maternal elimination of CDDs and, therefore, for exposure to offspring. In C57BL/6N mice administered a single oral dose of 30 µg ^{14}C-2,3,7,8-TCDD/kg on Gd 11 the levels of 2,3,7,8-TCDD-derived radioactivity in the embryos on Gd 12, 13, or 14 were below 0.5% of the total 2,3,7,8-TCDD dose (Weber and Birnbaum 1985). In the dams, the highest concentration of radioactivity was in the liver (50–67% of total dose), whereas embryos had a relatively higher concentration of radioactivity in the heads than in the rest of the body. Approximately 0.03% of the administered dose was delivered to each embryo. In a different study in NMRI mice, pregnant females were administered a single dose of 25 µg ^{14}C-2,3,7,8-TCDD (oral, intraperitoneal, or subcutaneous) on Gd 16 and the distribution of radioactivity was examined in the pups on postnatal days 7–36 (Nau et al. 1986). At all times, the highest concentration of radioactivity in the pups (per gram of tissue) was found in the liver; extrahepatic tissues such as intestines and skin had a concentration of radioactivity that was approximately one order of magnitude lower than the liver. During the first postnatal week, 2,3,7,8-TCDD concentrations increased considerably in the pups. It was also found that during the first two weeks the pups received doses of 2,3,7,8-TCDD through milk which were, on a body weight basis, similar to those

which had been administered to their mothers prior to birth. In pups raised by untreated foster mothers, 2,3,7,8-TCDD tissue concentrations decreased rapidly due to organ growth with concomitant dilution of 2,3,7,8-TCDD. Abbott et al. (1996) examined the distribution of 2,3,7,8-TCDD in embryonic tissues of mice at times earlier than previous studies. Pregnant mice were treated with 2,3,7,8-TCDD on Gd 12 and embryonic tissues were examined at various times from 0.5 to 24 hours after dosing. The rate of accumulation of 2,3,7,8-TCDD reached a maximum in placental tissue in about 3 hours and, following a slight decline, remained relatively constant between 8 and 24 hours. After 24 hours, 0.27% of the maternal dose was detected in the placenta. In embryonic liver, 2,3,7,8-TCDD peaked approximately 8 hours after dosing and decreased thereafter, as opposed to maternal liver, where it remained constant after achieving an apparent maximum. The relative decrease in the rate of concentration in the embryonic liver was attributed to a rapid growth of the tissue during that time period. Distribution of 2,3,7,8-TCDD to embryonic palates followed a pattern similar to that in embryonic liver. Twenty-four hours after dosing, the secondary palates had 0.0045% of the administered maternal dose.

Van den Berg et al. (1987b) examined the transfer of CDDs and CDFs through the placenta and via the milk in Wistar rats. Prenatal exposure of the fetus was studied by administering a diet containing a fly ash extract from a municipal incinerator to rats from day 8 until 17 of pregnancy, after which time the rats were sacrificed. Postnatal transfer was assessed in rats fed the same diet during the first 10 days after delivery while nursing their offspring. Of the 49 tetra- to octa-CDDs, only 7 CDD congeners were detected and all had a 2,3,7,8-chlorine-substitution pattern. In the fetus, 2,3,7,8-TCDD had the highest retention (0.13% of total dose, 0.0092% of the dose/g). Retention decreased with the number of chlorine atoms; HpCDDs and OCDD were not detected. In the liver of offspring, 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, and the three 2,3,7,8-substituted HxCDDs had the highest retention (5.3–8.1% of total dose, 0.74–1.13% of dose/g). The 2,3,7,8-penta- and hexa-substituted congeners had the highest retention in the livers of pregnant and lactating rats (53.9–80.2% of total dose, 2.9–5.2% of dose/g). No significant differences were found in liver retention of tetra- to octa-chlorinated congeners between pregnant and lactating rats, but lactating females stored less CDDs in their adipose tissue. Similar results were reported by Li et al. (1995c) in Sprague-Dawley rats. These authors administered a single intravenous dose of 5.6 µg ^{14}C-2,3,7,8-TCDD/kg to pregnant rats on Gd 18. Sacrifices were conducted on Gd 19 and 20, and postnatal days 1 and 5. Groups of neonates were also cross-fostered between treated and nontreated dams to differentially assess transfer of 2,3,7,8-TCDD through the placenta and through nursing. Only about 0.01% of the dose administered to the dams was found in whole livers of fetuses one and two days after dosing (0.04 and 0.07% of dose/g fetal liver), indicating limited placental transfer. In contrast, the

concentration of 2,3,7,8-TCDD in the liver of neonates after 1 day of lactation was 0.65% of the administered dose/g liver, and this increased to 2.88% after 4 days of nursing. Four days after nursing, the liver concentration of 2,3,7,8-TCDD in neonates from dams dosed 1 day after parturition was 4.1% of the administered dose/g of liver, and this was higher than in the dam's liver (3.32%). As in earlier studies, the results from the cross-fostering experiments confirmed that nursing is a major pathway for transfer of 2,3,7,8-TCDD to the offspring.

The transfer of CDDs and CDFs via placenta and through milk was also investigated in a marmoset monkey administered a defined mixture of CDDs and CDFs subcutaneously 11 weeks prior to delivery (Hagenmaier et al. 1990). Concentrations of CDDs and CDFs were measured in a newborn 1 day after birth and in an infant of the same litter after a period of 33 days of lactation. The highest deposition in newborn liver was observed for 2,3,7,8-TCDD and 1,2,3,7,8-PCDD (54 and 51 pg/g wet weight, respectively) and corresponded to about 0.15% of the administered dose/g tissue. The concentration of all other congeners was <10% of the corresponding concentrations in adults. In contrast to liver, the concentrations of 2,3,7,8-substituted CDDs in newborn adipose tissue were at least one third the levels in adults, and for OCDD, the concentration in adipose tissue was three times higher that in adult adipose tissue. Transfer of CDDs through milk was considerable, though selective. The concentration of 2,3,7,8-TCDD and 1,2,3,7,8-PCDD in the infant's liver was 395 and 611 pg/g wet tissue, respectively; the corresponding concentrations in the mother's liver were 107 and 326 pg/g. However, the concentration of OCDD in infant's liver was less than 10% that of the mother's liver. Bowman et al. (1989b) examined the transfer of 2,3,7,8-TCDD from mother to offspring in rhesus monkeys. Female monkeys had been exposed to 2,3,7,8-TCDD for about 4 years to a diet (5 or 25 ppt) that provided an estimated 0.0001–0.0006 µg 2,3,7,8-TCDD/kg/day before breeding. Breeding started 10 months after exposure ceased. At weaning (4 months), the offspring had a concentration of 2,3,7,8-TCDD in mesenteric fat 4.3 times higher than in subcutaneous fat from their respective mothers. Bowman et al. (1989b) estimated that the mothers excreted between 17 and 44% of their 2,3,7,8-TCDD burden by lactation. Based on measurements of 2,3,7,8-TCDD in fat at 4, 12, and 24 months of age, it was found that in the young monkeys the decline in 2,3,7,8-TCDD in fat followed first-order, single-compartment kinetics with a half-life of approximately 181 days (Bowman et al. 1990). For the purpose of comparison, the mean half-life in 7 adult female rhesus monkeys was 391 days with standard error of 88 days (Bowman et al. 1989b).

In summary, CDDs can be transferred to the fetus across the placenta and, although the amounts may be relatively small, the transfer may have great biological significance if it occurs during critical periods of

organogenesis. Due to their lipophilicity, CDDs can concentrate in human breast milk and can be transferred to infants through nursing. In general, the amount of individual congeners in breast milk decreases as chlorination decreases. Excretion via milk is highest during the first weeks after delivery. Also, the concentration of CDDs in milk is higher in mothers breast-feeding their first child than in those breast-feeding their second child. CDDs transferred to infants through nursing are readily absorbed by the infants. A pharmacokinetic model predicted that the increased body burden in infants that results from breast-feeding does not translate into raised lifetime body burden. Studies in animals have also shown transfer of CDDs across the placenta and via mother's milk.

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.

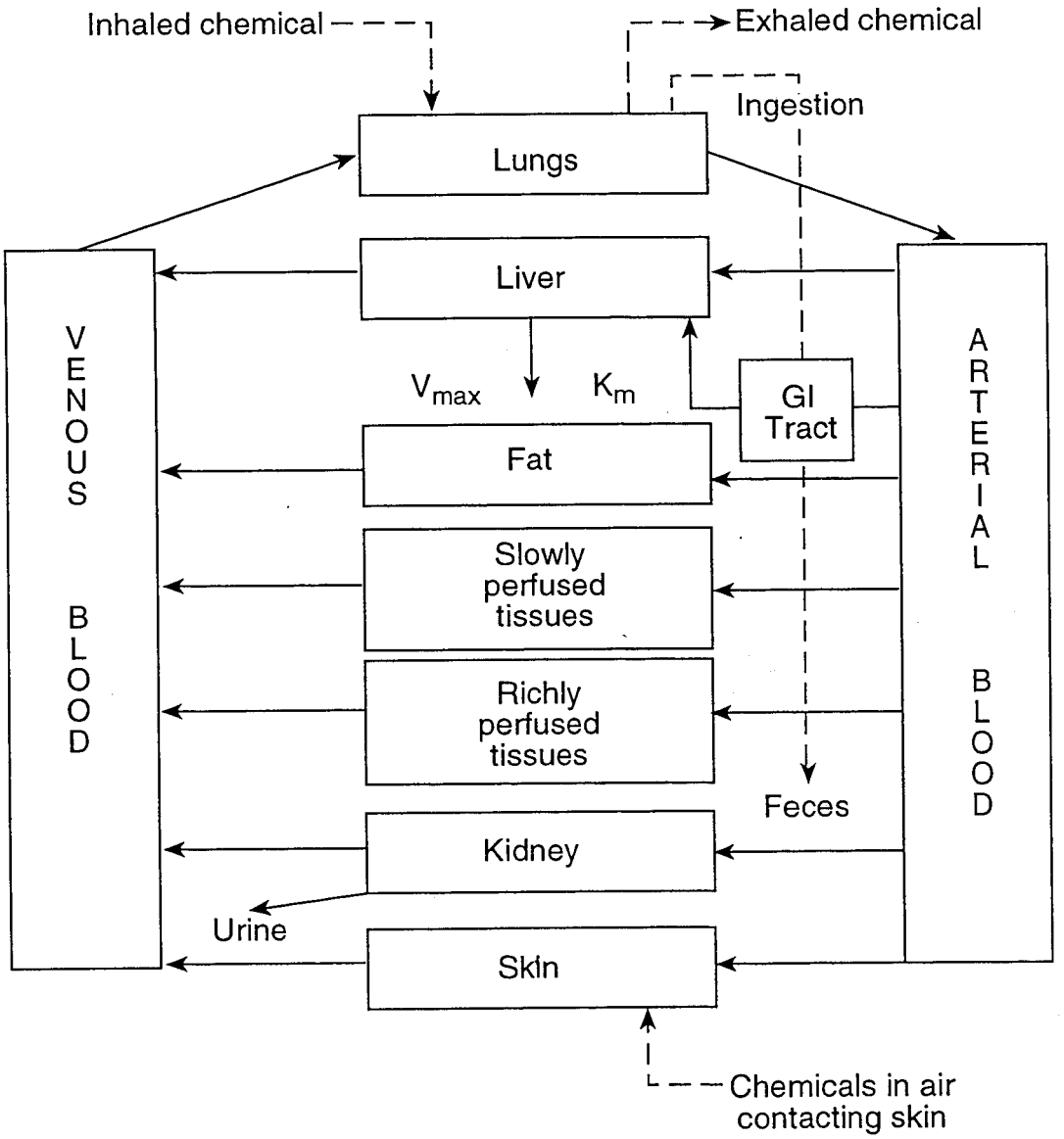
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many 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 2-4 shows a conceptualized representation of a PBPK model.

If PBPK models for CDDs 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.

PBPK models for 2,3,7,8-TCDD are discussed below. The pharmacokinetic behavior of 2,3,7,8-TCDD, especially distribution, has been shown to be dose-dependent and involves protein binding and enzyme induction in hepatic tissue. Thus, terms describing these interactions have been included in the animal models described below. Furthermore, since induction of these dioxin-binding proteins is a process mediated by the interaction of a dioxin-receptor (the Ah receptor) complex with specific binding sites on DNA additional terms were included in the models. For a detailed explanation regarding the Ah receptor and its involvement in the mechanism of action of 2,3,7,8-TCDD and structurally related halogenated aromatic hydrocarbons, see Section 2.4.2.

Figure 2-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

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.

2.3.5.1 Summary of PBPK Models.

The elimination of 2,3,7,8-TCDD from humans was evaluated using a PBPK model developed by Kissel and Robarge (1988). The steady-state adipose tissue concentration predicted by the model was similar to the lipid-based blood levels reported in the general population with no known special exposure to 2,3,7,8-TCDD. The model was also used to predict elimination of 2,3,7,8-TCDD from Ranch Hand Vietnam veterans. The predicted half-lives were similar to an experimental value based on analysis of 2,3,7,8-TCDD in blood of veterans with adipose burdens >10 ppt. The apparent half-lives increased as the adipose tissue concentration approached the steady-state level associated with background exposure. The model also predicted reasonably well the elimination of 2,3,7,8-TCDD from a volunteer who ingested a single 2,3,7,8-TCDD dose.

Leung et al. (1988) developed a five compartment PBPK model to describe the time course of 2,3,7,8-TCDD distribution in tissues of both the Ah-responsive C57BL/6J and Ah-less responsive DBA/2J mice (C57BL/6J mice respond to 2,3,7,8-TCDD with an increase in AHH activity, at a dose less than required to elicit this response in DBA/2J mice). The model also included binding in blood and two hepatic sites, one in the cytosol and the other in microsomes. It was found that the greater accumulation of 2,3,7,8-TCDD in the liver of C57BL/6J mice, relative to DBA/2J mice, was not attributed to the greater fat content in the DBA/2J mice, but to the more avid microsomal binding (CYP1A2) in the liver of the C57BL/6J mice. In the concentration range covered in the model simulations, the cytosolic receptor (Ah receptor) did not seem to play a major role in determining the overall tissue distribution pattern.

The same group of investigators (Leung et al. 1990b) developed a PBPK model to describe the tissue disposition of 2,3,7,8-TCDD in Sprague-Dawley rats. The description included the same compartments used in modeling the behavior of 2,3,7,8-TCDD in mice. The ratio of liver to fat concentration of 2,3,7,8-TCDD was found to be primarily determined by the dissociation constant of the microsomal binding protein (CYP1A2) and the basal and induced concentration of this protein in the liver. In general, there was agreement between the simulated data and experimental data from a single-dose study and a 7- and 13-week repeated-dosing study. However, the model underpredicted the concentration of 2,3,7,8-TCDD in the fat at low dose and overestimated the concentration at high dose for a 2-year feeding study. Induction of microsomal binding protein was necessary to account for the differences in disposition at low and high daily doses. Further refinements of Leung et al. (1988, 1990b) models were conducted by

Wang et al. (1997) and Santostefano et al. (1998) and included analyses of distribution and responses to 2,3,7,8-TCDD exposure at early times and in multiple tissues.

A receptor-mediated PBPK model was developed by Andersen et al. (1993). The model included interactions of the Ah-TCDD complex with DNA and was used to examine the tissue disposition of 2,3,7,8-TCDD and the induction of the dioxin-binding protein (presumably CYP1A2) and CYP1A1. It was found that tumor promotion correlated more closely with predicted induction of CYP1A1 than with induction of the hepatic binding proteins (CYP1A2, AhR). More recently, Andersen et al. (1997a, 1997b) developed a multicompartment geometric model of the liver that provided a better prediction of both total and regional induction of CYP450 proteins within the liver than conventional one-compartment models.

A mechanistic model (known as the NIEHS model) was constructed to describe 2,3,7,8-TCDD-mediated alterations in hepatic proteins in the rat (Kohn et al. 1993). This model included the tissue distribution of 2,3,7,8-TCDD and its effects on concentrations of CYP1A2 and CYP1A1 and the effects of 2,3,7,8-TCDD on the Ah, estrogen, and epidermal growth factor (EGF) receptors over a wide range of 2,3,7,8-TCDD doses. The model predictions were compared to experimental data from 2,3,7,8-TCDD promotion studies. The biochemical response curves for the proteins examined were hyperbolic, indicating a proportional relationship between target-tissue dose and protein concentration at low 2,3,7,8-TCDD doses. Also, the model successfully reproduced the observed tissue distribution of 2,3,7,8-TCDD, the concentration of CYP1A2 and CYP1A1, and the effects of 2,3,7,8-TCDD on the Ah, estrogen, and EGF receptors over a wide dose range.

Carrier et al. (1995a) developed a model that describes the distribution kinetics of 2,3,7,8-TCDD and related chemicals (with chlorine substitutions in positions 2,3,7, and 8) in various mammalian species, including humans. Their model takes into account cellular diffusion, binding of the chemicals with the Ah receptor and with proteins, and enzyme induction in the liver. The model was used to describe the distribution of CDDs between liver and adipose tissue as a function of overall body concentration. Model simulations showed that the fractions of the body burden contained in the liver and adipose tissue vary nonlinearly as a function of the overall body concentration; this was in agreement with published data in rodents, monkeys and humans. The authors further modeled the disposition kinetics of CDDs in liver, adipose tissue, and whole body as a function of time (Carrier et al. 1995b). The results showed that the rate of change in CDD tissue concentrations varies as a function of total body burden such that whole body

elimination rate decreases as body burden decreases, suggesting nonlinear disposition kinetics. This was also in agreement with published data on absorption and elimination kinetics of CDDs in rats and humans.

2.3.5.2 Comparison of PBPK Models for 2,3,7,8-TCDD.

Several models that describe the disposition of 2,3,7,8-TCDD in animals and one in humans were identified from the literature. In the Leung et al. (1988, 1990b) models in mice and rats, tissue 2,3,7,8-TCDD concentration ratios, particularly liver concentrations, were related both to intrinsic partitioning and to the presence of specific cytosolic and microsomal 2,3,7,8-TCDD-binding proteins. This is in contrast to other PBPK models developed for similar, persistent lipophilic chemicals, which distributed the chemicals between organs based on experimentally observed concentrations ratios under various dosing conditions. This could explain why two organs having the same partition coefficients contain very different 2,3,7,8-TCDD concentrations under a particular experimental condition. Carrier et al. (1995a, 1995b) developed a similar model, which also included other 2,3,7,8-substituted dioxins and furans and simulated experimental data on rodents, monkeys, and humans. The Andersen et al. (1993) model extended the Leung et al. (1988, 1990b) models by including induction of binding proteins/enzymes and of 2,3,7,8-TCDD metabolism in response to ternary interactions of 2,3,7,8-TCDD, the Ah receptor, and DNA binding sites and correlated various tissue dose measures with the promotional efficacy of 2,3,7,8-TCDD. The five-compartment liver model described by Andersen et al. (1997a, 1997b) provided a better description of mRNA production and regional localization of induced proteins, consistent with immunohistochemical information, than conventional one-compartment models. The model constructed by Kohn et al. (1993) suggested possible biochemical mechanisms which could explain a complex response to exposure to 2,3,7,8-TCDD such as cell proliferation in female rats. This model not only included enzyme induction and the Ah receptor, but also the estrogen and EGF receptors, all of which seem to be involved in a complex sequence of events that lead to cell proliferation as a result of 2,3,7,8-TCDD exposure. In contrast with the models developed for animals and described above, the Kissel and Robarge (1988) fugacity-based model for humans was used to predict vehicle-dependent uptake and elimination of 2,3,7,8-TCDD without including any Ah receptor-related terms. (Fugacity is defined as the “escaping tendency” of a substance in a phase.) Pharmacokinetic parameters used in the various models are listed in Table 2-7.

Table 2-7. Pharmacokinetic Parameters for 2,3,7,8-TCDD Used in PBPK Models

Parameters	C57BL/6J mouse[a]	DBA/2J mouse[a]	Sprague-Dawley rat[b]	Human[c]	Wistar rat[d]
Partition coefficient (tissue/blood)					
Liver	20	20	20	25	20
Fat	350	350	350	300	375
Rapidly perfused (kidney)	20	20	20	7–10	20
Slowly perfused (skin)	250	250	40	30	30
Slowly perfused (muscle)	250	250	40	4	30
Biochemical constants					
Binding capacity to hepatic cytosolic protein (nmol/liver)	0.0042	0.0042	0.054	–	0.0038
Binding affinity to hepatic cytosolic protein (nM)	0.29	2	0.015	–	0.035
Binding capacity to hepatic microsomal protein (nmol/liver)	20	20	–	–	–
Noninduced binding capacity to hepatic microsomal protein (nmol/liver)	–	–	25	–	10
Induced binding capacity to hepatic microsomal protein (µmol/liver)	–	–	175	–	85
Binding affinity to hepatic microsomal protein (nM)	20	75	7	–	6.5
First-order metabolic rate constant (per hour per kg liver)	3.25	1.75	2	–	1.65
Absorption constant from gastrointestinal tract into liver (per hour)	0.02	0.02	0.2	–	–
Binding to blood	2.5	2.5	2.5	–	–

[a] Leung et al. 1988
[b] Leung et al. 1990b
[c] Kissel and Robarge 1988
[d] Andersen et al. 1993

2.3.5.3 TCDD Models

The Kissel and Robarge Model

Description of the Model. The elimination of 2,3,7,8-TCDD from humans was described with a fugacity-based model using physiologically based parameters (Kissel and Robarge 1988). In this model, transport of 2,3,7,8-TCDD was assumed to be perfusion-limited (flow-limited) and 2,3,7,8-TCDD was assumed to be uniformly distributed within each tissue group or fluid phase, and tissue levels were considered to be in equilibrium with exiting fluids (blood, urine, bile). Because 2,3,7,8-TCDD appears to be poorly metabolized in humans, the model did not include terms for metabolites. Transport between gut lumen and gut tissue was described as a diffusive process. Included in the differential equations used to solve the system were data for several diets. Body compartment sizes and densities used in the simulations of background exposure and of elimination from individuals with body burdens similar to those of Ranch Hand veterans were based on reference-man data. Tissue perfusion rates and partition coefficients were obtained from the literature. The diet used in all simulations was adapted from the literature and also included a typical intake of added fats and oils. The fugacity capacity of the various diet components, gastric secretions, and fecal materials were either calculated or obtained from the literature. The model was used to predict tissue levels resulting from background exposures, elimination of 2,3,7,8-TCDD from Ranch Hand veterans, and elimination of 2,3,7,8-TCDD from a human volunteer.

Validation and Discussion. The steady-state adipose tissue concentrations predicted by the model, assuming no metabolism and a daily background exposure of 50 pg/day in North America, was 7.7 ppt. This value was similar to the lipid-based blood tissue levels reported in the general population with no known unusual exposure. The body burden projected for an intake of 100 pg/day fell outside the typical range associated with background sources. In simulating the elimination of 2,3,7,8-TCDD from Ranch Hand veterans the model assumed a background exposure of 50 pg/day and no metabolism. Under these conditions, apparent half-lives of 4.4, 5.2, 5.9, 7.2, 9.1, and 20 years were estimated for individuals with 2,3,7,8-TCDD adipose tissue concentrations of 100, 50, 30, 20, 15, and 10 ppt, respectively. This was in good agreement with a half-life of 7.1 years determined by analysis of blood lipids of veterans with adipose burdens >10 ppt (Pirkle et al. 1989). The results showed that the apparent half-lives increased greatly as tissue concentrations approached the steady-state level associated with background exposure. The model also approximated the uptake efficiency and elimination of 2,3,7,8-TCDD from a volunteer as reported by Poiger and Schlatter (1986). The fact that the predicted uptake efficiency was similar to that found

experimentally indicated that the estimated gut-lumen/gut-tissue mass transfer coefficient used was in the appropriate range. The reported half-life was 5.8 years and the model estimated a value of 6.7 years. Overall, the result suggested that a fugacity-based model can provide a viable method for describing overall elimination of 2,3,7,8-TCDD from humans, but it does not provide much insight regarding why elimination occurs in a particular manner.

The Leung et al. Model in Mice

Description of the Model. The model described by Leung et al. (1988) in mice provides quantitative descriptions of the time-course of elimination and levels of 2,3,7,8-TCDD in various organs of C57BL/6J mice and DBA/2J mice, a less responsive strain with higher body-fat content. The model contains five compartments: blood, liver, fat, richly perfused tissues, and slowly perfused tissues. To account for the 2,3,7,8-TCDD binding to receptor in the liver, the model contained two hepatic binding sites, one corresponding to the high affinity/low capacity cytosolic Ah receptor and the other to the inducible, low affinity/high capacity microsomal protein (CYP1A2). To simulate the intraperitoneal dose route used by Gasiewicz et al. (1983a), 2,3,7,8-TCDD was assumed to be absorbed into the liver compartment by a first-order uptake process. Bioavailability was assumed to be 100%. Partition coefficients, physiological parameters, and biochemical constants were obtained or calculated from the literature for each mouse strain. The kidney was assumed to be representative of the richly perfused tissue, whereas the slowly perfused tissue consisted mainly of muscle and skin. The binding capacity of the Ah-less responsive DBA/2J mice was set to equal that of the Ah-responsive mice even though the binding affinity is extremely low. Blood binding was described as a linear process with an effective equilibrium between bound and free 2,3,7,8-TCDD given by a constant. In blood, only one form of 2,3,7,8-TCDD is exchangeable in the tissues, which gives rise to kinetic behavior observed for diffusion-limited uptake into tissues.

Validation and Discussion. The simulation of the time-course of 2,3,7,8-TCDD concentration in the liver and fat of C57BL/6J mice after a single 10 μg/kg intraperitoneal injection generated by the model was in good agreement with the empirical data of Gasiewicz et al. (1983a). In trying to simulate the 3-times-higher liver/fat ratio of 2,3,7,8-TCDD in the C57BL/6L mice than in the DBA/2J mice, Leung et al. (1988) varied the fat content parameter in the C57BL/6J mice from 3 to 12% of body weight. The rationale was that the difference in hepatic concentration may have been due to greater capacity of the DBA/2J mouse to sequester the highly lipophilic 2,3,7,8-TCDD in adipose tissue. However, the results showed that 2,3,7,8-TCDD concentration in the liver was relatively insensitive to body fat content,

indicating that this was not an important factor influencing the disposition of 2,3,7,8-TCDD in the liver between the two strains of mice. The authors also found that the distribution of 2,3,7,8-TCDD was strongly influenced by the binding characteristics of the microsomal binding protein, especially the binding constant. The model gave good simulations of 2,3,7,8-TCDD excretion in both strains of mice. The simulation of the time-course of 2,3,7,8-TCDD concentration in the liver and fat of DBA/2J mice after a single 10 μg/kg intraperitoneal injection was not as good as that for the C57BL/6J mouse if the input was set to be consistent with the uptake and elimination. As with the C57BL/6J mouse, disposition of 2,3,7,8-TCDD in the liver of DBA/2J mice was greatly influenced by the microsomal protein binding constant and rather insensitive to changes in body fat content. The best fit of the empirical data was obtained with a binding constant of 75 nM (20 nM for the C57BL/6J mice), indicating that the 2,3,7,8-binding affinity to the hepatic microsomal protein in the DBA/2J mice was at least 3.5 times lower than that of the C57BL/6J mice.

The Leung et al. Model in Rats

Description of the model. This model in the Sprague-Dawley rat (Leung et al. 1990b) is an extension of the mouse model previously described and contains the same five compartments and two types of binding proteins: one corresponding to the high-affinity, low-capacity cytosolic 2,3,7,8-TCDD (Ah) receptor, and the other to the inducible, lower-affinity, high-capacity microsomal protein (CYP1A2). In the rat model, both types of binding proteins are defined with their own binding capacities and dissociation constants. The model was used to analyze experimental data for the single-dose studies of McConnell et al. (1984) and Rose et al. (1976), the 7-week Rose et al. (1976) study, the 13-week multiple-dose study of Kociba et al. (1978b), and the 2-year feeding study of Kociba et al. (1978a). In simulating the single-dose gavage study, 2,3,7,8-TCDD was assumed to be absorbed from the gastrointestinal tract by a first-order uptake process with a rate constant of 0.2/hour. In simulating the multidosing studies, bioavailability was assumed to be 100%. Physiological parameters, partition coefficients, and biochemical constants were calculated or obtained from the literature. Since there was no literature value for the binding capacity of the microsomal 2,3,7,8-TCDD-binding site in the rat, the value used was approximated by assuming it to be 10 times that of the mouse. The total microsomal binding capacity was apportioned between a basal level and an induced level. Also, AHH activity was taken to be the sum of a basal and induced level. A first-order metabolic rate constant for 2,3,7,8-TCDD metabolism in the liver was adjusted to provide a biological half-life of about 25–30 days.

Validation and Discussion. When the simulation of the McConnell et al. (1984) data for AHH induction included a term for induction of microsomal binding protein there was good agreement between the simulation and the empirical data. This had not been the case in an initial fitting which included a constant concentration of microsomal binding protein. Rose et al. (1976) examined the accumulation of 2,3,7,8-TCDD in adipose and liver tissues in rats administered 0.01, 0.1 and 1 µg 2,3,7,8-TCDD/kg/day 5 days a week for 7 weeks; sampling was done at weeks 1, 3, and 7. Model predictions of 2,3,7,8-TCDD concentrations were in good agreement with the experimental data except for concentration in fat at the 0.01 µg/kg/day dose level, in which case the model overpredicted the tissue concentration. Model formulations that had constant microsomal binding capacity overpredicted liver 2,3,7,8-TCDD concentrations at the lower-dose rates. Also, model formulations that contained final amounts of microsomal binding protein (CYP1A2) very different (much higher or lower) from the basal 200 nmol/liver could not simulate 2,3,7,8-TCDD concentration in liver at the highest-dose rate. Similar to the findings in mice, the liver/fat concentration ratio in rats was extremely sensitive to the dissociation constant of the microsomal binding protein. The model simulated well the data from the 7- and 13-week studies (Rose et al. 1976; Kociba 1978b), but not as well for data from the 2-year feeding study (Kociba et al. 1978a). There was underprediction of 2,3,7,8-TCDD concentration in fat and liver at the low dose (0.001 µg/kg/day) and overprediction of the liver concentration at the high-dose level (0.1 µg/kg/day). However, the ratios of the concentrations were consistent with those observed experimentally (1/1 at low doses, much higher in liver at high doses). According to Leung et al. (1990b), the underprediction at low dose may reflect the fact that the low-dose fat concentration in the 2-year study was close to the limit of detection and thus, subject to more error. At the high dose, physiological parameters such as tissue volume, metabolic constants, and amounts of binding proteins may have been altered by weight loss and changes in body composition, known effects of chronic exposure to 2,3,7,8-TCDD. Leung et al. (1990b) indicated that the overprediction at high dose could have been due to a loss of microsomal 2,3,7,8-TCDD-binding sites in the chronically exposed rats. The affinity of 2,3,7,8-TCDD for the microsomal binding protein appeared to be greater in the Sprague-Dawley rats than in C57BL/6J mice, which could account for the higher liver/fat concentration ratio in rats than in mice, assuming that the partitioning between tissues is approximately the same in the two species.

Wang et al. (1997) extended the work of Leung et al. (1988, 1990b) and Andersen et al. (1993) and developed an improved model to describe the disposition of 2,3,7,8-TCDD in multiple tissues from female Sprague-Dawley rats. The model of Wang et al. (1997) improved previous modeling attempts in some specific areas such as 1) providing information on distribution of 2,3,7,8-TCDD at early time points

(important for determining unique parameters related to mass transfer such as permeability), 2) better handling of mass balance when considering 2,3,7,8-TCDD binding to plasma proteins, and 3) improved estimation of physical and biochemical parameters. The Wang et al. (1997) model accurately described the time course distribution of 2,3,7,8-TCDD following a single oral dose, as well as the concentration of 2,3,7,8-TCDD in eight target tissues on day 3 after six different doses. The model described by Wang et al. (1997) was recently coupled to a biologically-based pharmacodynamic (BBPD) model to quantitatively describe the relationship between disposition and response in multiple tissues (Santostefano et al. 1998). This later model incorporated both pharmacokinetic and pharmacodynamic events to account for the ability to 2,3,7,8-TCDD to induce CYP1A1 and the fact that CYP1A2 is responsible for maintaining high concentrations of 2,3,7,8-TCDD in the liver. The results showed that the BBPD model accurately described the time course of CYP1A1 protein expression and EROD activity in the liver, skin, and kidneys. It also confirmed that EROD activity can be an appropriate marker for CYP1A1 protein expression, and the shape of the induction curves supported the hypothesis that similar time-dependent mechanism of 2,3,7,8-TCDD-induced CYP1A1 protein expression and associated EROD activity occurs in multiple tissues. This, in turn, suggested that parameter estimation in the study accurately described the Ah receptor-mediated mechanism on protein expression and enzymatic activities in multiple tissues.

The Andersen et al. Model

Description of the Model. This model (Andersen et al. 1993) is an extension of the earlier PBPK models developed by Leung et al. (1988, 1990b) for 2,3,7,8-TCDD. Like the earlier models, this model consists of five compartments. Each of the four tissue compartments has a specified blood flow, tissue compartment volume, and a tissue blood volume. Movement of chemical from blood to tissue was modeled to be proportional to the product of a permeation coefficient times surface area for the tissue. When this product is lower that the specified blood flow for the tissue, tissue uptake is diffusion-limited. Because of the diffusion-limited tissue compartments, the model did not require blood binding to match the time-course of tissue uptake. It was assumed that in the liver both the Ah receptor and the inducible binding protein act to sequester 2,3,7,8-TCDD through a capacity-limited binding process, and the binding protein was assumed to be CYP1A2. Binding interactions with CYP1A2 and CYP1A1 were described by reversible equilibrium relationships, which is valid as long as the rate constants for association/dissociation are large. It was also assumed that the DNA sites to which the Ah-2,3,7,8-TCDD complex binds are present at much lower concentrations than the Ah-ligand complex. For both CYP1A1 and CYP1A2 induction, it was assumed that the Ah-ligand complex formation was equivalent, but that the Hill term, *n*, (a measure of

interaction for multiple Ah-ligand complex binding sites) and the Hill binding constant were different for the two responses. The model also allowed for autoinduction of metabolism following 2,3,7,8-TCDD treatment. Data from Abraham et al. (1988) and Krowke et al. (1989) were analyzed. The former study provided dose-response characterization of concentrations of 2,3,7,8-TCDD in liver and of liver CYP1A1 activity and time-course characterization of 2,3,7,8-TCDD concentration in tissues and enzyme activities in female Wistar rats. Krowke et al. (1989) examined liver and fat concentrations in male Wistar rats dosed weekly for up to 6 months. In addition, Andersen et al. (1993) examined the potential correlation between several measures of dose estimated by the model and the promotional efficacy and carcinogenicity of 2,3,7,8-TCDD in Sprague-Dawley rats. Cancer data from Kociba et al. (1978a) and Pitot et al. (1980) were analyzed.

Validation and Discussion. Abraham et al. (1988) found that the disposition of 2,3,7,8-TCDD in liver and fat from rats administered a single subcutaneous dose (0.001–10 μg/kg) of the chemical was highly dose-dependent. The disproportionately higher concentration in the liver at higher doses appeared to be due to induction of a dioxin-binding protein, presumably CYP1A2. The model developed by Andersen et al. (1993) successfully simulated the experimental data. The affinity of the binding protein was estimated to be 6.5 nmol, while a value of 1 for *n* suggested little interaction among 2,3,7,8-TCDD-responsive DNA-binding sites involved in the expression of CYP1A2. For describing induction of CYP1A1, an *n* of 2.3 was required, which suggested possible interactions among DNA-binding sites for the Ah-ligand complex with this gene. The simulation of the time-course of elimination from liver and of induction of CYP1A1 was in good agreement with the empirical data, but required the inclusion of time-dependent growth parameters over the 100 days of the experiment. The model also successfully simulated the data from the repeated-dosing study by Krowke et al. (1989) after small adjustments were made to fat and slowly perfused tissue parameters. The measures of dose that were used for comparison with the promotional and carcinogenic properties of 2,3,7,8-TCDD were integrated total liver concentration during the treatment period, or integrated free liver 2,3,7,8-TCDD concentration. Also, measures of tissue dose related to enhanced expression of CYP1A1 and hepatic binding proteins were calculated and examined for correlation with promotional activity. Results of the analysis revealed that under the exposure conditions, the tumor promotional response of 2,3,7,8-TCDD in the rat liver most closely correlated with integrated expression of the CYP1A1 gene. However, Andersen et al. (1993) indicate that since there is no expectation of causality between tumor responses and induction of CYP1A1 (or CYP1A2), the correlation should be regarded cautiously. Consistent with the findings of Leung et al. (1988, 1990b), the results from

the Andersen et al. (1993) study showed that over a certain dose range (e.g., at doses several fold above background), protein (CYP1A2) induction greatly alters 2,3,7,8-TCDD disposition.

Recently, Andersen and co-workers developed a model of hepatic enzyme zonation that was combined with the PBPK model of protein induction (Andersen et al. 1993) to create a multicompartmental representation of the liver architecture that can be used to predict the degree of induction in both the whole liver and in specific regions (Andersen et al. 1997a, 1997b). A geometric representation was used to divide functional units (based on enzyme distribution) within the liver into five zones. The primary objective was to compare model predictions for regional induction with regional protein induction as visualized by immuno-histochemistry. The data set modeled included analysis of tissue distribution of 2,3,7,8-TCDD in the first days or weeks after a single dose, time course studies for about 100 days after a single dose, and initiation-promotion studies in rats dosed for up to 6 months. The results showed that the five-compartment model was more successful than conventional homogeneous one-compartment liver models not only in simulating low-dose behavior for mRNA in whole liver but also in representing immunohistochemical observations. Five or more compartments were required to give a sharp boundary between induced and noninduced regions of the liver. When the five-compartment liver model was used to account for CYP1A1 and CYP1A2 induction and regional distribution of induced enzymes, the low-dose behavior appeared to be nonlinear and was better described, with a large *n* value (Hill coefficient) and a range of affinities in the liver covering about 81-fold differences between centrilobular and periportal regions.

The Kohn et al. (NIEHS) Model

Description of the Model. Kohn et al. (1993) constructed a mathematical model (the NIEHS model) to describe 2,3,7,8-TCDD tissue distribution and 2,3,7,8-TCDD-mediated alterations in hepatic proteins in the rat. The model assumed that 2,3,7,8-TCDD mediates increases in liver concentration of transforming growth factor-α (TGF-α) by a mechanism which requires the Ah receptor. TGF-α subsequently binds to the EGF receptor, a process which is known to cause internalization of the receptor in hepatocytes. This is thought to be an early event in the generation of a mitogenic signal. The model included equations for the Ah receptor-dependent induction of CYP1A1 and CYP1A2 activity and of the Ah receptor itself. Because it was also assumed that estrogen action is required for 2,3,7,8-TCDD-mediated induction of TGF-α, production of the estrogen receptor, CYP1A2-catalyzed formation of catechol estrogens, and deactivation of estrogens by glucuronidation were included in the model. The model predictions were compared to the two-stage initiation-promotion data of Tritscher et al. (1992) and Sewall et al. (1993). Gavage doses

equivalent to 3.5–125 ng 2,3,7,8-TCDD/kg/day for 30 weeks were used in these studies. Data from Abraham et al. (1988) were also analyzed. Model parameters were obtained from the literature or calculated from experimental data and adjusted to make the model reproduce the observations of Tritscher et al. (1992) and Sewall et al. (1993).

Validation and Discussion. The model prediction for the percentage of absorption (>90%) from ingestion of 2,3,7,8-TCDD was in good agreement with experimental data of Rose et al. (1976). The model also predicted that 92.2% of the metabolite appears in the feces and 7.8% in the urine at a dose of 125 ng/kg/day. The dose of 2,3,7,8-TCDD did not have a significant effect on these predictions. From the fit to the data of Abraham et al. (1988), the model predicted an initial and overall half-time clearance from liver of 11.8 and 13.5 days, respectively, which is very close to the experimentally obtained 11.5 and 13.6 days. Similar good agreement was obtained for half-time elimination from fat (22.3 days versus 24.5 days). The model predicted a linear relationship between administered dose and the concentration of 2,3,7,8-TCDD in the liver at doses between 3.5 and 125 ng/kg/day, which was in good agreement with the data of Tritscher et al. (1992). The relationship between 2,3,7,8-TCDD dose and induction of both CYP1A1 and CY1A2 was best fit by an hyperbolic curve suggesting lack of cooperative interactions among binding sites. The hyperbolic curve was consistent with the experimental data for induction of these proteins from Tritscher et al. (1992). The model also predicted that the fractional occupancy of the Ah receptor by 2,3,7,8-TCDD rises from 13.4% at a dose of 3.5 ng/kg/day to 69.3% at 125 ng/kg/day. The model prediction of the degree of internalization of the EGF receptor as a function of the concentration of TGF-α was also hyperbolic in shape and successfully reproduced the experimental data of Sewall et al. (1993). Kohn et al. (1993) indicate that as this response may be involved in the mechanism of tumorigenesis in 2,3,7,8-TCDD-treated rats, it would be expected that it would correlate with tumor incidence better than does tissue dose. If so, extrapolation of effects at high dose to low doses may underestimate low-dose effects. However, extrapolation from low dose to extremely low dose would still be valid. The model predicted that 10 days after administration of a single dose of 1 μg 2,3,7,8-TCDD/kg there should be a greater decrease in plasma membrane EGF receptor in female rat liver than in male rat liver, which is consistent with the observed lower sensitivity of the male. Consistent with the experimental data, the model reproduced the decrease in hepatic estrogen receptor (ER) level resulting from exposure to 2,3,7,8-TCDD, and the relationship between concentration of 2,3,7,8-TCDD and amount of receptor was also hyperbolic. Overall, the model's success in reproducing the observed responses to 2,3,7,8-TCDD for the various proteins included in the model supports the proposed mechanism that internalization of the EGF

receptor in response to induction of TGF-α may be the origin of the mitogenic signal important for carcinogenesis.

The Carrier Model

Description of the Model. The first part of this model provides a quantitative description of the distribution of 2,3,7,8-substituted CDDs (and CDD-like compounds) between liver and adipose tissues as a function of overall body concentration at any given time (Carrier et al. 1995a). In a second step, differential equations were used to describe the disposition of CDDs in liver, adipose tissues, and whole body as a function of time (Carrier et al. 1995b). The first step of the model was based on several hypotheses: 1) changes in overall CDD concentration are slow relative to intertissue diffusion exchanges, protein induction, and binding of CDDs in the liver; 2) CDDs are mainly in adipose tissue and in the liver, but exchanges between these two sites are mediated via the blood; 3) the liver synthesizes proteins that bind free CDDs according to standard mass action association-dissociation mechanisms; 4) synthesis of binding proteins in the liver is linked to binding of free CDDs to the Ah receptor; 5) CDDs in fat deposits within the liver contribute to the overall liver burden and is taken into account; and 6) small amounts of CDDs are contained in organs other than the liver and adipose tissues and this fraction is assumed to be constant. In the second step, CDDs were assumed to be eliminated mainly by hepatic clearance; elimination by lactation or transplacental distribution was not considered. Model simulations of various experimental data sets, as specified below, were conducted. When not readily available, anatomical and physicochemical parameters were obtained from laboratory or clinical data.

Validation and Discussion. The model successfully simulated data from Abraham et al. (1988), who provided dose-response characterization of concentrations of 2,3,7,8-TCDD in the liver of rats after a single dose of the compound. Analysis of the data showed that the higher the body burden, the higher the proportion of the burden contained in the liver. However, the model predicted that a plateau is reached when body burden is >1 mg 2,3,7,8-TCDD/kg body weight. The model predictions were also in good agreement with experimental data from Van den Berg et al. (1986a), who administered a single dose of a mixture of CDDs and CDFs to rats and hamsters and with data in monkeys administered a single oral dose of 2,3,7,8-TCDD (McNulty et al. 1982). Results from simulations conducted on data from chronic studies in rats (Kociba et al. 1978a; Rose et al. 1976) and on human data from Yusho patients also showed that increasing the body burden results in an increase in the fraction of the body burden present in the liver and in an increase in the liver/adipose concentration ratio. These changes in fractional distributions were

attributed to the affinity of specific liver proteins for binding of free hepatic CDDs and the saturable capacity of the binding proteins at high concentration of free CDDs. Model simulations of elimination data in rats after single (Abraham et al. 1988) or repeated doses (Kociba et al. 1978a; Rose et al. 1976) of 2,3,7,8-TCDD, as well as data from a Yu-Cheng patient agreed well with the empirical data and showed that disposition kinetics of 2,3,7,8-substituted CDDs are nonlinear (i.e., as body burden decreases with time, liver and adipose tissue half-lives increase). According to the model, an additional factor that can influence the disposition kinetics of 2,3,7,8-CDDs is a rapid change in body weight and/or adipose tissue mass. A rapid loss of adipose tissue whether by dieting or in patients experiencing anorexia, would result in a higher concentration of the chemical in the remaining adipose tissue, particularly if the loss of tissue is much faster than whole body elimination via the liver.

2.3.5.4 Risk Assessment.

In early efforts to model the disposition of persistent halogenated aromatic hydrocarbons, disposition was described by simple partitioning between the blood and the various tissues with first-order metabolism in the liver. In those studies, the role that extensive tissue binding to particular cellular proteins might play in determining the overall disposition of the chemical was not accounted for. In contrast, the descriptions of Leung et al. (1988, 1990b) and Carrier et al. (1995a, 1995b) attempted to provide a biochemical basis for the observed tissue distribution. The use of this type of model may help explain interspecies differences in 2,3,7,8-TCDD sensitivity and carcinogenicity. The rodent PBPK model for 2,3,7,8-TCDD revealed very consistent behavior between species, and some of the predictions of high dose-low dose behavior were verified.

One advantage of a description that explicitly includes protein binding is the ultimate ability to develop pharmacodynamic models for 2,3,7,8-TCDD (and related chemicals) toxicity based on Ah receptor occupancy or Ah-TCDD complex concentration *in vivo* and to realistically couple it with the biologically based cancer models (or with models for other 2,3,7,8-TCDD responses). This was attempted by Andersen et al. (1993) and Kohn et al. (1993), who included estimates of binding constants between the Ah receptor and 2,3,7,8-TCDD and between the Ah receptor-dioxin complex and sites on DNA. Santostefano et al. (1998) extended previous modeling attempts by determining parameter values based on time course of CYP1A1 and CYP1A2 responses in multiple tissues using a simultaneous PBPK and BBPD models. However, as noted by Andersen et al. (1993), in order to develop a complete biologically motivated risk-assessment model, these dosimetry models need to be combined with quantitative descriptions of cell and

tissue responses. Kohn et al. (1993) used the NIEHS model to successfully predict tissue concentrations of 2,3,7,8-TCDD and of various induced proteins involved in the carcinogenic response to 2,3,7,8-TCDD and suggested that such a model might permit extrapolation of responses beyond the range obtained from experimental data and lead to scientifically sound approaches for estimating risks of adverse health effects of exposure to 2,3,7,8-TCDD. The importance of the results of Kohn et al. (1993) can be illustrated by the finding that the dose-response curves for various proteins were hyperbolic rather than sigmoid. Sigmoidicity in the response requires a higher concentration to produce a given response at low dose than does a hyperbolic response having the same concentration for half-maximal effect. This implies that the response is approximately linear at very low doses.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

The mechanism of absorption of CDDs by the inhalation and dermal routes of exposure is not known. Transfer of CDDs from the aqueous environment of the intestine across cell membranes is predominantly limited by molecular size and lipid solubility. The overall evidence indicates that 2,3,7,8-substituted tetra- and pentachlorinated congeners are well absorbed. In contrast, OCDD was poorly absorbed from the gastrointestinal tract of rats (Birnbaum and Couture 1988), but absorbed more on chronic exposure (Birnbaum et al. 1989a). Absorption is also vehicle-dependent (Poiger and Schlatter 1980). Highly chlorinated congeners, although absorbed in small amounts, can accumulate in the liver. Results from studies in thoracic duct-cannulated rats showed that 2,3,7,8-TCDD was transported primarily via the lymphatic route and was predominantly associated with chylomicrons (Lakshmanan et al. 1986). Several studies have examined the distribution of CDDs between blood and adipose tissue. Patterson et al. (1989d) showed that on a lipid basis the serum/adipose ratio for 2,3,7,8-TCDD in humans was approximately 1:1, and this correlation held over a concentration range of almost three orders of magnitude. They also found that in blood <10% of 2,3,7,8-TCDD was associated with red blood cells, which according to Patterson et al. (1989d), suggested that most of 2,3,7,8-TCDD in blood was bound to serum lipids and lipoproteins. However, the distribution between plasma lipid and adipose tissue increased with chlorine substitution, which indicated that higher chlorinated congeners have a higher binding affinity for plasma proteins (Patterson et al. 1989d; Schecter et al. 1990). Experiments of *in vivo* binding of CDD congeners to various serum fractions revealed that as chlorine content increased, binding to lipoproteins gradually decreased, 75% of 2,3,7,8-TCDD was found bound to lipoprotein compared to 45% for OCDD (Patterson et al.

1989b). However, binding to other proteins increased with chlorine content (20% for 2,3,7,8-TCDD versus 50% for OCDD). Also, fewer CDDs (<10%) were bound to the chylomicrons in serum. In studies *in vitro* with human whole blood, 80% of the applied amount of 2,3,7,8-TCDD was associated with lipoproteins, 15% with proteins, and 5% with cellular components (Henderson et al. 1988). Also, there is some evidence that 2,3,7,8-TCDD and related stereoisomers may be associated with plasma prealbumin (McKinney et al. 1985a; Pedersen et al. 1986). Within the lipoprotein fraction and per mole of lipoprotein, 2,3,7,8-TCDD has highest affinity for very low density lipoprotein (VLDL), followed by LDL and HDL (Marinovich et al. 1983). A study using cultured human fibroblasts presented some evidence that specific binding to LDL and the LDL receptor pathway may explain in part the rapid early uptake of 2,3,7,8-TCDD with LDL entry (Weisiger et al. 1981).

2,3,7,8-substituted CDDs are the predominant congeners retained in tissue and body fluids from rodents and monkeys (Abraham et al. 1989b; Van den Berg et al. 1983), although minor retention of non-2,3,7,8-substituted congeners has been reported in the rat (Abraham et al. 1989b). In general, the tissue distribution of CDDs is congener-specific and depends on the dose and route of administration (see Van den Berg et al. 1994 for review). In rats, for any particular organ or tissue, distribution within 24 hours of dosing depends on blood perfusion rate and relative tissue size, such that relatively high initial CDD concentrations are found in the adrenal glands and skeletal muscle (Pohjanvirta et al. 1990). Shortly thereafter, the liver and adipose tissue become the major storage sites (Allen et al. 1975; Lakshmanan et al. 1986; Rose et al. 1976). Data from studies in humans, marmoset monkeys and rats suggest that the distribution ratio between liver and adipose tissue increases with increasing degree of chlorination (Abraham et al. 1989c; Neubert et al. 1990a; Thoma et al. 1990), but also depends on the dose, metabolic rate, route of administration, and the time of observation after dosing. In non-human primates and in humans, the liver appears to be a less significant storage site than in rodents (Van Miller et al. 1976). In mice, the Ah receptor does not appear to play a significant role in 2,3,7,8-TCDD body distribution for adipose tissue, skin, kidney, and total-body concentration (Birnbaum 1986). However, it plays some role in liver retention (Birnbaum 1986; Gasiewicz et al. 1983a) and this was found to be related to inducibility of cytochrome P-450 (Leung et al. 1988), in particular CYP1A2. Distribution of 2,3,7,8-TCDD in mice has been shown to be age-dependent (Pegram et al. 1995). The greater fat content of some tissues in old mice enhances partitioning of 2,3,7,8-TCDD into the tissues, while decreased perfusion prolongs clearance (Pegram et al. 1995). Some acute- and chronic-duration studies in rats have demonstrated a disproportionate dose-dependent distribution of 2,3,7,8-TCDD in liver and adipose tissue (Abraham et al. 1988; Kociba et al. 1978b). The greater the dose, the greater the liver/adipose tissue ratio. A disproportionate

dose-dependent distribution has also been demonstrated in mice (Diliberto et al. 1995). 2,3,7,8-TCDD-derived radioactivity in the liver was found associated preferentially with the microsomal fraction (Allen et al. 1975). Information summarized by Van den Berg et al. (1994) suggests that the disproportionately greater hepatic concentration of 2,3,7,8-TCDD after exposure to higher doses may be explained in part by the induction of a hepatic-binding species, CYP1A2. This distribution parameter is also explained by the Carrier et al. (1995a,b) model in humans.

There is some experimental data to suggest that the fetal/neonatal period may be more sensitive to the toxicity of CDDs than the adult animal. Several studies have shown that limited placental transfer of CDDs takes place in rodents (Li et al. 1995c; Nau et al. 1986; Van den Berg et al. 1987b; Weber and Birnbaum 1985) and in humans (Schecter et al. 1996a). However, little is known about the mechanisms responsible for the transfer of CDDs across the placenta, the dependence of these mechanisms on the gestational period, and the distribution of these compounds in fetal tissue. However, CDDs and related chemicals are able to concentrate in breast milk, and limited human (Abraham et al. 1994; McLachlan 1993; Pluim et al. 1993b) and animal (Nau et al. 1986) data have indicated considerable absorption of these compounds by the nursing infant. Thus, while the *in utero* exposure of fetal tissues to CDDs may represent only a small percentage of the maternal body burden of CDDs, the breast-fed infants will receive a higher daily dose per body weight than adults. Further information regarding placental transfer and elimination of CDDs through breast milk is presented in Section 2.3.4.4.

As mentioned in Section 2.3.3, metabolic transformation of CDDs *in vivo* includes oxidation and reductive dechlorination as well as glutathione conjugation. Studies in two rat strains which differ greatly in sensitivity to 2,3,7,8-TCDD did not provide evidence for a role of toxicokinetics and metabolism in the difference in sensitivity (Pohjanvirta et al. 1990). Also, studies in various mice strains showed no significant Ah receptor-related differences in metabolic pathways (Gasiewicz et al. 1983a). While *in vitro* studies have shown similarities between most species regarding metabolite profile, the rate of 2,3,7,8-TCDD metabolism and the number of metabolites were reduced in hepatocytes in suspension culture from guinea pigs, a highly sensitive species (Wroblewski and Olson 1985). The overall evidence suggests that 2,3,7,8-TCDD can induce its own metabolism *in vivo*, but only at doses that could cause overt signs of toxicity (Van den Berg et al. 1994). It is important to consider the possibility of autoinduction at high doses because data obtained with exposure levels associated with a significant induction of CYP1A1 and CYP1A2 may not necessarily reflect toxicokinetic behavior at low-exposure levels.

Elimination of 2,3,7,8-substituted CDDs occurs mainly via the bile and the feces as polar metabolites; much smaller amounts are excreted via the urine. Moreover, in almost all mammalian species studied, the 2,3,7,8-TCDD-derived radioactivity in tissues is associated with the parent compound, suggesting that the hydroxylated and/or conjugated metabolites are rapidly eliminated from the body. Studies in mice showed that a strain of mice having a low affinity Ah receptor eliminate 2,3,7,8-TCDD at a much slower rate than mice with an Ah receptor of high affinity for the ligand (Gasiewicz et al. 1983a). These strain differences in body distribution and elimination could be explained not only by the differences in adipose tissue content, but also by the presence of a hepatic microsomal binding protein (Leung et al. 1988). Further studies in congenic mice suggested that the distribution and excretion of 2,3,7,8-TCDD is controlled primarily by the total genetic background and not by the allele present at the Ah-locus (Birnbaum 1986). Guinea pigs eliminate 2,3,7,8-TCDD considerably slower than other rodents (Olson 1986). This may reflect the relatively limited ability of the guinea pig to metabolize 2,3,7,8-TCDD and may contribute to the greater persistence and greater acute toxicity of 2,3,7,8-TCDD in this species. Results from a repeated-dosing study in rats showed that the rate-constant defining the approach to steady-state concentrations was independent of the dose over the range tested (Rose et al. 1976). This was consistent with evidence suggesting that autoinduction of 2,3,7,8-TCDD metabolism does not occur following exposure to sublethal doses. Autoinduction of metabolism could explain cases of dose-related excretion in which longer half-lives for elimination are seen at lower-exposure levels which are not associated with enzyme induction.

2.4.2 Mechanisms of Toxicity

The mechanism(s) of toxicity for CDDs is not completely understood but has been extensively studied, particularly for 2,3,7,8-TCDD, and numerous reviews are available on this subject (Birnbaum 1994a; Goldstein and Safe 1989; Kerkvliet 1995; Landers and Bunce 1991; Okey et al. 1994; Poland and Knutson 1982; Safe 1986, 1990; Silbergeld and Gasiewicz 1989; Whitlock 1987, 1993). Many CDDs, CDFs, coplanar PCBs, and other structurally related halogenated aromatic hydrocarbons are believed to share a common mechanism of action intimately related to similarities in their structural configuration. Most of what is known regarding the mechanism of action of these compounds is based on research in three main areas: structure-activity relationships for receptor binding and induction of a variety of biochemical and toxicological responses; genetic studies using inbred mouse strains; and studies at the molecular level which have elucidated key events in the actions of the receptor. Most of the studies providing this information used parenteral routes of exposure and/or *in vitro* tests systems. It is beyond the scope of this profile to discuss these studies in detail.

The extraordinary potency of 2,3,7,8-TCDD in evoking a dose-related induction of cytochrome P-450-associated AHH activity, the stereospecificity among related halogenated aromatic compounds to evoke this response, and the tissue specificity of enzyme induction, led Poland and Glover (1973b) to postulate the existence of an induction receptor. This receptor, the Ah receptor (Ah for aromatic hydrocarbon), was later identified in the cytosol of mouse liver cells (Poland et al. 1976) and, subsequently, in hepatic and extrahepatic tissues of a variety of laboratory animals, mammalian cell cultures, human organs and cell cultures, and also nonmammalian species (Okey et al. 1994). Results from structure-binding relationships for a series of CDD congeners using mouse hepatic cytosol showed that not all the congeners had the same affinity for the Ah receptor; affinity was found to be determined by the chlorine-substitution pattern (Mason et al. 1986; Poland et al. 1976, 1979). The most active compound was 2,3,7,8-TCDD, which is substituted in all four lateral positions. Addition of one, two, or four nonlateral chlorine substituents, or removal of lateral chlorine substituents, resulted in congeners with lower binding affinities. The stereospecific nature of the binding suggested the existence of a cytosolic receptor as a mediator in responses caused by 2,3,7,8-TCDD and related compounds.

2,3,7,8-TCDD and structurally related halogenated aromatic compounds induce a variety of microsomal enzymes primarily in the liver. The most widely studied of these responses are induction of hepatic AHH and EROD (markers of CYP1A1 activity) in mammalian cell cultures and in laboratory rodents (Goldstein and Safe 1989; Poland and Glover 1973a; Safe 1986, 1990). Several studies have examined the *in vitro* and *in vivo* structure-activity relationships for CDDs as inducers of hepatic and extrahepatic CYP1A1 activity (Bradlaw and Casterline 1979; Harris et al. 1990; Mason et al. 1986; Poland and Glover 1973a; Poland and Knutson 1982; Poland et al. 1979). The most active CDDs were substituted in their 2,3,7, and 8 positions, and the structure-activity relationships for induction and receptor binding assay were comparable. The molecular dimensions of the binding site was initially estimated to fit ligands that were approximately 3×10Ð (Poland and Knutson 1982), which would accommodate molecules such as 2,3,7,8-TCDD; however, approximate dimensions of 12x14x5Ð would be required to accommodate other chemicals (e.g., 3-MC or β-naphthoflavone), known to bind (Landers and Bunce 1991; Rannug et al. 1991; Waller and McKinney 1995). Although results from these experiments provided further evidence for a receptor-mediated mechanism of action, there was not strictly a linear correlation between Ah receptor binding and enzyme induction. Mason et al. (1986) suggested that a number of factors, including differential solubilities of the CDDs in the assay buffer system at higher concentrations, may contribute to the nonlinearity. They also suggested that structure-dependent receptor protein-ligand interactions which occur after the initial binding event may have played a role in the nonlinearity of the data sets.

Furthermore, differential rates of metabolism and elimination of particular CDDs also likely account for the comparative differences between studies *in vivo* and *in vitro* (Birnbaum 1985).

Numerous studies have examined the structure-toxicity relationships for CDDs. For example, examination of lethality data in guinea pigs revealed that the fully lateral-substituted tetra- to hexachloro-substituted isomers were the most toxic congeners, and the structure-activity relationships were comparable to those observed for their AHH-induction and receptor-binding activities (Eadon et al. 1986). Similar results have been reported for responses such as body weight loss and thymic atrophy (Mason et al. 1986; Safe 1987). Furthermore, there was an excellent correlation between the *in vitro* AHH induction potencies and the *in vivo* responses. Additional end points for which structure-toxicity relationships correlate well with structure-induction potencies and/or Ah receptor-binding affinities are epidermal responses such as the keratinization of the mouse teratoma XB cells and the production of skin lesions in genetically inbred haired and hairless mice (Knutson and Poland 1980, 1982), suppression of the splenic antibody response to SRBC (Kerkvliet et al. 1985), antiestrogenicity (Gierthy et al. 1987; Krishnan and Safe 1993), and teratogenicity (Weber et al. 1985). Taken together, these results, and others, strongly supported the role of the Ah receptor in mediating the toxicity of 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons.

As previously mentioned, 2,3,7,8-TCDD and structurally related compounds induce a wide range of biological responses, including alterations in metabolic pathways, body weight loss, thymic atrophy, impaired immune responses, hepatotoxicity, chloracne and related skin lesions, developmental and reproductive effects, and neoplasia. The expression of these responses is thought to be initiated by the binding of individual congeners (or ligands) with the Ah receptor. However, responsiveness of certain mouse strains to aromatic hydrocarbons is inherited in a simple autosomal-dominant mode and both enzyme induction and the toxic responses to 2,3,7,8-TCDD appear to segregate with the Ah locus (Poland and Glover 1980). For example, certain mouse strains, typified by C57XBL/6J, have an Ah receptor protein with a relatively high binding affinity for inducers of AHH such as 3-methylcholanthrene, β-naphthoflavone, 2,3,7,8-TCDD, and other isostereomers of 2,3,7,8-TCDD, and are sensitive to the toxic effects of these chemicals. In contrast, other mouse strains, such as DBA/2J, have an Ah receptor protein that has a lower ligand affinity (Okey et al. 1989), and are much less sensitive to the toxic effects of these compounds. The use of these mouse strains and strains differing only the Ah locus (congenic) has suggested that many of the responses elicited by these chemicals (e.g., enzyme induction, thymic involution, cleft palate formation, hepatic porphyria, and immunotoxicity) segregate with this Ah locus (Birnbaum et al. 1990; Kerkvliet et al. 1990b; Lin et al. 1991a, 1991b; Poland and Knutson, 1982; Swanson and

Bradfield 1993). More recent investigations using an Ah receptor-deficient mouse (Fernandez-Salguero et al. 1996) also support the role of this protein in mediating the toxicity of 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons.

These genetic data strongly support the role of the Ah receptor in mediating the toxicity of 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons. However, it has become clear that a comparison of the properties of the Ah receptor across species and tissues indicates that it is difficult to account for the species-specific sensitivity and diversity of the biological effects of 2,3,7,8-TCDD by characteristics of the receptor alone. There are several different forms of this protein in mice encoded by several different alleles of the same locus (Poland and Glover 1990; Poland et al. 1994). By analogy with the existence of multiple receptor forms in mice, it is reasonable to anticipate that the human population will also have different receptor forms. The extent to which these forms in mice and humans affect the types of responses elicited and the sensitivity to TCDD is not known. As indicated above, the Ah receptor has been identified in several human tissues and cell lines (Cook and Greenlee 1989; Harper et al. 1991; Harris et al. 1989a; Lorenzen and Okey 1991; Roberts et al. 1990). Although the general properties and function of the human Ah receptor (Harper et al. 1991) appear to be very similar to the rodent and other species (Denison et al. 1986a; Gasiewicz and Rucci 1984), some differences exist. For example, the molecular mass from a variety of human cell lines or tissues ranges from 106 to 110 kDa (Harper et al. 1991; Poland and Glover 1987; Wang et al. 1991), compared to approximately 95 kDa for C57XBL/6J mice or from Hepa-1 cells (Landers et al. 1989; Poland and Glover 1987, 1990; Prokipcak and Okey 1990), and 124 kDa from the hamster (Poland and Glover 1987). The same parameter for the nonresponsive DBA/2J mouse is approximately 104 kDa (Poland and Glover 1990). There is no known correspondence between molecular mass of the protein and its affinity for any ligand and/or ability to mediate a biological or toxicological response. Apparent affinity constants (measured under *in vitro* conditions) for 2,3,7,8-TCDD-human Ah receptor binding from various cell lines range from 3 to 15 nM compared with about 1 nM in cytosol from C57XBL/6J mice, 16 nM for the DBA/2J mouse, 0.1 nM for the guinea pig and 0.3 nM for the hamster (Cook and Greenlee 1989; Gasiewicz and Rucci 1984; Harper et al. 1991; Okey et al. 1989).

While the use of structure-activity relationships and mouse genetics are consistent with the notion that the binding of 2,3,7,8-TCDD and structurally-related chemicals to the Ah receptor is the initial event that leads to the induced synthesis of certain enzymes, it has only been through the work at the cellular and molecular biological levels that this has been substantiated. Furthermore, these investigations indicate that complex series of events regulate the activity of the receptor and it is likely that the differential regulation of these

may, at least in part, be responsible for the tissue- and species-specific nature of the response observed in mammals following the exposure to 2,3,7,8-TCDD and related compounds. Immunohistochemical studies have shown that in intact mouse hepatoma cells, the unliganded receptor resides in the cytoplasm, and that exposure to 2,3,7,8-TCDD leads to an accumulation of the receptor within the nucleus (Pollenz et al. 1994). However, the precise location of the unoccupied (i.e., without 2,3,7,8-TCDD bound) receptor in intact cells is still unresolved. The unoccupied AhR exists as a heteromeric complex with 2 molecules of another protein called 90 kDa heat-shock protein (hsp90) and another 43-kDa protein (Chen and Perdew 1994). Hsp90 appears to be necessary for maintaining the proper folding of the Ah receptor so it can bind ligand and limit the presence of another receptor form that is able to bind to DNA (Pongratz et al. 1992). The exact role of the 43-kDa protein is not yet known.

Binding of the Ah receptor by 2,3,7,8-TCDD initiates a series of as yet undefined events resulting in the dissociation of hsp90 and nuclear localization (Henry and Gasiewicz 1993; Pollenz et al. 1994; Pongratz et al. 1992). Results from experiments in genetically variant cells that respond poorly to 2,3,7,8-TCDD revealed a defect in 2,3,7,8-TCDD binding that results in an altered receptor. Other variants exhibited normal binding, but the liganded receptors do not bind DNA and do not accumulate in the nucleus (Hankinson 1979; Miller and Whitlock 1981). The finding that these variants have a defect in a protein, termed Arnt (Ah receptor nuclear transport protein) (Reyes et al. 1992), suggested that 2,3,7,8-TCDD responsiveness requires both a ligand-binding protein (the Ah receptor) and a second protein which mediates the binding of the liganded receptor to DNA (Whitlock 1993). Furthermore, the ligand-bound Ah receptor does not itself bind DNA (Gasiewicz et al. 1991). The Arnt protein does not bind 2,3,7,8-TCDD, nor does it bind to DNA in the absence of the liganded Ah receptor protein (Whitelaw et al. 1993). The role of the Arnt protein as a translocator of the receptor from cytoplasm to the nucleus has been questioned; instead, it has been shown that Arnt interacts with the liganded Ah receptor to form a heterodimeric DNA-binding protein complex that can bind DNA and activate gene transcription (Whitlock 1993). Other investigations have shown that phosphorylation/dephosphorylation of the Ah receptor and the Arnt protein may influence both heterodimerization and the binding of this complex to DNA (Berghard et al. 1993; Mahon and Gasiewicz 1995; Okino et al. 1992; Pongratz et al. 1991).

Most of the information regarding the sequence of events that follow 2,3,7,8-TCDD binding to the Ah receptor is based on analyses of induction of AHH activity, which results from enhanced transcription of the corresponding cytochrome P-450 1A1 (CYP1A1) gene. Stimulation of transcription occurs within minutes and does not require ongoing protein synthesis (Israel et al. 1985). These findings led to the

discovery of a dioxin-responsive regulatory DNA domain which has the properties of a transcriptional enhancer (Fisher et al. 1990; Jones et al. 1986; Neuhold et al. 1986). These specific DNA elements have been termed dioxin-responsive elements (DREs) and require both receptor protein and Arnt protein for enhancer function. DREs function in a chromosomal location distinct from that of the CYP1A1 gene (Fisher et al. 1989). In addition to the enhancer, the DNA upstream of the CYP1A1 gene has a second control element (a transcriptional promoter), which ensures that transcription is initiated at the correct site. Neither enhancer nor promoter functions in the absence of the other (Jones and Whitlock 1990). The fact that enhancer function requires both the receptor and Arnt protein, and that the liganded heteromeric form of the receptor shows increased affinity for the specific DNA sequence within the enhancer region suggested that the activation of the CYP1A1 gene involves the binding of the receptor heteromer to the DRE. This has been shown for the purified Ah receptor-Arnt protein complex (Henry et al. 1994). Analysis of the interaction of the Ah receptor with specific DNA domains indicates that the heteromer binds in a 1:1 ratio to the DRE (Denison et al. 1989). There is, however, no strict relationship between the affinity of the receptor heteromer for the DRE and the extent of enhancer activation (Neuhold et al. 1989; Shen and Whitlock 1992), which suggests that additional events, including DNA bending (Elferink and Whitlock 1990), must take place to activate transcription.

The use of many *in vitro* techniques for these studies has required removing the DNA regulatory elements from the chromosome environment, and this may produce misleading results. This led researchers to examine the protein-DNA interactions at the dioxin-responsive enhancer in intact cells. Results from these studies suggested that the inactive enhancer is relatively inaccessible to DNA-binding proteins in vivo and that exposure to 2,3,7,8-TCDD leads to a rapid binding of six receptor heteromers and other proteins to the enhancer upstream of the CYP1A1 gene (Wu and Whitlock 1993). It has also been shown that the CYP1A1 promoter, like the enhancer, is inaccessible in uninduced cells, and that exposure to 2,3,7,8-TCDD increases its accessibility to constitutively expressed proteins (Durrin and Whitlock 1989; Wu and Whitlock 1992). The 2,3,7,8-TCDD-induced change is not dependent on protein synthesis and is receptor- and Arnt-dependent. It has been suggested that the inaccessibility of the enhancer/promoter region in uninduced cells is due to its organization into nucleosomes (Ko et al. 1996; Morgan and Whitlock 1992). The mechanism by which the binding of liganded receptor heteromers to the enhancer alters chromatin structure leading to activation of transcription is unknown. Whitlock (1993) suggested that the DRE-bound receptor complex affects histones, thereby weakening the histone-DNA interactions and destabilizing nucleosomal structures. They also proposed that the receptor-enhancer interaction may alter the DNA structure of the enhancer/promoter region stabilizing it in a non-nucleosomal configuration.

The information resulting from the cloning and sequencing of the Ah receptor and Arnt has also expanded our knowledge of the molecular mechanisms whereby these proteins influence transcriptional activity (Hankinson 1995; Whitlock 1993). Both proteins are members of a class of transcription factors containing a basic helix-loop-helix (bHLH) structural motif as well as a PAS (Per-Arnt-Sim) domain. Both of these regions are involved in dimerization. The bHLH motif is also required for DNA sequence recognition, while the PAS domain contains the ligand-binding site (in the Ah receptor) and interacts with hsp90 (Coumaileau et al. 1995; Dolwick et al. 1993; Fukunaga et al. 1995; Whitelaw et al. 1993). Both the Ah receptor and Arnt have C-terminal regions that function in transcriptional activation, although their relative contributions may depend on the gene involved (Ko et al. 1996). Other members of the HLH-PAS family include hypoxia-inducible factor 1 alpha (HIF-a) and *Drosophila* protein Sim (Huang et al. 1993; Wang et al. 1995). All of the bHLH proteins identified to date are involved in transcriptional regulation, and have a variety of roles in tissue growth and differentiation processes. It is not yet clear whether the ligand-activated Ah receptor modulates gene expression only through its interaction with Arnt; it may have other dimerization partners. It is known that multiple heterodimerizations occur among several transcription factors, and that this multiplicity provides for the recognition of other DNA sequences and diversity of regulation of responsive genes. Arnt has been shown to dimerize with HIF-1a and Sim, and there appear to be several different isoforms of Arnt, two of which have been shown to interact with the Ah receptor (Henry et al. 1994; Hirose et al. 1996; Ireland et al. 1995; Swanson et al. 1995; Wang and Semanza 1995). However, as of yet, Arnt is the only protein that has been demonstrated to be a functional partner to the Ah receptor. Furthermore, the Ah receptor and Arnt appear to be co-expressed in a variety of tissues that have been examined (Abbott and Probst 1995; Abbot et al. 1995; Carver et al. 1994), suggesting co-dependence.

As indicated above, much of our understanding of the interaction of 2,3,7,8-TCDD with the Ah receptor and how it modulates gene expression has come mainly from the analysis of the regulation of the CYP1A1 gene. However, other studies have observed the presence of functional DREs in the genes that encode for CYP1A2 (Quattrochi et al. 1994), glutathione S-transferase Ya (Paulson et al. 1990), aldehyde-3-dehydrogenase (Takimoto et al. 1994), and NAD(P)H:quinone oxidoreductase (Favreau et al. 1991). In addition, an imperfect DRE is present in the regulatory region of the cathepsin D gene. In this case, the Ah receptor-Arnt complex may act as a repressor to prevent the binding of other transcription factors to nearby enhancer sequences (Safe 1995).

As discussed below, 2,3,7,8-TCDD-elicited activation of the Ah receptor has been shown to alter the transcription of a number of genes. However, only a few of these, as indicated above, are as yet known to contain functional DREs (Lai et al. 1996). While in some cases the regulatory regions of all of the genes known to be altered by 2,3,7,8-TCDD have not been thoroughly examined; in other cases, the regulatory regions are known not to contain the conserved consensus sequence for the DRE. It is possible that other dimerization partners exist and that different DNA sequences might be recognized. It is also possible that the 2,3,7,8-elicited modulation of many of these genes and processes may be secondary subsequent to the induction/repression of a DRE-containing gene. However, there is some evidence to suggest that other Ah receptor-dependent pathways may exist for the alteration of gene expression that may not be dependent upon the interaction of the Ah receptor with nuclear elements. It has been suggested that the interaction of 2,3,7,8-TCDD with the Ah receptor may initiate a phosphorylation/dephosphorylation cascade that may subsequently activate other transcription factors (Matsumura 1994). Enan and Matsumura (1995) reported an increase in protein kinase activity within 1–10 minutes following the addition of 2,3,7,8-TCDD to nuclear-free preparations of guinea pig adipose tissue. These results are consistent with previous investigations showing increased tyrosine kinase activity within minutes of 2,3,7,8-TCDD exposure (Bombick et al. 1988; Clark et al. 1991a; DeVito et al. 1994). Hsp90 has been found associated with a protein of 50 kDa (Chen and Perdew 1994; Whitelaw et al. 1993), and both have been shown to regulate the activity of pp60v-src, a tyrosine kinase (Brugge et al. 1983; Mimnaugh et al. 1995). c-Src has recently been reported to be a component of the unoccupied Ah receptor complex (Enan and Matsumura 1996). Thus, 2,3,7,8-TCDD may modulate signal transduction processes and gene expression by at least two pathways: through the direct interaction of the Ah receptor and its heterodimer partners with gene regulatory elements, and from the initiation of a phosphorylation/dephosphorylation cascade and the subsequent modulated activity of other nuclear transcription factors. It has yet to be determined which pathways may be more important in acute versus chronic responses to these compounds and/or during particular developmental periods. Nevertheless, together these data indicate that well regulated and conserved pathways exist for the transduction of cellular signals through the binding of 2,3,7,8-TCDD-like chemicals to the Ah receptor. Since the modulation of these pathways results in toxicity in response to 2,3,7,8-TCDD and related compounds, it is presumed that these chemicals cause these responses by either interfering with the normal function of some unknown endogenous ligand, and/or stimulating the signal transduction process at an inappropriate time and/or for an inappropriately long period of time.

As indicated in the preceding sections, cell/tissue death and necrosis are not prominent features of effects resulting from 2,3,7,8-TCDD exposure *in vivo* or *in vitro*. Hyperplasia, hypoplasia, metaplasia, and

dysplasia are the most common histopathological changes observed in animals (McConnell and Moore 1979). Likewise, under conditions *in vitro*, 2,3,7,8-TCDD-like compounds are potent in altering cellular differentiation and growth patterns for a number of different cell types including embryonic palatal epithelial cells (Abbott et al. 1989), keratinocytes (Gaido and Maness 1994), osteoblasts (Gierthy et al. 1994) and preimplantation embryos (Blankenship et al. 1993).

Despite the numerous tissue- and species-specific responses that have been observed and the elegant work on the molecular mechanisms mediating some of these, there exists a considerable gap between knowledge of these changes and the degree to which they are related to the biological and toxicological end points elicited by 2,3,7,8-TCDD and related compounds. These chemicals have been shown to alter the transcription and/or translation of a number of genes, including several oncogenes and those encoding growth factors, receptors, hormones, and drug-metabolizing enzymes (Birnbaum 1994a, 1994b). More recent investigations have noted effects on certain enzymes and proteins (e.g., kinases) involved in various signal transduction processes as well as cell cycle control (Birnbaum 1994a, 1994b; Weib et al. 1996). The elicited induction of certain drug metabolizing enzymes such as CYP1A1, CYP1A2, and CYP1B1 are some of the most sensitive responses observed in a variety of different animal species, including humans. Significantly increased levels of CYP1A1 mRNA have been observed as dosages as low as 0.1 ng/kg body weight (Kohn et al. 1993). However, the precise 2,3,7,8-TCDD-induced biochemical alterations that are causally responsible for the abnormal growth processes observed are not known. This is due predominantly to our incomplete understanding of the complex and coordinate molecular, biochemical, and cellular interactions that regulate tissue processes during development and under normal homeostatic conditions. Nevertheless, there is some evidence that many of these biochemical alterations may be relevant to altered growth responses observed. For example, changes in the EGF receptor have been seen in tissues from 2,3,7,8-TCDD-exposed animals and humans (Abbott and Birnbaum 1990a; Sewall et al. 1993; Sunahara et al. 1987). EGF and its receptor possess diverse functions relevant to cell transformation and tumorigenesis, and changes in these functions may be related to a number of dioxin-induced responses including neoplastic lesions, chloracne, and a variety of developmental effects. Likewise, the known ability of 2,3,7,8-TCDD directly or indirectly to alter the levels and/or activity of other growth factors and hormones, such as estrogen and thyroid hormone and their respective receptors, as well as enzymes involved in the control of cell cycle, may affect growth patterns in cells/tissues leading to adverse consequences. Thus, both the biochemical and biological data are consistent with the notion that 2,3,7,8-TCDD and related compounds are growth regulators.

2,3,7,8-TCDD and structurally related compounds elicit a wide range of adverse effects. Of the many adverse responses observed both in humans and experimental animals after exposure to 2,3,7,8-TCDD, the ones that appear at the lowest dose (more sensitive) are perhaps developmental/reproductive effects, alterations in the immune response, and neoplasia. An overview of the mechanism(s) involved in these effects is presented below. Detailed mechanistic explanations are beyond the scope of this profile. Some of the information has been extracted from recent reviews on these subjects (Kerkvliet 1995; Lucier et al. 1993a; Peterson et al. 1993).

Some common developmental effects attributed to 2,3,7,8-TCDD exposure in most laboratory mammals are thymic hypoplasia, subcutaneous edema, decreased prenatal growth, and prenatal mortality (Couture et al. 1990). In addition, there are other species-specific effects, such as cleft palate in mice. Any of these effects may result from actions on the mother, embryo/fetus, placenta, or any combination of these sites (Peterson et al. 1993). In general, developmental effects can be induced at exposure levels that are not maternally toxic; however, prenatal mortality appears to be associated with maternal toxicity. Structure-activity results for 2,3,7,8-TCDD and related halogenated hydrocarbons for overt fetotoxicity are consistent with an Ah receptor-mediated mechanism. Hydronephrosis is the most sensitive developmental response induced by 2,3,7,8-TCDD in mice and it can be observed at maternal doses that do not cause cleft palate or overt maternal toxicity (Abbott and Birnbaum 1989a; Abbott et al. 1987a, 1987b; Couture-Haws et al. 1991b; Neubert and Dillman 1972; Weber et al. 1985). Hydronephrosis *in vivo* is induced by a direct hyperplastic action of 2,3,7,8-TCDD on the uretic epithelium. This results in occlusion of the ureter and subsequent accumulation of urine in the kidney (Abbott et al. 1987a). As for cleft palate formation, 2,3,7,8-TCDD and related compounds seem to allow the palatal shelves to grow and make contact, but prevent the subsequent epithelial-to-mesenchyme transformation (Peterson et al. 1993; Pratt et al. 1984). Susceptibility to both hydronephrosis and cleft palate formation segregate with the Ah locus, and structure-activity relationships for dioxin-like compounds are consistent with those for Ah receptor binding (Safe 1990; Weber et al. 1985). Further details on the mechanism of 2,3,7,8-TCDD-induced hydronephrosis and cleft palate formation and the involvement of various growth factors in these responses can be found in Section 2.5.

Another sensitive system for 2,3,7,8-TCDD toxicity is the male reproductive system, and many of the effects observed were originally thought to be related to the ability of 2,3,7,8-TCDD to decrease plasma androgen concentrations (Mably et al. 1992a, 1992b, 1992c; Moore et al. 1985). The fact that 2,3,7,8-TCDD is transferred from mother to fetus and to neonates during lactation has a great impact on

the male reproductive system during early development. Testosterone and its metabolite dihydro-testosterone (DHT) are essential prenatally and/or early postnatally for imprinting and development of accessory sex organs and for initiation of spermatogenesis. Mably et al. (1992b) suggested that the demasculinization and feminization of sexual behavior and feminization by LH secretion is due to the fact that perinatal exposure to 2,3,7,8-TCDD impairs sexual differentiation of the central nervous system, which is dependent on the presence of androgens during early development. However, results from more recent studies suggest that the 2,3,7,8-TCDD-induced effects on the male reproductive system may be related to alterations in other systems such as brain amine content or in the expression of growth factors and receptors involved in urogenital cell system differentiation and proliferation (Bjerke et al. 1994a; Gray et al. 1995). Results from recent studies have also delineated the role of the Ah receptor in the development of 2,3,7,8-TCDD-induced alterations in the male reproductive tract (Roman and Peterson 1998; Roman et al. 1998a,b). A more detailed discussion of the mechanisms involved in these responses is presented in Section 2.5.

2,3,7,8-TCDD has been shown to block some estrogenic effects both *in vivo* and *in vitro*, and the relative potencies of 2,3,7,8-TCDD and related congeners are consistent with their relative binding affinities with the Ah receptor (Safe et al. 1991). Estrogens are necessary for normal uterine development and for maintenance of the adult uterus. The mechanism of these antiestrogenic effects seems to be related to a decrease in gonadal tissue responsiveness to estrogen (DeVito et al. 1992) rather than to increased metabolism of estrogen. Studies in cultured MCF-7 cells (estrogen-responsive cells derived from a human breast adenocarcinoma) revealed that the antiestrogenic activity of 2,3,7,8-TCDD could result from the increased metabolism of estrogens due to Ah receptor-mediated enzyme induction and/or a decreased number of estrogen receptors in the nucleus (Gierthy et al. 1987; Harris et al. 1989a, 1990; Safe et al. 1991; Zacharewski et al. 1991, 1992). More recent data indicates that in some cases 2,3,7,8-TCDD may block the effects of estrogen through the ability of the 2,3,7,8-TCDD-bound Ah receptor-Arnt complex to interfere with the estrogen receptors binding to enhancer elements within the regulatory regions of estrogen-responsive genes (Krishnan et al. 1995; Safe 1995). Thus, the mechanism by which 2,3,7,8-TCDD and related compounds may block certain effects of estrogen may be varied depending on the particular gene, response, and tissue. Under some conditions, 2,3,7,8-TCDD may also cause estrogen-like responses. For example, the treatment of mice with an appropriate dosage of 2,3,7,8-TCDD or estrogen results in thymic involution and modulation of particular bone marrow stem cell markers (Silverstone et al. 1994). However, the mechanism by which these compounds act are clearly different since potent antiestrogens block the effects of estrogen treatment without affecting 2,3,7,8-TCDD-elicited

responses (Frazier et al. 1994). Similarly, the effects of 2,3,7,8-TCDD on the development of external genitalia in rats are similar to the effects observed in animals exposed to potent estrogen-like chemicals (Gray and Ostby 1995).

Extensive evidence suggests that the immune system is a sensitive target for toxicity of 2,3,7,8-TCDD and structurally related halogenated aromatic hydrocarbons (Kerkvliet 1995). Exposure to 2,3,7,8-TCDD can increase susceptibility to bacterial (Thigpen et al. 1975; Thomas and Hinsdill 1979; White et al. 1986), viral (Clark et al. 1983; House et al. 1990), parasitic (Tucker et al. 1986), and neoplastic disease (Luster et al. 1980). However, the specific immunological functions affected by 2,3,7,8-TCDD in most of the host-resistance models have not been fully defined. Thymic involution is characteristic of exposure to 2,3,7,8-TCDD and structurally related chemicals in all species examined. There is experimental evidence showing that immune suppression in rodents occurs at lower doses of 2,3,7,8-TCDD when the animals are exposed perinatally as compared with rodents exposed as adults, and that the prenatal effects are selective for T-cell-mediated immunity (Clark et al. 1983; Faith and Moore 1977; Vos and Moore 1974). The mechanism for 2,3,7,8-TCDD-induced thymic atrophy is not completely understood. There is evidence in rats suggesting that the 2,3,7,8-TCDD-induced effect is not mediated by an effect on the pituitary or adrenal glands, or from decreased production of thymic hormones (Van Logten et al. 1980; Vos et al. 1978). There appear to be multiple mechanisms involving alterations in thymocyte differentiation (Blaylock et al. 1992; Cook et al. 1987a; Denker et al. 1985; Greenlee et al. 1985), thymocyte proliferation (Lundberg et al. 1990), and migration of lymphocyte stem cells (Fine et al. 1990).

A commonly used assay for immunotoxicity is the suppression of the antibody response to SRBC. The magnitude of the anti-SRBC response depends on the interactions of antigen-presenting cells (i.e., macrophages), regulatory T-lymphocytes (i.e., helper and suppressor T cells), and B-lymphocytes (i.e., antibody-producing cells). Results from experiments *in vivo* suggested that the target for 2,3,7,8-TCDD in the antibody response to either SRBC or tumor cells is the T-cell and/or macrophage components rather than the B-cell (Kerkvliet and Brauner 1987; Kerkvliet et al. 1996). Although the effects of 2,3,7,8-TCDD on B-cell function *in vivo* have not been examined, *in vitro* studies suggest that 2,3,7,8-TCDD inhibits the terminal differentiation of B cells via alteration of an early activation event, perhaps increased protein phosphorylation and tyrosine kinase activity (Clark et al. 1991a; Kramer et al. 1987; Luster et al. 1988; Morris et al. 1991). Macrophage functions, examined *ex vivo*, generally have been found to be resistant to suppression by 2,3,7,8-TCDD (Mantovani et al. 1980; Vos et al. 1978). There is also evidence suggesting

that inflammatory cells may be activated by 2,3,7,8-TCDD via enhanced production of inflammatory mediators such as interleukin 1 and tumor necrosis factor (Clark et al. 1991b; Taylor et al. 1992).

Extensive research has been conducted on the role of the Ah locus in immunotoxicity of 2,3,7,8-TCDD and related compounds, and overall, the data linking 2,3,7,8-TCDD-induced immunotoxicity to the Ah receptor are convincing. For example, Vecchi et al. (1983a) reported that the antibody response to SRBC was greatly suppressed by 2,3,7,8-TCDD in C57BL/6J mice, but not as much in DBA/2J mice. Results from structure-activity studies for the SRBC response with CDDs that contaminate technical grade pentachlorophenol supported an Ah receptor-mediated effect (Kerkvliet et al. 1985). The Ah receptor was also found to be involved in the suppression of the antibody response to lipopolysaccharide (Kerkvliet et al. 1990a); and the cytotoxic T-lymphocyte response, and suppression of the latter by dioxin-like PCBs, correlated with relative-binding affinities for the Ah receptor (Kerkvliet et al. 1990b). An additional response found to segregate with Ah-responsiveness was the cytotoxic response to activated neutrophils (Ackermann et al. 1989). It is important to mention that results from some studies suggest that suppression of the *in vitro* antibody response may not be Ah receptor-mediated. For example, Holsapple et al. (1986a) reported that the magnitude of the response was comparable using cells from responsive mice relative to nonresponsive mice. Also, 2,7-dichlorodibenzo-p-dioxin, a congener with little affinity for the Ah receptor, was equipotent with 2,3,7,8-TCDD in suppressing the *in vitro* response. A similar conclusion was reached by Davis and Safe (1991), who found that a series of halogenated aromatic hydrocarbons, which had a >14,900-fold difference in *in vivo* immunotoxic potency, were equipotent *in vitro* in suppressing the anti-SRBC response using cells from either responsive or nonresponsive mice. Although these results suggest a possible role of non-Ah receptor mechanisms, the studies fail to rule out a role of the Ah receptor. The variable effects of 2,3,7,8-TCDD *in vitro* may have been due to factors such as media components or procedures used to prepare cell suspensions. Kerkvliet (1994) suggested that "the difficulty in demonstrating consistent, direct effects of 2,3,7,8-TCDD *in vitro* on lymphocytes, the dependence of those effects on serum components, and the requirements for high concentrations of 2,3,7,8-TCDD are all consistent with an indirect mechanism of 2,3,7,8-TCDD on the immune system." One potentially important indirect mechanism operates through effects on the endocrine system. Glucocorticoids, sex steroids, T4, growth hormone, and prolactin have been shown to regulate immune responses, and 2,3,7,8-TCDD has been shown to alter the activity of all of them (see also sections on endocrine and reproductive effects).

There is sufficient evidence that 2,3,7,8-TCDD is carcinogenic in animals, and the overall epidemiological database suggests that the incidence of certain types of cancer may be increased in humans by exposure to

2,3,7,8-TCDD (Hardell et al. 1994; Lucier et al. 1993a). The mechanism of 2,3,7,8-TCDD carcinogenicity has not been fully elucidated, but there is considerable evidence indicating that it does not involve direct damage to DNA through formation of DNA adducts. The criteria for designating 2,3,7,8-TCDD as a nongenotoxic carcinogen are based on the following: studies using extraordinarily sensitive analytical methods have been unable to detect DNA adducts in rodent tissue after exposure to 2,3,7,8-TCDD (Randerath et al. 1988; Turteltaub et al. 1990), numerous studies have demonstrated that 2,3,7,8-TCDD is not mutagenic in the *Salmonella*/Ames test with or without an activating system (Giri 1986; Wassom et al. 1978), and 2,3,7,8-TCDD is a potent tumor promoter and a weak initiator or noninitiator in the two-stage models for liver (Flodstrom and Ahlborg 1989; Lucier et al. 1991; Pitot et al. 1980) and skin (Poland et al. 1982). Instead, it has been proposed that 2,3,7,8-TCDD might alter the capacity of both exogenous and endogenous substances to damage the DNA by inducing CYP1A1- and CYP1A2-dependent drug-metabolizing enzymes. In some cases, enzyme induction will lead to increased formation of DNA-damaging metabolites, as appears to be the case in the two-stage model in rat liver and mouse skin (Flodstrom and Ahlborg 1992; Hebert et al. 1990; Poland and Knutson 1982; Poland et al. 1982). A recent study suggested that the induction of CYP1A1 may also lead to an increase in oxygen radicals and consequent oxidative DNA damage that could lead to mutation and cancer (Park et al. 1996). However, in many cases in which induction leads to increased rate of detoxification, the opposite will occur, as demonstrated by Cohen et al. (1979) for benzo[a]pyrene. The protection afforded by preinduction of CYP1A1 by 2,3,7,8-TCDD appears to be Ah receptor-mediated since it does not occur in mice deficient with low-affinity Ah receptor (Kouri et al. 1978). It should be noted that results from structure-activity studies for 2,3,7,8-TCDD and related compounds strongly suggest that the hepatocarcinogenic actions of 2,3,7,8-TCDD are Ah receptor-dependent (Flodstrom and Ahlborg 1992; Hebert et al. 1990; Poland et al. 1982; Poland and Knutson 1982). The role of CYP1A2 induction is less clear than for CYP1A1. Some have suggested that the liver carcinogenicity of 2,3,7,8-TCDD in intact female rats, but not male rats or ovariectomized female rats, could be explained in part by the formation of toxic catechol estrogens from 17β-estradiol, a reaction catalyzed by CYP1A2 (Lucier et al. 1993a). This is also consistent with the finding that CYP1A2 is induced in liver but not in extrahepatic organs.

The role of the EGF receptor in 2,3,7,8-TCDD-induced carcinogenicity has also been examined. EGF is a mitogen that stimulates the generation of mitotic signals in both normal and neoplastic cells, and its receptor and ligands have a variety of functions involved in cell transformation and tumorigenesis. It has been shown that 2,3,7,8-TCDD decreases the binding capacity of the plasma membrane EGF receptor for its ligand without changing the affinity constant (Abbott and Birnbaum 1990a; Hudson et al. 1985; Lin et

al. 1991a; Madhukar et al. 1984). The mechanism involved is not completely understood, but it appears that 2,3,7,8-TCDD does not decrease EGF receptor mRNA (Lin et al. 1991a). The effects of 2,3,7,8-TCDD on the EGF receptor have been shown to require the Ah receptor (Lin et al. 1991a). The EGF receptor-like response produced by 2,3,7,8-TCDD is consistent with the idea that 2,3,7,8-TCDD increases the generation of cellular mitotic signals which may, in part, be responsible for the tumor-promoting actions of 2,3,7,8-TCDD.

The possible role of UDP-glucuronyltransferases (UDPGT) on the carcinogenicity of 2,3,7,8-TCDD has also been studied. UDPGTs are thought to be a deactivation pathway for many environmental chemicals and endogenous substances such as steroid hormones by increasing their water solubility, thereby facilitating excretion by a conjugation reaction. 2,3,7,8-TCDD induces synthesis of at least one UDPGT isozyme (Lucier et al. 1986) by a Ah receptor-mediated mechanism (Bock 1991). For example, the oncogenic effect of prolonged stimulation of the thyroid by TSH has been attributed to decreased levels of T4 due to UDPGT induction. Decreased T4 levels induce the pituitary gland to respond by secreting increased amounts of TSH. This is consistent with results from rodent studies in which 2,3,7,8-TCDD and other inducers of UDPGT decreased T4 levels in blood, which is associated with increased TSH levels (Henry and Gasiewicz 1987). Lucier et al. (1986) showed that in rats, the shape of the dose-response curve for induction of UDPGT by 2,3,7,8-TCDD is similar to that of CYP1A1 induction. Kohn et al. (1996) constructed a physiologically based model to investigate the hypothesis that induction of UDPGT by 2,3,7,8-TCDD may ultimately lead to a tumorigenic response in the thyroid of rats. The model included compartments for the thyroid and thyroxine-sensitive tissues, secretion and tissue uptake of thyroid hormones, binding of T3 and T4 to proteins in blood and tissues, iodination of iodothyronines, and glucuronidation of T4 by hepatic UDPGT. The model accurately predicted the effects of 2,3,7,8-TCDD on blood thyroid hormone concentrations, hepatic UDPGT activity, and the consequent increase of serum TSH. This was consistent with the observation that induction of UDPGT results in increased glucuronidation and biliary excretion of T4. The results of Kohn et al. (1996) provided further support to the hypothesis that induction of UDPGT is an early event in the generation of thyroid tumors by 2,3,7,8-TCDD in the rat.

There is evidence that some carcinogenic responses to 2,3,7,8-TCDD are related to effects of 2,3,7,8-TCDD on the estrogen receptor (ER) and on estrogen metabolism. The responses appear to be tissue-specific as illustrated by the fact that in rats 2,3,7,8-TCDD increases liver tumor incidence, but decreases tumor incidence in mammary glands, the uterus, and pituitary gland (Kociba et al. 1978a). In

rats, a single dose of 2,3,7,8-TCDD decreases the binding capacity of the hepatic ER for estrogens (Romkes and Safe 1988; Zacharewski et al. 1991, 1992). This response seems to be Ah receptor-mediated since 2,3,7,8-TCDD was much more effective in decreasing hepatic ER binding in C57BL/6J mice (responsive strain) than in congenic mice with low-affinity Ah receptor (Lin et al. 1991b). 2,3,7,8-TCDD also decreased rat hepatic ER in a 30-week-duration study (Clark et al. 1991b). The single ED_{50} for decreasing hepatic ER binding is similar to that for CYP1A1 induction, loss of plasma membrane EGF receptor, and induction of UDPGT (Lucier et al. 1993a). The relationship, however, between changes in concentration and cell proliferation have yet to be fully evaluated. In reproductive tract tissues, 2,3,7,8-TCDD decreases tumor incidences by a mechanism possibly involving increased estrogen metabolism as a consequence of UDPGT induction. Increased estrogen degradation was also observed in the MCF-7 breast cancer cell line after addition of 2,3,7,8-TCDD (Gierthy et al. 1988).

As indicated above, a substantial body of evidence is consistent with the premise that the Ah receptor mediates the biological effects of 2,3,7,8-TCDD. Furthermore, this evidence indicates that a response to this chemical requires the formation of a ligand-receptor complex. 2,3,7,8-TCDD-receptor binding appears to obey the law of mass action and, therefore, depends on the concentrations of both ligand and receptor in the target cell, and the binding affinity of the ligand for the receptor. In principle, and according to the law of mass action, some active 2,3,7,8-TCDD-Ah receptor complexes may form even at very low levels of exposure. In reality, however, it is likely that at some finite concentration of 2,3,7,8-TCDD, the formation of 2,3,7,8-TCDD-receptor complexes will be insufficient to elicit detectable or biologically relevant effects due to dependence on other factors (e.g., Arnt binding and DRE binding) and events (e.g., mRNA transcription and protein synthesis) necessary for the cascade of a signal transduction process to occur. Recent studies have indicated no evidence of a threshold for some relatively simple biochemical responses to 2,3,7,8-TCDD such as CYP1A1 induction (Kohn et al. 1993). However, this cannot yet be interpreted as an absence of a threshold since it is possible that either insufficiently low concentrations of 2,3,7,8-TCDD were used, or the background level of 2,3,7,8-TCDD equivalents (including any putative endogenous ligand) is already above the threshold level (even in experimental animals). Events leading to a toxic response that are subsequent to 2,3,7,8-TCDD-receptor binding and the induction of a particular biochemical event such as CYP1A1 activity, may or may not exhibit a linear response to 2,3,7,8-TCDD since these events are likely additionally dependent on multiple and complex biochemical, cellular, and tissue changes that may or may not be dependent on saturable processes. Further information will be required to determine if other responses to 2,3,7,8-TCDD, both biochemical and biological, do or do not demonstrate threshold behavior.

It is generally accepted that the toxicity of CDDs, including 2,3,7,8-TCDD, is due mainly to the parent compound. Hydroxylated metabolites lack the activity of the parent compound, suggesting that metabolism is a detoxification process necessary for the biliary and urinary excretion of these compounds. For instance, dog metabolites of 2,3,7,8-TCDD administered to guinea pigs had at least 100 times less acute toxic potency than the parent compound (Weber et al. 1982). In another study, two hydroxylated metabolites of 2,3,7,8-TCDD showed no significant effect on Ah receptor-mediated responses, such as body weight loss, thymus atrophy, or liver or spleen weight in male Wistar rats at doses as high as 5,000 µg/kg (Mason and Safe 1986a). One of the metabolites, 2-hydroxy-3,7,8-TrCDD, induced CYP1A1-related enzyme activities only at very high dose levels (1,000 and 5,000 µg/kg), whereas the other metabolite, 2-hydroxy-1,3,7,8-TCDD, lacked inducing capacity. Structure-activity studies of 7-substituted 2,3-CDDs, including the hydroxylated congeners, showed that the binding affinities of the hydroxylated congeners for the Ah receptor were significantly lower than those of the corresponding chlorine analogs (Denomme et al. 1985). Similar results were obtained in an additional study using hepatic cytosol from rat, mouse, hamster, and guinea pig (Romkes et al. 1987).

There is some evidence that hydroxylated metabolites of CDDs interfere with the transport of T4 in blood by a mechanism unrelated to the Ah receptor (Lans et al. 1993, 1994). These investigators showed that hydroxy-CDDs with chlorine substitution adjacent to the hydroxy group (e.g., 7-hydroxy-2,3,8-TrCDD, 2-hydroxy-1,3,7,8-TCDD, and 3-hydroxy-2,6,7,8-TCDD) showed similar or higher relative binding potency than T4 for the thyroid hormone transport protein transthyretin (TTR) in an *in vitro* assay using purified human TTR (Lans et al. 1993). In a subsequent study, they found that none of several hydroxylated CDDs tested inhibited T4 binding to thyroxin-binding globulin, the major T4-transporting plasma protein in humans, as opposed to TTR in rodents (Lans et al. 1994). This clearly indicated that hydroxylated CDDs may cause different effects in rodents and humans.

The possibility exists that reactive epoxide intermediates of 2,3,7,8-TCDD that may be formed as a result of metabolism are involved in 2,3,7,8-TCDD-induced carcinogenicity by covalently binding to DNA. However, this appears unlikely since, as previously mentioned, studies using extraordinarily sensitive analytical methods have been unable to detect DNA adducts in rodent tissue after exposure to 2,3,7,8-TCDD (Randerath et al. 1988; Turteltaub et al. 1990), and the fact that 2,3,7,8-TCDD is not mutagenic in the *Salmonella*/Ames test with or without an activating system (Giri 1986; Wasson et al. 1977).

2.4.3 Animal-To-Human Extrapolations

As discussed in the introduction to Section 2.1, there are a number of limitations in the human database; for most health effects, the data are inadequate to assess the potential for humans having a particular effect. Because the human data are incomplete, hazard and risk must be extrapolated across species. A large number of adverse effects have been observed in animals, and most have been observed in every experimental animal species tested, if the appropriate dose is administered. This is illustrated in Table 2-8 for 8 major effects associated with CDD toxicity (acute lethality, hepatotoxicity, wasting syndrome, chloracne, immunotoxicity, reproductive toxicity, developmental toxicity, and cancer). With the exception of acute lethality in humans, positive responses have been observed in each tested species, when a response has been investigated. Despite the similarities in hazard response between different species, large species differences in sensitivity have been observed. Comparisons of species sensitivity demonstrate that no species is consistently sensitive or refractory for all effects and, for some effects, there is a small range of species sensitivity. As presented in Table 2-9, the range of LD_{50} values for 6 commonly tested animal species spans several orders of magnitude. Guinea pigs have the lowest LD_{50} value (0.6 µg/kg) and hamsters have the largest (1,157 µg/kg). However, if these outliers are removed, the range of LD_{50} values for mice, monkeys, rabbits, and rats is less than an order of magnitude (22–115 µg/kg). In contrast, the range of LOAELs for reproductive toxicity (abortions, resorptions, pre- and post-implantation losses) spans approximately an order of magnitude with rats (0.125 µg/kg) being the most sensitive and guinea pigs the least sensitive (1.5 µg/kg; NOAEL of 0.15 µg/kg). These data suggest that even though some effects have wide ranges of sensitivity, for most of the effects, the LOAELs for the majority of species cluster within an order of magnitude (Table 2-9).

It is generally accepted that the Ah receptor plays a role in mediating many toxic responses attributed to exposure to CDDs (for additional information on the mechanisms of toxicity, see Section 2.4.2). For some responses, receptor binding appears necessary but may not be sufficient to result in downstream biological effects. Ah receptors have been found in most species, including humans, monkeys, rats, mice, hamsters, rabbits, and guinea pigs (Denison et al. 1986a; Landers and Bunce 1991). A simple way to explain sensitivity differences among species to 2,3,7,8-TCDD and related compounds, at least for Ah receptor-mediated responses, would be to assume that they are related to differences in receptor levels in target tissues and/or to differences in the affinity of binding of the specific CDD congeners. However, experimental data indicate that differences in such parameters cannot explain marked differences to CDD toxicity across species. For example, single dose LD_{50}s range from 0.6 µg/kg in guinea pigs to 1,157 µg/kg in

Table 2-8. Comparison of Health Effects Among Species Exposed to CDDs

Effect	Human	Monkey	Rat	Mouse	Hamster	Dog	Rabbit	Guinea pig	Mink
Acute lethality	–	+	+	+	+	+	+	+	+
Hepatotoxicity	+	+	+	+	ND	ND	ND	+	+
Wasting syndrome	**	+	+	+	+	ND	ND	+	+
Chloracne	+	+	ND	+	ND	ND	+	ND	ND
Immunotoxicity (thymic atrophy)	ND	+	+	+	+	ND	ND	+	ND
Reproductive toxicity (loss of pregnancy)	**	+	+	+	ND	ND	+	+	ND
Developmental toxicity (fetal toxicity and/or mortality)	**	+	+	+	+	ND	+	+	ND
Cancer	+	ND	+	+	+	ND	ND	ND	ND

+ = observed; – = not observed; ** = some effects have been observed but data limitations preclude drawing conclusions; ND = no data

Table 2-9. Comparison of LOAELs Among Animal Species Following a Single Oral Dose of 2,3,7,8-TCDD

	LOAEL (µg/kg)			
Species	Death (LD_{50})	Immunological effects (thymic atrophy)	Reproductive effects (abortions, resorptions, or pre- and post-implantation losses)	Developmental effects
Guinea pig	0.6 (Schwetz et al. 1973)	0.8 (Hanberg et al. 1989)	1.5 (Olson and McGarrigle 1992)	1.5 fetal mortality (Olson and McGarrigle 1992)
Hamster	1157 (Olson et al. 1980a)	48 (Hanberg et al. 1989)	ND	1.5 hydronephrosis (Olson and McGarrigle 1992)
Mouse	100 (Weber et al. 1995)	280 (Hanberg et al. 1989)	1.0 (Smith et al. 1976)	1 hydronephrosis (Moore et al. 1973)
Monkey	70 (1/3 died) (McConnell et al. 1978a)	70 (McConnell et al. 1978a)	1.0 (McNulty 1984)	1 fetal death (McNulty 1984)
Rabbit	115 (Schwetz et al. 1973)	ND	0.25 (Giavini et al. 1982)	ND
Rat	22 (Schwetz et al. 1973)	26 (Hanberg et al. 1989)	0.125 (Sparschu et al. 1971a)	0.064 impaired development of male reproductive system (Mably et al. 1992b, 1992c)

ND = no data

hamsters, but the affinity with which 2,3,7,8-TCDD binds to the Ah receptor from guinea pigs is not significantly different from the affinity with which 2,3,7,8-TCDD binds to the hamster Ah receptor (Denison et al. 1986a). In addition, there are no significant differences in the level of the hepatic Ah receptor between the two species, suggesting that in addition to species differences in receptor levels and in their affinities for the ligand, differences in species sensitivity to 2,3,7,8-TCDD may be determined by some event or events occurring after the initial binding of 2,3,7,8-TCDD to the Ah receptor. These late events may involve a complicated interplay between genetic and environmental factors which may be key determinants of 2,3,7,8-TCDD biological potency and toxicity. Factors unrelated to the Ah receptor, such as toxicokinetic differences, may also account for some of the observed species differences (for additional information, see Section 2.4.2). The Ah receptor has been identified in many human tissues and human cell lines (Okey et al. 1994). However, considerable individual differences in the expression levels of both Ah receptor and Arnt mRNAs have been found in human tissues (Hayashi et al. 1994). Furthermore, based on findings in inbred mice, polymorphism in the Ah receptor probably exists in humans, so that a concentration of TCDD that produces a response in one individual may not do the same in another (Whitlock 1993). This could explain why there was a wide range of serum 2,3,7,8-TCDD levels among Seveso residents where the occurrence of chloracne was sporadic over a generally wide range of doses (Mocarelli et al. 1991).

The weight of evidence from animal species comparisons and mechanistic data indicates that caution should be exercised when extrapolating from animals to humans. Some theoretical models indicate a basis for extrapolating from animals to humans, but such models have not been validated; there is wide variation in the results of different models; and a great deal of uncertainty remains regarding whether valid, predictive extrapolations can be made. It is reasonable to assume that humans will not be the most sensitive responder or be refractory to all effects, and that they will have a wider range of response due to increased heterogeneity. Levels of exposure to CDDs that produce toxicity in experimental animals cannot be directly compared to levels associated with adverse health effects in humans because most epidemiologic studies do not provide adequate data to estimate CDD exposures in the studied populations. However, the CDD body-burden history can sometimes be estimated in epidemiology studies from reported serum or adipose concentrations and empirically based assumptions regarding whole-body elimination kinetics of CDDs (as discussed in the introduction to Section 2.1). Comparisons between estimated adverse body burdens of CDDs and related compounds (CDFs, PCBs) in experimental animals and humans have shown that humans and animals appear to respond to similar body burdens (DeVito et al. 1995). As presented in Table 2-10, the adverse effect levels identified in humans are typically within a factor of 10 of the body

Table 2-10. Comparison of Body Burden Effect Levels Among Humans and Animals

Category	Effect	Species	Duration of exposure	Body burden[a,b] (ng/kg)	Reference
Dermal	Chloracne	Human	<1 year	2,876	Mocarelli et al. 1991
	Chloracne	Human	NS	1,480 262	Schecter et al. 1993
	Chloracne	Human	11 years	646	Jansing and Korff 1994
	Chloracnegenic effects	Monkey	3 weeks	530[c]-N 1,650-L	McNulty 1984
	Chloracnegenic effects	Monkey	9 months	1,910[c]	Allen et al. 1977
Immunological	Immunosuppression	Human	6.5 years	207–244	Tonn et al. 1996
	Decreased cell-mediated immunity	Mouse	4 weeks	9,100[d]	Vos et al. 1973
	Decreased humoral immunity	Guinea pig	8 weeks	670	Vos et al. 1973
	Decreased effector and memory T cells	Mouse	14.5 months	60[d]	Oughton et al. 1995
Reproductive	Change in sex ratio of children	Human	<1 year	119 174	Mocarelli et al. 1996
	Increased prevalence of high luteinizing hormone and low testosterone levels	Human	≥15 years	31-6,600	Egeland et al. 1994
	Increased incidence of abortions	Monkey	3 weeks	330[c]-N 1650-L	McNulty 1984
	Increased incidence of abortions	Monkey	4 years	270[c]	Bowman et al. 1989b; Hong et al. 1989
	Decreased plasma testosterone levels	Rat	once	12,500[f]	Moore et al. 1985
Cancer	Increased cancer mortality risk	Human	≥1 year	310-1,858	Fingerhut et al. 1991
	Increased cancer mortality risk	Human	NS	≥1,000	Ott and Zober 1996
	Increased cancer mortality risk	Human	≥20 years	71-945	Manz et al. 1991
	Liver, lung, hard palate cancer	Rat	2 years	2,770[f]	Kociba et al. 1978a

[a] Calculations of human body burdens are described in the footnotes to Table 2-1

[b] Animal body burdens were estimated as follows: $Cb = (I \cdot a \cdot f)/k$, $f = 1-(e^{-kt})$; where, C_b is the TCDD body burden (ng/kg bw), I is TCDD intake (ng/kg-day), a is the gastrointestinal absorption fraction, f is the fraction of steady-state body burden, k is the whole body elimination rate constant for TCDD (day^{-1}, $ln2/t_{1/2}$), and t is the exposure duration (day)

[c] Assumed parameter values for monkeys: a = 0.8 (value for rats from Van den Berg et al. 1994), $t_{1/2}$= 391 days (Bowman et al. 1989b)

[d] Assumed parameter values for mice: a = 0.8 (Curtis et al. 1990), $t_{1/2}$= 11 days (Birnbaum 1986)

[e] Assumed parameter values for guinea pigs: a=0.5 (Van den Berg et al. 1994), $t_{1/2}$ = 94 days (Olson 1986)

[f] Assumed parameter values for rats: a=0.8 (Van den Berg et al. 1994, $t_{1/2}$ = 24 days (Van den Berg et al. 1994)

L = LOAEL; N = NOAEL;NS = not specified

burdens associated with similar effects in animals. The data in Table 2-10 should be interpreted cautiously and should not be taken to suggest that humans are more sensitive than experimental animals. The human body burdens were estimated from serum 2,3,7,8-TCDD levels measured many years after exposure termination using empirically based assumptions; small differences in one or more assumption can result in large differences in the estimated body burdens. For example, the body burden of 945 ng/kg in the Manz et al. (1991) study was estimated using a half-life of 8.5 years; if the half-life of 7.1 years were used, the estimated body burden would have been 1606 ng/kg. Additionally, individual serum 2,3,7,8-TCDD levels and length of time between exposure termination and measurement of serum 2,3,7,8-TCDD levels (latency) were not available for a few epidemiology studies, and mean serum levels and latency periods were used to estimate body burdens. The use of mean values rather than individual values and empirically based assumptions may have resulted in an over- or underestimation of actual body burdens. Conversely, in the animal studies, actual exposure levels were known and there is greater confidence in the estimated body burdens. An acute high-dose exposure would produce higher peak serum lipid 2,3,7,8-TCDD and target tissue levels than chronic exposure to lower levels, although the body burdens may be similar. Thus, it may be misleading to compare adverse-effect body burdens from acute studies to those identified in chronic studies. Another issue which needs to be considered in comparing the human and animal adverse-effect body burdens is that this is comparison of LOAELs not a comparison of threshold levels, and free-standing LOAELs may not accurately predict threshold levels.

2.5 RELEVANCE TO PUBLIC HEALTH

Overview

The primary route of exposure to CDDs for the general population is the food supply. This type of exposure is the main contributor to the background exposure. Background exposure refers to exposure of the general population who are not exposed to readily identifiable point-sources of CDDs that result in widespread, low-level circulation of CDDs in the environment. It is generally accepted that the contribution of inhalation and direct contact with CDDs to the body burden of the general population is not more than a few percent. However, inhalation and direct contact represent major exposure routes in cases of occupational or accidental exposures. A background exposure level of approximately 0.7 pg 2,3,7,8-TCDD/kg/day (assuming a 70 kg reference body weight) has been estimated for the general population in the United States (Travis and Hattemer-Frey 1987). If other CDD and CDF congeners are included, the background exposure level increases to approximately 18–192.3 pg TEQ/day (0.26–2.75

pg/kg/day using a 70 kg reference body weight) (Schecter et al. 1994b) (for additional information on TEQ, see the Toxic Equivalency Factor [TEF] and Toxic Equivalents [TEQ] subsections). The inclusion of dioxin-like PCBs further raises the estimate to 3–6 pg TEQ/kg/day (Beck et al. 1989a; WHO 1991). The average concentration of 2,3,7,8-TCDD in the adipose tissue of the U.S. population is 5.8 pg/g lipid (Orban et al. 1994). For all TEQ congeners, excluding dioxin-like PCBs, the national average was approximately 28 pg TEQ/g lipid. In humans, the partitioning ratio of 2,3,7,8-TCDD between adipose tissue lipid and serum lipid is approximately 1 and remains near unity over at least a 1,000-fold concentration range over background levels (Patterson et al. 1988). This makes serum lipid an accurate and more practical measure of body burden than adipose tissue lipid.

Data on human health effects of CDDs are derived from a variety of sources, including case reports and epidemiologic studies using case-control, cross-sectional, and cohort designs. While case-control and cohort studies have been used to investigate increases in the incidence of cancers among populations exposed to 2,3,7,8-TCDD, nonmalignant effects have been examined in cross-sectional medical studies. In many of the earliest studies, the magnitude of exposure-response relationships could not be adequately assessed for a number of reasons, including small sample size, poor participation, selection of inappropriate controls, the inability to identify confounding exposures, and short latency periods (especially important for assessment of cancer). A long interval between exposure and examination (up to 40 years in some cases) is a serious limitation when assessing noncancer responses since responses that resolve with time might not be detected at the time of the examination. On the other hand, health conditions that may be present at the time of examination may be totally unrelated to past exposure to 2,3,7,8-TCDD. An additional limitation was the inability to quantify exposure. However, serum or adipose tissue levels of 2,3,7,8-TCDD have been measured in more recent cross-sectional studies of U.S. chemical workers (Sweeney et al. 1989), Ranch Hand veterans (USAF 1991), and Missouri residents (Webb et al. 1989). Using a standard half-life equation and assuming a one-compartment model and first-order kinetics, the half-life for 2,3,7,8-TCDD in humans has been estimated to be 8.7 years (Michalek et al. 1996). By knowing the half-life, estimates of body burdens at the time of exposure can be back-calculated. These estimates, however, should be used with caution since little information exists regarding the metabolism of 2,3,7,8-TCDD in humans. In addition, there are considerable differences in the elimination half-lives for these chemicals among individual humans.

For 2,3,7,8-TCDD, the majority of the effects have been reported among occupationally exposed individuals such as producers or users of chemicals in which 2,3,7,8-TCDD might have occurred as

impurities, and among residents of communities contaminated with 2,3,7,8-TCDD. Effects that have been associated with exposure to materials contaminated with 2,3,7,8-TCDD in some studies include cancer; dermal, hepatic and thyroid effects; effects on serum lipids; diabetes; and cardiovascular, respiratory, immunologic, neurologic, and reproductive effects. A number of studies have consistently found increases in cancer mortalities (all types combined) in the highest exposed workers with long latency periods, but the data on site-specific cancer are inconclusive. Among the dermal effects, chloracne is clearly a response associated with exposure to 2,3,7,8-TCDD and structurally related chemicals, but the threshold level of 2,3,7,8-TCDD at which it occurs has not been established. Moreover, there seems to be a great deal of innate human variability in the chloracne response between individuals (see Section 2.1.2). Hepatic changes observed in exposed populations include hepatomegaly, increased hepatic enzyme (GGT, AST, ALT) levels, induced hepatic microsomal activity (measured as increased D-glucaric acid excretion), alterations in porphyrin metabolism, and increases in serum lipid (cholesterol, triglycerides) levels. With the exception of long-lasting changes in GGT (Calvert et al. 1992; USAF 1991) and in serum cholesterol (USAF 1991) in some exposed groups, hepatic effects were transient and appeared to have been associated with acute exposure to high 2,3,7,8-TCDD concentrations. Few long-term thyroid effects were found in Ranch Hand veterans (USAF 1991), but a recent study of nursing infants suggests that ingestion of breast milk containing CDD and CDF levels somewhat higher than those reported in most general population studies, may alter thyroid function (these data are not conclusive because the measure thyroid hormone levels were within the normal range) (Koopman-Esseboom et al. 1994; Pluim et al. 1992). Slightly increased risk of diabetes and abnormal glucose tolerance tests have been reported in populations exposed to high 2,3,7,8-TCDD concentrations (Sweeney et al. 1992; USAF 1991). In the former study, however, age and body mass index, both known risk factors for diabetes, appear to have a greater influence than 2,3,7,8-TCDD level. Dose-related trends for deaths from cardiovascular disease and ischemic heart disease were observed in individuals exposed to CDDs during the BASF accident (Flesch-Janys et al. 1995). However, other studies found no relationship between 2,3,7,8-TCDD exposure and cardiovascular deaths (Bertazzi et al. 1989b) or other cardiovascular effects (Hoffman et al. 1986; Wolfe et al. 1985). A few case reports indicate that acute exposure to high 2,3,7,8-TCDD levels can produce respiratory irritation, but there is no indication that exposure to 2,3,7,8-TCDD produces chronic respiratory effects. Although there have been some reports of alterations in some immune end points in populations exposed to 2,3,7,8-TCDD, there has not been a consistent pattern, and the clinical significance of the effects is not totally clear. The overall evidence for neurologic effects suggests that although neurologic effects are reported to have occurred shortly after exposure in occupationally exposed individuals, even high exposure to 2,3,7,8-TCDD caused no long-term sequelae (Goetz et al. 1994; Sweeney et al. 1993). More recent data

suggests that exposure to 2,3,7,8-TCDD and related chemicals in humans during the pre- and neonatal periods may affect neurological development (Huisman et al. 1995a), but these data need to be interpreted cautiously because the neurological optimality score in infants was within the normal range and CDD/CDF levels may have only contributed a small amount to the variance in scores. Of the many reproductive end points studied in populations exposed to 2,3,7,8-TCDD, the available data provide suggestive evidence of altered sex ratios in children of exposed parents (Basharova 1996; Dimich-Ward et al. 1996; Mocarelli et al. 1996) and possibly alterations in reproductive hormone levels in males (Egeland et al. 1994) are associated with increased serum 2,3,7,8-TCDD levels.

Most of the toxicity studies of 2,3,7,8-TCDDs in animals have involved oral exposure, and numerous effects have been documented after short- and long-term exposure including lethality, and cardiovascular, gastrointestinal, hematological, hepatic, renal, endocrine, dermal, body weight, immunologic, reproductive, and developmental effects. In addition, 2,3,7,8-TCDD is a potent carcinogen in various species, and produces tumors in multiple sites in rodents of both sexes. However, as shown in Table 2-2, these effects occurred at doses several orders of magnitude higher than estimates of background exposure for CDDs. The most reliable and consistent sign of 2,3,7,8-TCDD toxicity in experimental animals is weight loss or decreased weight gain in growing rodents. Animal responses to 2,3,7,8-TCDD exposure are species- and strain-dependent, although almost all responses can be induced in every species and strain if the appropriate dose is used. The animal data suggest that the most sensitive effects of 2,3,7,8-TCDD exposure are immunotoxicity, and reproductive and developmental toxicity.

In recent years considerable advances have been made regarding the mechanisms of toxicities of 2,3,7,8-TCDD and related chemicals, as well as the pharmacokinetics of dioxins in experimental animals. For CDDs, toxicity and toxicokinetics cannot be dealt with separately. Based on results from research in these fields, it has become apparent that the comparison of responses from animals to humans (or even between animal species) should be done on the basis of body-burden or target-tissue dose, rather than on the basis of administered dose. By doing so, species-specific toxicokinetic considerations such as dose-dependent distribution, the existence of tissue-specific sequestering chemical entities (i.e., CYP1A2), and body composition (i.e., percent fat) can be taken into account. A discussion of relationships between administered dose, body burden, and biological responses is presented below.

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

Toxic Equivalency Factors (TEFs) and Toxic Equivalents (TEQs). Humans are exposed to complex mixtures of CDDs and other halogenated aromatic hydrocarbons such as CDFs and PCBs which are found in the environment (including food). The toxicological concerns resulting from exposure to these mixtures, as well as the gaps in available information with which to evaluate the potential risks from such exposures, led the EPA Chlorinated Dibenzo-p-dioxins/Chlorinated Dibenzofurans Technical Panel of the Risk Assessment Forum to recommend an interim method for assisting in estimating the risk from exposure to these mixtures that can be used until the data gaps are filled (Barnes 1991; EPA 1989e). Since for many of these chemicals very limited data on toxicity exist, TEFs were developed and validated in studies in animals (Eadon et al. 1986; Silkworth et al. 1989a; Viluksela et al. 1998a, 1998b).

The TEF approach involves assessment of the comparative effects of individual halogenated aromatic hydrocarbons congeners on various biological end points and derivation of TEFs based on the upper range of potency data for these effects The key assumptions unifying the diverse types of data that are considered in the derivation of TEFs are: that congeners exert toxicity through a common receptor-mediated mechanism, and that the effects of mixtures are additive (Safe 1990). The TEF approach compares the relative toxicity of individual congeners to that of 2,3,7,8-TCDD, which is the most extensively studied of the halogenated aromatic hydrocarbons that interact with the Ah receptor. The TEF for 2,3,7,8-TCDD is defined as unity; and TEFs for all other CDD congeners, CDFs, and dioxin-like PCBs are less than one, thus reflecting their lower toxic potency (see Kennedy et al. [1996] for an exception to this general rule). TEFs proposed earlier by EPA (1989) are presented in Table 2-11; recently revised values are presented in Table 2-12 (WHO 1998). The recent update also assigned a TEF of 1 to 1,2,3,7,8-PeCDD. The toxic potency of a mixture of congeners (i.e., the TEQ) is the sum of the products of the TEFs for each congener and its concentration in the mixture. Thus, TEQs represent 2,3,7,8-TCDD toxic equivalents for mixtures of CDDs, CDFs, and/or dioxin-like PCBs.

The TEF approach facilitates site-specific assessments that account for changes in congener composition due to differential environmental partitioning and transformation, as well as differences in congener profiles between sites and co-exposure to related halogenated aromatic hydrocarbons. The TEF approach, however, has several shortcomings. One problem is that very little data may be available for estimating the TEF and the available data are often from *in vitro* or single-exposure acute *in vivo* studies. Furthermore, there is a wide range in relative potency estimates derived from the literature. For example, Safe (1990) estimated 2,3,7,8-TCDD/1,2,3,4,7,8-HxCDD potency ratios of 33/1 for rat body weight loss, 12/1 for rat thymic atrophy, and 8/1 for AHH induction in cultured rat liver cells. One further problem is that

Table 2-11. Toxicity Equivalency Factors (TEFs) for Halogenated Hydrocarbons

CDFs	EPA current recommended values[a]	CDDs	EPA current recommended values[a]	PCBs	WHO/IPCS interim value[b]
monoCDFs	0	monoCDDs	0	3,3'4,4'-tetraCB	0.0005
diCDFs	0	diCDDs	0	3,3',4,4',5-pentaCB	0.1
triCDFs	0	triCDDs	0	2,3,3',4,4'-pentaCB	0.0001
2,3,7,8-tetraCDF	0.1	2,3,7,8-TCDD	1	2,3,4,4',5-pentaCB	0.0005
other tetraCDFs	0	other tetraCDDs	0	2,3',4,4',5-pentaCB	0.0001
1,2,3,7,8-pentaCDF	0.05	2,3,7,8-pentaCDD[c]	0.5	2',3,4,4',5-pentaCB	0.0001
2,3,4,7,8-pentaCDF	0.5	other pentaCDDs	0	3,3',4,4',5,5'-hexaCB	0.01
other pentaCDFs	0			2,3,3',4,4',5-hexaCB	0.0005
2,3,7,8-hexaCDF[c]	0.1	2,3,7,8-hexaCDD[c]	0.1	2,3,3',4,4',5'-hexaCB	0.0005
other hexaCDFs	0	other hexaCDDs	0	2,3',4,4',5,5'-hexaCB	0.00001
2,3,7,8-heptaCDF[c]	0.01	2,3,7,8-heptaCDD[c]	0.01	2,3,3',4,4',5,5'-heptaCB	0.0001
other heptaCDFs	0	other heptaCDDs	0	2,2',3,3',4,4',5,-heptaCB	0.0001
octaCDF	0.001	octaCDD	0.001	2,2',3,4,4',5,5'-heptaCB	0.00001

[a]Derived from EPA 1989e
[b]Derived from Ahlborg et al. 1994
[c]Any isomer that contains chlorine in the 2,3,7,8-positions

CDDs = chlorinated dibenzo-*p*-dioxins; CDFs = chlorinated dibenzofurans; PCBs = polychlorinated biphenyls; TCDD = tetrachlorodibenzo-*p*-dioxin

Table 2-12. World Health Organization (WHO)-TEFs for Humans, Mammals, Fish, and Birds

Compound	Humans/mammals	Fish[a]	Birds[a]
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PeCDD	1	1	1[f]
1,2,3,4,7,8-HxCDD	0.1[a]	0.5	0.05[f]
1,2,3,6,7,8-HxCDD	0.1[a]	0.01	0.01[f]
1,2,3,7,8,9-HxCDD	0.1[a]	0.01[e]	0.1[f]
1,2,3,4,6,7,8-HpCDD	0.01	0.001	<0.001[f]
OCDD	0.0001[a]	–	–
2,3,7,8-TCDF	0.1	0.05	1[f]
1,2,3,7,8-PeCDF	0.05	0.05	0.1[f]
2,3,4,7,8-PeCDF	0.5	0.5	1[f]
1,2,3,4,7,8-HxCDF	0.1	0.1	0.1[c,f]
1,2,3,6,7,8-HxCDF	0.1	0.1[c]	0.1[c,f]
1,2,3,7,8,9-HxCDF	0.1[a]	0.1[c,e]	0.1[c]
2,3,4,6,7,8-HxCDF	0.1[a]	0.1[c]	0.1[c]
1,2,3,4,6,7,8-HpCDF	0.01[a]	0.01[b]	0.01[b]
1,2,3,4,7,8,9-HpCDF	0.01[a]	0.01[b,e]	0.01[b]
OCDF	0.0001[a]	0.0001[b,e]	0.0001[b]
3,3',4,4'-TCB (81)	0.0001[a,b,c,e]	0.0005	0.1[e]
3,4,4',5-TCB (77)	0.0001	0.0001	0.05
3,3',4,4',5-PCB (126)	0.1	0.005	0.1
3,3',4,4',5,5'-HxCB (169)	0.01	0.00005	0.001
2,3,3',4,4'-PeCB (105)	0.0001	<0.000005	0.0001
2,3,4,4',5-PeCB (114)	0.0005[a,b,c,d]	<0.000005[b]	0.0001[g]
2,3',4,4',5-PeCB (118)	0.0001	<0.000005	0.00001
2,3,4,4',5-PeCB (123)	0.0001[a,c,d]	<0.000005[b]	0.00001[g]
2,3,3',4,4',5,-HxCB (156)	0.0005[b,c]	<0.000005	0.0001
2,3',4,4',5'-HxCB (157)	0.0005[b,c,d]	<0.000005[b,c]	0.0001
2,3',4,4'5,5'-HxCB (167)	0.00001[a,d]	<0.000005[b]	0.00001[g]
2,3,3',4,4'5,5'-HpCB (189)	0.0001[a,c]	<0.000005	0.00001[g]

[a] limited data set
[b] structural similarity
[c] quantitative structure activity relationships (QSAR) modeling prediction from CYP1A induction (monkey, pig, chicken, or fish)
[d] no new data from 1993 WHO review
[e] *in vitro* CYP1A induction
[f] *in vivo* CYP1A induction after *in ovo* exposure
[g] QSAR modeling prediction from class-specific TEFs

– = no TEF because of lack of data; HpCDD = heptachlorodibenzo-*p*-dioxin; HpCDF = heptachlorodibenzofuran; HxCB = hexachlorobiphenyl; HxCDD = hexachlorodibenzo-*p*-dioxin; HxCDF = hexachlorodibenzofuran; ND = not detected; OCDD = octachlorodibenzo-*p*-dioxin; OCDF = octachlorodibenzofuran; PeCDD = pentachlorodibenzo-*p*-dioxin; PeCDF = pentachlorodibenzofuran; TCB = tetrachlorobiphenyl; TCDD = tetrachlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzofuran; TEF=Toxic equivalency factor.

Source: Van den Berg et al. 1998

differences in pharmacokinetics between two chemicals result in different estimates of the relative potency depending upon the exposure protocol (DeVito and Birnbaum 1995). These investigators demonstrated that published TEFs for 2,3,7,8-TCDD (TEF 1) and 2,3,7,8-TCDF (TEF 0.1) accurately estimated the relative potencies for liver EROD induction in female B6C3F_1 mice after 4 weeks of treatment, but failed to do so after 13 weeks of treatment. The inability to estimate relative potencies after the longer treatment duration was attributed to the difference in half-lives between the two compounds (2 days for 2,3,7,8-TCDF and 15 days for 2,3,7,8-TCDD). Steady-state levels of 2,3,7,8-TCDF were achieved within 4 weeks and, thus, EROD remained constant from 4 to 13 weeks. Steady-state levels of 2,3,7,8-TCDD were not attained within 4 weeks, which explained the increased hepatic EROD between 4 and 13 weeks. The results showed that TEFs for congeners with a short half-life may overestimate their potency and that the opposite may be true for congeners with a long half-life. Based on their results, DeVito and Birnbaum (1995) suggested that "present TEFs should be reevaluated to determine whether values have adequately incorporated pharmacokinetic differences between the test compound and 2,3,7,8-TCDD." In a more recent study, the same group of investigators compared the relative potencies for enzyme induction of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, OCDF, and 2,3,7,8-tetrabromodibenzo-*p*-dioxin (2,3,7,8-TBDD) in mice based on daily administered or final tissue dose following gavage dosing for 90 days (DeVito et al. 1997). The enzymes monitored were EROD (liver, lung, and skin) and ACOH (liver). After the 90-day administration period, the chemicals were assumed to be at or approaching steady-state conditions. Since ED_{50} values could not be estimated for all the congeners, the authors used an alternative method of comparison that fitted a function to the 2,3,7,8-TCDD dose-response data. The function was then used to predict the 2,3,7,8-TCDD equivalent dose of a chemical based on the enzymatic activity induced at a given dose of the test compound. A linear regression of the predicted dose of 2,3,7,8-TCDD and the actual congener dose provided the relative potency estimate. The results showed that, when based on administered dose, the relative potencies for the specific congeners did not vary substantially among tissues. However, for congeners with a much shorter half-life than 2,3,7,8-TCDD, the relative potencies increased for all enzymes when estimated from tissue concentrations. For example, for 2,3,7,8-TCDF the relative potency based on administered dose varied by less than a factor of 2 between endpoints, ranging from 0.0076 for skin EROD to 0.014 for ACOH. The relative potency increased by 4- to 14-fold when based on tissue dose and varied between tissues by a factor of 4, from 0.028 to 0.11. Because 2,3,7,8-TCDF is metabolized much faster than 2,3,7,8-TCDD, to achieve an equivalent tissue concentration of these chemicals, higher doses of 2,3,7,8-TCDF must be administered relative to 2,3,7,8-TCDD. Overall, the results confirmed their previous observations that differences in absorption and metabolism modulate the relative potency of this class of chemicals. DeVito et al. (1997)

suggested that it might be useful to derive two sets of TEF values, one used for estimating intake equivalents and the other for estimating tissue equivalents.

Viluksela et al. (1998a, 1998b) recently examined a wide range of endpoints in rats administered either a mixture of CDDs with a given TEQ or single CDD congeners at the same TEQ dose level as the mixture. The TEFs for the various congeners were derived from acute experiments. The dosing period was 13 weeks. The results showed effects of similar magnitude in response to administration of the CDD mixture or single CDD congeners. This supported the validity of the TEF method and the notion of additive toxicity for the CDDs evaluated. Moreover, the concentration ratios for the various congeners in the liver were very similar to the ratios at which the congeners were administered.

Neubert and coworkers (Neubert et al. 1992c) have also examined the issue of TEFs and proposed that a number of prerequisites need to be fulfilled in order to consider the TEF approach from a scientific point of view:

- The actions of the congeners must be strictly additive in the dose range to be evaluated.
- The organotropic manifestations in different species must be identical over the relevant dose ranges.
- Dose-response curves for various toxicological end points for a given congener must run parallel.
- The dose-response curves for a given toxicological end point must run parallel for the various congeners.
- For extrapolations between species, the kinetics must be identical, or differences have to be taken into consideration.
- With respect to a risk assessment relevant to humans, toxic or biological manifestations in the lower dose ranges are of special interest, and LD_{50} or ED_{50} values or effects induced by highly toxic dose are of minor importance.
- Effects to be expected at low exposures must be identical with those observed at the high doses studied.

After discussing each one of these seven points, Neubert et al. (1992c) concluded that the toxicological background for using the TEF approach for risk assessment must be increased considerably. A similar conclusion was reached by a scientific panel that examined the feasibility of developing a TEF approach that would be applicable to PCB mixtures (Barnes et al. 1991). In the case of PCBs, the study group concluded that “the application of TEF approach for PCBs would be less straightforward than it was in the case of chlorinated dibenzo-p-dioxins and dibenzofurans.”

In 1992, a group of scientists met in Belgium under the auspices of the European Environmental Research Organization to discuss the impact of CDDs, CDFs, and PCBs on human and environmental health with special emphasis on application of the TEF concept. The main conclusions, relevant to the TEF concept,

were that TEFs may be useful for risk management (i.e., quantitative estimation of Ah receptor-mediated toxic potential) of mixtures of CDDs, CDFs and the coplanar non-ortho and mono-ortho PCBs, but that the TEF concept is not applicable for the various toxic responses whose mechanisms do not involve the Ah receptor (Ahlborg et al. 1992, 1994).

The TEF approach in relation to cancer risk estimation has also been examined Rao and Unger (1995). First, the authors used the standard approach of multiplying TEF doses by the cancer slope factor for 2,3,7,8-TCDD to estimate lifetime incremental cancer risks for a mixture of CDDs and CDFs. This method was compared with a modified approach in which the TEF dose was adjusted for differences in the probability of formation of bound receptor-ligand complexes. Briefly, using algorithms from a competitive binding model, the fractions of Ah receptor bound to congeners were derived. This fraction was defined as competitive binding ratios (CBR) in mixtures and represents the maximum likelihood estimate for the formation of a congener-receptor bound complex in the presence of other competing ligands. Two distinct risk scenarios were used for comparison: (1) human adipose tissue residue data from the national human adipose tissue survey (Stanley et al. 1986) were used to generate potential lifetime incremental cancer risks, and (2) lifetime cancer risk was characterized for a potentially exposed population ingesting contaminated carp. In the modified TEF approach, CBR values for individual congeners computed from the competitive binding algorithms were used to derive the tissue concentrations. The main findings of this analysis were that TEF doses calculated by using the model algorithms were lower than the combined TEF dose for all congeners estimated by the TEF method without considering the competitive binding. In addition, the combined incremental cancer risks for all congeners were generally lower when model algorithms were used in the dose-response analysis. Also, the standard TEF method tended to overestimate the risks of higher congeners with low toxicity and underestimated the risk of more toxic congeners.

One further concern regarding the use of TEQs for risk assessment is the fact that the human diet also contains Ah receptor agonists, such as indole-3-carbinol and related compounds in vegetables, polynuclear aromatic hydrocarbons (PAHs), aromatic amines formed during cooking. These natural Ah receptor agonists elicit responses in humans that are consistent with a receptor-mediated pathway, but the response-specific potencies of natural Ah receptor versus xenodioxins are unknown. Therefore, a TEF/TEQ approach based solely on intake of xenodioxins does not take into account the background of natural dioxins that may influence responses associated with persistent low-level occupation of the Ah receptor (see Safe 1998a, 1998b for review on this issue).

Minimal Risk Levels for 2,3,7,8-TCDD

It is ATSDR's policy (see Appendix B) to use health guidance values (i.e., MRLs, EMEGs) derived for 2,3,7,8-TCDD for other dioxin-like compounds, expressed in total TEQs.

Inhalation MRLs

MRLs were not derived for inhalation exposure.

Oral MRLs

- An MRL of 0.0002 (2×10^{-4}) µg/kg/day has been derived for acute-duration oral exposure (14 days or less) to 2,3,7,8-TCDD.

The acute duration oral MRL was based on a NOAEL of 0.005 µg/kg and a LOAEL of 0.01 µg/kg for immunological effects in female mice (Burleson et al. 1996). In this study, groups of 20 female B6C3F_1 mice were administered a single gavage dose of 0, 0.001, 0.005, 0.01, 0.05, or 0.1 µg/kg 2,3,7,8-TCDD in corn oil. Seven days after 2,3,7,8-TCDD exposure, the mice were infected intranasally with influenza A/Hong Kong/8/68 (H3N2) virus diluted at 10^{-48}, 10^{-50}, 10^{-52}, or 10^{-54}. In a separate experiment, groups of 18 female mice received a single gavage dose of 0, 0.001, 0.01, or 0.1 µg/kg 2,3,7,8-TCDD and were infected 7 days later with influenza A virus at a dose not known to cause mortality (10^{-54} and 10^{-58}) or were sham-infected. Body weight, thymus weight, and wet lung weights were measured 3, 9, or 12 days postinfection. Pulmonary virus titers were determined in groups of 72 mice exposed to 0, 0.001, 0.01, or 0.01 µg/kg 2,3,7,8-TCDD and infected with influenza A virus seven days later. For the virus titer study, groups of mice were killed 2 hours, 1, 4, 6, 7, 8, 9, 10, and 11 days post-infection.

Statistically significant increases in mortality were observed in the influenza A infected mice exposed to 0.01, 0.05, or 0.1 µg/kg 2,3,7,8-TCDD. However, no between group differences in mortality were observed at these 2,3,7,8-TCDD dosages. Mortality in mice receiving 0.001 or 0.005 µg/kg did not significantly differ from the mortality in the control group. Exposure to 2,3,7,8-TCDD did not enhance the increase in relative lung weight normally seen in mice infected with influenza A virus. As compared to controls, no significant alterations in thymus weights were observed in 2,3,7,8-TCDD-exposed mice sham-infected or those infected with influenza A virus. 2,3,7,8-TCDD exposure did not result in a significant increase in viral titers in the lung, as compared to titers from the control group. The authors noted that the

lack of dose-response in mortality and the lack of effect on the relative lung weight, thymus weight, and viral titers suggest that 2,3,7,8-TCDD may be exerting an effect via an indirect mechanism such as through an effect on cytokines. The 0.005 µg/kg dose was considered a NOAEL for immunotoxicity. As described in the footnote to Table 2-2, an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability) and modifying factor of 0.7 (to adjust for the difference in higher bioavailability of 2,3,7,8-TCDD from an oil gavage vehicle than from food) were used to derive the MRL from the NOAEL value.

- An MRL of 0.00002 (2×10^{-5}) µg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to 2,3,7,8-TCDD.

The intermediate-duration oral MRL was based on a NOAEL of 0.0007 µg/kg/day for immunological effects in Hartley guinea pigs fed 2,3,7,8-TCDD in the diet for 90 days (DeCaprio et al. 1986). In that study, groups of weanling Hartley guinea pigs (10 per sex) were administered a diet that provided an average of 0.0001, 0.0007, 0.005, or 0.028 µg 2,3,7,8-TCDD/kg/day. This corresponds to 2, 10, 76, and 430 ppt 2,3,7,8-TCDD in the food. A control group was fed a diet without added 2,3,7,8-TCDD. The recovery following treatment was studied in groups of 10 guinea pigs fed a diet containing 430 ppt 2,3,7,8-TCDD for 11, 21, or 35 days and allowed to recover for 79, 69, or 55 additional days, respectively. The highest dietary level of 2,3,7,8-TCDD caused net body weight loss and mortality. Four males and four females died, and additional animals had to be sacrificed due to poor health. Food consumption was significantly reduced in the highest-dose group only. Body weight gain in the 0.0007 and 0.005 µg/kg/day male groups was reduced by 9 and 20%, respectively. In the corresponding female groups, body weight gain was reduced by 6 and 15%. Gross lesions were observed only in the highest-dose group and included thymic atrophy, depletion of body fat, and liver enlargement. Significant changes in organ weights included a decrease in absolute kidney weight and in absolute and relative thymus weight in males dosed with 0.005 µg/kg/day, increase in relative liver weight in males and females at the 0.005 µg/kg/day level, and increase in relative brain weight in males at this same dose level. Organ weights from high-dose animals were not monitored. Administration of 2,3,7,8-TCDD did not cause any significant hematological effect (blood was not collected from the highest-dose group). In the 0.005 µg/kg/day groups, serum ALT was significantly reduced in females, and triglycerides were elevated in males. No other significant changes in clinical chemistries were observed. Treatment-related histological alterations were observed only in the two higher-dose groups and consisted of hepatocellular cytoplasmic inclusion bodies and atrophy of the thymic cortex. In the recovery study there was 10% mortality in the groups treated for 11 and 21 days, and 70% mortality in the group treated for 35 days. Surviving animals in all groups exhibited significantly

reduced body weight gain. The 0.0007 µg/kg/day dose represents a NOAEL for decreased thymus weight, and the 0.005 µg/kg/day dose is a LOAEL. As described in the footnote to Table 2-2, an uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability) was used to derive the MRL from the NOAEL.

- An MRL of 0.000001 (1×10^{-6}) µg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to 2,3,7,8-TCDD.

The chronic-duration oral MRL is based on a LOAEL of 0.00012 µg/kg/day for developmental toxicity in rhesus monkeys (Schantz et al. 1992). In this study, groups of 8 female rhesus monkeys were fed a diet containing 0, 5, or 25 ppt 2,3,7,8-TCDD for a total of 16.2 months (the results of the neurodevelopmental portion of this study were published in papers by Bowman et al. [1989a], Schantz and Bowman [1989], Schantz et al. [1992]). After 7 months of exposure, the monkeys were mated with unexposed males. (Only 1 monkey in the 25 ppt group delivered a viable offspring; this offspring was not studied behaviorally.) The monkeys were fed the 2,3,7,8-TCDD diet throughout the mating period, gestation, and lactation. When the offspring (3 males and 3 females per exposure group) were 8.6 months of age, they were placed in 3 peer groups of 4 monkeys and allowed to play for 1.5 hours without interference. The peer groups consisted of two 2,3,7,8-TCDD-exposed monkeys and two control monkeys. Behavioral patterns (social interactions and other behaviors such as vocalization, locomotion, self-directed behavior, and environmental exploration) were monitored 4 days a week for 9 weeks. No overt signs of toxicity were observed in the mothers or offspring, and birth weights and growth were not adversely affected by 2,3,7,8-TCDD exposure. Significant alterations were observed in play behavior, displacement, and self-directed behavior in the 2,3,7,8-TCDD-exposed offspring. 2,3,7,8-TCDD-exposed monkeys tended to initiate more rough-tumble play bouts and retreated less from play bouts than controls, were less often displaced from preferred positions in the playroom than the controls, and engaged in more self-directed behavior than controls. No other significant alterations in behavior or alterations in reflex development, visual exploration, locomotor activity, or fine motor control were found (Bowman et al. 1989a). In tests of cognitive function, object learning was significantly impaired, but no effect on spatial learning was observed (Schantz and Bowman 1989). As described in the footnote to Table 2-2, an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability) was used to derive the MRL.

It should be also noted that 10 years after termination of 2,3,7,8-TCDD exposure in the Schantz et al. (1992) study, Rier et al. (1993) reported a dose-related increase in the incidence and severity of

endometriosis in these same rhesus monkeys. Rier et al. (1993) identified a less serious LOAEL of 5 ppt (0.00012 µg/kg/day) for moderate endometriosis. However, monkeys appear to be more susceptible to endometriosis, based on a background incidence of endometriosis in monkeys of 30% (Rier et al. 1993) compared to a background incidence of 10% in humans (Wheeler 1992). Thus, derivation of a chronic oral MRL based on endometriosis would necessitate using an uncertainty factor of less than 1 (or at most, 1) to account for the increased sensitivity of monkeys to endometriosis as compared to humans. If the Rier et al. (1993) study were used to calculate an oral MRL, the LOAEL of 0.00012 µg/kg/day would be divided by an uncertainty factor of 100 (10 to extrapolate from a LOAEL, 10 for human variability and 1 for interspecies differences). This would result in a computed MRL essentially the same as the chronic oral MRL of 1 pg/kg/day based on developmental toxicity as described in the preceding paragraph. Moreover, (1) the clinical history for these rhesus monkeys during the 10-year period between the Schantz et al. (1992) study and examination by Rier et al. (1993) is unknown (not reported); (2) Boyd et al. (1995) did not find an association between exposure to CDDs, CDFs, or PCBs and endometriosis in a clinical study in women; and (3) the EPA (1997) concluded that "the evidence for supporting the hypothesis that CDDs and PCBs are causally related to human endometriosis via an endocrine-disruption mechanism is very weak." So, even though there is information to indicate that endometriosis may also be a sensitive toxicological end point for 2,3,7,8-TCDD exposure, the developmental end point (altered social behavior) reported in the Schantz et al. (1992) study was determined to be the most appropriate end point for derivation of an MRL for chronic oral 2,3,7,8-TCDD exposure.

Comparison of Estimated Body Burdens Associated with Effects in Experimental Animals and Humans. Estimated average body burdens of 2,3,7,8-TCDD in human populations in which various health effects of 2,3,7,8-TCDD are suspected range from 31 to 6,600 ng/kg (estimated body burdens at the time of exposure termination). See Table 2-1 for more information. The human body burden expected in populations exposed to background environmental levels of 2,3,7,8-TCDD has been estimated to be 1 ng TCDD/kg body weight (DeVito et al. 1995; Orban et al. 1994). This would suggest that effects of 2,3,7,8-TCDD in humans may occur at body burdens that are 30 to 6,600 times greater than background burdens for 2,3,7,8-TCDD.

The similarities in response of humans and experimental animals to similar body burdens of CDDs and related chemicals (Table 2-10), along with our understanding of common mechanisms of actions of CDDs in humans and experimental animals lends support to both the relevance of experimental animal toxicology to humans and the use of experimental animal data for establishing MRLs (see Section 2.4.3 for more

information on the animal-to-human extrapolations). Acute, intermediate and chronic MRLs for 2,3,7,8-TCDD were derived from experimental animal studies that identified the highest NOAELs or lowest LOAELs for the respective exposure-duration category (Table 2-2). The MRLs, and the NOAELs and LOAELs on which they are based, have been converted to their corresponding equivalent body burdens and are compared to background 2,3,7,8-TCDD body burdens in humans and body burdens associated with adverse health effects (Table 2-13). This comparison shows that the NOAELs for acute, intermediate, and LOAEL for chronic exposure to 2,3,7,8-TCDD in experimental animals are generally below the low end of the 31–6,600 ng TCDD/kg of body weight range of body burdens that is suspected to be adverse to humans based on the epidemiologic evidence. Body burdens that correspond to the MRLs are 2-6 times lower than the estimated background body burden in humans, suggesting that adverse health effects are unlikely in humans exposed to background levels of 2,3,7,8-TCDD. However, because of the magnitude of uncertainty in dose-response relationships for 2,3,7,8-TCDD, the possibility that current background exposures may be sufficient to contribute to a risk of adverse health effects in human populations cannot be completely excluded.

Death. Information regarding mortality in humans after exposure to CDDs is limited to epidemiological studies in populations exposed occupationally or environmentally (Bertazzi et al. 1989b; Cook et al. 1986, 1987b; Fingerhut et al. 1991; Ott et al. 1980, 1987; Pesatori et al. 1998; Thiess et al. 1982; Vena et al. 1998; Wolfe et al. 1985; Zack and Suskind 1980; Zober et al. 1990). These studies did not find a significant increase in the overall mortality rate in populations exposed to 2,3,7,8-TCDD or other CDD congeners for acute or chronic durations. However, several studies did find significant increases in cause-specific mortality (i.e., cancer and cardiovascular disease). These increases in cause-specific mortality are discussed under the specific effect.

Several studies provided data regarding lethality following CDDs exposure in animals. Oral LD_{50} values for 2,3,7,8-TCDD were calculated in rats (NTP 1982b; Schwetz et al. 1973; Walden and Schiller 1985), minks (Hochstein et al. 1988), rabbits (Schwetz et al. 1973), guinea pigs (McConnell et al. 1984; Schwetz et al. 1973), and hamsters (Henck et al. 1981) following gavage doses in corn oil or corn oil:acetone vehicle. Doses that produced death were in the µg/kg range. Differences in the susceptibility to the lethality of 2,3,7,8-TCDD were observed not only among different species, but also among different strains

Table 2-13. Estimated Body Burdens of 2,3,7,8-TCDD That Correspond to MRLs

Duration	Risk or effect level	Exposure (ng/kg/day)	Body burden (Cb)[a] (ng/kg bw)
Acute	MRL	0.2	0.16[b]
	NOAEL	5	4[b]
	LOAEL	10	8[b]
Intermediate	MRL	0.02	0.66[c]
	NOAEL	0.7	23[c]
	LOAEL	5	164[c]
Chronic	MRL	0.001 (maternal dose)	0.26 (peak maternal)[d] 0.76 (offspring at weaning)[e]
	LOAEL	0.12 (maternal dose)	32 (peak maternal)[d] 68 (offspring at weaning)
Human health effects	—	—	31–6,600[f]
Human background	—	—	1[g]

[a] Estimated as follows: Cb = (I·a·f)/k, f = 1-(e^{-kt}); where, C_b is the 2,3,7,8-TCDD body burden (ng/kg bw), I is 2,3,7,8-TCDD intake (ng/kg-day), a is the gastrointestinal absorption fraction, f is the fraction of steady-state body burden, k is the whole body elimination rate constant for 2,3,7,8-TCDD (day^{-1}, $ln2/t_{1/2}$), and t is the exposure duration (day)

[b] Assumed parameter values for mice in Burleson et al. (1996) study: a = 0.8 (Curtis et al. 1990), $t_{1/2}$ = 11 days (Birnbaum 1986)

[c] Assumed parameter values for guinea pigs in DeCaprio et al. (1986) study: a = 0.5 (Van den Berg et al. 1994), $t_{1/2}$ = 94 days (Olson 1986)

[d] Assumed parameter values for monkeys in Schantz et al. (1992) study: a = 0.8 (value for rats from Van den Berg et al. 1994), $t_{1/2}$ = 391 days (Bowman et al. 1989b)

[e] At 5 months (weaning), the reported mean 2,3,7,8-TCDD concentration of adipose tissue in offspring was 377 ng/kg; this is equivalent to approximately 68 ng/kg bw, assuming that adipose tissue was 72% lipid and 13% of the body weight was lipid (Bowman et al. 1989b) ([377 x 0.13]/0.72=68). Assuming a reported linear regression relating 2,3,7,8-TCDD in adipose at weaning and maternal 2,3,7,8-TCDD in adipose at parturition, (Bowman et al. 1989b), 377 ng/kg adipose in offspring is equivalent to 84 ng/kg maternal adipose, or 17 ng/kg bw, assuming maternal adipose is 72% lipid and maternal body weight is 15% lipid (Bowman et al. 1989b). At the MRL exposure level, the 2,3,7,8-TCDD body burden in the offspring was calculated by dividing the 68 ng/kg body burden by the uncertainty factor used to calculate the chronic MRL (90).

[f] From Table 2-1

[g] From Orban et al. (1994) and DeVito et al. (1995)

within the same species, and even in the same strain of rat bred in different laboratories (Walden and Schiller 1985). The use of rats of different ages may have played a role in the interlaboratory differences in susceptibility between rats of the same strain. Toxicity results from acute- and intermediate-duration categories indicated that the guinea pig is the most sensitive species to 2,3,7,8-TCDD toxicity leading to death (DeCaprio et al. 1986; McConnell et al. 1984; Schwetz et al. 1973; Vos et al. 1973), and that the hamster is the most resistant (Hanberg et al. 1989; Henck et al. 1981). Experiments with mice that were injected with 2,3,7,8-TCDD intraperitoneally showed that the C57BL/6J mice responsive to 2,3,7,8-TCDD-induced toxicity were twice as sensitive to 2,3,7,8-TCDD-induced lethality as the less-responsive DBA/2J strains (Gasiewicz et al. 1983a). Increased mortality was also recorded in mice following intermediate-duration dermal exposure to 2,3,7,8-TCDD (NTP 1982a).

Toxicity data in animals indicated that similar effects occur after exposure to CDDs by oral, dermal, or parenteral routes. Toxicokinetic data in mice showed that 2,3,7,8-TCDD hepatic levels were similar following oral, intraperitoneal, and subcutaneous exposure (Nau and Bass 1981). However, recent data in rats showed that intratracheal administration of a 2,3,7,8-TCDD dose resulted in a relatively higher accumulation of 2,3,7,8-TCDD in the liver than after oral administration of the same dose (Diliberto et al. 1996). Intraperitoneal administration of 2,3,7,8-TCDD was less toxic than oral dosing in acute-exposure experiments with hamsters (Olson et al. 1980a).

Following acute oral exposure to 2,3,7,8-TCDD, death occurred within 6–42 days depending on the dose and species tested, indicating a delayed type of toxicity. Death was usually preceded by significant weight loss in all duration categories. However, weight loss did not appear to be the only cause of death. A total parenteral nutrition fluid given to 2,3,7,8-TCDD-exposed rats and guinea pigs protected the animals against wasting syndrome, but not against 2,3,7,8-TCDD-induced lethality (Gasiewicz et al. 1980; Lu et al. 1986). Specifically, biochemical changes indicative of severe liver damage were found in moribund rats.

2,3,7,8-TCDD was found not only to be more toxic than its isomer 1,2,3,4-TCDD (Courtney 1976) but also more toxic than any other congener tested (2,7-DCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD) (Courtney 1976; NCI/NTP 1980; NTP 1982b; Viluksela et al. 1994, 1998a). LD_{50} values for acute oral exposure to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD derived for rats and mice (NCI/NTP 1980) were higher by more than 2 orders of

magnitude than LD_{50} values for 2,3,7,8-TCDD. However, 1,2,3,4,6,7,8-OCDD and 2,7-DCDD did not cause death in mice even at doses as high as 4,000 and 2,000 mg/kg, respectively.

Systemic Effects.

Respiratory Effects. No exposure-related respiratory effects were found in a group of Air Force Vietnam veterans exposed to 2,3,7,8-TCDD during aerial spraying studied sometime after exposure (Wolfe et al. 1985). No respiratory effects clearly attributable to 2,3,7,8-TCDD have been found in workers potentially exposed (Calvert et al. 1991). In rhesus monkeys, intermediate-duration exposure to a lethal oral dose of 2,3,7,8-TCDD caused nose bleeding (McNulty 1984), hemorrhage, and hyperplasia of the bronchial epithelium (Allen et al. 1977). Bronchiolar adenomatoid changes were seen in mice chronically exposed dermally (NTP 1982a). Furthermore, hyperplastic changes in the lungs were recorded in rats exposed orally to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980). However, mostly negative results were obtained in other oral studies in animals regardless of duration of exposure (Holsapple et al. 1986b; Kociba et al. 1978a; NCI/NTP 1979a; NTP 1982a, 1982b). The relevance of the animal findings to human health is unclear. Intense acute exposure to 2,3,7,8-TCDD can produce respiratory irritation, but the findings from controlled epidemiologic studies do not support an association between 2,3,7,8-TCDD exposure and chronic respiratory disease. It should be noted, however, that chronic bronchitis and related effects were observed in many Yusho and Yu-Cheng patients, who were exposed to the structurally related CDFs (ATSDR 1994).

Cardiovascular Effects. While some studies have found an association between CDD exposure and cardiovascular disease, most studies have not found a clear association between exposure to 2,3,7,8-TCDD and diseases of the heart and circulatory system (Bond et al. 1983; Calvert et al. 1998; Hoffman et al. 1986; Moses et al. 1984; Reggiani 1980; Suskind and Hertzberg 1984; Wolfe et al. 1985). However, human studies have suffered from limitations such as examination of the cohorts after exposure has ended, thus allowing for tissue repair to occur; lack of good exposure data; and inability to examine the relationship between serum 2,3,7,8-TCDD levels and cardiovascular disease in most studies. In the Ranch Hand study (USAF 1991), a weak association was found between decreased mean diastolic blood pressure, cardiac arrhythmias, and decreases in peripheral pulses and exposure to 2,3,7,8-TCDD. Bertazzi et al. (1989b) and Pesatori et al. (1998) found increases in deaths from ischemic heart disease and cardiovascular disease in men and chronic rheumatic heart disease in women in the 10-year period following the Seveso accident. However, the authors attributed these findings to post-accident stress rather than the 2,3,7,8-TCDD

exposure. In workers exposed occupationally to 2,3,7,8-TCDD and other CDD congeners at the Boehringer Hamburg plant, a statistically significant trend for increased risk of cardiovascular disease and ischemic heart disease mortalities with increasing serum lipid levels of 2,3,7,8-TCDD or TEQ (CDDs and CDFs) was found (Flesch-Janys et al. 1995). An international study of more than 20,000 workers followed from 1939 to 1992 found an increased risk for death from cardiovascular disease, especially ischemic heart disease, among exposed workers, but the authors did not rule out the influence of risk factors such as cigarette smoking, high fat diet, obesity, physical inactivity, and serum lipids (Vena et al. 1998).

Experiments in animals demonstrated that exposure to relatively high doses of 2,3,7,8-TCDD can cause various pathophysiological effects. Acute oral exposure of rats increased the basal tension of the left cardiac atria (Kelling et al. 1987) or decreased the basal rate for spontaneous beating, depending on the dose used (Hermansky et al. 1988; Kelling et al. 1987). Reduced blood pressure and increased myocardial peroxidase activity were also recorded. All of the effects on the heart in these two studies were attributed to a hypothyroid condition caused by near-lethal doses (Hermansky et al. 1988). Other studies have suggested a direct effect of 2,3,7,8-TCDD on cardiac muscle, as for example an intraperitoneal injection of 2,3,7,8-TCDD to guinea pigs reduced the contractive responsiveness of isolated myocardium (Brewster et al. 1987; Canga et al. 1988).

Reports of histological findings are few. Myocardial degenerative changes were reported in rats after chronic oral exposure to a lethal dose of 2,3,7,8-TCDD (Kociba et al. 1978a), and hemorrhages were reported in monkeys after intermediate-duration dietary exposure to near-lethal doses (Allen et al. 1977). However, most studies did not find any histopathological changes in rats and mice following chronic oral exposure to 2,3,7,8-TCDD (NTP 1982b), 2,7-DCDD (NCI/NTP 1979a), or a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980). Similarly, no changes were reported after chronic dermal exposure of mice to 2,3,7,8-TCDD (NTP 1982a). There is no conclusive evidence that the cardiovascular system is a target for 2,3,7,8-TCDD toxicity.

Gastrointestinal Effects. Limited information is available regarding gastrointestinal effects of 2,3,7,8-TCDD in humans. Earlier studies of individuals with exposure to substances contaminated with 2,3,7,8-TCDD found significant elevations in self-reported ulcers (Bond et al. 1983; Suskind and Hertzberg 1984), but a study of Vietnam veterans (USAF 1991) failed to find such effect. A more recent cross-sectional medical study of workers employed more than 15 years earlier in the production of

2,3,7,8-TCDD-contaminated chemicals found no association between 2,3,7,8-TCDD exposure (body burden) and gastrointestinal disease (Calvert et al. 1992).

Only a few of the numerous animal studies found any effects. Gastrointestinal ulcerations were reported in minks after an acute oral exposure to a lethal dose of 2,3,7,8-TCDD (Hochstein et al. 1988), and hemorrhages were reported in rats following chronic exposure (Van Miller et al. 1977). Ileitis and peritonitis were observed in hamsters receiving a single lethal dose of 2,3,7,8-TCDD (Olson et al. 1980a). A trophic effect on the antral mucosa was found in 2,3,7,8-TCDD treated rats, in contrast to atrophy found in pair-fed control animals (Theobald et al. 1991). Although these authors attempted to relate the mechanism of action to hormonal effects, a definitive mechanism was not established. Changes progressing from epithelial hyperplasia and metaplasia of gastric mucosa to stomach ulcerations were observed in rhesus monkeys with prolonged oral exposure (Allen et al. 1977; McConnell et al. 1978a; McNulty 1984). These data indicate that primates are particularly sensitive to 2,3,7,8-TCDD-induced gastrointestinal toxicity; however, the effects are seen at doses which caused severe toxicity at multiple sites. In contrast, most studies in rodents did not find any gastrointestinal effects after oral or dermal exposure to 2,3,7,8-TCDD (Christian et al. 1986a; Henck et al. 1981; Kociba et al. 1978a; NTP 1982a, 1982b), or after oral exposure to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980) and 2,7-DCDD (NCI/NTP 1979a). The available information suggests that the gastrointestinal tract is not a target for 2,3,7,8-TCDD toxicity in humans.

Hematological Effects. Limited human data were located regarding hematological effects following exposure to CDDs. Increases in leukocyte and platelet counts were reported in Vietnam veterans involved in Operation Ranch Hand (USAF 1991), which suggested the presence of a low-level, chronic inflammatory response related to higher levels of 2,3,7,8-TCDD exposure. Increased prevalence of high white blood cell counts was found in a population exposed to 2,3,7,8-TCDD in the environment, but the increase was not of clinical importance (Hoffman et al. 1986), and other epidemiological studies reported negative results (Stehr et al. 1986; Wolfe et al. 1985).

Increased packed-cell volume was found in guinea pigs following a single intraperitoneal injection of 2,3,7,8-TCDD; however, this was considered to be secondary to progressive dehydration of exposed animals with decreased water consumption (Gasiewicz and Neal 1979). Several studies in animals reported hematological effects after intermediate-duration exposure to 2,3,7,8-TCDD. Among the effects observed were reduced leukocytes in guinea pigs at 0.001 µg/kg/day (Vos et al. 1973), thrombocytopenia and

hemoconcentration in rats at 0.8–1 µg/kg/day (Viluksela et al. 1994; Zinkl et al. 1973), and anemia and bone marrow hypoplasia in rhesus monkeys at 0.1 µg/kg/day (McNulty 1984). Reduced erythrocytes was reported in rats chronically exposed to 0.1 µg 2,3,7,8-TCDD/kg/day in the feed (Kociba et al. 1978a).

Splenic changes include reduced germinal centers after acute (Christian et al. 1986a) and splenic atrophy after chronic (Van Miller et al. 1977) oral exposures in rats. Furthermore, splenic hyperplasia was found in rats orally exposed to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD for an intermediate duration (NCI/NTP 1980). Whether or not the splenic changes were secondary to hematopoietic effects is unclear.

No hematological effects were found after acute oral exposure in minks (Hochstein et al. 1988) and mice (Holsapple et al. 1986a), intermediate oral exposure in guinea pigs (DeCaprio et al. 1986), chronic oral exposure in rats and mice (NTP 1982a; Oughton et al. 1995), chronic dermal exposure in mice (NTP 1982a), and acute intraperitoneal exposure in rats (Mason and Safe 1986a). Similarly, no effects were reported in rodents exposed chronically to 2,7-DCDD (NCI/NTP 1979a) or a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980) by the oral route, but thrombocytopenia was reported in male Sprague-Dawley rats exposed for 13 weeks to 1,2,3,4,6,7,8-HpCDD (Viluksela et al. 1994). Decreased hematocrit and reduced platelet counts were reported in rats administered 1,2,3,7,8-PeCDD or 1,2,3,4,7,8-HxCDD for 13 weeks at dose levels that caused lethality (Viluksela et al. 1998a).

No clear picture regarding hematologic effects of 2,3,7,8-TCDD emerges from the studies in animals. From the limited data, it appears, however, that mice are less sensitive than other species. The relevance of the findings in animals to human health is difficult to ascertain.

Musculoskeletal Effects. No relevant information was located regarding musculoskeletal effects in humans exposed to CDDs. However, evidence from case reports in the Yu-Cheng incident (which involved oral exposure to the structurally related CDFs and PCBs) indicate that musculoskeletal effects may occur after oral exposure to CDDs. Guo et al. (1994) reported that Yu-Cheng children were smaller and had less total lean mass and soft-tissue mass compared to matched control subjects. Hemorrhages in the musculoskeletal system of monkeys were observed following an oral intermediate-duration exposure to 2,3,7,8-TCDD (Allen et al. 1977). However, monkeys in this experiment were in terminal stages, and hemorrhages were found in several other systems. There is no evidence that would indicate that the musculoskeletal system is a target for 2,3,7,8-TCDD toxicity in humans or in animals.

Hepatic Effects. Exposure to 2,3,7,8-TCDD induces liver microsomal enzymes in both humans and animals, regardless of the route or duration of exposure. Increased urinary δ-glucaric acid (UGA) excretion, an indirect index of enzyme induction, was found in children with chloracne living in the Seveso area following the 1976 industrial accident (Ideo et al. 1982). Biochemical changes (increased cholesterol and bilirubin levels, induced GGT and ALT activities) indicated liver effects in exposed humans (Hoffman et al. 1986; Mocarelli et al. 1986). Biochemical changes indicative of a subclinical effect on lipid metabolism were found in Vietnam veterans involved in Operation Ranch Hand (USAF 1991). Biochemical examinations found disorders in the metabolism of porphyrins, lipids, carbohydrates, and plasma proteins in workers exposed to 2,3,7,8-TCDD during the manufacture of herbicides (Jirasek et al. 1976; Pazderova-Vejlupkova et al. 1981). In addition, histopathological changes (steatosis, fibrosis) were also documented. A more recent and better-designed study of workers employed at 2 chemical plants in the manufacture of sodium trichlorophenol and its more than 15 years earlier derivatives found no evidence of an elevated risk for long-term clinical hepatic disease (Calvert et al. 1992). Exposure was assessed by measuring lipid-adjusted serum 2,3,7,8-TCDD levels, and exposed workers had serum 2,3,7,8-TCDD levels significantly higher than unexposed controls. The negative findings, however, are not necessarily inconsistent with results from earlier studies, but suggest that hepatic effects observed in humans immediately after exposure probably resolve with time. A follow-up study of the same cohort found a positive association between serum 2,3,7,8-TCDD levels and the concentration of triglycerides and a negative correlation with HDL cholesterol; these associations were small when compared with the influence of many other factors (Calvert et al. 1996).

Studies in animals have shown that exposure to 2,3,7,8-TCDD can induce hepatotoxicity in several species administered the chemical by various exposure routes for several exposure durations. The severity of the lesion is dependent not only on the species, but also on the strain. In general, it appears that rats exhibit more signs of hepatotoxicity than guinea pigs and hamsters. Histological alterations of the liver are common findings observed in animals exposed to 2,3,7,8-TCDD. These effects have been reported after acute exposure in rats (Christian et al. 1986; Hermansky et al. 1988), mice (Greig 1984; Greig et al. 1987; Kelling et al. 1985), and guinea pigs (Turner and Collins 1983); after intermediate-duration exposure in rats (NTP 1982b; Van Miller et al. 1977), mice (Thigpen et al. 1975), guinea pigs (DeCaprio et al. 1986), and monkeys (Allen et al. 1977; McNulty 1984); and after chronic exposure in rats (Kociba et al. 1978a). Hepatic lesions in rats are characterized by degenerative and necrotic lesions with the appearance of mononuclear cell infiltration, multinucleated giant hepatocytes, and increased number of mitotic figures and intracytoplasmic lipid droplets. Markers of hepatic damage such as serum ALT and AST activities usually

increase in animals that exhibit altered liver histology (Greig 1984; Rosenthal et al. 1989; Smith et al. 1981). DBA/2J mice developed hepatic necrosis and inflammation without fatty changes after acute intraperitoneal exposure to 2,3,7,8-TCDD (Shen et al. 1991). Only slight lipid accumulation was found after exposure to a high dose (600 µg/kg). In contrast, severe fatty changes were observed in C57BL/6J mice, indicating that the steatitic effect may depend on the Ah locus. Histological lesions may be severe enough to be a contributing factor in death. Dose-related increases in intracellular and paracellular permeability of the biliary tree was observed in rats administered ip doses of 2,3,7,8-TCDD (Davidson and Fujimoto 1987).

2,3,7,8-TCDD has been found to be porphyrogenic in both rats and mice (Cantoni et al. 1981; Goldstein et al. 1977, 1982; Jones and Sweeney 1977, 1980). The mechanism of induction of porphyria is not known. 2,3,7,8-TCDD is a potent inducer of the initial and rate-limiting enzyme involved in heme synthesis, ALA-synthetase, but no increased activity was seen in mice which exhibited porphyria after treatment with 2,3,7,8-TCDD for 11 weeks (Jones and Sweeney 1980). A more likely explanation is that the primary event in 2,3,7,8-TCDD-induced porphyria is inhibition of hepatic porphyrinogen decarboxylase (Jones and Sweeney 1980). Crossbreeding experiments have shown that porphyrinuria was inherited together with AHH inducibility (Jones and Sweeney 1980), indicating that the Ah locus is involved in the porphyrogenic response to 2,3,7,8-TCDD.

Enzyme induction is one of the most sensitive responses to 2,3,7,8-TCDD exposure, and has been one of the most extensively studied biochemical responses produced by 2,3,7,8-TCDD. The MFO system is the most thoroughly investigated, and AHH and EROD (CYP1A1 markers) and acetanilide-4-hydroxylase (ACOH) (CYP1A2 marker) are the most frequently assayed enzyme activities. The lowest single oral dose of 2,3,7,8-TCDD shown to induce AHH activity in rats was 0.002 µg/kg (Kitchens and Woods 1979). Similarly, the induction of EROD was observed in the liver in Wistar rats (Abraham et al. 1988) and C57BL/6 mice (Harris et al. 1990) following subcutaneous and intraperitoneal injection, respectively. In female $B6C3F_1$ mice, administration of a single oral dose of $0.1 µg 2,3,7,8-TCDD/kg significantly increased liver, lung, and skin EROD activities and liver acetanilide-4-hydroxylase activity (CYP1A2 marker) (Diliberto et al. 1995). In the three tissues examined, induction of EROD was dose-dependent. Also in $B6C3F_1$ female mice, repeated oral administration of doses as low as 1.5 ng 2,3,7,8-TCDD/kg day significantly increased liver, lung, and skin EROD activities and liver acetanilide-4-hydroxylase activity (DeVito et al. 1994). In both studies (DeVito et al. 1994; Diliberto et al. 1995), liver, lung, and skin exhibited different sensitivities for enzyme induction. In male C57BL/6J and DBA/2J mice, the ED_{50}

values for induction of hepatic EROD after a single dose of 2,3,7,8-TCDD were 1.1 and 16 µg/kg, respectively (Weber et al. 1995). In an intermediate-duration dietary study in Sprague-Dawley rats, doses as low as 0.014 µg/kg/day induced both EROD and acetanilide-4-hydroxylase (Van Birgelen et al. 1995). Enzyme induction is a reversible process dependent on the dose and the dosing regime (Fan and Rozman 1995; Li and Rozman 1995). In male C57BL/6N mice, Pegram et al. (1995) showed that induction of acetanilide-4-hydroxylase by 2,3,7,8-TCDD was age-dependent, as it was significantly greater in old than in young mice.

In addition to altering the activities of enzymes from the MFO system in the liver, 2,3,7,8-TCDD also alters the activities of some key liver enzymes of the intermediary metabolism. These effects are intimately related with the wasting syndrome as discussed below (see Body Weight Effects). For example, 2,3,7,8-TCDD decreased the activities of hepatic PEPCK and G-6-Pase (key enzymes of gluconeogenesis) in mice and rats (Fan and Rozman 1995; Li and Rozman 1995; Viluksela et al. 1994; Weber et al. 1995) and also reduced the activity of TdO (key enzyme of tryptophan metabolism) in rats (Li and Rozman 1995; Viluksela et al. 1994), but not in mice (Weber et al. 1995).

Vitamin A (retinol) is essential for normal growth and cell differentiation, particularly for epithelial cells. 2,3,7,8-TCDD has been shown to decrease the storage of vitamin A in rodents. Decreased ability to store vitamin A (retinol) was found in rats and guinea pigs; however, partial recovery of the retinol content by week 16 postexposure was reported only in rats. A single oral dose of 2,3,7,8-TCDD caused a 70% reduction in the liver storage of retinol in rats when measured 2 months postexposure (Thunberg et al. 1979). The reduction was dose-related within the dose range studied (0.1–10 µg/kg) (Thunberg et al. 1980). Reduction of hepatic retinol by 2,3,7,8-TCDD was greater (87%) in younger rats with lower initial weights (Thunberg et al. 1984) than in more mature rats (60%) (Thunberg et al. 1979, 1980). In a 13-week dietary study in female Sprague-Dawley rats, dose of $0.014 µg/kg/day produced a dose-dependent reduction in hepatic retinol (Van Birgelen et al. 1995). In addition to reducing hepatic retinol storage, 2,3,7,8-TCDD exposure induced the activity of UDPGT and AHH in the rat, results obtained with other compounds (polychlorinated phenols, methylcholanthrene, phenobarbital) suggest that the effect is not mediated by the Ah receptor (Thunberg et al. 1984). Experiments with Sprague-Dawley rats showed that pretreatment with 2,3,7,8-TCDD influenced not only the storage, but also urinary and fecal excretion of a subsequent dose of radioactively labeled retinyl acetate. Retinol-content was also altered in various tissues; liver, intestine, and epididymis content decreased by 39–53, 19–67, and 18–44%, respectively, while renal content increased 3–30 times (Håkansson et al. 1989b). The 2,3,7,8-TCDD pretreated rats, though not

retinol-deficient, used a subsequently administered dose of retinyl acetate in a manner similar to retinol-deficient animals.

Mild-to-moderate hepatic effects of 2,3,7,8-TCDD exposure were also found after intermediate-duration dermal exposure in mice (Hebert et al. 1990; NTP 1982a), after acute intratracheal instillation in rats (Nessel et al. 1990, 1992), after acute intraperitoneal exposure in rats (DiBartolomeis et al. 1986; Mason and Safe 1986a), hamsters (Olson et al. 1980a), and guinea pigs (Gasiewicz and Neal 1979; Holcomb et al. 1988; Lu et al. 1986), and after acute subcutaneous exposure in mice (Courtney 1976). Some effects (increased plasma albumin, total protein) were considered to be secondary to progressive dehydration of exposed animals with decreased water consumption (Gasiewicz and Neal 1979).

Hepatotoxicity was observed in rats and mice chronically exposed by gavage to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980). Liver effects were also found in rats exposed by diet to HpCDD (Viluksela et al. 1994), 1,2,3,7,8-PeCDD and 1,2,3,4,7,8-HxCDD (Viluksela et al. 1998b), and OCDD for subchronic durations (Birnbaum et al. 1989a; Couture et al. 1988), and to 2,7-DCDD for a chronic duration (NCI/NTP 1979a).

It is clear that the liver is a target for 2,3,7,8-TCDD toxicity in animals. In humans, hepatic alterations have been observed sometimes following exposure to high 2,3,7,8-TCDD levels. In general, the effects are mild and transient, which might explain the negative findings of Calvert et al. (1992).

Renal Effects. The overall evidence from studies of populations exposed to high concentrations of 2,3,7,8-TCDD suggests that the kidney is not a target for 2,3,7,8-TCDD toxicity in humans (Stehr et al. 1986; USAF 1991; Wolfe et al. 1985).

Likewise, the kidney is not a target organ in adult animals. No effects were found in mice exposed acutely (Holsapple et al. 1986a; Weber et al. 1995) and in rats exposed chronically (Kociba et al. 1978a; NTP 1982b) to 2,3,7,8-TCDD by the oral route or in mice exposed by the dermal route (NTP 1982a). Similarly, no changes were found in rodents exposed chronically to 2,7-DCDD (NCI/NTP 1979a) or a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980) by the oral route.

Renal effects which were seen at near-lethal doses and considered secondary to frank toxicity included pale kidneys in minks after acute oral exposure to 2,3,7,8-TCDD (Hochstein et al. 1988), and enlarged

convoluted tubules and Bowman's spaces together with epithelial hyperplasia in rats (Christian et al. 1986) and monkeys (McConnell et al. 1978a) orally exposed for acute durations and in monkeys orally exposed for intermediate durations (Allen et al. 1977). An increased incidence of renal inflammatory changes recorded in mice after chronic oral exposure to 2,3,7,8-TCDD (NTP 1982b) was not a primary effect. Decreased glomerular filtration rate (Anaizi and Cohen 1978; Pegg et al. 1976) and increased tubular filtration rate (Anaizi and Cohen 1978) were reported in rats treated with a single intraperitoneal dose of 2,3,7,8-TCDD. The authors concluded that observed renal effects were probably secondary to the general toxic reaction to 2,3,7,8-TCDD (Pegg et al. 1976). However, renal effects (mainly hydronephrosis) were found in pups of 2,3,7,8-TCDD-exposed pregnant rodents (Abbott et al. 1987a, 1987b; Courtney 1976; Schwetz et al. 1973) indicating special ability of CDDs to induce effects in the developing kidneys.

Endocrine Effects. No biochemical evidence of thyroid dysfunction, as evaluated by serum levels of T4, triiodothyronine, and TSH, were reported in a group of 18 workers examined 17 years after an industrial accident during the manufacture of 2,4,5-T (Jennings et al. 1988). The small sample size, the fact that no measure of exposure was provided, and the long period of time between exposure and examination preclude any conclusion regarding possible effects of 2,3,7,8-TCDD. Zober et al. (1994) found a significant increase in the incidence of thyroid disease (no further details provided) 35 years after the BASF accident. An increased incidence of diabetes and subclinical decreases in thyroid function were found in Vietnam veterans who participated in operation Ranch Hand (USAF 1991).

A strong positive association was found between glucose intolerance or increased risk of diabetes and 2,3,7,8-TCDD serum levels (USAF 1991). The diabetes finding remained significant even after adjusting for body fat. Furthermore, subclinical effects in thyroid function (significant decrease in mean T3 % uptake and increases in mean TSH) were reported for Operation Ranch Hand veterans with high 2,3,7,8-TCDD serum levels (USAF 1991). However, the magnitude of the differences was not considered physiologically significant. Diabetes and glucose intolerance were also found in workers exposed occupationally (Pazderova-Vejlupkova et al. 1981; Sweeney et al. 1992). However, in the Sweeney et al. (1992) study, age and body mass index, both known risk factors for diabetes, appear to have a greater influence on the increase in both the risk of diabetes and elevated fasting serum glucose levels than 2,3,7,8-TCDD level. A follow-up study of Operation Ranch Hand veterans confirmed earlier findings of glucose abnormalities and increased risk of diabetes mellitus in exposed subjects (Henriksen et al. 1997). Furthermore, a follow-up of Seveso residents found a significant increase in deaths from diabetes among women from zone B (Pesatori et al. 1998).

The evidence available from epidemiological studies suggests that exposure to high concentrations of CDDs may induce long-term alterations in glucose metabolism and subtle alterations (of unknown clinical relevance) in thyroid function.

Numerous studies in rodents have reported alterations in thyroid status after exposure to 2,3,7,8-TCDD. End points commonly examined included serum levels of T4, T3, TSH, and activity of hepatic microsomal UDPGT, an enzyme which increases glucuronation of T4 and clearance. The effects on T4 levels are dose-dependent and also appear to be species-dependent. For example, serum T4 was decreased in rats after acute (Bastomsky 1977; Fan and Rozman 1995; Gorski and Rozman 1987; Henry and Gasiewicz 1987; Hermansky et al. 1988; Potter et al. 1986) and intermediate exposure (Li and Rozman 1995; Sewall et al. 1995; Van Birgelen et al. 1995; Viluksela et al. 1994). In contrast, the response of serum T3 levels ranged from increased (Bastomsky 1977; Potter et al. 1986) to no change or inconsistent change (Fan and Rozman 1995; Henry and Gasiewicz 1987; Potter et al. 1983; Sewall et al. 1995) to decreased (Pazdernik and Rozman 1985). In hamsters, a species less susceptible to 2,3,7,8-TCDD toxicity than rats, T4 serum levels were increased by 2,3,7,8-TCDD even though hepatic microsomal UDPGT activity was significantly increased, suggesting that mechanisms other than induction of this enzyme must account for the species-specific alterations in T4 (Henry and Gasiewicz 1987). A further species-specific response was noted by Weber et al. (1995) who reported that in C57BL/6J and DBA/2J mice, both T4 and T3 levels were decreased in a parallel fashion as a result of a single dose of 2,3,7,8-TCDD. According to Weber et al. (1995), the decrease in T3 in mice would reduce the *de novo* synthesis of fatty acids, thus improving the balance of metabolic energy which might explain, at least in part, the reduced susceptibility of mice to 2,3,7,8-TCDD toxicity compared to rats. It is interesting to note that Long Evans rats exhibited a dose-related increase in T4 and T3 90 days after single doses of 2,3,7,8-TCDD that decreased T4, but did not significantly alter T3 4 days after dosing (Fan and Rozman 1995). The significance of this finding was not entirely clear, but according to the authors it indicated that sustained effects of 2,3,7,8-TCDD on thyroid homeostasis trigger adaptive responses which persist even after most of the 2,3,7,8-TCDD has been cleared. Decreased levels of serum T4 have also been reported in rats administered other CDD congeners such as 1,2,3,7,8-PeCDD and 1,2,3,4,7,8-HxCDD in intermediate exposure duration studies (Viluksela et al. 1998b).

The reduction in circulating T4 levels observed in rats appears, in part, to be due to the increased activity of UDPGT (Bastomsky 1977; Sewall et al. 1995), but other possibilities have also been discussed. McKinney et al. (1985b) proposed that T4 and T3 might be endogenous ligands for the Ah receptor, and

that 2,3,7,8-TCDD might be an agonist for the T4 receptor. Although some evidence was presented in support for this hypothesis, the weight of evidence, summarized by Goldstein and Safe (1989), argues against 2,3,7,8-TCDD being a thyroid agonist. Lans et al. (1993, 1994) have explored the possibility that hydroxylated 2,3,7,8-TCDD metabolites competitively interact with plasma thyroid hormone transport proteins, thus facilitating clearance and excretion of T4. They tested various hydroxy-CDDs in an *in vitro* competitive-binding assay using purified human TTR and found that those with chlorine substitution adjacent to the hydroxy group (7-OH-2,3,8-TrCDD and 2-OH-1,3,7,8-TCDD) showed similar or higher relative-binding potency than T4 (Lans et al. 1993). 8-OH-2,3-DCCD, which did not contain chlorine substitution adjacent to the OH group, did not displace T4. In a subsequent study, Lans et al. (1994) studied the displacement of T4 from globulin, the major T4-binding protein in humans by hydroxy CDD metabolites (in contrast to TTR in rodents). The results showed than none of the tested hydroxylated CDD metabolites inhibited binding of T4 by T4-binding globulin and suggested that hydroxylated CDD metabolites can cause different effects in rodents and humans.

The overall evidence suggests that in rodents, thyroid hormones modify 2,3,7,8-TCDD toxicity, but a reduction in T4 (at least in rats) does not mediate toxicity.

Administration of 2,3,7,8-TCDD to rodents was also shown to reduce blood corticosterone levels (Balk and Piper 1984; DiBartolomeis et al. 1987; Mebus and Piper 1986). This effect has been attributed to decreased corticosterone synthesis by decreasing cholesterol side-chain cleavage in the adrenal gland. More recent studies suggested that 2,3,7,8-TCDD may interfere with secretion or synthesis of appropriate, bioactive ACTH from the anterior pituitary gland, which could compromise adrenal steroidogenenesis (Bestervelt et al. 1993).

Administration of 2,3,7,8-TCDD to animals results in a wide range of endocrine responses which are not only species-dependent, but also exhibit variability within species. Endocrine effects observed in humans have not been limited to thyroid effects and diabetes; alterations in levels of reproductive hormones, as summarized in the sections on reproductive effects have also been observed. The wide array of endocrine effects induced by CDDs and structurally-related chemicals has triggered increased interest within the scientific community and the term “endocrine disruptors” is currently being used to describe some members of this class of chemicals. The available information suggests that CDDs may cause adverse endocrine effects in humans.

Dermal Effects. Chloracne is the most easily recognized effect of exposure to 2,3,7,8-TCDD and structurally related chlorinated organic chemicals. Chloracne is a high-dose response in animals and humans; and its presence in humans indicates exposure to CDDs and other chlorinated organic compounds, but its absence does not preclude such exposure. Furthermore, the variability of the response in more highly exposed individuals suggests that susceptibility varies among individuals. Chloracne can first occur on the face, particularly under the eyes and behind the ears. With increasing exposure, the rest of the face and neck, upper arms, chest, back, abdomen, outer thighs, and genitalia may be affected. When severe, chloracne can cover the entire body. Clinically, changes vary from an eruption of comedones to occurrence of papules and pustules. Histologically, the lesions consist of keratinous cysts caused by squamous metaplasia of sebaceous glands. The acute stage is followed by vermiculite skin atrophy. The incidence of other dermal effects, including hyperpigmentation and hirsutism, correlates with the intensity of chloracne (Poland et al. 1971). Chloracne has been reported to have occurred in at least a small number of workers in all accidents at TCP-production facilities (Jansing and Korff 1994; May 1973; Schecter et al. 1993; Suskind 1985; Zober et al. 1990); among subjects involved in production of 2,3,7,8-TCDD-contaminated products (Bond et al. 1989a; Moses and Prioleau 1985; Pazderova-Vejlupkova et al. 1981; Poland et al. 1971; Suskind and Hertzberg 1984); in laboratory workers exposed to 2,3,7,8-TCDD (Oliver 1975); and among a small percentage of Seveso residents (Assennato et al. 1989; Caramaschi et al. 1981; Mocarelli et al. 1986; Reggiani 1980). Chloracne, however, was not observed among Missouri residents (Hoffman et al. 1986; Webb et al. 1989) examined 10 years after exposure or among Ranch Hand veterans (Burton et al. 1998; USAF 1991).

The dermal changes induced by 2,3,7,8-TCDD may appear as soon as 2 days after exposure (Ott et al. 1993; Zober et al. 1990) or within months (Caramaschi et al. 1981; Reggiani 1980). The lesions may heal within a few months after cessation of exposure (Assennato et al. 1989) despite high serum 2,3,7,8-TCDD levels (Mocarelli et al. 1991) or persist for over 15 years, depending upon severity (Crow 1978; Jansing and Korff 1994; Moses and Prioleau 1985; Schecter et al. 1993; Suskind and Hetzberg 1984). Children exposed to 2,3,7,8-TCDD appear to be more sensitive than adults, and individuals similarly exposed have variable susceptibility to chloracne (Mocarelli et al. 1991). Data from analyses of cases among chemical workers suggested that the risk for developing chloracne was highest among workers who were exposed at younger ages, among those who had been exposed for the longest periods, and among workers whose jobs rated at the highest intensity of exposure (Ott et al. 1987). The variability in the chloracneic response can be illustrated the following evidence from the Seveso incident: no chloracne was observed in subjects with initial serum lipid 2,3,7,8-TCDD levels of <800 ppt, chloracne was present at serum lipid levels of

>12,000 ppt; and between 800 and 12,000 ppt the occurrence of chloracne was sporadic (Mocarelli et al. 1991); this suggested that, in this population, 8,000 to 10,000 ppt 2,3,7,8-TCDD in blood was necessary for expression of chloracne. German workers involved in TCP production who had chloracne had estimated adipose levels $200 ppt 2,3,7,8-TCDD and $2,000 ppt HxCDD at the time of diagnosis (Beck et al. 1989a). In another study of German workers, 80% of the severe chloracne cases had 2,3,7,8-TCDD levels of $250 ppt; however, 26% of the workers without chloracne also had 2,3,7,8-TCDD levels of $250 ppt (Ott et al. 1993). Schecter et al. (1993) provided the first reported incidence of chloracne in females with elevated dioxin blood levels from occupational exposure. Their observation that one worker diagnosed with chloracne in their study had the lowest 2,3,7,8-TCDD blood concentration, whereas two workers with the higher levels did not display chloracne, confirmed the view that chloracne indicates exposure to dioxin, but its absence does not preclude such exposure.

No studies were located regarding dermal effects in humans exposed specifically to CDDs by the oral route. Evidence from human case reports in the Yusho/Yu-Cheng incidents (which involved exposure to CDFs, PCBs, and CDDs) and from animal studies, however, indicates that dermal effects could occur after exposure by the oral route (ATSDR 1994).

Oral studies of 2,3,7,8-TCDD showed the development of rough hair in hamsters (Henck et al. 1981) and skin thickening in A2G-hr/+ mice (Greig 1984) after acute exposure. Chronic oral exposure to 2,3,7,8-TCDD caused dermatitis in B6C3F_1 mice (Della Porta et al. 1987) and amyloidosis in Swiss mice (Toth et al. 1979). Rhesus monkeys proved to be very sensitive to 2,3,7,8-TCDD-induced dermal effects. The changes consisted of swollen eyelids, nail loss, facial alopecia, and acneform lesions after both acute- (McConnell et al. 1978a) and intermediate-duration oral exposures (Allen et al. 1977; McNulty 1984). Dermal exposure to 2,3,7,8-TCDD induced hyperkeratosis and epidermal hyperplasia in hairless HRS/J mice after acute- (Puhvel and Sakamoto 1988) and intermediate-duration (Puhvel et al. 1982) exposures. While acneiform lesions were reported in CD-1 mice after intermediate-duration dermal exposure to 2,3,7,8-TCDD (Berry et al. 1978, 1979), no effects were found in Swiss Webster mice chronically exposed to lower levels (NTP 1982a).

The data available suggest that 2,3,7,8-TCDD is a dermal toxicant both in humans and animals. Erythematous skin rashes and chloracne are considered one of the hallmarks of 2,3,7,8-TCDD toxicity, although it can be caused also by exposure to other polyhalogenated aromatic compounds. It is also worth

mentioning that although in humans chloracne indicates exposure to chlorinated or halogenated aromatics, lack of chloracne does not indicate that exposure has not occurred.

Ocular Effects. The incidence of eye irritation correlated with the intensity of chloracne in a study of workers employed in a 2,4,5-T factory (Poland et al. 1971), but the role of 2,3,7,8-TCDD, if any, cannot be established. No studies were located regarding ocular effects in humans exposed specifically to CDDs by the oral route. Evidence from the human case reports in the Yusho/Yu-Cheng incidents (which involved exposure to CDFs, PCBs, and less so CDDs) and from animal studies, however, indicates that ocular effects could occur after exposure by the oral route (ATSDR 1994). Ocular effects observed in the Yusho and Yu-Cheng victims included hypersecretion of the Meibomian glands, abnormal pigmentation of the conjunctiva, and swelling of the eyelids (Hsu et al. 1994; Masuda 1994).

Topical application of 2,7-DCDD, with no toxic 2,3,7,8-chlorine pattern, 2,3,7,8-TCDD, and mixed HxCDD, or OCDD into the conjunctival sac of rabbits caused transient pain and conjunctival inflammation (Schwetz et al. 1973).

Based on adverse ocular effects observed in humans and animals exposed to chemicals structurally-related to CDDs and animals (monkeys) exposed to 2,3,7,8-TCDD itself, it is reasonable to assume that CDDs will cause similar effects under similar exposure conditions.

Body Weight Effects. A transient weight loss was reported in a small number of subjects exposed to 2,3,7,8-TCDD in the workplace (Jirasek et al. 1976; Oliver 1975). However, due to the lack of data from controlled studies, the role of 2,3,7,8-TCDD, if any, is difficult to ascertain. Although weight loss has not been well documented in humans following exposure to 2,3,7,8-TCDD, numerous animal studies provide evidence that exposure to CDDs causes the wasting syndrome. Acute oral exposure to 2,3,7,8-TCDD induced weight loss in rats (Christian et al. 1986a; Moore et al. 1985; Roth et al. 1988; Seefeld and Peterson 1984; Seefeld et al. 1984a, 1984b; Walden and Schiller 1985), mice (Hanberg et al. 1989; Kelling et al. 1985; Smith et al. 1976; Weber et al. 1995), guinea pigs (Hanberg et al. 1989; Umbreit et al. 1985), hamsters (Hanberg et al. 1989), and monkeys (McConnell et al. 1978a). Similarly, body weight changes were found after intermediate-duration oral exposure to 2,3,7,8-TCDD in rats (Diliberto et al. 1996; NTP 1982b; Van Birgelen et al. 1995; Viluksela et al. 1994; Vos et al. 1973), guinea pigs (DeCaprio et al. 1986; Vos et al. 1973), mice (Thigpen et al. 1975; Vos et al. 1973), and monkeys (McNulty 1984), and after chronic exposure in rats (Kociba et al. 1978a; NTP 1982b; Van Miller et al. 1977) and mice (Della

Porta et al. 1987). In addition, milder changes, represented usually by decreases in body weight gain, were seen after oral exposure to other congeners (NCI/NTP 1979a, 1980; Schwetz et al. 1973). The wasting syndrome does not appear to be a route-specific effect since body weight changes were observed in mice exposed to 2,3,7,8-TCDD dermally (NTP 1982a; Puhvel et al. 1982), and numerous studies reported body weight changes in animals that were injected with 2,3,7,8-TCDD subcutaneously or intraperitoneally (Canga et al. 1988; Chahoud et al. 1989; Della Porta et al. 1987; Gorski et al. 1988b; Holcomb et al. 1988; Kelling et al. 1985; Lu et al. 1986; McConkey and Orrenius 1989; Pohjanvirta et al. 1989).

The mechanism of the wasting syndrome has been extensively investigated. Results of studies in C57BL/6 mice, guinea pigs, and Fischer 344 rats showed that 2,3,7,8-TCDD exposure induces appetite suppression resulting in loss of adipose and lean tissue, and eventually death (Kelling et al. 1985). However, by using pair-fed animals as controls, it was clear that body weight loss alone was not the cause of death. This is also supported by the fact that the weight loss, but not the lethality of 2,3,7,8-TCDD, can be prevented by parenteral feeding of rats and guinea pigs (Gasiewicz et al. 1980; Lu et al. 1986). Seefeld and Peterson (1984) showed that in rats, fecal energy loss as a percentage of daily feed energy uptake was not significantly altered by treatment with 2,3,7,8-TCDD. Furthermore, the percentage of feed energy absorbed by the gastrointestinal tract was not changed by 2,3,7,8-TCDD, which ruled out the possibility of a 2,3,7,8-TCDD-induced gross malabsorption syndrome. The same group of investigators (Seefeld et al. 1984a, 1984b) showed that 2,3,7,8-TCDD does not impair the animals' capacity to feed since rats that lost weight prior to treatment with 2,3,7,8-TCDD ate and gained weight after treatment with 2,3,7,8-TCDD. Based on their results, Seefeld and coworkers (Seefeld and Peterson 1984; Seefeld et al. 1984a, 1984b) proposed that 2,3,7,8-TCDD lowers a “set point” for regulated body weight, and hypophagia serves as a secondary response to reduce the animal’s body weight to the lower regulation level determined by the dose of 2,3,7,8-TCDD administered. The ability of an animal to recover from the 2,3,7,8-TCDD-induced hypophagia may be species- and/or strain-specific (Tuomisto and Pohjanvirta 1991). Within 1-2 weeks after a single dose of 2,3,7,8-TCDD, feed intake increased in Hans/Wistar rats but not in Long Evans rats; the Long Evans rats died by week 3. Although Hans/Wistar and Long Evans rats have similar Ah receptor binding and cytochrome P-450 induction properties, the wide differences in sensitivity to 2,3,7,8-TCDD suggest that other mechanisms may be involved in the wasting syndrome.

Numerous studies have examined the possibility that the wasting syndrome results from 2,3,7,8-TCDD-induced alterations in intermediate metabolism. For example, it has been shown that in male Sprague-Dawley rats, lethal doses of 2,3,7,8-TCDD severely alter glucose homeostasis (Gorski and Rozman 1987;

Gorski et al. 1990; Potter et al. 1983). Hypoglycemia was not the result of hyperinsulinemia since insulin levels were also depressed with 2,3,7,8-TCDD treatment (Potter et al. 1983). Also, hypophagia did not account for hypoglycemia since pair-fed rats also exhibited hypoglycemia (Potter et al. 1983). Further studies showed that decreased gluconeogenesis was the result of significantly reduced activity of hepatic PEPCK, the rate-determining enzyme in the pathway (Weber et al. 1991a). Other gluconeogenic enzymes such as glucose-6-phosphatase and pyruvate carboxylase were also decreased by treatment with 2,3,7,8-TCDD, but pyruvate kinase, a glycolytic enzyme, was not affected (Weber et al. 1991a). It was also shown that changes in gluconeogenic enzyme activities preceded hormonal changes (insulin, corticosterone) by at least 2 days (Weber et al. 1991b), which led the authors to suggest that 2,3,7,8-TCDD-induced changes in hormonal homeostasis are adaptive responses of the organism to stimulate gluconeogenesis. Reduced liver PEPCK activity as a result of 2,3,7,8-TCDD treatment has also been observed in Long Evans rats (Fan and Rozman 1995) and in C57BL/6J and DBA/2J mice (Weber et al. 1995).

Some investigators suggested that the wasting syndrome may be linked to 2,3,7,8-TCDD-induced effects on the thyroid (Rozman 1984; Rozman et al. 1985). In thyroidectomized rats, the weight loss after 2,3,7,8-TCDD exposure was slow, suggesting that the lack of thyroid hormone reduced the rate of stored fat utilization (Rozman et al. 1985). Thyroidectomy protected rats from immunotoxicity induced by an intraperitoneal dose of 2,3,7,8-TCDD (Pazdernik and Rozman 1985). Replacement therapy with T4 partially reversed the effects of thyroidectomy on T4 and triiodothyronine serum levels, body weight, and immune function. The authors suggested that 2,3,7,8-TCDD-induced hypothyroidism may be a protective mechanism against 2,3,7,8-TCDD-induced wasting syndrome and lethality. Thyroid hormones regulate fat mobilization and use of fatty acids in adipose tissue and influence norepinephrine-mediated nonshivering thermogenesis that is also linked to brown adipose tissue. It was also suggested that the effect of 2,3,7,8-TCDD on the thyroid causes activation of thyrotropin-releasing hormone, which results in anorexia (Aust 1984). Anorexia and 2,3,7,8-TCDD-induced retinol depletion would then lead to the body weight loss.

Based on the findings that 2,3,7,8-TCDD administered into the lateral cerebral ventricles does not cause death or decreased feed intake in rats (Stahl and Rozman 1990), Rozman et al. (1991) examined the possibility that 2,3,7,8-TCDD suppresses appetite via peripheral mechanisms acting on the central nervous system. The results of experiments of transfusion of blood from rats with 2,3,7,8-TCDD-induced appetite suppression and normal satiated rats suggested that 2,3,7,8-TCDD-treated rats are not satiated, rather than

that they are not hungry. In a second experimental series, the possible role of norepinephrine, dopamine, and serotonin as central mediators of appetite suppression induced by 2,3,7,8-TCDD was investigated. No changes were found in epinephrine and dopamine in the hypothalamus or in dopamine and its metabolites in the striatum. However, tryptophan (a precursor of serotonin) levels in plasma and brain were increased and this was paralleled by increases in brain serotonin and 5-hydroxyindolacetic acid (the major serotonin metabolite) (Rozman et al. 1991). Based on the results of these experiments, Rozman et al. (1991) proposed that decreased PEPCK activity decreases gluconeogenesis and leads to increased plasma concentrations of glycogenic amino acids, such as tryptophan. Increased tryptophan leads to increase in serotonin release in the brain and to appetite suppression. It was subsequently shown, however, that lethal doses of 2,3,7,8-TCDD reduces the activity of TdO, the key enzyme of the major tryptophan degradation pathway (Weber et al. 1992c, 1994). Whether due to reduction in TdO activity, reduced glyconeogenesis, or both, Weber et al. (1994) proposed that an initial increase in tryptophan levels result in some initial feed refusal, which in turn initiates the wasting of body mass and increases the supply of tryptophan with which the animals cannot deal. A vicious cycle develops which results in strongly elevated tryptophan levels and increased serotonin turnover, which acts as an appetite suppressant.

Alternative explanations for the increased levels of plasma-free tryptophan in 2,3,7,8-TCDD-treated rats have been offered. Four possibilities were discussed by Unkila et al. (1994): 2,3,7,8-TCDD may reduce the binding capacity of the blood, i.e., may decrease plasma albumin levels; 2,3,7,8-TCDD may stimulate the production of some competing factor in the blood (e.g., nonesterified fatty acids or bilirubin) which are also bound to albumin; 2,3,7,8-TCDD might affect the binding properties of the albumin molecule; and 2,3,7,8-TCDD might inhibit tryptophan catabolism. Of the four factors examined that might affect the binding of tryptophan to albumin, Unkila et al. (1994) indicated that the most important is probably plasma bilirubins and suggested that disturbances in liver function may be involved in the changes in tryptophan metabolism.

The wasting syndrome is a characteristic effect of exposure to 2,3,7,8-TCDD in animals and, in its most severe form, is usually associated with lethality, particularly in rodents. The fact that the wasting syndrome has not been demonstrated in humans does not necessarily indicate that humans are insensitive to this effect of dioxins, but may indicate that human exposure has not approached acutely high enough levels.

Immunological and Lymphoreticular Effects. No consistent exposure-related effects on the immune system have been observed in human populations exposed to above-background levels of 2,3,7,8-TCDD (Ernst et al. 1998; Jansing and Korff 1994; Jennings et al. 1988; Jung et al. 1998; Mocarelli et al. 1986; Neubert et al. 1993, 1995; Svensson et al. 1994; Tonn et al. 1996; USAF 1991; Webb et al. 1989; Zober et al. 1994). The immunological effects of 2,3,7,8-TCDD were recently reviewed by Kerkvliet (1995) who identified a number of factors on which the results of immunological assessments may be dependent. One of these factors, perhaps the most notable, is the inherent difficulty in assessing subclinical immunological effects in an outbred human population. In addition, the wide range of normal responses of most immunological assays diminishes the sensitivity to detect small changes. Another factor to consider is that assays used to assess immune function in humans exposed to 2,3,7,8-TCDD and related chemicals have been based for the most part on what was clinically feasible rather than on assays proven to be sensitive in animal studies (i.e., the antibody response to SRBC). Therefore, the lack of consistent or significant immunotoxic effects in humans exposed to 2,3,7,8-TCDD may be a function of both the type of assay and the immune status of the population studied. Furthermore, often the cohort exposure is not validated and the immune status has been examined long after exposure allowing for recovery from any immunotoxic effect that may have occurred shortly after exposure.

A potentially useful approach to studying the sensitivity of the human immune system to 2,3,7,8-TCDD has been to examine the direct *in vitro* effects of 2,3,7,8-TCDD on human cell cultures. For example, Cook et al. (1987a) observed concentration dependent immunosuppressive responses of cultured human thymic epithelial cell and thymocytes exposed to 2,3,7,8-TCDD. The proliferative response of human lymphocytes *in vitro* to stimulation with mitogens is extremely sensitive to 2,3,7,8-TCDD. Concentrations of 2,3,7,8-TCDD as low as 10^{-12} to 10^{-14} M reduced the percentage of $CD20^+$ B cells and $CD4^+CDw29^+$ T cells (Neubert et al. 1991). However, these results could not be corroborated in a similar study by Lang et al. (1994) who used 2,3,7,8-TCDD concentrations ranging from 10^{-7} to 10^{-11} M. In another study, proliferation of human tonsillar lymphocytes (HTLs) cultured *in vitro* was inhibited by 3×10^{-8} M 2,3,7,8-TCDD, but pokeweed mitogen (PWM) induced proliferation was not affected by 2,3,7,8-TCDD concentrations ranging from 3×10^{-8} to 10^{-10} M (Wood et al. 1992). However, when low density ß cells from HTLs were purified and cultured *in vitro* in the same laboratory and stimulated with lipopolysaccharide and TRF (T-cell replacing factor), 3×10^{-8} to 10^{-10} M 2,3,7,8-TCDD suppressed the IgG secretion in a dose related manner. HTLs possess the Ah receptor as indicated by the induction of 7-ethoxycoumarin-o-deethylase (EROD) in a dose-related manner at the above doses when the HTLs are stimulated with phytohemagglutenin (PHA) or PWM (Wood et al. 1993). A promising animal model for

assessing the potential immunotoxicity of CDDs in humans is the SCID mice, which can be engrafted with human fetal thymus and liver tissue fragments under the kidney capsule. Using the SCID mice model, it was shown that human thymus cells are as sensitive to 2,3,7,8-TCDD as the thymus of Wistar rats (De Heer et al. 1995).

The immune system appears to be one of the most sensitive targets for CDDs in animals. However, it is difficult to make interspecies or congener comparisons due to interlaboratory variability of functional tests and use of different end points in various studies. Thymic atrophy was observed in rats, mice, guinea pigs, (De Heer et al. 1994a; Hanberg et al. 1989), hamsters (Hanberg et al. 1989; Olson et al. 1980a), and monkeys (McConnell et al. 1978a) after acute exposure; in guinea pigs (Vos et al. 1973) after intermediate-duration exposure; and in rats (Kociba et al. 1978a) after chronic oral exposure to 2,3,7,8-TCDD. Furthermore, lymph node atrophy (Allen et al. 1977) and bone marrow degeneration (Hong et al. 1989) were reported in monkeys after intermediate- and chronic-duration exposure to 2,3,7,8-TCDD, respectively. In support of these data, thymic atrophy was also induced by a single intraperitoneal injection of 2,3,7,8-TCDD in Sprague-Dawley rats (Gorski et al. 1988b), Syrian hamsters (Olson et al. 1980a), and C57BL/6J mice (Poland and Glover 1980). Only increased thymus/body weight ratio was found in HRS/J mice exposed to 2,3,7,8-TCDD dermally (Hebert et al. 1990). Effects on peripheral lymphocytes following acute subcutaneous and *in vitro* exposure, particularly changes in percentages of lymphocyte subpopulations, suggest that marmoset monkeys may be particularly sensitive to immunologic effects of 2,3,7,8-TCDD (Neubert et al. 1990a, 1991). In general, relatively high doses cause lymphoid depletion, lower doses cause thymic cellular depletion in young animals, and much lower doses affect specific immune receptor functions.

Administration of total parenteral nutrition did not protect rats from thymic atrophy with decreased numbers of cortical lymphocytes that developed after acute intraperitoneal 2,3,7,8-TCDD exposure (Gasiewicz et al. 1980). 2,3,7,8-TCDD-induced thymic atrophy in BALB/CJ and DBA/2J mice correlated with a reduction in thymic and bone marrow terminal deoxynucleotidyl transferase synthesis (Fine et al. 1990b). The prothymocyte activity was severely damaged by 2,3,7,8-TCDD exposure. The authors concluded that 2,3,7,8-TCDD produces atrophy by damaging the capability of bone marrow prethymic stem cells to seed the thymus. In addition to bone marrow effects, 2,3,7,8-TCDD may also inhibit normal thymocyte maturational processes. When $B6C3F_1$ mice were exposed *in utero* by dosing the dam at 3 µg/kg/day between Gd 6–14 and foster-nursing the offspring with unexposed females, thymic atrophy was seen at Gd 18 or on postnatal day 6, but the thymic effects were no longer seen by day 14. *In utero*

effects on the thymus were at much lower doses than effects in animals exposed postnatally. Rodents are born with an immature immune system which develops in the first few days after birth. These mice were tested for immune function at 7–8 weeks of age; the cytotoxic T-lymphocyte was still suppressed, but the mitogen response to SRBC was not suppressed (Holladay et al. 1991). Humans, in contrast to rodents, have a more mature immune system at birth. The role of the thymus is important in prenatal and perinatal development of the immune system, but its role in adult life has not been established. Concentrations of 140 fg 2,3,7,8-TCDD/mg in the thymus of mice at Gd 18 were associated with thymic atrophy (Fine et al. 1990a).

In addition to causing lymphoid organ weight changes, 2,3,7,8-TCDD has been shown to cause functional alterations in the immune response (Vecchi et al. 1980a). Studies have shown that suppressed antibody response (Holsapple et al. 1986a; Vecchi et al. 1980a, 1983b) decreased host resistance to *Streptococcus pneumoniae* or influenza A virus (Burleson et al. 1996; White et al. 1986), and suppressed serum complement activity (White et al. 1986) occur in $B6C3F_1$ mice after single or repeated oral dose(s) of 2,3,7,8-TCDD. Immunological effects occurred at the lowest LOAEL in acute- and intermediate-duration exposure studies and indicated that the immunological system is very sensitive to 2,3,7,8-TCDD-induced toxicity. The dose of 0.01 µg/kg for impaired resistance was the lowest LOAEL for acute oral exposure (Burleson et al. 1996). The NOAEL of 0.005 µg/kg identified in this study was used to derive an acute oral MRL of 2×10^{-4} µg/kg/day. The 0.0007 µg/kg/day dose for reduced thymus weight for intermediate-duration oral exposure (DeCaprio et al. 1986) was used to derive an intermediate-duration oral MRL for 2,3,7,8-TCDD of 2×10^{-5} µg/kg/day. Immunosuppression as evidenced by increased mortality when challenged with bacteria was demonstrated in C57BL/6J mice after administration of a dose of 1 µg 2,3,7,8-TCDD/kg/week over a period of 4 weeks (Thigpen et al. 1975); this occurred without any other apparent signs of toxicity. In addition, an intermediate-duration exposure to 2,3,7,8-TCDD induced decreased cell-mediated (mice and guinea pigs) and humoral (guinea pigs) immunity (Vos et al. 1973). The results indicated that guinea pigs are the most sensitive species tested. 2,3,7,8-TCDD-induced suppression of humoral immunity was also reported in animals exposed parenterally.

In general, the route of exposure does not affect the immune response. Several tests of immunotoxicity dosed the animals by parenteral routes (intravenous, subcutaneous, or intraperitoneal). Acute intraperitoneal 2,3,7,8-TCDD exposure inhibited the primary and secondary humoral response to T-dependent (SRBC) and T-independent (pneumococcal polysaccharide) antigens in C57BL/6 mice (Vecchi et al. 1980b, 1983b); doses were comparable to those causing effects by the oral route. A dose-

related suppression of IgM and IgG antibody-forming cells was induced by exposure to a single intraperitoneal injection of 2,3,7,8-TCDD in B6C3F_1 mice (House et al. 1990). Furthermore, doses as low as 0.1 µg/kg decreased survival after influenza virus infection, and exposure at 10 µg/kg suppressed production of antibody to viral hemagglutinin. Cytolytic and NK-cell-mediated immunity was impaired in C57BL/6 mice after a single intraperitoneal injection of 2,3,7,8-TCDD due to the decreased number of peritoneal macrophages and splenocytes (Mantovani et al. 1980). However, the immune function per unit was not damaged. In addition, an *in vitro* study demonstrated that 2,3,7,8-TCDD induces tumor necrosis factor in human keratinocytes that could affect tumor promotion and affect immune parameters (Choi et al. 1991).

The role of the Ah receptor in the immune responses to CDDs has been examined in several studies. A correlation between the AHH inducibility and suppression of humoral immunity caused by 2,3,7,8-TCDD injection was observed in several strains of mice (Vecchi et al. 1983a). Similarly, when three strains of Ah responsive mice (C57BL/6nQdj, BALB/cCrj, C3H/HeNQdj) were compared with nonresponsive (AKR/JSea, DBA/2JCrj, DDD:Qdj) strains of mice, decreased thymus weight was found only in the responsive animals (Nagayama et al. 1989). The C57 strain also had decreased lymphocyte counts. Results of an *in vitro* experiment supported these observations (Dencker et al. 1985). Thymus cultures from Ah locus responsive C57BL/6 mice were very sensitive to the toxicity of 2,3,7,8-TCDD compared with thymus cultures from the nonresponsive DBA/2J mice. 2,3,7,8-TCDD exposure of cultures of thymic epithelial cells from responsive C57BL/6 mice indicated that 2,3,7,8-TCDD alters the maturation of thymocytes (Greenlee et al. 1985). It was further demonstrated that 2,3,7,8-TCDD toxicity in human thymic epithelial cells was mediated by a protein receptor (Cook and Greenlee 1989). Similarly, *in vitro* studies with lymphocytes, spleen cells, and bone marrow cells from 2,3,7,8-TCDD-pretreated mice indicated that 2,3,7,8-TCDD acts by an Ah locus-dependent mechanism to obstruct the formation of cytotoxic T cell generation from their precursors (Dooley et al. 1990; Holladay et al. 1991; Nagarkatti et al. 1984). A brief summary of the possible mechanisms of 2,3,7,8-TCDD immunotoxicity can be found in Section 2.4.2.

Oral experiments with other congeners reported suppressed antibody response in B6C3F_1 mice after acute exposure to 2,7-DCDD, a non 2,3,7,8-chlorine substituted CDD (Holsapple et al. 1986b) and splenic hyperplasia in rats after intermediate-duration exposure to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980).

Although human exposure studies to date found no conclusive evidence of immunotoxicity, the animal data show that the immune system is a target for CDD toxicity in many species. However, a defined 2,3,7,8-TCDD-induced immune deficiency syndrome has not emerged largely because in animals, the immune response depends on the species studied, the dose of 2,3,7,8-TCDD, and the antigen and exposure protocol studied.

Neurological Effects. Some psychological effects were reported in Vietnam veterans potentially exposed to 2,3,7,8-TCDD-contaminated herbicides. These included depression in Air Force and ground troop veterans (Levy 1988; Wolfe et al. 1985) and hypochondria and hysteria in Air Force veterans (Wolfe et al. 1985). In contrast, a more recent study did not find any association between 2,3,7,8-TCDD exposure and neurological or psychological diseases in Air Force personnel (USAF 1991). These psychological effects could be due to a number of stress-related factors in the veterans. Recently, a group of 16 scientific experts from the National Academy of Sciences' Institute of Medicine, who evaluated the strength of evidence for human health effects among veterans exposed to herbicides used in Vietnam, found no strong evidence establishing an association between herbicide use in Vietnam and clinical neurologic disorders (Goetz et al. 1994). However, psychological changes were reported in relatively small cohorts of exposed individuals (Oliver 1975; Pazderova-Vejlupkova et al. 1981; Peper et al. 1993). Subclinical peripheral neuropathy, encephalopathy, and sensory impairment were reported in workers exposed to higher levels of 2,3,7,8-TCDD (Goldman 1973; Jirasek et al. 1976; Pazderova-Vejlupkova et al. 1981) and in the general population exposed to 2,3,7,8-TCDD after an industrial accident (Barbieri et al. 1988; Filippini et al. 1981; Pocchiari et al. 1979). Decreased nerve conduction velocity was observed in phenoxy herbicide production workers (Singer et al. 1982). In contrast, exposure to 2,3,7,8-TCDD (confirmed by elevated serum levels) was not related to chronic peripheral neuropathy in a group of workers exposed 15–37 years earlier compared to referent controls (Sweeney et al. 1993). These authors suggest that the finding of peripheral neuropathy in the earlier studies indicate that this condition may occur shortly after exposure and resolve over time.

Data regarding neurological or neurophysiological effects following exposure to CDDs in animals are limited. Decreased motor activity was seen in rats at 2,3,7,8-TCDD dose levels of #5 µg/kg (Giavini et al. 1983; Seefeld et al. 1984a). Time-dependent increases in tryptophan (amino acid precursor of the neurotransmitter serotonin) levels in plasma and brain (hypothalamus, striatum) correlated with elevations in brain serotonin and 5-hydroxyindoleacetic acid levels in rats after a single intraperitoneal injection of 50 or 120 µg 2,3,7,8-TCDD/kg (Rozman et al. 1991; Tuomisto et al. 1990). Furthermore, slight changes

were observed in levels of noradrenaline, dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) (Tuomisto et al. 1990). Results from a more recent study in rats showed that 2,3,7,8-TCDD increases neuronal serotonin turnover in TCDD-susceptible Long Evans rats, but not in TCDD-resistant Han/Wistar rats or in food-restricted Long Evans rats (Unkila et al. 1994). By using much lower intraperitoneal doses of 2,3,7,8-TCDD (2.2–8.8 µg/kg) in adult male Han/Wistar rats, thus avoiding manifestation of the wasting syndrome, Grahmann et al. (1993) and Grehl et al. (1993) observed electrophysiological (decreased conduction velocity) and histological signs (nerve degeneration) of peripheral neuropathy several months after a single injection of 2,3,7,8-TCDD. The possible mechanism of 2,3,7,8-TCDD neurotoxicity was not discussed. The existing information suggests that 2,3,7,8-TCDD causes minor alterations in brain neurotransmitter systems.

The overall evidence suggests that adverse neurological effects may occur in subjects exposed to relatively high levels of dioxins, or at least to levels that cause frank dermal effects. The neurological effects, however, may be transient and therefore, difficult to diagnose if examination is conducted several years after exposure. The nervous system in adults does not seem to be a particularly sensitive target for CDDs toxicity, but CDDs may represent a neurological hazard to the developing organism by, for example, altering hormone levels at critical times during the maturation of the central nervous system.

Reproductive Effects. The weaknesses of the epidemiology studies examining reproductive end points limits drawing conclusions regarding the reproductive toxicity of 2,3,7,8-TCDD in humans. Some common weaknesses include lack of exposure data (many of the studies rely on self-reported 2,3,7,8-TCDD exposure; CDC (1987) found that 2,3,7,8-TCDD blood levels of Vietnam veterans reporting direct or indirect exposure to Agent Orange were not significantly different from levels in non-Vietnam veterans), concomitant exposure to other chemicals, and lack of data on 2,3,7,8-TCDD levels at the time of conception. Several studies looked for an association between 2,3,7,8-TCDD exposure and an increased risk of spontaneous abortions, most did not find any statistically significant alterations following paternal exposure to 2,3,7,8-TCDD (Aschengrau and Monson 1989; Smith et al. 1982; Wolfe et al. 1995). An increased incidence of spontaneous abortions, was observed in women living near an herbicide manufacturing facility (Forsberg and Nordstrom 1985). However, this study has been criticized for its small sample size, inadequate discussion of sample selection, and concomitant exposure to other chemicals, including dibenzofurans (Sweeney 1994). In Vietnamese residents living in areas sprayed with Agent Orange, an increased incidence of hydatiform moles was observed (Phuong et al. 1989a). A later case-

control study by Ha et al. (1996) did not confirm the results of the Phuong et al. (1989a) study. In the 7½-year period after the Seveso accident, the number of female children born to parents living in area A was significantly higher than the number of male children (48 versus 26) (Mocarelli et al. 1996). An increased ratio of female to male children was also reported in workers of a 2,4,5-T production facility in Ufa, Russia (Basharova 1996) and in men exposed to chlorophenate wood preservatives contaminated with CDD (Dimich-Ward et al. 1996; James 1997). No alterations were found in the Missouri cohort of women living in 2,3,7,8-TCDD-contaminated areas (Stockbauer et al. 1988). Although several studies provide suggestive evidence of a relationship between CDD exposure and alterations in the sex ratio, the data are inadequate to establish a causal relationship. Additionally, it is not known how 2,3,7,8-TCDD affects the sex ratio. It has been postulated that the effect may be due to an alteration in hormonal balance or a disproportional number of miscarriages of male fetuses.

Data on 2,3,7,8-TCDD-induced alterations in gonads and reproductive endocrine function in humans are limited to effects observed in males. Decreased testicular size without any hormonal changes was found in Air Force Vietnam veterans exposed to 2,3,7,8-TCDD during Operation Ranch Hand (USAF 1991). This finding (decreased testicular size) was not confirmed when a more sensitive measurement device (ultra-sound) was used (Henriksen et al. 1996). Wolfe et al. (1985) found no alterations in sperm count or morphology in veterans involved in Operation Ranch Hand. Henriksen et al. (1996) assessed the possible relationship between 2,3,7,8-TCDD exposure and alterations in testosterone levels, FSH, LH, testicular abnormalities, sperm abnormalities, and sperm counts in the Operation Ranch Hand cohort (reproductive parameters were assessed in 1982, 1987, and 1992) and found no consistent, statistically significant alterations. Increases in FSH and LH levels and decreases in testosterone levels were observed in males working in 2,4,5-trichlorophenol manufacturing facilities (NIOSH cohort); however the magnitude of the changes in hormone levels was small (Egeland et al. 1994). The study authors note that increases in LH levels and decreases in testosterone levels were not found in the same men, suggesting that 2,3,7,8-TCDD may result in subtle alterations rather than primary gonadal failure.

A number of reproductive effects, including decreased fertility, damage to the gonads, and alterations in hormone levels, have been observed in male and female animals orally exposed to 2,3,7,8-TCDD. In male rats, a dose- and time-dependent reduction of serum testosterone and dihydrotestosterone levels was observed after acute oral exposure to 2,3,7,8-TCDD (Mebus et al. 1987; Moore et al. 1985, 1991). Furthermore, male rats had decreased weight of seminal vesicles following oral exposure to 2,3,7,8-TCDD (Al-Bayati et al. 1988; Moore et al. 1985) and reduced spermatogenesis after oral and subcutaneous

exposure (Al-Bayati et al. 1988; Chahoud et al. 1989; Van Miller et al. 1977). Biochemical changes in rat testes included dose- and time-dependent decreases in 17-hydroxylase activity and 20-lyase activity and reduced microsomal cytochrome P-450 (Mebus et al. 1987). Decreases in testicular superoxidase dismutase and glutathione peroxidase activities, and increases in protein kinase C activity and lipid peroxidation were also found in 2,3,7,8-TCDD-exposed rats (Al-Bayati et al. 1988). On the basis of the above data, it was postulated that the androgen deficiency is due to decreased androgen synthesis. It was further suggested that the morphological changes in rat testes may be due to changes in lipid peroxidation.

Pre- and/or postimplantation losses have been observed in rats (Giavini et al. 1983; Sparschu et al. 1971a), mice (Neubert and Dillman 1972; Smith et al. 1976), and rabbits (Giavini et al. 1982) following acute oral exposure to 2,3,7,8-TCDD. A single intraperitoneal injection of 2,3,7,8-TCDD (100 µg/kg) given between Gd 2–6 caused a high incidence of resorptions in C57BL/6J mice (Pratt et al. 1984). Similarly, increased resorptions were reported in rats exposed to mixed HxCDD during gestation, but not in those exposed to 2,7-DCDD or OCDD (Schwetz et al. 1973). In addition, abortions were observed in monkeys exposed to 2,3,7,8-TCDD for 3 weeks by gavage (McNulty 1984), and reduced reproduction was observed in those exposed chronically in the feed (Bowman et al. 1989b; Hong et al. 1989; Schantz et al. 1992). Finally, significantly decreased fertility in F_1 and F_2 generations was reported in a 3-generation reproductive study in rats exposed to 2,3,7,8-TCDD (Murray et al. 1979).

Investigations into the mechanism of CDD-induced decreased fertility revealed blocked estrous cycle in female mice exposed orally to 2,3,7,8-TCDD for an intermediate duration (Umbreit et al. 1987) and dose-dependent decreases in uterine and hepatic cytosolic, and nuclear estrogen and progesterone receptor levels in rats after intraperitoneal 2,3,7,8-TCDD injection (Romkes and Safe 1988). Furthermore, 2,3,7,8-TCDD antagonized the estradiol-mediated increases in these levels. In addition, a dose-related reduction of uterine peroxidase activity and decreased uterine wet weight were seen after a single 2,3,7,8-TCDD injection in rats (Astroff and Safe 1990). 2,3,7,8-TCDD application also antagonized the treatment with estradiol. The authors concluded that 2,3,7,8-TCDD antagonized the estrogen-induced uterine response and that the Ah receptor was involved in mediating the reaction. Other authors suggest that the anti-estrogen effect is mediated by 2,3,7,8-TCDD-induced metabolism of estrogens (Gierthy et al. 1987).

In non-pregnant female rats, decreases in ovarian weight, estrous cyclicity, ovulation rate, and the number of ova released were observed following a single dose of 2,3,7,8-TCDD (Li et al. 1995a, 1995b). Increases in LH and follicle stimulating hormone levels were also observed. The mechanisms involved in these

effects are thought to involve direct effects on the ovaries and effects on the hypothalamus/pituitary axis. The normal preovulatory surge of LH was not observed in the 2,3,7,8-TCDD-exposed rats, suggesting that 2,3,7,8-TCDD inhibited the positive feedback action of 17β-estradiol at the hypothalamic-pituitary axis (Li et al. 1995a). In hypophysectomized rats, 2,3,7,8-TCDD exposure resulted in a reduction of ovulation; Li et al. (1995a) suggests that this may be the result of a direct effect on the ovary, although the mechanism has not been elucidated.

Endometriosis has been observed in monkeys chronically exposed to 2,3,7,8-TCDD in the diet (Rier et al. 1993). A possible association between 2,3,7,8-TCDD and endometriosis is supported by rat and mouse studies using surgically induced models of endometriosis (Cummings et al. 1996; Johnson et al. 1997). In contrast, Foster et al. (1997) found that 2,3,7,8-TCDD exposure diminished endometrial tissue growth in mice. These studies used different models of surgically induced endometriosis and highlight the complexity of the disease. In the Cummings et al. (1996) and Johnson et al. (1997) studies, the animals were exposed to 2,3,7,8-TCDD prior to the development of endometriosis, and immune suppression probably facilitated the growth of endometrial tissue. In the Foster et al. (1997) model, 2,3,7,8-TCDD was administered after endometriosis development and 2,3,7,8-TCDD, via its anti-estrogenic effects, inhibited tissue growth. The relationship between CDD exposure and endometriosis in humans has not been adequately studied. In humans, the etiology of endometriosis likely involves a complex interplay between a number of diverse physiological factors including altered cell-mediated immunity and increased levels of growth hormone.

Although the human data regarding reproductive effects are inconsistent, a number of reproductive effects have been observed in animals, including deceased fertility, altered hormone levels, and gonad damage in males and females. The similarity between some of the effects observed in humans and animals suggest that reproductive effects may also occur in humans.

Developmental Effects. The developmental toxicity of 2,3,7,8-TCDD has been investigated in several human populations, with conflicting results. Most studies did not find increases in the number of birth defects in the children of men exposed to 2,3,7,8-TCDD in a chlorophenols manufacturing facility (Townsend et al. 1982) or during the Vietnam war (Aschengrau and Monson 1990; Erickson et al. 1984; Wolfe et al. 1995); or the children of parents living in Seveso, Italy (Bisanti et al. 1980; Mastroiacovo et al. 1988). Some studies did find increases in the incidence of specific defects (e.g., talipes, ventricular septal defect) in the infants of exposed fathers or mothers and fathers (Aschengrau and Monson 1990; Erickson et al. 1984; Hanify et al. 1981; Wolfe et al. 1995), but there was little consistency regarding the

type of defect or the target organ/system. The lack of exposure data, small sample sizes, and the lack of reliable data for birth defect rates prior to 2,3,7,8-TCDD exposure precludes drawing conclusions from these human studies. A section below summarizes information on health effects in humans associated with exposure to CDDs *in utero* and/or via breast milk.

Developmental toxicity has been observed in rats, mice, rabbits, hamsters, and monkeys exposed to 2,3,7,8-TCDD and other CDD congeners. Perinatal exposure to 2,3,7,8-TCDD results in structural malformations, functional alterations, decreased growth, and fetal/newborn mortality. Many of the effects occurred at 2,3,7,8-TCDD doses which were not maternally toxic. Acute oral exposure to 2,3,7,8-TCDD during gestation caused an increased incidence of cleft palate and skeletal anomalies in offspring of rats (Giaviani et al. 1983; Huuskonen et al. 1994), mice (Abbott and Birnbaum 1989a; Courtney 1976; Dasenbrock et al. 1992; Neubert and Dillman 1972; Smith et al. 1976; Weber et al. 1985), and rabbits (Giavini et al. 1983). These effects were also observed in fetuses of mice that received subcutaneous injections of 2,3,7,8-TCDD during gestation (Courtney 1976; Poland and Glover 1980). The 2,3,7,8-TCDD-induced cleft palate is caused by the failure of the opposing palatal shelves to fuse (Pratt et al. 1984); 2,3,7,8-TCDD does not alter the size of the palatal shelves or interfere with the opposing shelves coming into contact. Under normal conditions, there is a cessation of medial cell proliferation, a degeneration of peridermal medial cells, and a transformation of basal cells to mesenchymal cells as the opposing palatal shelves come into contact and fuse (Abbott and Birnbaum 1989b). 2,3,7,8-TCDD exposure alters medial cell proliferation and differentiation resulting in the formation of stratified squamous epithelium. Abbott and Birnbaum (1990a) suggest that the altered proliferation and differentiation of the medial cells is due to 2,3,7,8-TCDD-induced reductions of several growth factors (EGF, TGF-α, and TGF-β1) and increases in EGF receptor expression. EGF and TGF-α (which both bind to the EGF receptor) stimulate epithelial proliferation and differentiation and TGF-β1 inhibits epithelial proliferation. The increased levels of EGF receptor appear to compensate for the decreased EGF and TGF-α levels resulting in continued proliferation. Abbott et al. (1994a, 1994b) suggest that the altered expression of growth factors may be mediated by the Ah receptor. Exposure to 2,3,7,8-TCDD resulted in a dose-dependent downregulation of the Ah receptor throughout the palate; this probably occurs at the transcriptional level as decreases in mRNA were also observed (Abbott et al. 1994b). There is no evidence for direct Ah regulation of growth factors; rather, transcriptional regulation of related genetic activity may indirectly influence growth factor expression. Data which support an association between Ah receptor and cleft palate include a correlation between 2,3,7,8-TCDD binding to the Ah receptor and altered growth factor expression (Abbott et al. 1994b); finding of 2,3,7,8-TCDD-induced altered Ah receptor expression

and altered growth factor expression at doses which do not induce cleft palate (Abbott et al. 1994b); and the inability of 2,3,7,8-TCDD to induce cleft palate in strains of mice which have low affinity for Ah receptors (Pratt et al. 1984; Silkworth et al. 1989b).

Kidney malformations, particularly hydronephrosis, were observed in the offspring of rats (Giavini et al. 1983; Huuskonen et al. 1994), mice (Abbott et al. 1987a, 1987b; Courtney 1976; Moore et al. 1973; Silkworth et al. 1989b), and hamsters (Gray et al. 1995) orally exposed to 2,3,7,8-TCDD during gestation. Kidney defects were also observed in mouse offspring following *in utero* subcutaneous exposure to 2,3,7,8-TCDD (Courtney 1976) and in mice postnatally exposed to 2,3,7,8-TCDD via contaminated mothers' milk (Couture-Haws et al. 1991b). The hydronephrosis observed in these offspring is the result of occlusion of the ureter and subsequent accumulation of urine in the kidney (Abbott et al. 1987a). Prenatal exposure to 2,3,7,8-TCDD results in hyperplasia of the epithelium in the ureter, obstruction of the ureteric lumen, and a restriction of the flow of urine. Abbott and Birnbaum (1990b) found that 2,3,7,8-TCDD interfered with the normal decline in EGF receptors in the ureteric epithelia, resulting in excessive proliferation. In the bladder, 2,3,7,8-TCDD exposure also resulted in an increase in the epithelial thickness and continued expression of EGF receptors. 2,3,7,8-TCDD also appears to directly damage the kidney. Under normal conditions, there is an increase in laminin and type IV collagen levels and a thickening of the *lamina densa* of the glomerular basement membrane, which is important in establishing the filtration barrier. Following exposure to 2,3,7,8-TCDD, there is a decreased expression of laminin and type IV collagen and a diminished thickening of the lamina densa (Abbott et al. 1987b). This immature filtration barrier is likely to result in proteinuria and may result in increased urine volume.

A number of recently published studies have shown that the developing reproductive system is very sensitive to the toxicity of 2,3,7,8-TCDD. In female rats, exposure to 2,3,7,8-TCDD on Gd 8 caused functional reproductive toxicity (accelerated onset of constant estrus, shortened reproductive lifespan, reduced ovarian weight, and cystic hyperplasia of the endometrium) (Gray and Ostby 1995). Although there were no effects on fertility or estrous cyclicity when 2,3,7,8-TCDD exposure occurred after organogenesis (exposure on Gd 15) (Gray and Ostby 1995), external urogenital malformations (clefting, hypospadias, vaginal thread, and delayed vaginal opening) were observed (Flaws et al. 1997; Gray and Ostby 1995; Gray et al. 1997a; Heimler et al. 1998). These malformations to external genitalia are likely to interfere with mating (Gray and Ostby 1995). The authors note that the effects on the external genitalia are similar to effects observed in animals exposed to potent estrogen-like chemicals (e.g., DES, estradiol), although it likely that these effects occur by a different mechanism.

In male rats, perinatal exposure to 2,3,7,8-TCDD resulted in alterations in androgen status (decreased plasma testosterone levels, delay in testes descent, delay in external signs of puberty, and decreased ventral prostate and seminal vesicle weights), testes and cauda epididymis weights, and spermatogenesis (decreased daily sperm production, amount of mature sperm in cauda epididymis, and amount of sperm ejaculated), and in demasculinization and partial feminization of sexual behavior following exposure on Gd 15 (Bjerke and Peterson 1994; Bjerke et al. 1994a, 1994b; Gray et al. 1995; 1997b; Mably et al. 1992a, 1992b, 1992c; Sommer et al. 1996). In most of these studies, the experimental protocol involved gavaging the dams with a single dose of 2,3,7,8-TCDD on Gd 8 (Gray et al. 1995) or 15 (Bjerke and Peterson 1994; Bjerke et al. 1994a, 1994b; Gray et al. 1995; Mably et al. 1992a, 1992b, 1992c) and assessing a number of indices of reproductive development and function in newborn, juvenile, pre-pubescent, post-pubescent, and mature male rats. Because 2,3,7,8-TCDD is lipophilic and has a relatively long half-life, a single dose on Gd 15 will result in transplacental exposure from Gd 15 to birth and exposure via contaminated milk. Bjerke and Peterson (1994) compared the reproductive effects of 2,3,7,8-TCDD in rats exposed *in utero* to the effects observed in rats exposed to 2,3,7,8-TCDD only during lactation. Both *in utero* and lactational exposure resulted in decreased plasma testosterone level, decreased seminal vesicle and ventral prostate growth, and decreased epididymal sperm reserves. Exposure *in utero* only also resulted in decreased daily sperm production and delayed puberty; and exposure by lactation only resulted in partial feminization of sexual behavior. These data suggest that the timing of the 2,3,7,8-TCDD exposure is important. The mechanism by which 2,3,7,8-TCDD disrupts the development of the reproductive system and whether all of the reproductive effects have similar mechanisms is not known. Early investigators of the effects of 2,3,7,8-TCDD on sexual behavior suggested that perinatal exposure to 2,3,7,8-TCDD resulted in impaired sexual differentiation of the central nervous system (Mably et al. 1992b). The results of the Bjerke et al. (1994b) study suggest that the 2,3,7,8-TCDD-induced alterations in sexual behavior were not due to 2,3,7,8-TCDD acting as an estrogen antagonist or altering ER capacities of hypothalamic nuclei. The volume of the sexually dimorphic nucleus in the preoptic area of the hypothalamus (SDN-POA), which is dependent upon testosterone-derived estradiol in the brain during perinatal development, was not altered in 2,3,7,8-TCDD-exposed rats. Additionally, the sexual differentiation of ER concentration in brain nuclei which exhibit sexual dimorphism (ventromedial nuclei, medial preoptic nuclei, bed nucleus of the stria terminalis, periventricular preoptic area nucleus, cortical and medial amygdala, and arcuate nucleus) were not affected by 2,3,7,8-TCDD. Thus, 2,3,7,8-TCDD effects did not parallel those of either estrogen or androgen antagonists. Gray et al. (1995) also support the theory that 2,3,7,8-TCDD does not interfere with testosterone- and estrogen-dependent central nervous system sexual differentiation. In their study, no alterations in mounting behavior were observed in male hamsters perinatally exposed to 2,3,7,8-TCDD (in

hamsters, masculinization of the central nervous system requires perinatal exposure to testosterone). Bjerke et al. (1994b) proposed that 2,3,7,8-TCDD may affect other systems, such as brain amine content or growth factor expression of function, which would indirectly impact sexual differentiation. Similarly, Gray et al. (1995) suggested that 2,3,7,8-TCDD-induced alterations in the growth factors and receptors involved in urogenital system cell differentiation and proliferation may result in alterations in morphological sexual differentiation. Bjerke et al. (1994a) also found that the 2,3,7,8-TCDD-induced inhibition of ventral prostate weight and protein content imprinting was not due to perinatal reductions in plasma androgen levels because no effect on imprinting of the seminal vesicle, penis, or pituitary were observed in the 2,3,7,8-TCDD-exposed rats. Using a treatment regime that consisted of administration of a loading subcutaneous dose of 2,3,7,8-TCDD to female rats prior to mating, followed by weekly maintenance subcutaneous doses during mating, pregnancy, and lactation, Faqi et al. (1998) reported that sperm parameters were the most susceptible end points in male offspring examined at puberty (70 days old) and adulthood (170 days old). Based on pharmacokinetic considerations, the authors estimated that the lowest effective dose was <0.8 ng/kg/day. The sperm parameters that were altered were sperm number from cauda epididymis, daily sperm production, sperm transit rate, and percent abnormal sperm (more so in adults than in pubertal rats). No significant and/or consistent effects were observed on litter size, sex ratio, body weights, developmental landmarks, weight of sex organs, and sexual behavior. Testosterone levels were significantly reduced at age 170 days but not at age 70 days. In spite of sperm alterations, all exposed males exhibited normal reproductive performance and successfully impregnated untreated female to produce viable fetuses.

Recent studies have also focused on the role of the Ah receptor in the 2,3,7,8-TCDD-induced alterations in the development of the male reproductive system. Roman et al. (1998a) recently demonstrated the presence of both the Ah receptor and the receptor nuclear translocator (Arnt) in the testis, epididymis, vas deferens, ventral and dorsolateral prostate, and seminal vesicles from adult Holtzman rats. Arnt was localized in all organs in a variety of cell types; subcellular localization varied across organs and cell types within these organs. Unfortunately, technical difficulties precluded the evaluation of the Ah receptor distribution in the various organs. The authors also showed that a single oral dose of 25 μg 2,3,7,8-TCDD/kg produced significant induction of CYP1A1 in the ventral and dorsolateral prostate. CYP1A1 expression was localized in the epithelial cells of the ventral and lateral lobes of the prostate. Less CYP1A1 induction was seen in selected epithelial cells from other tissues, and no induction was detected in the testis. Also, 2,3,7,8-TCDD had no effect on Arnt protein expression, but Ah receptor expression was significantly reduced in all organs examined. In another study from this series, Roman and Peterson (1998) found that,

relative to controls, *in utero* exposure to 2,3,7,8-TCDD (1 µg/kg) transiently decreased the amount of several prostate-specific androgen-regulated mRNAs, all of which are markers of a differentiated ductal epithelium. This was in contrast with observations in adults, in which 2,3,7,8-TCDD induced CYP1A1 mRNA without altering the amount of prostate-specific, androgen-regulated mRNAs. These results suggested that the developing prostate can directly respond to *in utero* and lactational exposure to 2,3,7,8-TCDD, and that this exposure not only impairs prostate growth but also delays prostate luminal epithelial cell differentiation. In yet an additional study from this series, Roman et al. (1998b) reported that in the most severely affected animals, 2,3,7,8-TCDD produced alterations in the histological arrangement of epithelial and stromal cells and in the spatial distribution of androgen receptor expression.

Other developmental effects that have been observed in animals include immunotoxicity (thymic atrophy, immunosuppression, and alterations in thymocyte phenotypes) (Fine et al. 1989; Gehrs et al. 1997a, 1997b; Håkansson et al. 1987; Huuskonen et al. 1994; Luster et al. 1980; Madsen and Larsen 1989; Thomas and Hinsdill 1979), decreased fetal and newborn body weight (Abbott et al. 1992; Bjerke et al. 1994a; Bjerke and Peterson 1994), fetal/newborn mortality or decreased survival (Bjerke et al. 1994a; Bjerke and Peterson 1994; Huuskonen et al. 1994; McNulty 1984; Murray et al. 1979; Nau et al. 1986), and altered social behavior (Schantz et al. 1992).

Developmental toxicity has also been observed in animals exposed to other CDDs. These effects include heart defects in rats exposed to 2,7-DCDD (Schwetz et al. 1973); decreased thymic weight in rats exposed to 1,2,3,7,8-PCDD (Madsen and Larsen 1989); subcutaneous edema, decreased fetal growth, delayed ossification, dilated renal pelvis, and cleft palate in rats exposed to HxCDD (Schwetz et al. 1973); and subcutaneous edema in rats exposed to OCDD (Schwetz et al. 1973).

The animal database provides strong evidence that developmental toxicity is a sensitive end point following 2,3,7,8-TCDD exposure. Structural malformations, functional alterations (including impaired development of reproductive system), decreased growth, and fetal/newborn mortality have been observed in several animal species. Limited human data on the developmental toxicity of CDDs is available. Most of these studies examined the occurrence of birth defects in children of males exposed to 2,3,7,8-TCDD. Deficiencies in the human data preclude drawing firm conclusion on the potential of 2,3,7,8-TCDD to induce developmental effects in humans. However, the animal data suggest that 2,3,7,8-TCDD is a likely human developmental toxicant.

Health Effects Associated with Exposure to CDDs in Breast Milk. The developing organism is very susceptible to the toxicity of CDDs, in particular 2,3,7,8-TCDD. Prenatal or perinatal exposure has resulted in structural malformations (e.g., cleft palate, hydronephrosis), functional alterations (e.g., damage to the immune system, impaired development of the reproductive system), decreased growth, and fetal/newborn mortality in several animal species. Additionally, several animal studies (summarized in Table 2-14) provide evidence that lactation-only exposure to 2,3,7,8-TCDD can adversely affect the developing animal. Impaired development of the reproductive system (Bjerke and Peterson 1994), increased incidence of hydronephrosis (Couture-Haws et al. 1991a, 1991b), decreased weight gain, thymic atrophy (Faith and Moore 1977; Håkansson et al. 1987), and suppression of cell-mediated immunity (Faith and Moore 1977) have been observed in rats and mice exposed to 2,3,7,8-TCDD during lactation but not during gestation. The authors of these studies noted that most of the effects observed following lactation-only exposure were similar to those observed in animals exposed to 2,3,7,8-TCDD during gestation.

Because CDDs are efficiently absorbed following ingestion of breast milk (approximately 95% absorption efficiency for most congeners, see Section 2.3.4.4 for more information) and animal studies have found that lactation-only exposure can result in developmental effects, there is concern that breast-fed infants of women with high background levels of CDDs may be at risk. Ayotte et al. (1996) predicted that exposure to CDDs and related chemicals from breast milk will strongly influence the body burden of these chemicals during childhood and adolescence. Several human studies have examined the possible association between background CDD and CDF levels from *in utero* exposure and exposure from breast milk, and adverse health effects in infants. These studies (summarized in Table 2-15) found alterations in the levels of some markers of liver function (plasma ALT and AST) (Pluim et al. 1994a), thyroid function (thyroxine, thyroid stimulating hormone) (Koopman-Esseboom et al. 1994; Pluim et al. 1993b), and immune function (T-cell markers [TcR$\gamma\delta^+$, $CD3^+CD8^+$, and TcR$\alpha\beta^+$] and monocyte) (Weisglas-Kuperus et al. 1995), and the neurological optimality score in infants (Huisman et al. 1995a) which significantly correlated with CDD and CDF TEQ levels in breast milk. In follow-up studies and studies of older infants or children, no relationship between high levels of CDDs, CDFs, and PCBs in breast milk and neurological development, neurological optimality score, and/or reflexes was found at ages 6 (Pluim et al. 1996), 18 (Huisman et al. 1995b), or 31 months (Ilsen et al. 1996). Although the Ilsen et al. (1996) study of 31-month-old children did not find any alterations in overall neurological optimality or suboptimality scores, significant alterations, indicative of enhanced neuromuscular maturation and higher reflexes, were found in some tests (results were still within the normal range). Hypomineralization of teeth was found in 6- to 7-year-old children who

Table 2-14. Health Effects in Animals Following Lactation-Only Exposure to 2,3,7,8-TCDD

Health effect	Orally administered maternal dose of 2,3,7,8-TCDD	Reference
Feminization of sexual behavior, decreased plasma testosterone concentration, decreased ventral prostate and seminal vesicle weight, protein and DNA content, and decreased sperm reserves in male Holtzman rats	1 µg/kg on gestation day 15 (cross fostered)	Bjerke and Peterson 1994b
Increased incidence of hydronephrosis in newborn C57BL/6N mice	3 or 12 µg/kg on gestation day 6 (cross fostered)	Couture-Haws et al. 1991a
Increased incidence of hydronephrosis in newborn C57BL/6N mice	3 or 12 µg/kg on postnatal day 1 or 4	Couture-Haws et al. 1991b
Decreased weight gain, liver enlargement, thymus atrophy, and reduced ability to store vitamin A in young Sprague Dawley rats	10 µg/kg on postnatal day 0	Häkansson et al. 1987
Decreased body weight and thymic atrophy in 25- and 39-day old F344 rats, and suppression of cell-mediated immunity in young rats	5 µg/kg/day on postnatal days 0, 7, and 14	Faith and Moore 1977

Table 2-15. Health Effects in Humans Associated with CDD and CDF Levels in Breast Milk

Health effect	Levels of CDD and CDF in breast milk (ng TEQ/kg milk fat)	Reference
Increased plasma AST and ALT activities and decreased platelet levels correlated with increased cumulative CDD and CDF intake in 11-week-old infants. No effect on plasma GGT activity or plasma cholesterol, total and conjugated bilirubin, or leukocyte levels	8.7–62.7 (mean of 28.1); cumulative intake: 5.7–123.7 ng TEQ (mean of 44.7)	Pluim et al. 1994a
Increased total thyroxine level, thyroid stimulating hormone level, and ratio of total thyroxine level to thyroxine binding globulin level in 11-week-old infants in high-exposure group	High-exposure group: 37.5 Low-exposure group: 18.6	Pluim et al. 1993b
Increased thyroid stimulating hormone level in breastfed infants aged 2 weeks or 3 months. Lower total thyroxine levels and higher thyroid stimulating hormone levels in high exposure group	12.44–76.43 (mean of 32.06) high-exposure group: >30.75 low-exposure group: ≤30.75 ng	Koopman-Esseboom et al. 1994
Increased T-cell marker (TcRγδ$^+$) in newborns correlated with TEQ for CDDs and CDFs in breast milk. Decreased monocyte level in 3-month -olds correlated with TEQ for CDDs and CDFs in breast milk and cumulative TEQ intake for CDDs, CDFs, and dioxin-like PCBs (concentration in breast milk x number of days of breast feeding) Increased T-cell markers (CD3$^+$CD8$^+$ and TcRαβ$^+$) in 18-month olds correlated with TEQ for CDDs and CDFs in breast milk, but not with cumulative TEQ intake	Not reported	Weisglas-Kuperus et al. 1995
Decreased neurological optimality score in newborns correlated with breast milk concentration of 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, and 1,2,3,4,6,7,8-HpCDD	Breast milk levels (median concentration): 1,2,3,7,8-PeCDD=10.25, 1,2,3,4,7,8-HxCDD=8.71, 1,2,3,6,7,8-HxCDD=45.98, 1,2,3,7,8,9-HxCDD=6.72, and 1,2,3,4,6,7,8-HpCDD=57.38 ng/kg fat	Huisman et al. 1995a
No adverse effect on neurological development, neurological optimality score, number of abnormal reflexes, or tonus score at 6 months of age	high-exposure group: 29.2-62.7 (mean= 37.4) ng TEQ/kg low-exposure group: 8.7-28.0 (mean= 18.1) ng TEQ/kg	Pluim et al. 1996

Table 2-15. Health Effects in Humans Associated with CDD and CDF Levels in Breast Milk (continued)

Health effect	Levels of CDD and CDF in breast milk (ng TEQ/kg milk fat)	Reference
No adverse effect on neurological optimality score at 18 months of age	(same children as Huisman et al. 1995a)	Huisman et al. 1995b
No adverse effect on overall neurological optimality or suboptimality scores at 31 months of age. Some alterations in individual test scores which were indicative of enhanced neuromuscular maturation and higher reflexes (altered test scores were within normal range)	high-exposure group: median concentration of 67.7 ng TEQ low-exposure group: median=13.7 ng TEQ	Ilsen et al. 1996
Increase in frequency and severity of hypomineralization of teeth in 6–7-year-old children	high-exposure group: >16.0 pg TEQ/g milk fat medium-exposure group: 8.0-16.0 pg TEQ/g low-exposure group: <8.0 pg TEQ/g	Alaluusua et al. 1996
No correlation between birth weight of primiparae children and CDD and CDF TEQ levels in breast milk	10.8-96.3 pgTEQ/g fat	Vartiainen et al. 1998
No adverse effect on birth weight, body weight and head circumference at ages 1, 10, or 26 weeks, growth weight, or liver size	high-exposure group: 29.2-62.7 (mean= 37.4) ng TEQ/kg low-exposure group: 8.7-28.0 (mean= 18.1) ng TEQ/kg	Pluim et al. 1996
No correlation between decarboxylated prothrombin and vitamin K levels in 11 week old infants and CDD and CDF TEQ levels in breast milk	13.7–62.6 (mean of 29.4)	Pluim et al. 1994b
2,3,7,8-TCDD content of total breast milk was significantly higher in 4 of 14 mothers with children exhibiting abnormal bleeding	Breast milk level of 2,3,7,8-TCDD: 5.35–17.0 ng/kg milk fat (mean of 9.79)	Koope et al. 1991

ALT = alanine aminotransferase; AST = aspartate aminotransferase; TEQ = toxicity equivalency

received higher than background levels of CDDs and CDFs in breast milk (Alaluusua et al. 1996). Other studies have not found a relationship between higher background levels of CDDs, CDFs, and PCBs in breast milk and adverse health effects (summarized in Table 2-15); decarboxylated prothrombin and vitamin K levels in 11-week-old infants (Pluim et al. 1994b); birth weight (Pluim et al. 1996; Vartiainen et al. 1998), head circumference, and body weight at 1, 10, and 26 weeks of age (Pluim et al. 1996); or liver size (Pluim et al. 1996) were not adversely affected. Although significant correlations were found, the data should be interpreted cautiously because the levels of these markers were within the normal range and the correlation coefficients were low suggesting that only a small amount of the variance in the marker concentrations can be attributed to CDD and CDF levels.

The animal data suggest that lactation-only exposure to relatively high concentrations of CDDs can result in serious health effects. However, the human data show that the risk of CDD-induced health effects in infants exposed to background levels of CDDs and CDFs in breastmilk is small and this risk, in most cases, does not outweigh the benefits of breast-feeding.

Genotoxic Effects. *In vivo* genotoxicity studies are summarized in Table 2-16. Human studies have been conducted on populations exposed to 2,3,7,8-TCDD. An increased incidence of chromosomal aberrations was found in the fetal tissues, but not in the maternal tissues, following induced abortions in a group of women exposed to 2,3,7,8-TCDD in the Seveso accident (Tenchini et al. 1983). However, cases treated for chloracne in the area did not have an elevated frequency of chromosomal aberrations (Reggiani 1980). Results of a higher incidence of chromosomal aberrations were inconsistent in groups of Vietnam veterans (Kaye et al. 1985) or no cytogenetic changes were reported (Mulcahy et al. 1980). Fewer birth defects due to chromosomal abnormalities in children of Vietnam veterans were reported in another study (Erickson et al. 1984). Human studies cited above were limited by several factors. Generally, the levels of exposure to 2,3,7,8-TCDD were not known and coexposure to other potentially active compounds occurred in all studies. In the case of Vietnam veterans, a long postexposure period passed before the cytogenetic analysis was done. Furthermore, most of the studies used groups that were too small (less than 20 individuals) to have the statistical power to detect any changes.

Table 2-16. Genotoxicity of 2,3,7,8-TCDD *In Vivo*

Species (test system)	End point	Results	Reference
Drosophila melanogaster	Recessive lethals	–	Zimmering et al. 1985
Rats, bone marrow	Chromosomal aberrations	–	Loprieno et al. 1982
Mice, bone marrow	Chromosomal aberrations	+	Loprieno et al. 1982
Rats, bone marrow	Chromosomal aberrations	+	Green et al. 1977
Mice, bone marrow	Chromosomal aberrations, SCE, micronucleus test	–	Meyne et al. 1985
Monkeys, peripheral lymphocytes	Chromosomal aberrations, SCE	–	Lim et al. 1987
Rats	Dominant lethals	–	Khera and Ruddick 1973
Rats, liver	DNA adducts	–	Randerath et al. 1989
Rats, liver	DNA-single strand breaks	+	Wahba et al. 1989
Rats, liver	DNA adducts	–	Poland and Glover 1979
Human, aborted tissues	Chromosomal aberrations	+	Tenchini et al. 1983
Human, peripheral lymphocytes	Chromosomal aberrations	–	Reggiani 1980
Human, peripheral lymphocytes	Chromosomal aberrations	+	Kaye et al. 1985
Human, peripheral lymphocytes	Chromosomal aberrations	–	Mulcahy et al. 1980
Human, peripheral lymphocytes	Chromosomal aberrations, SCE	–	Zober et al. 1993

– = negative result; + = positive result; DNA = deoxyribonucleic acid; SCE = sister chromatid exchanges

In a study in which current 2,3,7,8-TCDD blood levels of previously exposed workers were approximately 25 times higher than in referents, there was no evidence of increased incidence of chromosomal aberrations or sister chromatid exchanges (Zober et al. 1993).

Animal studies on the genotoxicity of CDDs are inconclusive. When Osborne-Mendel rats were given 2,3,7,8-TCDD (0.25, 0.5, 1, 2, or 4 µg/kg) by gavage twice a week for 13 weeks, increased incidence of chromosomal aberrations was observed in the highest-exposure group (Green et al. 1977). Increased incidences of gaps and chromatid aberrations were observed in bone marrow cells of CD-1 mice following an intraperitoneal injection of 10 µg/kg 2,3,7,8-TCDD (Loprieno et al. 1982). Positive results were obtained at 96 hours, but not 24 hours, posttreatment. In contrast, no induction of structural chromosomal changes was found in CD-COBS rats orally exposed to 1.0, 0.1, or 0.01 µg/kg 2,3,7,8-TCDD once a week for 45 weeks (Loprieno et al. 1982). In addition, no differences in the frequency of sister chromatid exchanges and chromosomal aberrations in peripheral lymphocytes were observed in a group of rhesus monkeys receiving 0.001 µg/kg 2,3,7,8-TCDD in the feed for 4 years and their matching controls (Lim et al. 1987). Furthermore, no induction of chromosomal aberrations or sister chromatid exchanges, or increases in the frequency of micronuclei, were found in bone marrow cells of C57BL/6J (with high-affinity 2,3,7,8-TCDD receptor) or DBA/2J mice (with low-affinity 2,3,7,8-TCDD receptor) following a single intraperitoneal injection of 2,3,7,8-TCDD at doses of 50, 100, or 150 µg/kg (Meyne et al. 1985). The samples were examined within 8–48 hours. The negative results may, however, have been due to the time-dependent detectability of chromosomal changes after CDD exposure reported earlier (Loprieno et al. 1982).

In addition to studies dealing with structural chromosomal changes, effects on DNA were also investigated. Oral exposure to 1 µg/kg/week of 2,3,7,8-TCDD or 1,2,3,7,8-PCDD for up to 6 months did not increase the formation of DNA adducts in Sprague-Dawley rats (Randerath et al. 1989). A single oral dose of 2,3,7,8-TCDD (25–100 µg/kg) caused time-dependent increases in the induction of DNA single-strand breaks (and lipid peroxidation) in hepatic cells of Sprague-Dawley rats terminated within 3–14 days after the treatment (Wahba et al. 1989).

Negative results were obtained in reproductive tests including a dominant-lethal test following 7 daily oral doses of 2,3,7,8-TCDD (4, 8, or 12 µg/kg/day) to male Wistar rats (Khera and Ruddick 1973) and a sex-linked recessive-lethal test with 2,3,7,8-TCDD in *Drosophila melanogaster* (Zimmering et al. 1985).

In vitro genotoxicity studies are summarized in Table 2-17. Eukaryotic cell systems were used for detecting the effects of 2,3,7,8-TCDD exposure on DNA. Exposure to 2,3,7,8-TCDD did not stimulate the unscheduled DNA synthesis in cultural human cells (Loprieno et al. 1982), but inhibited DNA, ribonucleic acid (RNA), and protein synthesis in mouse lymphocytes (Luster et al. 1979); caused gene mutations in mouse lymphoma cells (Rogers et al. 1982); and induced sister chromatid exchanges in Chinese hamster cells (Toth et al. 1984).

Several researchers used the Ames test with *Salmonella typhimurium* to assess the mutagenicity of 2,3,7,8-TCDD in prokaryotic organisms. Predominantly negative results were obtained with tester strains G46, TA 1530, TA 1535, TA 100, TA 1950, and TA 1975, revealing base pair substitutions; and with strains TA 1531, TA 1532, TA 1534, TA 1538, TA 98, and TA 1978, revealing frame shift mutations (Geiger and Neal 1981; Gilbert et al. 1980; Mortelmans et al. 1984; Toth et al. 1984). However, some of the studies were limited by using 2,3,7,8-TCDD concentrations in excess of its solubility in water. Only two early studies reported positive results (Hussain et al. 1972; Seiler 1973). However, the results were limited by failure to demonstrate a dose-response relationship and by low bacterial survival rates. In addition, 2,3,7,8-TCDD exposure induced reverse mutations in *Escherichia coli* (Hussain et al. 1972) and in *Saccharomyces cerevisiae* (Bronzetti et al. 1983). The conflicting data obtained in the above studies may result from technical difficulties in testing 2,3,7,8-TCDD rather than from a lack of biological activity. Testing difficulties arise from an extreme insolubility of this compound and a high toxicity observed in some test systems, which would be anticipated to result in a very narrow window for effective genotoxic doses.

Considering the inconclusive results reported above and the severe limitations of some studies, there is no strong evidence for 2,3,7,8-TCDD genotoxicity. The information regarding the mutagenic potential of other CDDs is even more limited.

Cancer. Numerous epidemiological studies investigated the effects of 2,3,7,8-TCDD exposure on the development of cancer. A number of large-scale retrospective cohort mortality studies (Becher et al. 1996; Fingerhut et al. 1991; Hooiveld et al. 1998; Kogevinas et al. 1993, 1997; Manz et al. 1991; Ott and Zober 1996; Zober et al. 1990) have found significant increases in cancer mortalities (all types of cancers combined). These increases were typically found in the highest exposed workers and in workers with the longest latency periods. In general, the SMRs were low (less than 1.5); however, the high degree of consistency between studies suggests that the increases in mortalities were not due to chance. The possible

Table 2-17. Genotoxicity of 2,3,7,8-TCDD *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium				
TA 1530	Reverse mutations	NA	–	Hussain et al. 1972
TA 1532	Reverse mutations	NA	+	Hussain et al. 1972
TA 1535	Reverse mutations	NA	–	Seiler 1973
TA 1531	Reverse mutations	NA	–	Seiler 1973
TA 1532	Reverse mutations	NA	(+)	Seiler 1973
TA 1537	Reverse mutations	NA	(+)	Seiler 1973
TA 1535	Reverse mutations	–	NA	Geiger and Neal 1981
TA 100	Reverse mutations	–	NA	Geiger and Neal 1981
TA 1537	Reverse mutations	–	–	Geiger and Neal 1981
TA 1538	Reverse mutations	–	NA	Geiger and Neal 1981
TA 98	Reverse mutations	–	NA	Geiger and Neal 1981
TA 100	Reverse mutations	–	NA	Mortelmans et al. 1984
TA 1535	Reverse mutations	–	NA	Mortelmans et al. 1984
TA 1537	Reverse mutations	–	NA	Mortelmans et al. 1984
TA 98	Reverse mutations	–	NA	Mortelmans et al. 1984
TA 1530	Reverse mutations	–	NA	Gilbert et al. 1980
TA 1535	Reverse mutations	–	NA	Gilbert et al. 1980
TA 100	Reverse mutations	–	NA	Gilbert et al. 1980
TA 1537	Reverse mutations	–	NA	Gilbert et al. 1980

Table 2-17. Genotoxicity of 2,3,7,8-TCDD *In Vitro* (continued)

Species (test system)	End point	Results		Reference
		With activation	Without activation	
TA 1538	Reverse mutations	–	NA	Gilbert et al. 1980
TA 98	Reverse mutations	–	NA	Gilbert et al. 1980
TA 1535	Reverse mutations	–	–	Toth et al. 1984
TA 100	Reverse mutations	–	–	Toth et al. 1984
TA 1537	Reverse mutations	–	–	Toth et al. 1984
TA 1538	Reverse mutations	–	–	Toth et al. 1984
TA 98	Reverse mutations	–	–	Toth et al. 1984
Escherichia coli	Reverse mutations	NA	–	Hussain et al. 1972
Saccharomyces cerevisiae	Reverse mutations	+	–	Bronzetti et al. 1983
S. cerevisiae	Gene conversion	+	–	Bronzetti et al. 1983
S. cerevisiae	Host mediated assay	+	–	Bronzetti et al. 1983
Eukaryotic organisms: EUE human cells	UDS	NA	–	Loprieno et al. 1982
Mouse lymphocytes	DNA, RNA synthesis inhibition	NA	–	Luster et al. 1979
L51784 mouse lymphoma cells	Gene mutations	NA	+	Rogers et al. 1982
Chinese hamster cells	SCE	–	+	Toth et al. 1984

– = negative result; + = positive result; (+) = weakly positive result; NA = not applicable; RNA = ribonucleic acid; SCE = sister chromatid exchanges; UDS = unscheduled DNA synthesis; 2,3,7,8-TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

risks of several specific types of cancer have also been found, but the data are somewhat inconsistent. The site-specific cancers with elevated possible risks include soft-tissue sarcoma (Eriksson et al. 1981, 1990; Fingerhut et al. 1991; Hardell and Eriksson 1988; Hardell and Sandrom 1979; Hardell et al. 1995; Kogevinas et al. 1995, 1997; Saracci et al. 1991; Smith et al. 1984a), non-Hodgkin's lymphoma or malignant lymphoma (Becher et al. 1996; Cantor 1982; Hardell et al. 1981; Kogevinas et al. 1995), respiratory tract cancer (Fingerhut et al. 1991; Kogevinas et al. 1997; Manz et al. 1991; Zober et al. 1990), and gastrointestinal organ cancers (Axelson et al. 1980; Thiess et al. 1982). Furthermore, an increased risk of benign systemic neoplasms was reported in Vietnam Air Force veterans involved in Operation Ranch Hand (USAF 1991). There is some uncertainty regarding the interpretation of the epidemiology study results. In most studies, the cohort was also exposed to chemicals other than 2,3,7,8-TCDD and exact levels of exposure were not known. Furthermore, in some studies the exposure data were based solely on questionnaires and some recall bias could have been present. Other studies suffered from examining small cohorts or investigating the effects after a short latency period. The long latency period is important for detecting increases in soft-tissue sarcomas, presumably a major cancer outcome of CDD exposure in humans.

Several studies provided evidence of CDD-related carcinogenicity in animals. In general, the effects were dependent on the congener, species, sex, and route of administration, and were seen at doses that were close to doses that are toxic in the same animal species. Intermediate- and chronic-duration oral exposure to 2,3,7,8-TCDD induced multiple-site carcinomas and/or sarcomas in rats (Kociba et al. 1978a; NTP 1982b) and mice (Della Porta et al. 1987; NTP 1982a, 1982b; Toth et al. 1979). Similarly, chronic oral exposure to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD induced carcinomas in mice and rats (NCI/NTP 1980), and exposure to 2,7-DCDD caused carcinomas and sarcomas in mice (NCI/NTP 1979a). However, no cancer effects were found following chronic exposure to 2,7-DCDD in rats (NCI/NTP 1979a). Furthermore, squamous cell carcinoma developed in hamsters (Rao et al. 1988) following intermediate-duration intraperitoneal exposures.

Short-term dermal studies with 2,3,7,8-TCDD had controversial results. Some studies reported its inhibitory effects on the development of skin tumors in mice otherwise initiated by 13-dimethylbenz-(*o*)anthracene (Berry et al. 1978, 1979). Others cited its ability to promote tumors initiated by N-methyl-N-nitro-N-nitrosoguanidine (Hebert et al. 1990; Poland et al. 1982). Further, intraperitoneal injection of 2,3,7,8-TCDD given 2 days prior to or concurrently with methylcholanthrene did not affect methyl-cholanthrene-induced carcinogenicity in C57BL/6 mice (Kouri et al. 1978); in contrast, 2,3,7,8-TCDD

pretreatment (intraperitoneal or subcutaneous) of DBA/2 mice slightly increased the carcinogenic index. In support of these data, promotion of GGT-positive hepatic foci and/or development of tumors was observed after initiation with nitrosodiethylamine in rats (Flodstrom and Ahlborg 1989; Flodstrom et al. 1991; Pitot et al. 1980) that were injected with 2,3,7,8-TCDD for an intermediate duration. A recent study of the promoting activity of 2,3,7,8-TCDD in the liver from female Sprague-Dawley rats showed that increased tissue burden of 2,3,7,8-TCDD, which correlated with increased CYP1A1 activity, did not necessarily lead to increased cell proliferation (Walker et al. 1998). Experimentally, cell proliferation was increased after 30 or more weeks of treatment, but not after only 14 weeks of treatment, whereas both tissue burden and CYP1A1 activity exhibited similar significant increases at both time points. Walker et al. (1998) noted that a dose metric such as the area under the curve, which measures total dose over time, did not correspond to either 2,3,7,8-TCDD-induced changes in cell proliferation or changes in CYP1A1 expression. This, according to the authors, suggested that for a number of 2,3,7,8-TCDD-induced responses, particularly those involving integrated signal transduction pathways such as altered cell/tissue growth and differentiation, dose metrics that incorporate not only magnitude of exposure, but also duration of exposure and temporal windows of sensitivity for the response, may be more appropriate.

The available data provided sufficient evidence that 2,3,7,8-TCDD is a carcinogen in animals and its action is not solely dependent upon initiation by other substances. This is in conflict with the inconclusive genotoxicity data. Significant binding of radioactivity derived from labeled 2,3,7,8-TCDD to liver proteins was observed in several studies. However, covalent binding to hepatic DNA was four times less than the levels of binding with other carcinogens (Poland and Glover 1979). This indicates that the typical mutation mechanism model (covalent binding/DNA alteration) may not be applicable in the case of CDDs. In addition, there is an evidence that 2,3,7,8-TCDD acts as a tumor promoter (Hebert et al. 1990; Poland et al. 1982), which is consistent with the increases in multiple-site tumors observed in exposed humans and animals.

2,3,7,8-TCDD is an atypical chemical because of its accumulation and long persistence in the body. Several studies demonstrated that 2,3,7,8-TCDD affects the adrenals, thymus (DiBartolomeis et al. 1987; Gorski et al. 1988b; Greenlee et al. 1985; Hochstein et al. 1988), and thyroid (Henry and Gasiewicz 1987; Hermansky et al. 1988; Hong et al. 1987; Lu et al. 1986; Rozman et al. 1985) and also alters the estradiol (Umbreit et al. 1987), testosterone, and dihydrotestosterone (Mebus et al. 1987; Moore et al. 1985) levels in the organism. A study with intact and ovariectomized rats indicated that ovarian estrogens are involved in 2,3,7,8-TCDD induced hepatocarcinogenesis (Lucier et al. 1991). Assuming that there is a relationship

between the 2,3,7,8-TCDD Ah receptor protein function and the steroid and thyroid receptor protein functions, 2,3,7,8-TCDD would interact with various hormone receptors (Holder and Menzel 1989). It has been proposed that 2,3,7,8-TCDD is a hormonal carcinogen causing effects in targeted organs and in secondary targets through hormonal imbalance. Furthermore, 2,3,7,8-TCDD may also promote the metabolism of procarcinogens to active intermediates by the induction of metabolizing enzymes as demonstrated *in vitro* in cultured human lymphocytes (Jaiswal et al. 1985; Kouri et al. 1974, 1978). The induction of these enzymes appears to be the subject of genetic polymorphism such that individuals who are highly inducible may be at high risk for the development of tumors.

Taken together, the results of the epidemiology studies and the animal studies suggest that 2,3,7,8-TCDD may be a human carcinogen. This is consistent with conclusions of several regulatory agencies. NTP (1998) considers 2,3,7,8-TCDD to be a substance that may reasonably be anticipated to be a carcinogen (limited evidence in humans, sufficient evidence in animals); NTP is currently considering a reclassification of 2,3,7,8-TCDD and the decision is pending. IARC (1997) has recently classified 2,3,7,8-TCDD in Group 1 based on limited evidence of carcinogenicity in humans and sufficient evidence in animals. EPA had classified 2,3,7,8-TCDD as a Group B2 carcinogen when considered alone and a Group B1 carcinogen when considered in association with phenoxyherbicides and/or chlorophenols (EPA 1985d, 1989d, 1991a). The Group B2 classification indicates that although evidence in humans is inadequate, the evidence in animals is sufficient to consider 2,3,7,8-TCDD a probable human carcinogen. The Group B1 classification indicates that there are not only sufficient animal data but also limited human data to support the consideration that 2,3,7,8-TCDD, in association with phenoxyherbicides and/or chlorophenols, is a probable human carcinogen. Moreover, in a proposed rule to add "Dioxin and Dioxin-Like Compounds" to the list of chemicals subject to release reporting requirements, EPA reiterated that, "Based on the EPA weight of evidence classification criteria, there is sufficient evidence to conclude that 2,3,7,8-TCDD is a probable human carcinogen" (EPA 1997c).

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 (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns 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 (Komori 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in the newborn who has a low glomerular filtration rate and has not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer 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).

There is a limited amount of information available on the toxicity of CDDs in children. Most of the available data come from a series of studies on children living in Seveso during the accidental release of airborne trichlorophenol contaminated with 2,3,7,8-TCDD. Shortly after the accident, early irritative dermal lesions (this effect may not have been related to 2,3,7,8-TCDD exposure) and chloracne were observed in a number of children. Erythema and edema, the main clinical features of the early irritative lesions, were only observed in children and young adults (less than 20 years old) (Caputo et al. 1988). Chloracne was observed in 187 individuals, 88% of them were children aged 0 to 14 years (Bisanti et al. 1980). Based on serum 2,3,7,8-TCDD levels measured in 30 Seveso residents with and without chloracne, Mocarelli et al. (1991) suggested that children may develop chloracne at lower 2,3,7,8-TCDD body burdens than adults following acute exposure to 2,3,7,8-TCDD. Other effects observed in the exposed children include a significant increase in the number of children with chloracne having clinical and electrophysiological signs of peripheral nervous system involvement (assessed 6 years after the accident) (Barbieri et al. 1988) and slight transient increases in serum γ-glutamyltransferase and alanine aminotransferase levels in boys aged 6-10 years (Mocarelli et al. 1986). Although the serum enzyme levels were higher than in non-exposed children, the values were within the normal range and were elevated 1, 2, and 3 years after the accident, but not after 4 or 5 years. Increased risks of Hodgkin's lymphoma, myeloid leukemia, and thyroid cancer were also reported among children who were 0–19 years old at the time of the Seveso accident (Pesatori et al. 1993). However, the differences in relative risks (RRs) for these cancer types between the Seveso residents and the control population did not reach statistical significance. Similar results were found in a 15-year follow-up study of this cohort (Bertazzi et al. 1997).

A wide variety of effects have been observed in adults exposed to 2,3,7,8-TCDD at work or following an accidental release of 2,3,7,8-TCDD into the environment. The primary targets appear to be the skin, liver, thyroid, and cardiovascular, endocrine, and immune systems; an increased cancer risk has also been observed. In the absence of data to the contrary, it is likely that these organs/systems will also be sensitive targets in children.

A number of human studies have investigated the potential of 2,3,7,8-TCDD to induce developmental effects. No significant increases in the incidence of birth defects have been observed in the children of parents living in Seveso at the time of the accident or during the next 6-year period (Bisanti et al. 1980;

Mastroiacovo et al. 1988) or in the children of men involved in the manufacture of chlorophenols (Townsend et al. 1982). In contrast, other studies have found increases in specific types of defects, although the total number of defects was not significantly altered. It is difficult to interpret these data because there is little consistency regarding the type of defect or the target organ/system. For example, a significant association between nervous system defects and paternal serum 2,3,7,8-TCDD levels was observed in the Ranch Hand cohort (Wolfe et al. 1995) and facial clefts were observed in Arkansas residents exposed to sprayed 2,4,5-T. The lack of exposure data, small sample sizes, and the lack of reliable data for birth defect rates prior to 2,3,7,8-TCDD exposure limits the power of the human studies to determine if an association between 2,3,7,8-TCDD exposure and developmental toxicity exists in humans.

The toxicity of 2,3,7,8-TCDD has been extensively examined in animal oral toxicity studies, and effects have been observed in most organs/systems. The animal studies clearly demonstrate that the developing organism is very sensitive to the toxicity of 2,3,7,8-TCDD. The types of effects observed in the offspring of animals exposed to 2,3,7,8-TCDD include fetal/newborn mortality, decreased growth, structural malformations, kidney anomalies, immunotoxicity, thymic atrophy impaired development of the reproductive system, and neurodevelopmental effects. The LOAELs for developmental effects are among the lowest identified in animals, and the chronic oral MRL is based on a developmental effect. The most sensitive developmental effects are impaired development of the reproductive system and neurobehavioral effects. *In utero* exposure to 2,3,7,8-TCDD adversely affects the development of the reproductive system in male and female offspring; studies have shown alterations in androgen levels, secondary sex organs, spermatogenesis, fertility, and sexual behaviors (Bjerke and Peterson 1994; Bjerke et al. 1994a, 1994b; Chaffin et al. 1996, 1997; Flaws et al. 1997; Gray and Ostby 1995; Gray et al. 1995, 1997a, 1997b; Heimler et al. 1998; Mably et al. 1992a, 1992b, 1992c). Gray and Ostby (1995) found decreased fertility in the female offspring exposed on Gd 8; no effects on fertility have been observed in female offspring exposed on Gd 15 (Gray et al. 1997a) or in male offspring (Gray et al. 1995; Mably et al. 1992c). Schantz and Bowman (Bowman et al. 1989b; Schantz et al. 1986, 1992; Schantz and Bowman 1989) found neurobehavioral alterations in the offspring of monkeys chronically exposed to dietary 2,3,7,8-TCDD (7 months prior to mating and during mating and lactation). Altered peer group behavior, cognitive deficits, and prolonged maternal care were observed. There are some data to suggest that other CDDs (2,7-DCDD, 1,2,3,7,8-PCDD, OCDD, and HxCDD) are also toxic to the developing organism (Madse and Larsen 1989; Schwetz et al. 1973). The observed developmental effects appear to be similar to those observed following *in utero* exposure to 2,3,7,8-TCDD. More details about these studies can be found in Sections 2.2.2.6, Developmental Effects, and 2.4.2, Mechanisms of Toxicity.

There is a limited amount of data on the toxicokinetic properties of CDDs in children or immature animals. A toxicokinetic model was constructed that accurately predicted the lifetime concentrations of 2,3,7,8-TCDD in adipose tissue, blood, liver, and feces at different ages (Kreuzer et al. 1997). In formula-fed infants, the model predicted that 2,3,7,8-TCDD lipid levels would decrease during the first year and subsequently increase, reaching a maximum at 16 years of age. In contrast, the model predicted an initial increase in 2,3,7,8-TCDD lipid levels in exclusively breast-fed infants followed by a 3-year decrease after weaning and merging at about 7 years with concentrations in formula-fed individuals. The half-life of nonmetabolic elimination (unchanged 2,3,7,8-TCDD) was calculated to be 0.42 years in newborns and 9.5 years in 40-year-old adults. The half-life of the fraction metabolized by the liver ranged from 1.5 years for newborns to approximately 10 years for a 40-year-old individual. The three times greater elimination half-life for the metabolized fraction relative to the nonmetabolized fraction in infants suggests that metabolic elimination does not play a major role in the elimination of 2,3,7,8-TCDD in infants. 2,3,7,8-TCDD accumulates preferentially in liver and adipose tissue. Accumulation in the liver is due to sequestration by the microsomal binding protein, CYP1A2. To the extent that this protein is developmentally regulated (Leeder and Kerns 1997), infants (<4 months old) might accumulate relatively less 2,3,7,8-TCDD in their livers than adults. Little is known about the metabolism of 2,3,7,8-TCDD in humans and it is unknown whether the metabolism of 2,3,7,8-TCDD or other CDDs differs between adults and children. In animals, phase II enzymes play an important role in the biotransformation and elimination of 2,3,7,8-TCDD. If this were the case in humans, it would be expected that very young infants would metabolize and eliminate 2,3,7,8-TCDD slower than adults since glucuronosyltransferase activity achieves adult levels by 6–18 months of age (Leeder and Kearns 1997).

CDDs are transferred from mother to offspring through the placenta and breast milk. Although there are human data indicating placental transfer of 2,3,7,8-TCDD (Kreuzer et al. 1997; Schecter et al. 1996b), quantitative data are not available. A study in mice administered a single dose of 2,3,7,8-TCDD on Gd 12 showed that the rate of accumulation of 2,3,7,8-TCDD in placental tissue reached a maximum in about 3 hours (Abbott et al. 1996); after 24 hours, 0.27% of the maternal dose was detected in the placenta. This issue is discussed in more detail in Section 2.3.4.4, Transfer of CDDs Through the Placenta and Breast Milk.

CDDs are lipophilic compounds that can concentrate in maternal milk and be transferred to the nursing infant. Numerous studies have examined the transfer of 2,3,7,8-TCDD and related chemicals to infants via breast milk and for the most part, the results showed that infants may absorb up to 95% of the administered

dose (Abraham et al. 1994, 1996; Dahl et al. 1995; McLachlan 1993; Pluim et al. 1993b). This percentage is similar to the percent of 2,3,7,8-TCDD absorbed (>87%) by an adult volunteer after ingestion of a single oral dose of 2,3,7,8-TCDD (Poiger and Schlatter 1986). As stated previously, it has also been shown that breast-fed infants have a larger 2,3,7,8-TCDD burden during the first year of life compared to formula-fed infants (Kreuzer et al. 1997). However, this initial higher burden does not translate into a higher lifetime burden. A number of human studies have examined breast-fed infants of mothers with high background levels of CDDs. These studies have found alterations in some markers of liver, thyroid, and immune function and neurodevelopment (neurological optimality score) (Huisman et al. 1995a; Koopman-Esseboom et al. 1994; Pluim et al. 1993b, 1994a; Weisglas-Kuperus et al. 1995); however, all of the markers were within the normal range. The impaired neurological optimality score that was observed in newborns was not significantly altered in children aged 6, 18, or 31 months (Ilsen et al. 1996; Huisman et al. 1995b; Pluim et al. 1996).

Subsequent sections of this chapter (Sections 2.7, 2.8, and 2.10) discuss the available information on biomarkers, interactions, and methods for reducing toxic effects. Most of the available information is from adults and mature animals; no child-specific information was identified, with the possible exception of biomarker data. However, there are some data to suggest that interactions with PCBs and CDFs may influence the developmental toxicity of 2,3,7,8-TCDD. Data from children living in Seveso suggest that serum 2,3,7,8-TCDD levels are reflective of exposure levels and are a sensitive indicator of past exposure. Likewise, it is likely that the available information in adults on interactions and methods for reducing toxic effects will also be applicable to children.

As discussed previously, children appear to be unusually susceptible to the dermal toxicity of 2,3,7,8-TCDD. The data are inadequate to assess whether they will also be more sensitive to other CDD effects. Additionally, the available animal data suggest that the developing fetus is very sensitive to 2,3,7,8-TCDD-induced toxicity. 2,3,7,8-TCDD appears to interfere with the development of the reproductive, immune, and nervous systems; the mechanisms of action for these toxic effects have not been elucidated.

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 (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 CDDs 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 CDDs 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 CDDs

Methods for measuring CDDs in biological fluids and tissues are available. Adipose tissue and liver are the primary storage site for CDDs and tissue samples have been analyzed in several studies. It was demonstrated that the relative (lipid-based) levels of 2,3,7,8-TCDD are similar in hepatic and adipose tissues (Leung et al. 1990a) and between adipose tissue and serum (Patterson et al. 1988; Schecter et al. 1990) from the same patients. However, this was not the case for more highly chlorinated dioxins; for example, for OCDD there is a 2:1 ratio between serum and adipose tissue lipid fractions (Schecter et al. 1990) and a 12:1 ratio between liver and adipose tissue levels (Thoma et al. 1990). However, the important TEQ variable was close to 1:1 ratio.

In the general population, adipose tissue levels of 2,3,7,8-TCDD ranged from non-detectable to 20.2 ppt in 128 Kansas City, St. Louis, and Springfield, Missouri, residents with no known special exposure to CDDs (Andrews et al. 1989). Similarly, 2,3,7,8-TCDD levels in adipose tissues were between 5 and 10 ppt in a sample of the general population in Canada, while OCDD levels ranged from 600 to 800 ppt in the cohort (Ryan et al. 1985a). Increased environmental exposure to CDDs was reflected by increased levels in adipose tissues. Residents of two California households who had eaten dioxin-contaminated beef and eggs had significantly elevated serum levels of 2,3,7,8-TCDD, PCDD, and HxCDD, compared with rural Californians who did not eat contaminated beef and eggs (Goldman et al. 1989). 2,3,7,8-TCDD serum lipid levels ranged from 2.8 to 750 ppt in individuals with possible recreational, residential, or occupational exposure in Missouri (Patterson et al. 1986a). Several other studies reported increased concentrations of 2,3,7,8-TCDD in adipose tissues (Beck et al. 1989c; Fingerhut et al. 1989; Patterson et al. 1989b) or serum lipid (Fingerhut et al. 1989; Patterson et al. 1989b) of occupationally exposed workers. The highest 2,3,7,8-TCDD levels reported ranged between 42 and 750 ppt in adipose tissue lipid and between 61 and 1,090 ppt in the serum lipid of Missouri chemical workers (Patterson et al. 1989b). Based on a mean of 208 ppt 2,3,7,8-TCDD in the serum lipid of chemical workers measured at least 17 years postexposure, and a mean biological half-life level of 7 years, it was estimated that the serum lipid level shortly after exposure would have been approximately 2,313 ppt (range, 6.4–14,673) (Fingerhut et al. 1989). The major disadvantage of these studies is a lack of information regarding actual 2,3,7,8-TCDD exposure. Studies in a population exposed to 2,3,7,8-TCDD in the Seveso industrial accident found serum lipid

2,3,7,8-TCDD levels in exposed individuals as high as 56,000 ppt in the highly contaminated zone where soil samples ranged from 956 to 1,185 µg 2,3,7,8-TCDD/m^2 (Mocarelli et al. 1991); these serum samples were collected about a month after exposure and were recently analyzed. Chloracne did not always develop in the individuals with highly elevated serum 2,3,7,8-TCDD levels, although it did occur in all who had levels above 12,000 ppt. In the most recent National Human Adipose Tissue Survey (NHATS), conducted in fiscal year 1987, it was found that the average concentration of 2,3,7,8-TCDD in the adipose tissue of the U.S. population was 5.38 ppt, increasing from 1.98 ppt in children under 14 years of age to 9.4 ppt in adults over 45 (Orban et al. 1994). The average concentration of 2,3,7,8-TCDD was found to increase at an average rate of 0.83 ppt per decade for individuals under age 31, and at an average rate of 1.52 ppt per decade for the older population. The study also found no statistical evidence of differences in the average levels for populations representing different sexes and racial groups nationwide. Furthermore, a comparison of mean concentrations of 2,3,7,8-TCDD and OCDD between the 1982 and 1987 NHATS revealed no statistically significant differences between the two surveys. For OCDD the values were 768 and 724 ppt in the 1982 and 1987 surveys, respectively (Orban et al. 1994).

Similarly, no exact exposure data were available in Vietnam veteran studies. In general, tissue samples for analyses were taken several (approximately 10–20) years after exposure, which represents another limitation of these studies. Increased adipose 2,3,7,8-TCDD levels (up to 99 ppt) were recorded in Vietnam War veterans involved in Operation Ranch Hand (Gross et al. 1984; Schecter et al. 1989a). In a small group of potentially exposed Vietnam veterans, adipose tissue 2,3,7,8-TCDD levels ranged from non-detectable to 11 ppt with a mean of 5.8 ppt (Schecter et al. 1989a). Schecter et al. (1989a) noted that the veterans with adipose tissue levels of $8 ppt were considered to have slightly elevated values or values within the normal range. In another study of 646 ground troop veterans, only two individuals had serum 2,3,7,8-TCDD levels above 20 ppt in the lipid fraction (CDC 1988). In the rest of the cohort, the median 2,3,7,8-TCDD levels ranged from 3.2 to 4.3 ppt and did not differ significantly from the levels found in the control group of non-Vietnam veterans (CDC 1988; MMWR 1987). It was concluded that those who did not handle or spray herbicides were not highly exposed to 2,3,7,8-TCDD (CDC 1988). With regard to the long time period between exposure and serum analysis, the authors argued that, assuming first-order kinetics and 2,3,7,8-TCDD's half-life of 7 years in humans, the study had enough statistical power to detect differences between the exposed and control groups (CDC 1988). Elevated CDD levels were also measured in some patients treated in a hospital in South Vietnam (Phiet 1989; Phuong et al. 1989b). However, these reports involved too few patients to give any conclusive results. In a more recent expanded half-life study of 337 Vietnam veterans, a median observed half-life of 11.5 years was calculated for

2,3,7,8-TCDD (Wolfe et al. 1994). The nonparametric 95% CI was 10–14.1 years. A review of CDD levels in human tissues from various populations can be found in Schecter et al. (1994c).

CDDs have also been detected in breast milk of women exposed to high levels of CDDs and in women presumably exposed to background levels. High 2,3,7,8-TCDD levels (mean of 484 pg/g milk fat; 18 pg/g whole milk) were found in the milk of mothers from South Vietnam in 1970; the levels dropped to a mean of 12 pg/g milk fat (0.47 pg/g milk) by 1985 (Schecter et al. 1987a) and 7.5 pg/g milk fat in samples collected between 1984 and 1992 (Schecter et al. 1995). Mean 2,3,7,8-TCDD levels in breast milk samples (collected in 1984) in mothers from South Vietnam, North Vietnam, and the United States were 0.68, not detectable, and 0.19 pg/g whole milk (Schecter and Gasiewicz 1987b). The total CDD and CDF levels (expressed as TEQs) in these samples were 1.11, 0.065, and 1.04 pg/g milk. Results from the analysis of 526 individual milk samples from the German general population revealed a mean 2,3,7,8-TCDD concentration of 3.2 pg/g milk fat (Fürst et al. 1994). The analysis also showed the presence of only 2,3,7,8-chlorine-substituted congeners. OCDD was the most concentrated congener with a mean level of 208 pg/g milk fat. In general, the levels in milk decreased with decreasing degree of chlorination from octa- to tetra-CDD. The total TEQs, including CDFs, was 29.3 pg/g milk fat. Fürst et al. (1994) estimated that the average daily intake of 2,3,7,8-TCDD via human milk for an infant weighing 5 kg is 15.4 pg/kg/day, and the mean total dioxin equivalents amounted to 140.6 pg/kg/day. Both parity and the length of time the woman has been lactating influence the CDD concentration in breast milk.

A reverse transcriptase polymerase chain reaction (RT-PCR) method was developed to quantitate CYP1A1 mRNA levels on total RNA extracts from mitogen-stimulated human blood lymphocytes cultured in the presence or absence of 10 nM 2,3,7,8-TCDD (Van den Heuvel et al. 1993). Although CYP1A1 gene expression can be monitored by measuring EROD activity (CYP1A1) or mRNA expression (using conventional RNA hybridization), RT-PCR is a much more sensitive approach. The average CYP1A1 mRNA levels in the cultured, 2,3,7,8-TCDD-treated cells was approximately 21 times higher than that in the non-2,3,7,8-TCDD-treated cells. In uncultured, nonstimulated lymphocytes from volunteers, CYP1A1 mRNA could be reproducibly measured at levels that were 10–40-fold lower than in mitogen-stimulated lymphocytes. In comparison, EROD activity measured in uncultured, nonstimulated lymphocytes was indistinguishable from measurements on reagents controls, which proved the high sensitivity of the RT-PCR approach. In a group of 6 smokers, the average level of CYP1A1 message was approximately 2 times higher than in a group of 6 nonsmokers, although there was great variability in the group of smokers. Based on these preliminary results, Van den Heuvel et al. (1993) suggested that CYP1A1 gene expression

in peripheral blood lymphocytes may be used as a human exposure marker for 2,3,7,8-TCDD and related compounds.

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

2.7.2 Biomarkers Used to Characterize Effects Caused by CDDs

Chloracne is one effect that is clearly associated with exposure to high levels of CDDs and other halogenated organic chemicals, and has been observed in some individuals who were exposed occupationally or in the environment to increased levels of 2,3,7,8-TCDD or chemicals contaminated with 2,3,7,8-TCDD. However, while the presence of chloracne indicates exposure to CDDs or other halogenated organic compounds, its absence does not preclude such exposure. For example, in a cohort from the Seveso incident, no chloracne was observed below an initial serum lipid 2,3,7,8-TCDD level of 800 ppt (body burden of 2.5 µg/kg, assuming 22% body fat and 70 kg body weight); above 12,000 ppt (body burden of 38 µg/kg) chloracne was always observed; and between 800 and 12,000 ppt the occurrence of chloracne was sporadic (Mocarelli et al. 1991). In the Yu-Cheng population, chloracne was associated with a body burden in 2,3,7,8-TCDD equivalents of 2–3 µg/kg body weight, or about 140–210 µg for a 70-kg adult (Ryan et al. 1990).

Biochemical changes (raised serum hepatic enzyme levels, disorders of lipid and carbohydrate metabolism, unbalanced porphyrin metabolism) and/or an enlarged liver can indicate effects induced by 2,3,7,8-TCDD exposure, but these effects are not specific for this or other compounds. Light and electron microscope changes in the liver (e.g., lipid droplets in parenchymal cells, increased endoplasmic reticulum, enlarged and pleomorphic mitochondria) are also sensitive but nonspecific biomarkers for exposure to CDDs (Schecter et al. 1985b). When biochemical changes in the placenta of women exposed in the Yu-Cheng incident were evaluated for use as possible biomarkers, the EGF receptor autophosphorylation effect was found to be associated with decreased birth weight in the neonates (Lucier et al. 1986). The authors suggested using this response as a biomarker of effect for all toxic chlorinated aromatic compounds.

2.8 INTERACTIONS WITH OTHER CHEMICALS

Several studies were located regarding interactions that affect the toxicity of CDDs. Probably the most important interactions that have an impact on human health are those involving CDDs, CDFs, and PCBs. It has been recognized that chloroaromatics cause a complex of similar effects that vary in severity depending on the number of chlorine atoms, positional substitution, and species susceptibility. Sufficient information is available for assessment of risk associated with exposure to 2,3,7,8-TCDD. However, exposure to a mixture of chloroaromatics is common in the general environment. The assessment of health risk resulting from exposure to chemical mixtures of chloroaromatics was enabled by the development of TEFs (2,3,7,8-TCDD equivalence factors) that relate the relative toxic potency for CDDs and CDFs to that of 2,3,7,8-TCDD (EPA 1989). It was assumed based on previous literature data (Eadon et al. 1986) and in animal dosing studies (Van den Berg et al. 1989), that CDDs and CDFs have an additive effect in the organism when weighted for relative toxicity compared to 2,3,7,8-TCDD (for further information see Sections 2.4 and 2.5). The assumption of additivity was later supported by experimental data. The concept of TEFs was used, for example, to assess the potential toxicity of background levels of CDFs and CDDs in general populations based on body burdens of indicator CDDs that were associated with chloracne and other effects in the Yusho and Yu-Cheng rice oil poisoning incidents (Ryan et al. 1990).

However, some recent studies further investigated the interactions of various chloroaromatics and indicated that the interactions may be more complicated. *In vitro* studies compared relative toxicity of various chloroaromatics in human cell lines monitoring enzyme induction and binding to the Ah receptor that mediates the induced responses (Nagayama et al. 1985; Safe 1987). *In vivo* studies concentrated on monitoring of enzyme induction, inhibition of body weight gain and immunotoxic and teratogenic effects. Coexposure of Long Evans rats to 6-methyl-1,3,8-trichlorodibenzofuran (MCDF) and 2,3,7,8-TCDD induced a partial inhibition of the monooxygenase enzyme-induction response caused by 2,3,7,8-TCDD treatment alone (Harris et al. 1989b). Although MCDF did not decrease the levels of occupied nuclear 2,3,7,8-TCDD Ah receptors, it inhibited the effects of 2,3,7,8-TCDD on the cytosolic Ah receptor (Harris et al. 1989b).

Other studies further indicated that PCBs may antagonize Ah receptor-mediated responses to 2,3,7,8-TCDD. In a recent review, Van den Berg et al. (1994) suggested that toxicokinetic factors contribute to the observed nonadditive toxicological and biological effects. Co-treatment of C57BL/6 mice with various commercial Aroclors (PCB mixtures) and 2,3,7,8-TCDD resulted in antagonizing the

2,3,7,8-TCDD-mediated inhibition of the splenic plaque-forming cell response (Bannister et al. 1987; Davis and Safe 1989). Similarly, significant antagonism of 2,3,7,8-TCDD and Aroclor 1254 was observed in the induction of cytochrome P-450-dependent monooxygenases in C57BL/6J mice (Bannister et al. 1987). The effects were dependent on the dose of both 2,3,7,8-TCDD and Aroclor 1254 and on their respective ratios. The ratios of Aroclor 1254/2,3,7,8-TCDD that induced antagonist reactions were comparable to the ratios of PCBs/CDDs found in human tissues and environmental samples. The authors speculated that less-toxic chlorinated compounds may have a protective effect against the more-toxic compounds in the environment. However, by comparing the immune sensitivities of both Ah responsive and Ah less-responsive mouse strains, it was demonstrated that a complex mixture of contaminants taken from the Love Canal site was immunosuppressive and that this effect was primarily due to the 2,3,7,8-TCDD component of the mixture, although 2,3,7,8-TCDD was a very minor component, and there was little interaction with the other hydrocarbon components of the mixture (Silkworth et al. 1989a).

Experimental studies have shown that interactions of 2,3,7,8-TCDD and CDFs or PCBs resulted in fetotoxic and teratogenic effects in the offspring of exposed animals. Exposure of pregnant mice to 2,3,7,8-TCDF resulted in cleft palates and hydronephrosis in the offspring (Hassoun et al. 1984). The results obtained in different strains of mice indicated an association with the Ah locus. Comparable results were obtained previously in mice exposed to 2,3,7,8-TCDD (Abbott and Birnbaum 1989a; Abbott et al. 1987a, 1987b; Courtney 1976). When C57BL/6N mice were treated orally with 2,3,7,8-TCDD and 2,3,7,8-TCDF on gestational day (Gd) 10, hydronephrosis and cleft palates were observed in the offspring (Weber et al. 1985). The effects of both chemicals were additive. Similarly, an increased incidence (10-fold) of cleft palates was observed in offspring of C57BL/6N mice after a combined treatment with 2,3,7,8-TCDD and 2,3,4,5,3',4'-hexachlorobiphenyl during gestation, as compared with those treated with 2,3,7,8-TCDD alone (cleft palate was not observed when 2,3,4,5,3',4'-hexachlorobiphenyl was administered alone) (Birnbaum et al. 1985). In contrast, no potentiation of CDD-mediated effect was found with 2,4,5,2',4',5'-hexachlorobiphenyl. Furthermore, co-treatment of pregnant C57BL/6J mice with Aroclor 1254 and 2,3,7,8-TCDD resulted in a sharp decrease in the incidence of cleft palate per litter (8.2%) compared with those treated with 2,3,7,8-TCDD alone (62%) (Haake et al. 1987).

Similarly, 2,3,7,8-TCDD-induced fetotoxicity and teratogenicity were altered by co-exposure to other chemicals. A synergistic effect on the induction of cleft palates was observed in offspring of C57BL/6N mice treated orally with 2,3,7,8-TCDD and retinoic acid on Gd 10 or 12 (Abbott and Birnbaum 1989b; Birnbaum et al. 1989b). However, the co-administration of retinoic acid did not influence the incidence of

2,3,7,8-TCDD-induced hydronephrosis, nor did 2,3,7,8-TCDD affect the incidence or severity of limb-bud defects induced by retinoic acid (Birnbaum et al. 1989b). A synergistic effect was observed when 2,3,7,8-TCDD (orally) and hydrocortisone (subcutaneously) were administered to C57BL/6N mice on Gd 10–13 (Birnbaum et al. 1986). The incidence of cleft palate in the offspring increased to 100% following the combined treatment. Pretreatment of pregnant NMRI mice with benzo(*a*)pyrene subcutaneously 5 hours prior to an intraperitoneal injection of 2,3,7,8-TCDD caused an increase in CDD-induced lethality but did not alter the rate of cleft palate formation (Hassoun 1987). Offspring of male mice, treated with chlorinated phenoxy acids and 2,3,7,8-TCDD in their feed for 8 weeks before the mating, did not differ in their development or survival from offspring in the control group (Lamb and Moore 1981).

Results in $B6C3F_1$ mice indicated that α-naphthoflavone antagonizes 2,3,7,8-TCDD in induction of splenocyte EROD activity (Blank et al. 1987). It was further suggested that α-naphthoflavone impedes 2,3,7,8-TCDD suppression of B lymphocyte differentiation by competing for binding to the Ah receptor. The mechanism of interaction of these chemicals was studied *in vitro* using rat hepatic cytosol or mouse hepatoma cells (Gasiewicz and Rucci 1991). The results indicated that α-naphthoflavone acts as a 2,3,7,8-TCDD antagonist by binding to the Ah receptor and forcing on it a conformation that cannot identify the DNA recognition sequence contained in the dioxin-responsive enhancer element of the CYP1A1 gene. In contrast, *in vitro* experiments showed that co-exposure of a thymus organ culture with the weakly toxic β-naphthoflavone and 2,3,7,8-TCDD results in a significant increase in the lymphoid inhibitory effect mediated by 2,3,7,8-TCDD (Hassoun 1987).

Hexachlorobenzene acted like a weak Ah receptor agonist and caused an up to 40% decrease in specific hepatic cytosol binding of 2,3,7,8-TCDD in rat cells (Hahn et al. 1989b). Similarly, 2,3,7,8-TCDD-induced myelotoxicity and enzyme induction was antagonized by 1-amino-3,7,8-trichlorodibenzo-p-dioxin in $B6C3F_1$ mice presumably by competitive binding to the cytosolic Ah receptor (Luster et al. 1986). Comparable effects were observed *in vitro* in murine bone-marrow-cells cultures. Treatment of Fischer 344 rats orally with di(2-ethylhexyl)phthalate (DEHP) before or after oral administration of 2,3,7,8-TCDD reduced the hyperlipidemia induced by the latter compound (Tomaszewski et al. 1988). Furthermore, DEHP pretreatment followed by daily doses of this hypolipidemic substance was partially protective against 2,3,7,8-TCDD-induced mortality, wasting, and liver fatty changes.

The addition of activated charcoal or dehydrocholic acid to the feed, protected animals (C57BL/6J mice, CD-COBS rats, and guinea pigs) from increased mortality caused by a single lethal dose of 2,3,7,8-TCDD (Manara et al. 1984). In the case of the former agent, the effect was probably due to the general high binding ability of superactivated charcoal; since no other antidote is known, its use for therapeutic purposes was recommended. Protective effects of ascorbic acid (administered orally) and butylated hydroxyanisole (BHA) (administered orally) against 2,3,7,8-TCDD given by gavage were investigated in Sprague-Dawley rats (Hassan et al. 1987). BHA administration partially protected rats from losses in organ weights and 2,3,7,8-TCDD-induced lipid peroxidation and inhibition of glutathione peroxidase activity. In contrast, ascorbic acid had no protective effects.

Data regarding interactions affecting the toxicity or toxicokinetics of other chemicals by 2,3,7,8-TCDD were limited. Dermal pretreatment with 2,3,7,8-TCDD inhibited the induction of skin tumors by subsequently applied benzo(*a*)pyrene or dimethylbenz(*a*)anthracene in Sencar mice (Cohen et al. 1979). It was proposed that 2,3,7,8-TCDD caused qualitative alteration of hydrogen binding to DNA. In addition, 2,3,7,8-TCDD may also promote the metabolism of procarcinogens (e.g., 3-methylcholanthrene) to active metabolites by the induction of metabolizing enzymes (Kouri et al. 1974, 1978).

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to CDDs than will most persons exposed to the same level of CDDs in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxification or excretion of CDDs, or compromised function of target organs affected by CDDs. Populations who are at greater risk due to their unusually high exposure to CDDs are discussed in Section 5.6, Populations With Potentially High Exposure.

No data were located regarding unusually susceptible subpopulation in humans. Animal data showed developmental effects of 2,3,7,8-TCDD in fetuses and newborns exposed *in utero* and via breast-feeding, respectively (Abbott and Birnbaum 1989b; Giavini et al. 1982, 1983; Håkansson et al. 1987; Weber et al. 1985) (see Section 2.2.2.6). The experimental data suggest that the prenatal and postnatal population may be more sensitive to 2,3,7,8-TCDD-induced effects; however, the levels of exposure necessary to induce such effects are not known. Data in mice indicated that strain differences in sensitivity to 2,3,7,8-TCDD toxicity exist and are associated with the Ah receptor (Poland and Glover 1980). It has been shown that

the Ah receptor exists in human lymphoid tissue and that its concentration is variable between persons (Hayashi et al. 1994; Lorenzen and Okey 1991). As noted in Section 2.4, 2,3,7,8-TCDD may promote the metabolism of procarcinogens (e.g., contained in cigarette smoke) to active intermediates by the induction of metabolizing enzymes. The induction of these enzymes in humans appears to be subject to genetic polymorphism so that individuals who have an Ah receptor with high affinity for 2,3,7,8-TCDD and related chemicals may be at the highest risk for the development of lung tumors (Antilla et al. 1991; Bartsch et al. 1990; Kawajiri et al. 1990; McLemore et al. 1990; Uematsu et al. 1991).

2.10 METHODS FOR REDUCING TOXIC EFFECTS

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

No texts were found that provided specific information about treatment following exposures to CDDs.

2.10.1 Reducing Peak Absorption Following Exposure

No specific information was located regarding the reduction of peak absorption of CDDs by the oral and inhalation routes of exposure in humans. Weber et al. (1992d) examined the effect of four decontamination protocols on the residency time of 2,3,7,8-TCDD in intact or damaged human post-mortem skin *in vitro*. Damage was simulated by stripping of the stratum corneum. 2,3,7,8-TCDD was applied to the skin for 100 minutes and one of the following protocols was performed: the sample was wiped with dry, adsorbent material (cotton balls); a 10-minute topical treatment with mineral oil was followed by dry wiping with cotton balls; a 10-minute topical treatment with mineral oil was followed by wiping with acetone-soaked cotton balls; and the sample was washed with water and soap. In intact skin, mineral oil treatment and acetone wipes reduced by about two-fold the amount of 2,3,7,8-TCDD in the stratum corneum. Mineral oil plus dry wipes reduced the amount of 2,3,7,8-TCDD in the stratum corneum by about 33%, whereas dry wiping alone was ineffective. However, all protocols were equally effective in reducing the amount of 2,3,7,8-TCDD in the epidermis and upper dermis by factors of up to 10. In damaged skin, by dry wiping with adsorbent material 2,3,7,8-TCDD was rubbed into the skin, leading to increased concentrations in the

various layers of the skin. In contrast, mineral oil treatment followed either by dry wipes or by acetone wipes, and washing with water and soap decontaminated the damaged skin quite effectively. The authors (Weber et al. 1992d) noted that the effect of decontamination was most pronounced at a skin depth between 160 and 500 μm, which is the depth at which vascularization begins and, therefore, the protocols discussed should be particularly effective in reducing systemic absorption of 2,3,7,8-TCDD from the skin.

2.10.2 Reducing Body Burden

Limited information was located regarding reducing body burden following exposure to CDDs in humans. A recent study examined the influence of short-term dietary measures on CDD and CDF concentrations in human milk (Pluim et al. 1994c). The authors hypothesized that mobilization of fatty acids from adipose tissue cause the concomitant release of CDDs and CDFs, which will then be eliminated in the breast milk. Two diets were tested for their ability to reduce the concentration of CDDs and CDFs in human milk: a low-fat/high carbohydrate/low CDD and CDF diet (16 women), and a high-fat/low carbohydrate/low CDD and CDF diet (18 women). The authors also analyzed the fatty acid pattern of the milk to determine whether the dietary changes were sufficient to change the milk-fat composition. The test diets were followed for 5 consecutive days in the fourth week after delivery. Body weights were not affected by the experimental diets. The results showed that the fat content of the milk did not decrease in either group during the test diet. Furthermore, there was no significant change in CDD and CDF concentration in milk fat after treatment with the experimental diets. However, the percentage of medium-chain fatty acids (MCFA) changed significantly. In the low-fat/high carbohydrate diet group, the percentage of MCFA increased while the percentage of C_{18}:1ω9 fatty acids (fatty acid with 18 straight-chain carbon atoms, 1-methylene-interrupted double bond and 9 carbon atoms from the terminal methyl group to the nearest double bond) decreased. In the high-fat/low carbohydrate diet group the changes were in the opposite direction. According to the authors, the results would indicate that the concentration of CDDs and CDFs in milk fat may be independent of the source of the fatty acids. Alternatively, they indicate that the dieting period may have been too short or the dietary changes in fat and carbohydrate intake may have not been large enough.

Using a fugacity-based PBPK model to evaluate elimination of 2,3,7,8-TCDD from humans, assuming a background 2,3,7,8-TCDD intake of 50 pg/day, Kissel and Robarge (1988) estimated that daily consumption of 10 g of a nonabsorbable oil would reduce the steady-state adipose tissue concentration of 2,3,7,8-TCDD from 7.7 ppt to 3 ppt. At an adipose tissue level of 50 ppt, the apparent half-life of

2,3,7,8-TCDD would be decreased by consumption of the nonabsorbable oil from 5.2. to 2.1 years. However, this is a theoretical assumption based on the PBPK model.

Zober and Päpke (1993) reported that serum lipid 2,3,7,8-TCDD levels increased from 17 ppt to 32 ppt in a patient who lost >7 kg of weight in a 5-month period. The serum lipid concentrations of 1,2,3,6,7,8-HxCDD, 1,2,3,4,5,7,8-HpCDD, and OCDD also increased during this period. Fasting appeared to relieve signs and symptoms of intoxication in a group of patients who ingested rice oil contaminated with the structurally related PCBs and CDFs (Yu-Cheng incident) (Imamura and Tung 1984). The authors suggested that fasting may stimulate mobilization of the chemicals from adipose tissue to the liver where they are then metabolized, which would facilitate excretion and reduce body burden. However, the findings of that study should be interpreted with caution because a control group was not used, small number of subjects were evaluated, the patients volunteered for the study, and some of the end points that were evaluated were subjective. Promotion of fecal excretion of CDFs and PCBs by cholestyramine, a hypercholesterolemia therapeutic agent used in the treatment of poisoning by chlorinated organic agricultural chemicals, was inconclusive in a clinical trial with six Yusho patients (Iida et al. 1991; Murai et al. 1991).

In experimental animals, administration of a diet containing 2.5 or 5% activated charcoal substantially reduced mortality due to a single lethal oral dose of 2,3,7,8-TCDD in rats, mice, and guinea pigs (Manara et al. 1984). Also, feed with 0.25 or 0.5% cholic acid had a similar protective action in mice (Manara et al. 1984). The effect of activated charcoal was attributed to increased clearance of unabsorbed 2,3,7,8-TCDD from the body; the mechanism of protection by cholic acids was unclear.

2.10.3 Interfering with the Mechanism of Action for Toxic Effects

There are no established methods for interfering with the mechanism of action of CDDs. Many of the toxic effects of 2,3,7,8-TCDD and structurally related halogenated aromatic hydrocarbons have been shown to be mediated through the Ah receptor (see Section 2.4 for details). The sequence of events associated with the receptor-mediated mechanism involve entry of 2,3,7,8-TCDD into the cell, binding to the cytosolic Ah receptor, binding of the receptor-ligand complex to DNA recognition sites, and expression of specific genes and the translation of their protein products. Although speculative, it is possible that interference with this mechanism may lead to a more specific treatment for reducing some of the toxic effects of 2,3,7,8-TCDD and structurally related chemicals. Future research on Ah receptor antagonists may provide new insights

for clinical treatment of the Ah receptor-mediated toxicity of 2,3,7,8-TCDD and other Ah receptor agonists.

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 CDDs 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 CDDs.

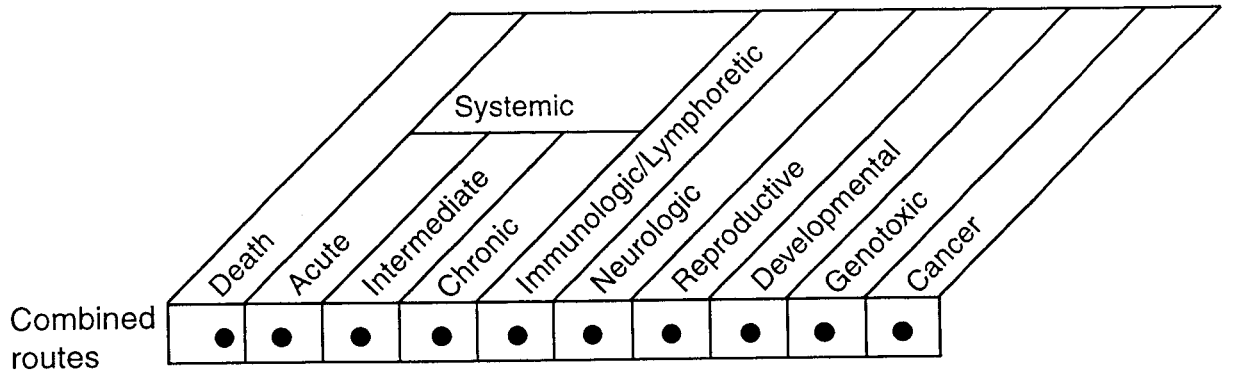
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean 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 CDDs

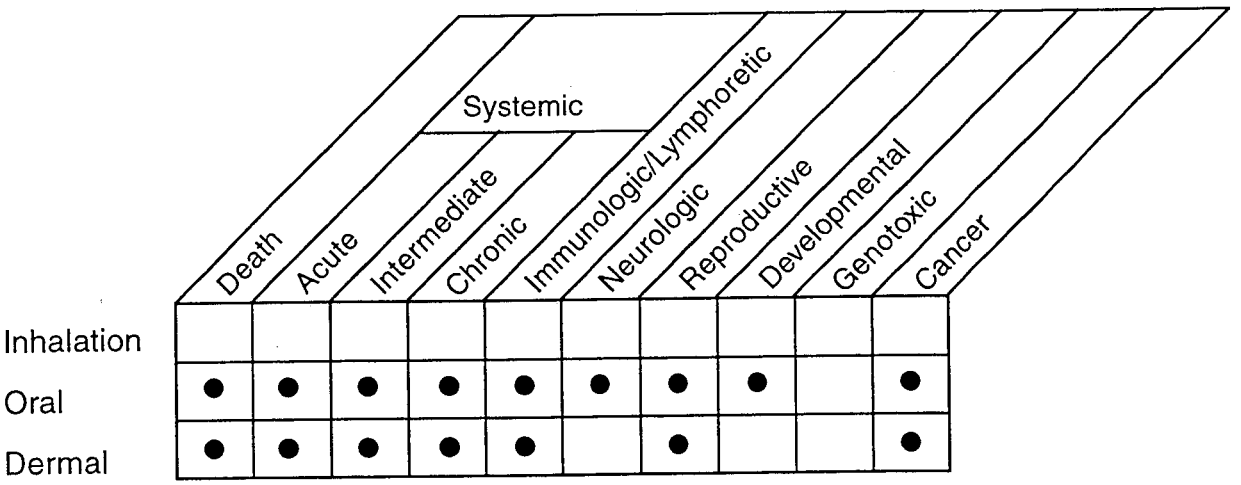
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to CDDs are summarized in Figures 2-5 and 2-6. The purpose of this figure is to illustrate the existing information concerning the health effects of CDDs. Each dot in the figure indicates that one or more 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.

As seen in Figures 2-5 and 2-6, information is available regarding death, systemic, immunological, neurological, developmental, reproductive, and genotoxic effects and cancer in humans. Most of the available information is for 2,3,7,8-TCDD. Most of this information is negative or inconclusive except for

Figure 2-5. Existing Information of Health Effects of 2,3,7,8-TCDD



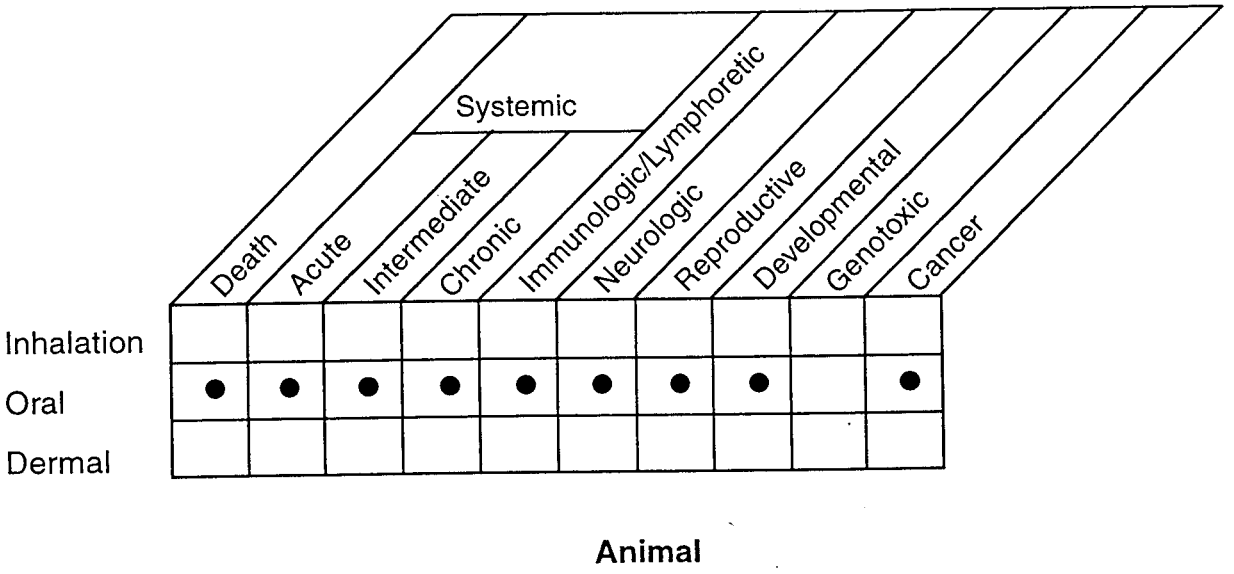
Human



Animal

● Existing Studies

Figure 2-6. Existing Information of Health Effects of Other CDDs



● Existing Studies

dermal effects and cancer. As previously mentioned, exposure to humans probably occurs by a combination of the inhalation, oral, and dermal routes. No information is available regarding effects of a single route of exposure in humans. However, food is the major source of human exposure in the general population; therefore, the oral route is the most significant exposure route.

Oral and dermal studies in animals provide data on death, systemic effects after acute-, intermediate-, and chronic-duration exposure, and immunological, reproductive, and cancer effects. Furthermore, data exist regarding neurological, developmental, and genotoxic effects after oral exposure. No data were located regarding effects in animals after inhalation exposure to CDDs.

2.11.2 Identification of Data Needs

As discussed in Section 2.5, the EPA and other agencies and scientists are using the TEF scheme as an alternative interim approach for hazard evaluation of CDDs and CDFs. Since toxicological data for other CDD congeners is more limited, additional congener-specific studies would provide valuable data for validating the TEF approach. *In vitro* and short-term parenteral injection studies using sensitive end points (i.e., enzyme induction, immune alterations) have been used for this purpose, but studies using other end points, the oral route, and/or longer durations of exposure would be more informative. Since the database for CDD effects not mediated through the Ah receptor is limited, additional studies may be relevant to understanding whether acute versus chronic responses to 2,3,7,8-TCDD occur by different mechanisms.

CDDs and the structurally related CDFs and dioxin-like PCBs are of concern to ATSDR because of the potential of these chemicals to harm health at relatively low doses. As discussed in Section 2.5, many of the toxic effects of these compounds appear to be mediated by a common mechanism, and CDDs frequently occur with CDFs in the environment. Therefore, due to the common mechanism of toxicity, total toxicity of a CDD/CDF mixture probably results from the added contribution (not necessarily linear) of both classes of chemicals. Because of this, the complex issue of appropriate methodology for quantitatively assessing health risks of CDDs and CDFs is currently being evaluated by ATSDR. Additional information on toxic interactions between CDDs and CDFs, as well as PCBs, would facilitate health risk assessment of this class of chemicals.

Acute-Duration Exposure Acute exposure of humans to 2,3,7,8-TCDD can cause chloracne and hepatic effects (Goldman 1973; Reggiani 1980). Specifying the route of exposure in these human cases is

difficult because the individuals were probably exposed by a combination of routes. Furthermore, human data did not provide any information regarding exposure levels and co-exposure to other chemicals confounds the results. Also, in most cases, the exposed subjects were examined long after exposure occurred. Acute oral exposure to 2,3,7,8-TCDD caused delayed type of death in all animal species tested, and LD_{50} values have been determined for rats (NTP 1982b; Schwetz et al. 1973; Walden and Schiller 1985), minks (Hochstein et al. 1988), rabbits (Schwetz et al. 1973), guinea pigs (McConnell et al. 1984; Schwetz et al. 1973), and hamsters (Henck et al. 1981). Furthermore, an acute LD_{50} was calculated for rats and mice exposed to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980). No deaths were observed with other congeners (2,7-DCDD, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8,9-OCDD) (NTP 1982b) tested, and 2,3,7,8-TCDD proved to be the most toxic CDD. However, interspecies and interstrain differences were found in the susceptibility to CDDs. Systemic effects observed in animals after acute oral exposure to 2,3,7,8-TCDD included cardiovascular (Hochstein et al. 1988; McConnell et al. 1978b), gastrointestinal (Theobald et al. 1991), hematological (Christian et al. 1986), hepatic (Christian et al. 1986; Kelling et al. 1985; Walden and Schiller 1985), renal (Christian et al. 1986; McConnell et al. 1978b), endocrine (Bastomsky 1977; Bestervelt et al. 1993; Fan and Rozman 1995; Potter et al. 1986; Weber et al. 1995), dermal effects (Greig 1984; McConnell et al. 1978b), and body weight loss (Kelling et al. 1985; Moore et al. 1985; Seefeld and Peterson 1984; Weber et al. 1994, 1995). Hepatic and body weight effects were the main signs of 2,3,7,8-TCDD toxicity and occurred also after exposure to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980). Furthermore, immunological effects were observed following low oral doses of 2,3,7,8-TCDD (Burleson et al. 1996; White et al. 1986), and an acute oral MRL was based on a NOAEL for immunological effects (Burleson et al. 1996). In addition, the dermal LD_{50} for 2,3,7,8-TCDD has been determined in rabbits (Schwetz et al. 1973). Since only dermal changes were investigated following acute dermal exposure (Puhvel et al. 1982), further studies could provide useful information regarding additional endpoints; dermal contact is a relevant route of exposure at waste sites where CDDs may be stored. Limited data were located regarding effects in animals after acute inhalation exposure to CDDs (Diliberto et al. 1996; Nessel et al. 1992). Further studies by the inhalation route of exposure would be useful since toxicokinetic data in rats suggest that this could be an important route for systemic absorption of CDDs (Diliberto et al. 1996).

No information was located regarding health effects of other congeners in humans, and limited data exist about effects caused by an acute exposure to these congeners in animals. The information would be useful for populations living near hazardous waste sites who may be exposed to CDDs for acute durations. Should a case of high acute exposure to 2,3,7,8-TCDD occur in humans, prompt comprehensive

examination of those exposed would provide greatly needed information. Furthermore, follow-up medical surveillance of such a population should be conducted for as long as possible.

Intermediate-Duration Exposure. Intermediate-duration exposure of humans to CDDs has occurred after industrial accidents or in population groups (e.g., Vietnam veterans, Vietnamese, and pesticide production workers and applicators) exposed to CDD-contaminated herbicides. As stated above, the route of exposure and exposure levels cannot be exactly determined. Hepatic and dermal changes were the main effects noted, and an association between incidence of diabetes and exposure to 2,3,7,8-TCDD has been reported (Jirasek et al. 1976; USAF 1991). More toxicokinetic data for various routes of exposure with relevant congeners would be useful. These data would help in extrapolation from one route of exposure to another, since no information is available in humans on exposure via the oral route, which is the major exposure route to CDDs. The main adverse effects in animals following intermediate-duration oral and dermal exposure to 2,3,7,8-TCDD included chloracne (Allen et al. 1977; Berry et al. 1978; McNulty 1984), wasting syndrome (DeCaprio 1986; NTP 1982b; Vos et al. 1973), and liver effects (Hebert et al. 1990; NTP 1982a). Similar effects were observed with a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (NCI/NTP 1980), and 1,2,3,7,8-PeCDD and 1,2,3,4,7,8-HxCDD (Viluksela et al. 1998a, 1998b). As with acute-duration exposure, the immune system proved to be a very sensitive end point for intermediate-duration exposure to 2,3,7,8-TCDD, and an intermediate-duration oral MRL was derived from a NOAEL value for immunological effects (DeCaprio et al. 1986). No data were located regarding toxicity or toxicokinetics in animals after intermediate-duration inhalation exposure to CDDs. Information obtained from a 90-day inhalation exposure study would be relevant to people living near hazardous waste sites who may be exposed to CDDs for similar durations or much longer time periods.

Chronic-Duration Exposure and Cancer. A number of epidemiology studies have examined the toxicity of CDDs following chronic exposure to phenoxy herbicides and chlorophenols contaminated with 2,3,7,8-TCDD (Calvert et al. 1991, 1992, 1996, 1998; Cook et al. 1987b; Egeland et al. 1994; Henriksen et al. 1997; Pesatori et al. 1998; Sweeney et al. 1993). Although a number of effects have been observed, interpretation of the results is confounded by a number of factors including lack of adequate exposure information, long postexposure periods, concomitant exposure to other chemicals, and small cohorts. Follow-up medical surveillance of subjects with known past high exposure to 2,3,7,8-TCDD would provide information on the possibility that adverse effects could manifest themselves later in adult life when compounded by normal age-related changes. In addition, further research is needed in areas for which the animal data have demonstrated exposure related effects, but the human data are inconclusive. Such

research in exposed humans should focus on diseases of the circulatory system, reproductive effects, immunological effects, effects on serum lipids, and effects on thyroid function. Hepatic effects were observed in animals after chronic exposure to CDDs (including 2,3,7,8-TCDD, mixed HxCDD isomers, and 2,7-DCDD) by the oral route (NCI/NTP 1979a, 1980; NTP 1982b) and to 2,3,7,8-TCDD by the dermal route (Schwetz et al. 1973). The 2,3,7,8-TCDD congener was the most toxic. Studies in monkeys demonstrated their high susceptibility to 2,3,7,8-TCDD-induced toxicity. Developmental behavioral effects were seen in offspring of monkeys chronically exposed to low oral doses (Bowman et al. 1989a; Schantz and Bowman 1989; Schantz et al. 1992). The lowest dose tested in this series of studies was used to derive a chronic-duration oral MRL.

No studies were located regarding chronic effects of CDD exposure by the inhalation route. Toxicokinetic inhalation data and chronic-duration studies would be useful for assessing the risk levels for people living near municipal, medical, and industrial waste incinerators who can be exposed for chronic durations to CDDs by this route.

Several epidemiological studies of phenoxy herbicide and chlorophenol producers found increases in cancer mortality in populations exposed to 2,3,7,8-TCDD (Fingerhut et al. 1991; Kogevinas et al. 1993; Manz et al. 1991; Zober et al. 1990). 2,3,7,8-TCDD exposure has been especially associated with the development of soft-tissue sarcoma after a prolonged latency period (Eriksson et al. 1981, 1990; Fingerhut et al. 1991; Hardell and Eriksson 1988; Hardell and Sandrom 1979; Kogevinas et al. 1995; Smith et al. 1984a). The human data suggest that 2,3,7,8-TCDD may be a human carcinogen; however, the interpretation of many of these studies is limited by confounding factors (e.g., small cohorts, short latency periods, co-exposure to other chemicals, inadequate exposure data). Since these factors are inherent to epidemiological studies, it is unlikely that new human studies would clarify this issue. There are no reliable human studies on the carcinogenicity of other CDDs. Animal studies provided sufficient evidence that 2,3,7,8-TCDD is a carcinogen after oral (Kociba et al. 1978a; NTP 1982b; Toth et al. 1979) and dermal (Della Porta et al. 1987; Rao et al. 1988) exposure. Furthermore, 2,3,7,8-TCDD has promoting ability on tumors initiated by diethylnitrosourea (Hebert et al. 1990; Poland et al. 1982). Similarly, chronic oral exposure of rodents to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD or to 2,7-DCDD resulted in carcinogenic effects (NCI/NTP 1979a, 1980). No studies were located regarding cancer effects in animals following inhalation exposure to CDDs. However, at this time, it is unlikely that such a study would add any new information regarding the potential carcinogenicity of CDDs in animals.

Genotoxicity. Inconclusive results were obtained regarding genotoxicity of CDDs in human as well as in animal studies. Structural chromosomal changes were found in some groups of exposed individuals (Kaye et al. 1985). However, the studies were confounded by small cohorts and unknown exposures. Positive and negative results at the chromosomal level (Green et al. 1977; Loprieno et al. 1982; Meyne et al. 1985) as well as at the gene level (Randerath et al. 1989; Wahba et al. 1989) were reported in animal studies. Furthermore, negative results were obtained in dominant-lethal tests (Khera and Ruddick 1973) and sex-linked recessive-lethal tests in rats and *Drosophila* (Zimmering et al. 1985), respectively. In addition, mostly negative results were obtained in prokaryotic organisms (Geiger and Neal 1981; Gilbert et al. 1980; Toth et al. 1984). Some studies indicated that the covalent binding of 2,3,7,8-TCDD to DNA is low, and that this mechanism does not operate in CDD genotoxicity. Further studies on the mechanism of CDDs would be useful to evaluate the best possible method for detecting CDD genotoxicity.

Reproductive Toxicity. Data from studies on reproductive effects in humans (Aschengrau and Monson 1989; Egeland et al. 1994; Forsberg and Nordstrom 1985; Henriksen et al. 1996; Phuong et al. 1989a; Smith et al. 1982; USAF 1991; Wolfe et al. 1985, 1995) are inconclusive and are limited by confounding factors such as small cohorts, co-exposure to other chemicals, and inadequate exposure data. Better controlled epidemiological studies measuring 2,3,7,8-TCDD exposure levels or 2,3,7,8-TCDD body burdens would be useful to assess the human reproductive toxicity risk. Reproductive effects have been observed in oral animal studies. Increased incidences of pre- and post-implantation losses were observed in 2,3,7,8-TCDD-exposed rodents (Giavini et al. 1983; Neubert and Dillman 1972; Smith et al. 1976; Sparschu et al. 1971a), rabbits (Giavini et al. 1982), and monkeys (McNulty 1985). Adverse effects have also been observed in the reproductive organs (decreased weight), hormone levels, and gametes of male rats (Khera and Ruddick 1973; Moore et al. 1985) and non-pregnant female rats (Li et al. 1995a, 1995b). None of the acute-duration exposure studies assessed the potential of CDDs to impair fertility; data on fertility would be useful in assessing potential effects in humans exposed to CDDs for a short period of time. Reduced fertility (Bowman et al. 1989b; Hong et al. 1989; Murray et al. 1979; Schantz et al. 1992), increased incidence of abortions (Bowman et al. 1989b; Hong et al. 1989; McNulty 1984; Schantz et al. 1992), altered estrus cycle (Umbreit et al. 1987), and endometriosis (Rier et al. 1993) were observed in animals exposed for intermediate or chronic durations. Reproductive effects have also been observed in animals exposed to mixed HxCDD (Schwetz et al. 1973), but not following exposure to 2-MCDD, 2,3-DCDD, 2,7-DCDD, 1,2,3,4-TCDD, or OCDD (Khera and Ruddick 1973). Data on the reproductive toxicity of CDD following dermal exposure is limited to a single animal study which found no adverse effects on reproductive organs of mice chronically exposed to 2,3,7,8-TCDD (NTP 1982a). No animal

inhalation reproductive toxicity studies were located. Additional animal inhalation and dermal reproductive studies, particularly studies which assessed reproductive performance, would be useful to assess the possible risk in humans exposed to CDDs by these routes.

Developmental Toxicity. Studies in humans and animals indicated that 2,3,7,8-TCDD can cross the placenta and is excreted in milk (Fürst et al. 1989b; Schecter et al. 1989d, 1989g, 1990). Studies on the developmental toxicity of 2,3,7,8-TCDD in humans are inconclusive. Some studies have found significant increases in the risk of certain birth defects (Aschengrau and Monson 1990; Erickson et al. 1984; Hanify et al. 1981; Nelson et al. 1979; Phuong et al. 1989a; Wolfe et al. 1985, 1995), while other studies have found no significant alterations (Bisanti et al. 1980; Mastroiacovo et al. 1988; Townsend et al. 1982). However, a number of limitations (e.g., lack of exposure data, small sample sizes, and the lack of reliable data for birth defects prior to 2,3,7,8-TCDD exposure) limits the interpretation of the results of these studies. Epidemiology studies which measure exposure concentrations or body burdens would be useful to determine if 2,3,7,8-TCDD and other CDD congeners are human developmental toxicants. Developmental toxicity has been observed in animals orally exposed to 2,3,7,8-TCDD (Abbott and Birnbaum 1989a; Abbott et al. 1992; Bjerke and Peterson 1994; Bjerke et al. 1994a, 1994b; Bowman et al. 1989a, 1989b; Brown et al. 1998; Courtney 1976; Couture-Haws et al. 1991b; Giaviani et al. 1983; Gordon et al. 1995; Gray and Ostby 1995; Gray et al. 1995; Håkansson et al. 1987; Huuskonen et al. 1994; McNulty 1985; Moore et al. 1973; Neubert and Dillman 1972; Roman et al. 1998a, 1998b; Schantz et al. 1992; Silkworth et al. 1989b; Smith et al. 1976; Thomas and Hinsdill 1979; Weber et al. 1985), 2,7-DCDD (Khera and Ruddick 1973; Schwetz et al. 1973), mixed HxCDD (Schwetz et al. 1973), and OCDD (Schwetz et al. 1973). The most common effects were cleft palate, hydronephrosis, impaired development of the reproductive system, immunotoxicity, and death. No studies were located regarding developmental effects in animals after inhalation and dermal exposure. Such studies would be useful for extrapolating the possible risk to human populations exposed environmentally by these routes.

Immunotoxicity. Studies in humans did not provide conclusive evidence regarding immunotoxicity of CDDs (Ernst et al. 1998; Jansing and Korff 1994; Jennings et al. 1988; Jung et al. 1998; Mocarelli et al. 1986; Neubert et al. 1993, 1995; Reggiani 1980; Stehr et al. 1986; Svensson et al. 1994; Tonn et al. 1996; USAF 1991; Webb et al. 1989; Wolfe et al. 1985). Studies in animals indicated that CDDs are immuno-suppressive (Kerkvliet 1995). 2,3,7,8-TCDD induced thymic atrophy or thymic weight changes after oral (Hanberg et al. 1989; McConnell et al. 1978b), dermal (Hebert et al. 1990), and parenteral exposure (Gorski et al. 1988b; Olson et al. 1980a). Bone marrow degeneration was reported in orally exposed

monkeys (Hong et al. 1989). Suppressed cell-mediated and humoral immunity was found in rodents after intermediate-duration exposure (Vos et al. 1973). Similarly, immunotoxic effects were found after oral exposure of rodents to 2,7-DCDD or to a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD (Holsapple et al. 1986b; NCI/NTP 1980). At least in mice, differences in responsiveness to CDDs' immunotoxicity *in vivo* segregated with the Ah locus (Nagayama et al. 1989; Vecchi et al. 1983a).

Studies in animals aimed at identifying 2,3,7,8-TCDD-sensitive immune end points that can also be measured in humans would be valuable to determine correlative changes in the biomarker and immune function. However, this can be done only after establishing a database of normal values for the clinical immunology end points that may be used as biomarkers of immune-function in immunotoxicity assessments. It also important to determine in animals how well changes in lymphoid organs correlate with changes in the expression of lymphocyte subset/activation markers in peripheral blood. The role of the Ah receptor in the immunotoxicity of 2,3,7,8-TCDD needs to be researched in species other than mice. In addition, the role of Ah receptor-independent processes in 2,3,7,8-TCDD-induced immunotoxicity needs to be examined further. Such actions may include changes in intracellular calcium or in the activity of kinase/phosphatase systems, or interactions with hormone systems. A battery of immune function tests in human cohorts exposed to CDDs would be useful for detecting the immunotoxic responses in exposed individuals. The ability of CDD-exposed individuals to mount an integrated functional response to a novel antigen, such as hepatitis B vaccine, would provide a broad measure of immune function in exposed human populations.

Neurotoxicity. Studies in Vietnam veterans could not conclusively demonstrate cognitive or other central nervous system deficits (Goetz et al. 1994). Neurological examinations revealed neurological effects in humans exposed to a CDD-contaminated environment (Pocchiari et al. 1979) and in occupational settings (Goldman 1973; Jirasek et al. 1976; Klawans 1987; Pazderova-Vejlupkova et al. 1981) shortly following exposure, but reports with comparison groups do not offer clear evidence that exposure to 2,3,7,8-TCDD is associated with chronic peripheral neuropathy (Suskind and Hertzberg 1984; Sweeney et al. 1993). No notable neurological effects were found in laboratory animals after oral and dermal exposure. The existing information suggests that in adults, no long-term neurologic affects were even caused by high exposure to 2,3,7,8-TCDD-contaminated materials. However, the possibility exists that subtle central nervous system changes acquired in early adulthood could manifest themselves later in adult life when compounded by normal age-related changes in the central nervous system (Goetz et al. 1994). Thus, it would be of interest to include tests of neurological function in ongoing prospective studies of

2,3,7,8-TCDD-exposed populations to determine if neurological effects occur as the exposed population ages.

Epidemiological and Human Dosimetry Studies. Epidemiology studies have investigated the toxicity of 2,3,7,8-TCDD in populations exposed in the workplace or in the contaminated environment (after industrial accidents or herbicide spraying) (Bertazzi et al. 1993; Calvert et al. 1992, 1996, 1998; Egeland et al. 1994; Eriksson et al. 1981, 1990; Fingerhut et al. 1991; Flesch-Janys et al. 1995; Hardell and Eriksson 1988; Hardell and Sandrom 1979; Manz et al. 1991; Mastoiacovo et al. 1988; Mocarelli et al. 1991; Pesatori et al. 1993, 1998; Saracci et al. 1991; Smith et al. 1984a; Sweeney et al. 1993; Vena et al. 1998; Zober et al. 1990) and in Vietnam veterans exposed to Agent Orange (Burton et al. 1998; Henriksen et al. 1997; USAF 1991; Wolfe et al. 1985, 1995). The interpretation of the results of most of these studies is confounded by such factors as unknown levels of exposure, too short or too long postexposure periods, and small cohorts. Well conducted epidemiological and occupational studies that quantify exposure levels would be useful to assess the risk for the main end points of concern (i.e., reproductive, developmental, immunotoxic effects, and cancer). Some of the more recent studies have measured the levels of 2,3,7,8-TCDD and related compounds in serum lipid; these levels can then be used to estimate body burden at the time of exposure. There are a number of drawbacks associated with extrapolating body burdens back to the time of the original exposure using current serum 2,3,7,8-TCDD levels; these include uncertainty associated with 2,3,7,8-TCDD half-life in humans and having to use average serum 2,3,7,8-TCDD levels and average exposure durations and reference body weights and percentage of body fat. There is a lack of consensus on the half-life of 2,3,7,8-TCDD in humans, half-lives ranging from 5 to 12 years have been estimated (Pirkle et al. 1979; Schecter et al. 1994b; Wolfe et al. 1994). Additional human studies measuring 2,3,7,8-TCDD half-life would be useful in establishing dose-response relationships for human effects. All of the above limitations for assessing the body burden of 2,3,7,8-TCDD also apply to other CDDs where far less human toxicokinetic data are available. Thus, it would be useful to have congener-specific human toxicokinetic data on other CDDs and related compounds. Furthermore, human dosimetry studies would be useful in occupational settings to obtain results regarding levels of CDDs in the environment as opposed to levels in serum or adipose tissues.

Biomarkers of Exposure and Effect.

Exposure. Several studies reported results of measurements of CDD levels in the lipid fraction of adipose tissue, milk, and serum from members of the general population with unknown CDD exposure (Andrews et

al. 1989; Ryan et al. 1985a; Schecter et al. 1987b). The gas chromatography-mass spectrometry (GS/MS) tests used to detect CDD levels are sensitive and specific. Analytical testing for levels in biological fluids and tissues can be used for monitoring exposed populations. While chloracne is a known, readily identifiable effect of exposure to CDDs, it is not useful as a biomarker of exposure because of its variable expression in individuals with even very high levels of exposure to these agents. Further information on how aging and changes in body composition can influence the distribution of CDDs in tissues and body fluids would be valuable. A reverse transcriptase polymerase chain reaction method has been used to quantify CYP1A1 mRNA levels on total RNA extracts from human blood lymphocytes (Van den Heuvel et al. 1993). This method was found to be much more sensitive than, for example, measuring EROD activity, and could potentially be used as a human exposure marker for CDDs and structurally related compounds. However, EROD activity measurements can be useful as a marker of exposure to the agents.

Effect. There are no specific biomarkers of effects for CDDs. Exposure to relatively high concentrations of CDDs can lead to the development of chloracne in humans. However, while the presence of chloracne indicates CDD or similar halogenated-chemical exposure, lack of chloracne does not indicate that exposure has not taken place, as evidenced in a cohort from the Seveso incident (Mocarelli et al. 1991). Additional studies could evaluate the feasibility of using body burden as a biomarker for predicting other effects of CDDs. Although the results of an earlier study suggested that 2,3,7,8-TCDD may form adducts with DNA, albeit at an extremely low rate (Poland and Glover 1979), more recent studies that have rigorously looked for 2,3,7,8-TCDD-DNA adducts have been negative (Randerath et al. 1988; Turteltaub 1990). Expression of CYP1A1 mRNA, protein, and/or activity are sensitive biological responses in human tissues which can be observed following exposure to 2,3,7,8-TCDD and related compounds, and may be useful biomarkers of effects. Further studies to identify biomarkers of effects of CDDs would facilitate medical surveillance leading to early detection of potentially adverse health effects and possible treatment.

Absorption, Distribution, Metabolism, and Excretion. There are no quantitative data regarding absorption in humans by the inhalation and dermal routes, but data from accidentally exposed individuals suggest that exposure by these routes may lead to a significant increase in body burden of CDDs (Patterson et al. 1994; Schecter 1994b). Results from one human study indicated that more than 87% of an oral 2,3,7,8-TCDD dose in an oil vehicle was absorbed (Poiger and Schlatter 1986). Also, results from studies of absorption of CDDs from maternal milk by nursing infants showed that 90–95% of the dose of CDDs can be absorbed; hepta-substituted congeners and OCDD exhibited lower absorption rates (Abraham et al. 1994, 1996; Dahl et al. 1995; McLachlan 1993; Pluim et al. 1993b). The data indicate that 2,3,7,8-TCDD

is effectively absorbed and that absorption is vehicle-dependent (Fries and Marrow 1975; Lucier et al. 1986; Poiger and Schlatter 1980); oil vehicles were most effective (Olson et al. 1980b; Piper et al. 1973). Transpulmonary absorption of 2,3,7,8-TCDD also occurs in animals (Diliberto et al. 1996; Nessel et al. 1992). Dermal absorption of 2,3,7,8-TCDD in rats was found to be age-dependent (Banks et al. 1993). In rats, following single equivalent intratracheal, oral, and dermal 2,3,7,8-TCDD doses, absorption was calculated as 95, 88, and 40% of the administered dose, respectively (Diliberto et al. 1996). The available information shows that absorption of 2,3,7,8-TCDD has been fairly well characterized in animals.

Based on analysis of CDDs in adipose tissue, milk, and blood, it appears that humans store exclusively 2,3,7,8-chlorine substituted congeners (Fürst et al. 1987; Rappe et al. 1987; Van den Berg et al. 1986b). Data are available on tissue distribution of 2,3,7,8-TCDD in rats after inhalation, oral, and dermal exposure (Diliberto et al. 1996). The liver and adipose tissue are the major storage sites in animals. In general, distribution of CDDs is congener specific, and depends on the dose and route of administration (Diliberto et al. 1996; Van den Berg et al. 1994). Age was also a factor in the distribution of 2,3,7,8-TCDD in mice (Pegram et al. 1995). The distribution of 2,3,7,8-TCDD-derived radioactivity in subcellular liver fractions has also been studied (Santostefano et al. 1996). 2,3,7,8-Chlorine substituted CDDs are the predominant congeners retained in tissue and body fluids from humans, rodents, and monkeys (Abraham et al. 1989c; Van den Berg et al. 1983). Further dosimetry studies of various durations in which levels of 2,3,7,8-TCDD and related compounds are monitored in tissues suspected of being targets for 2,3,7,8-TCDD toxicity would provide valuable information. These data can be used to establish correlations between target-tissue doses and adverse effects.

Data regarding the biotransformation of CDDs in humans are limited to a self-dosing experiment that provided some evidence that 2,3,7,8-TCDD is partially excreted in the feces in the form of metabolites (Wendling et al. 1990). The use of human cell systems in culture might be considered a useful addition to whole-animal studies for examining the metabolic fate of CDDs. Biotransformation of CDDs has been examined in several species, but the structure of metabolites has been elucidated only in the rat and dog (Poiger and Buser 1984). Although information regarding metabolism following inhalation or dermal exposure is lacking, there is no reason to believe that different pathways would operate after exposure by these routes.

Only one study was located that provide limited evidence of fecal excretion of 2,3,7,8-TCDD metabolites in adult humans (Wendling et al. 1990). Several studies provided information regarding fecal excretion of

CDDs in infants exposed through breast milk (Abraham et al. 1994; McLachlan 1993; Pluim et al. 1993b). Elimination of CDDs through maternal milk is well documented (Fürst et al. 1994; Rappe et al. 1985; Schecter and Gasiewicz 1987a; Schecter et al. 1989d, 1989e). Fecal excretion is the main route of excretion of CDDs in animals after all routes of exposure (Diliberto et al. 1996). Estimates of 2,3,7,8-TCDD half-life in humans are available (Pirkle et al. 1989; Poiger and Schlatter 1986; Wolfe et al. 1994), but further information regarding the relationships between aging, fat redistribution, and half-lives in humans would be valuable.

Comparative Toxicokinetics. CDDs are efficiently absorbed from the gastrointestinal tract of mammals, but the vehicle plays an important role (Olson et al. 1980b; Piper et al. 1973; Poiger and Schlatter 1986; Van den Berg et al. 1987c). Distribution data in orally exposed rodents indicated that the highest postexposure levels were in the liver followed by the fat (Diliberto et al. 1996; Khera and Ruddick 1973; Olson 1986), but distribution is highly dose- and species-dependent. The studies to date suggest that compared with rodents, primates, including humans, accumulate significantly less CDDs in the liver than in adipose tissue (Neubert et al. 1990a; Ryan et al. 1986; Van Miller et al. 1976). With the exception of the guinea pig, mammals retain only 2,3,7,8-substituted congeners. The high liver retention of 2,3,7,8-substituted congeners by rodents has been attributed to the presence of inducible storage sites, presumably CYP1A2 (Leung et al. 1990b). In all mammalian species studied, exposure by breast-feeding has a much greater contribution to the offspring 2,3,7,8-TCDD body burden than placental transfer. Metabolic capacities are species-dependent. Rats, hamsters, and mice metabolize and eliminate CDDs much faster than the guinea pig. The metabolites were excreted predominantly via the bile and feces, with minor amounts excreted in the urine in all species (Diliberto et al. 1996; Fries and Marrow 1975; Weber and Birnbaum 1985). Whole-body half-lives ranged from 11 days in hamsters (Olson et al. 1980b) to more than 1 year in monkeys (Bowman et al. 1989b; McNulty et al. 1982), and approximately 7–12 years in humans (Wolfe et al. 1994). The toxicity of CDDs has been associated with the parent compound and not the metabolites (Mason and Safe 1986a; Weber et al. 1982); therefore, metabolism and excretion represent a detoxification process. The data collected in recent years indicate differences in species susceptibility to CDDs cannot be explained by differences in toxicokinetics alone; it is likely that genetic factors have an important role. Based on this information, species-, congener-, and dose-specific toxicokinetic data need to be factored in human risk assessment for CDDs. Several models that describe the disposition of 2,3,7,8-TCDD in animals and humans were identified from the literature (Andersen et al. 1993, 1997a, 1997b; Carrier et al. 1995a, 1995b; Kissel and Robarge 1988; Kohn et al. 1993; Leung et al. 1988,

1990b). Although each new model that is published usually fills data gaps identified in earlier models, further research is necessary to increase their reliability for use in human risk assessment.

Methods for Reducing Toxic Effects. The mechanism by which CDDs enter the blood stream in humans is not known; consequently, there are no established methods for reducing absorption. In experimental animals, however, administration of a diet containing activated charcoal reduced mortality in an acute-duration study presumably by preventing gastrointestinal absorption and the reabsorption of the chemical from biliary secretions (Manara et al. 1984). Identification of additional substances that could prevent or delay absorption and that do not represent a toxic risk *per se* would be valuable. Increasing the fact content of the diet by ingesting non-absorbable lipids has been suggested as a method for increasing the elimination rate (Rohde et al. 1997). These authors estimated that if the normal feces excretion of 5 g fat/day was quadrupled and the lipid based distribution of CDDs/CDFs between the body and the intestine stayed the same, the overall elimination rate would at least double. There are no established methods for reducing body burden in humans, but data from a study of Vietnam veterans suggested that persons with more fat tend to eliminate 2,3,7,8-TCDD more slowly (Wolfe et al. 1994). It was suggested that metabolic or other factors that change with age (i.e., redistribution of fat stores) affect 2,3,7,8-TCDD elimination. Studies examining the effect of fasting in animals exposed to CDDs would provide useful information that can be used to characterize the effectiveness of this approach better. Although, in recent years, great advances have been made related to the understanding of the mechanism of action of CDDs, no methods exist to block the toxic response due to exposure to CDDs. Further characterization of the Ah receptor protein and understanding of physiological effects of interfering with the chain of events that follow binding of CDDs to the Ah receptor would be useful for the possible identification of blockers of those events. Further studies aimed at elucidating the non Ah receptor-mediated mechanisms of action of CDDs would also be valuable. There are no established methods for mitigation of health effects resulting from exposure to CDDs.

Children's Susceptibility. A limited number of human studies have examined health effects of CDDs in children. Data from the Seveso accident suggest that children may be more susceptible to the dermal toxicity of 2,3,7,8-TCDD (chloracne), but it is not known if this would be the case for other effects. Follow-up medical surveillance of the Seveso children (including measurement of serum 2,3,7,8-TCDD levels) would provide information on whether childhood exposure would pose a risk when the individual matures and ages. The available human and animal data provide evidence that 2,3,7,8-TCDD can cross the placenta and be transferred to an infant via breast milk. Although information on the developmental

toxicity of CDDs in humans is limited, there are extensive animal data that the developing organism is very sensitive to the toxicity of 2,3,7,8-TCDD. Several human studies have found significant alterations in markers of liver, thyroid, immune, and neurological function in young breast-fed infants of mothers with higher current background or general population CDD levels. Recent data suggest that the neurological effects are reversible; prospective studies of the breast-fed individuals would provide useful information on whether these children are at risk of developing additional effects as they age. Further data needs relating to developmental effects are discussed above under Developmental Toxicity.

In general, the available toxicokinetic data did not examine potential differences between adults and children; toxicokinetic studies examining how aging and changes in body composition can influence distribution and turnover rates would be useful in assessing children's susceptibility to CDD toxicity. Most of the available mechanism of action data suggest that the toxicity of 2,3,7,8-TCDD is mediated through the Ah receptor. We do not know if there are any age-related differences in receptor binding or expression; studies in animals would be valuable to fill this information gap. No age-specific biomarkers of exposure or effect were identified for CDDs; the long half-life of 2,3,7,8-TCDD in humans, suggests that there may not be a way to assess whether adults were exposed as children to 2,3,7,8-TCDD. Additionally, there are no data to determine whether there are any interactions with other chemicals which would be specific for children. There is very little available information on methods for reducing 2,3,7,8-TCDD toxic effects or body burdens; it is likely that research in adults would also be applicable to children.

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

2.11.3 Ongoing Studies

Ongoing studies regarding the health effects of CDDs were reported in the Federal Research in Progress File (FEDRIP 1998). Table 2-18 presents a summary of this information.

Table 2-18. Ongoing Studies on CDDs

Investigator	Affiliation	Research description	Sponsor
RL Allen	Hybrizyme Corp., Research Triangle Park, NY	This project will complete the development of a cost-effective assay system for the detection of dioxin-like compounds that incorporates the human Ah-receptor	NIEHS
NZ Alsharif	Creighton University, Omaha, NE	Data from this study will provide a clear indication of the role of oxidative stress in the chronic toxicity of TCDD including its most sensitive target, the immune system	NIEHS
DA Bell	NIEHS	Explore genetic variability in the metabolism of carcinogens such as 2,3,7,8-TCDD	
L Bernstein	University of Southern California	Conduct a case-control study which examines the association of serum organochlorine residue levels with the risk of breast cancer among African-American women	
L F Bjeldanes	University of California San Diego	Identification and characterization of mechanisms of action of food-borne antitoxicants in rats, trout, and murine hepatoma cell lines	
JA Boyd	National Institute of Environmental Health Sciences (NIEHS	Perform a molecular genetic analysis of pathologic conditions of the human uterus, resulting from exposure to chemicals such as 2,3,7,8-TCDD	
CA Bradfield	Northwestern University	Develop new models of the Ah receptor signaling pathway and using transgenic mouse lines to identify the molecular basis of species dependent responses to 2,3,7,8-TCDD; cloning the cDNAs which encode the Ah receptor from a variety of murine strains and animal models	
TP Brent	St. Jude Children's Research Hospital	Develop histochemical assays for MGMT (O6-alkylguanine-DNA alkylt-ransferase, previously called GATase) expression in human tumor and tissue preparations Detailed analysis of the structure, function, and regulation of MGMT activity will ultimately enable the to prediction of tumor resistance, as well as an individual's susceptibility to carcinogenesis induced by chemicals such as 2,3,7,8-TCDD	

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
AL Bunge	Colorado School of Mines, Golden, CO	Develop algorithms which predict the absorbed dose following dermal exposure to chemically contaminated soils	NIEHS
SW Burchiel	University of New Mexico Albuquerque, Albuquerque, NM	Examine the influence of environmental chemicals on human breast epithelial cell growth and signaling associated with endogenous growth-factor receptors	
DL Busbee	Texas A&M University	Evaluate the induction of cytochrome P-4501A1 in human and animal cells by a series of aromatic and halogenated aromatic hydrocarbons; he is also examining whether prior exposure to PAHs significantly alters the amount and type of DNA damage initiated by mutagens in human and rodent cells	
KW Brown	Texas A&M University	Utilize bioassays, mammalian cell cultures, and human lymphocyte cultures to measure the genotoxicity, immunotoxicity, developmental toxicity, and 2,3,7,8-TCDD-induced toxicity of sample extracts from Superfund sites;	
KW Brown and KC Donnelly	Texas A&M University	Develop a comprehensive laboratory testing procedure for evaluating the acute and chronic toxicity of complex environmental mixtures	
MJ Connor	University of California Los Angeles, Los Angeles, CA	Investigation of the mechanisms involved in the expression of 2,3,7,8-TCDD induced toxicity in congenic haired and hairless HRS/J mice	
K Cooper	Rutgers University, New Brunswick, NJ	Evaluate the affects of a number of different compounds: dioxins, dibenzofurnas, oil and estrogenic compounds on both invertebrates and vertebrate species	U. S. Department of Agriculture Cooperative State Research Service
MS Denison	University of California San Diego	Investigations of the molecular mechanisms of action of 2,3,7,8-TCDD in mouse hepatoma cells	
RL Dickerson, GP Cobb, and G Birrenkott	Clemson University	Determine the impact of low levels of 2,3,7,8-TCDD on productivity of White Leghorns chickens and are developing non-lethal techniques for measuring effects in chickens	

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
B Eskenazi	University of California Berkeley, Berkeley, CA	Follow-up the Seveso cohort and examine their risk of endometriosis and other related reproductive endpoints hypothesized to be dose-related to serum levels of TCDD	NIEHS
VJ Feil, JK Huwe, and H Hakk	Agricultural Research Service, Fargo, ND	Identify and quantitate residues of chlorinated organics (congeners of 2,3,7,8-TCDD, furans, and PCBs) in beef and milk, and in animal feeds and certain forages	U. S. Department of Agriculture Agricultural Research Service
PK Freeman	Oregon State University	Determine the mechanistic features of the photodegradation of 2,3,7,8-TCDD in mice	
GF Fries and LS Willett	Ohio Agricultural Research and Development Center	Develop models of the transport of 2,3,7,8-TCDD contained in feeds and other environmental matrices to beef intended for human consumption	
GF Fries	Beltsville Agricultural Research Center	Refine a model for simulation of persistent residues such as 2,3,7,8-TCDD in pigs from weaning through marketing; in growing beef cattle, including the fattening phase; and in dairy cattle during lactation	
TA Gasiewicz	University of Rochester Medical Center	Utilize an *in vivo* bone marrow-thymus reconstitution model and mouse strains with arrested T-cell development to define the cellular and molecular targets of 2,3,7,8-TCDD that lead to thymic atrophy, and determine how these events relate to its overall action on the immune system. Dr. Gasiewicz is also determining what controls the functional activity of the Ah receptor, what target genes are affected in sensitive tissues, and how the modulated expression of these genes leads to the toxic responses observed after exposure to 2,3,7,8-TCDD	
J P Giesy	Michigan State University	Assess the acute and chronic effects of trace contaminants on aquatic organisms using mechanistic and statistical models for predicting the fates of trace contaminants	U. S. Department of Agriculture Cooperative State Research Service

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
WF Greenlee	University of Massachusetts Medical School	Examine the role of the Ah receptor and its known differentiation and growth regulatory genes in the development and function of the thymic microenvironment. The studies use early gestation C57BL/6 and neonatal SCID mice and fetal thymic organ cultures as models.	
M Hahn	Boston University	Utilize fish to investigate the mechanisms of animal sensitivity and resistance to 2,3,7,8-TCDD and related planar halogenated aromatic hydrocarbons (PHAH), especially the resistance which develops after long-term (multi-generation) exposure associated with hazardous waste sites	
ME Hahn	Woods Hole Oceanographic Institution, Woods Hole, MA	Characterize the structure, function, regulation, and evolutionary relationships of the Ah receptor in non-mammalian species, particularly fish	
O Hankinson	University of California Los Angeles, Los Angeles, CA	Use transgenic mouse technology to address the potential role of the Ah receptor nuclear translocator (Arnt) protein in carcinogenesis and in developmental processes	
O Hankinson	University of California Los Angeles, Los Angeles, CA	*In vitro* mutagenesis experiments performed on the Ah receptor nuclear translocator (Arnt) protein in order to identify putative functional domains, including domains for nuclear translocation, for binding the ligand-binding subunit, for binding the XRE, and for transcriptional activation	
EA Hassoun	Creighton University	Study the teratogenicity and fetotoxicity of two polyhalogenated cyclic hydrocarbons (PCH) (endrin and lindane), as compared with that induced by 2,3,7,8-TCDD, in the fetuses of pregnant C57BL/6J (TCDD-responsive) and DBA/2J (TCDD-non-responsive) mice	
M Hejtmancik	Battelle Memorial Institute, Columbus, OH	Test the hypotheses: The USEPA interim TEFs for dioxins, dibenzofurans and PCBs can predict the relative carcinogenic potency of single congeners in female Sprague-Dawley rats	NIEHS

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
A Hendricks	University of California Davis	Test the utility of *in vivo* biomarkers for detecting and characterizing reproductive toxicity of 2,3,7,8-TCDD in experiments with a non-human primate model	
MH Hooper	University of Washington	Characterize biomarkers for toxic substances in wildlife populations inhabiting hazardous waste sites	
RJ Hutz	University of Wisconsin-Milwaukee, Milwaukee, WI	Utilize female rats to determine whether 2,3,7,8-TCDD exerts its antifertility effects by modulating the action of estrogen at the ovary	
AK Jaiswal	Fox Chase Cancer Center	Elucidate the molecular mechanisms that control the basal level of expression in normal and tumor cells, induction and transcription due to xenobiotics such as 2,3,7,8-TCDD, and tissue-specific and developmental expression of NQOI and NQO2 genes in rat tissues	
MO James	University of Florida, Gainesville, FL	Test the hypotheses that two Ah receptor agonists found in Superfund sites, namely 2,3,7,8-TCDD and 3,3',4,4'-TCB cause alterations in the physiological and structural make-up of intestinal cells that affect the intestinal bioavailability and biotransformation of lipophilic chemicals	NIEHS
CR Jeffcoate	University of Wisconsin-Madison, Madison, WI	Elucidate mechanisms of regulation of CYP1B1 gene transcription and see if the AhR is involved in this regulation	National Cancer Institute
NE Kaminski	Michigan State University	Determine 2,3,7,8-TCDD's relative effects on B-cell proliferation and differentiation using B-cells from $B6C3F_1$ mice	
HK Kang	Department of Veterans Affairs Medical Center, Washington, DC	Perform a retrospective cohort mortality study to determine the overall mortality rate as well as the cause-specific mortality rates associated with Vietnam service or exposure to Agent Orange in 10,000 Marines who served in Vietnam and an equal number of those who served elsewhere	Department of Veterans Affairs Research and Development
NI Kerkvliet	Oregon State University	Perform a series of studies investigating cytokine production in 2,3,7,8-TCDD-treated mice	

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
NI Kerkvliet	Oregon State University	Characterize the enhanced T cell activation induced by 2,3,7,8-TCDD as it relates to the suppression of antigen-specific immune responses	
AT Kong	University of Illinois at Chicago, Chicago, IL	Isolate and characterize the genes encoding for two major types of UDP-glucuronosyltransferases (UGTs) in mice to gain insights into the biological mechanisms of detoxification and/or toxicity of different carcinogens catalyzed by UGTs in the mouse	
CA Lamartiniere	University of Alabama at Birmingham, Birmingham, AL	Investigate the potential of environmental chemicals for altering susceptibility for breast cancer in Sprague Dawley CD rats exposed during three critical periods of development	NIEHS
RG Lindahl	University of South Dakota	Elucidate and characterize the mechanisms responsible for tissue-specific differential gene expression in rat in liver and hepatoma cells so as to understand the molecular basis of gene expression under normal and pathophysiological (i.e., following both xenobiotic exposure [to 2,3,7,8-TCDD or 3-methylcholantherene] and during hepatocarcinogenesis) conditions	
G Lucier	NIEHS	Determine dose-response relationships for 2,3,7,8-TCDD following chronic exposure in rodent models and, accidental or occupational exposure in humans	
MI Luster	NIEHS	Employ a variety of *in vivo* and *in vitro* techniques to study the adverse effects on the immune system resulting from exposure to environmental chemicals such as 2,3,7,8-TCDD	
BV Madhukar	Michigan State University	Use embryo and fetal cell cultures to determine if remediation of several classes of mixtures of environmental toxicants (PCBs, HAHs, PAHs) decreases the toxicities of the parent mixtures or actually enhances the toxicities	

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
F Matsumura	University of California Davis	Study the acute and chronic effects of 2,3,7,8-TCDD on adipose lipoprotein lipase (LPL) in guinea pigs	
F Matsumura	University of California Davis	Determine the toxicological consequences of elevated protein tyrosine kinase activities in guinea pigs and mice and why 2,3,7,8-TCDD causes such an effect on EGF receptors	
JL Napoli	State University of New York (SUNY) at Buffalo, NY	Determine whether 2,3,7,8-TCDD causes functional vitamin A abnormalities by altering the steady-state concentrations of retinoic acid and/or RAR/RXR in male and female rat and male Syrian golden hamster tissues	
DW Nebert	University of Cincinnati, Cincinnati, OH	Utilize human transgenic mouse lines to define the precise role of the human Ah receptor in studies of toxicity and cancer caused by 2,3,7,8-TCDD and other environmental chemicals	
JL Newsted	University of Massachusetts Medical School	Develop methodologies utilizing white sucker fish to evaluate the impact of xenobiotics on ecosystem health	
PW O'Keefe	State University of New York SUNY, Albany, NY	Determine the complete range of toxic compounds in the sediment collected near an aluminum plant in the Massena area of the St. Lawrence River	
JR Olson	State University of New York (SUNY) at Buffalo, NY	Develop mechanism-based molecular and biochemical markers of exposure, effect, and/or susceptibility specific to 2,3,7,8-TCDD in maternal and fetal rat liver, placenta, and lymphocytes	
GH Perdew	Purdue University	Use tissue and cell cultures to examine the multiple mechanisms of Ah receptor regulation and seeks to determine the biochemical events from initial synthesis and assembly to translocation into the nucleus. Dr. Perdew is also examining the biochemical properties of the Ah receptor-ligand complex that affect its overall regulation.	
DH Petering	University of Wisconsin-Milwaukee, Milwaukee, WI	Utilize aquatic organisms to study the mechanism of action (i.e, target-organ specificity, regulation of cytochrome P-450 expression) of 2,3,7,8-TCDD	

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
RE Peterson	University of Wisconsin-Madison, Madison, WI	Determine the mechanisms by which 2,3,7,8-TCDD adversely affects the male reproductive system of rats exposed in adulthood or during fetal and neonatal development;	
AP Poland	University of Wisconsin-Madison, Madison, WI	Seek to characterize the Ah receptor in the mouse and is screening lower vertebrate and invertebrate species for the presence of the Ah receptor	
CJ Portier	NIEHS	Increase the use and application of mathematical and statistical models in toxicology and biochemistry and to implement new mathematical models to help explain current research findings relating to carcinogenesis and suppressed immune function following exposure to toxicants such as 2,3,7,8-TCDD	
A Puga	University of Cincinnati, Cincinnati, OH	Elucidate the biological responses (i.e., gene expression patterns) to 2,3,7,8-TCDD in mice	
SM Puhvel	Department of Veterans Affairs Medical Center	Study the effect that 2,3,7,8-TCDD has on human skin (i.e., the biochemical mechanisms involved in the induction of chloracne)	
K Randerath	Texas A&M University	Measure chemical DNA alterations in exposed experimental animals, humans, and cultured cells induced by exposure to aromatic and halogenated aromatic hydrocarbons, reconstituted mixtures from these compound classes, and field extracts of wood-preserving and oily waste dump sites;	
DJ Reed	Oregon State University	Examine the mechanisms of toxicity of selected bioenvironmental chemicals, especially halocarbons	
RH Rice	University of California San Diego	Establish a new short-term test for toxic agents using human epidermal cells	
RH Rice	University of California Davis	Utilize guinea pigs to develop bioassays for the detection of 2,3,7,8-TCDD and other hazardous agents	

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
RH Rice	University of California Davis	Utilize human tissues to investigate the posttranslational modifications to which the enzyme keratinocyte transglutaminase (TGK) is subject, and its transcriptional regulation by effectors such as 2,3,7,8-TCDD	
AB Rifkind	Cornell University Medical Center, Ithaca, NY	Use a chick embryo model to investigate whether 2,3,7,8-TCDD-induced P-450 participates in 2,3,7,8-TCDD toxicity by metabolizing endogenous compounds, such as the membrane fatty acid, arachidonic acid (AA), to biologically active metabolites that can affect cell signals and thereby modulate toxicity	
RA Roeder and MJ Garber	University of Idaho	Develop baseline data regarding the incidence and quantities of PCDD/PCDFs in soft tissues and milk from cattle and determination of the relationship between feeding practices and the presence of PCDD/PCDFs in soft tissues and milk	
SH Safe	Texas A&M University	Conduct several interrelated studies with individual polycyclic aromatic hydrocarbons (PAHs) and reconstituted PAH mixtures to determine the interactions of these compounds and the role of these interactions in PAH-induced carcinogenicity	NIEHS
AE Silverstone	State University of New York SUNY, Syracuse, NY	Determine how activation of the Ah receptor or the ER can lead to thymic atrophy and the appearance of T-cells that could promote autoimmune disease in selected mouse strains	
KT Shiverick	University of Florida, Gainesville, FL	Investigate mechanisms by which the chlorinated hydrocarbons 2,3,7,8-TCDD and PCBs have disruptive effects on placental- uterine function	NIEHS
P Sinclair	Department of Veterans Affairs Medical Center, White River Junction, VT	Determine the mechanism by which 2,3,7,8-TCDD and related planar PAHs cause massive liver accumulation of uroporphyrin (URO)	

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
PR Sinclair	Dartmouth College	Investigate the mechanisms of uroporphyrinogen (UROgen) oxidation and the inhibition of UROgen decarboxylase (URO-D), which are among the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and other planar polyhalogenated hydrocarbons (in hepatocyte cultures and in intact animals, mice and guinea pigs)	
G S Smith	New Mexico State University	Utilize rats, sheep, and/or cattle to study the disposition of selected xenobiotics such as 2,3,7,8-TCDD and protocols that might enhance tolerance	
MB Solomon and LB Willett	Ohio Agricultural Research and Development Center, Wooster, OH	Develop models of the transport of dioxins contained in feeds and other environmental matrices to beef intended for human consumption	U. S. Department of Agriculture Agricultural Research Service
GM Stancel	University of Texas	Examine how developmental exposure of rodents to toxicants such as 2,3,7,8-TCDD disrupt gene expression and produce cellular defects	
TR Sutter	Johns Hopkins University	Elucidate the events that occur in humans exposed to Ah receptor agonists and determine whether effects of 2,3,7,8-TCDD are caused by the altered expression of specific subsets of Ah receptor-regulated genes	
WA Toscano	Tulane University	Determine the biochemical basis underlying 2,3,7,8-TCDD toxicity	
JW Tracy	the University of Wisconsin-Madison, Madison, WI	Determine whether 2,3,7,8-TCDD and oltipraz induce glutathione S-transferase gene expression in *S. mansoni*	
RH Tukey	University of California San Diego	Investigate the cellular events involved in the regulation of the mouse AhR *in vivo*	

Table 2-18. Ongoing Studies on CDDs (continued)

Investigator	Affiliation	Research description	Sponsor
C Vandevoort	University of California Davis	Evaluate the function of human and macaque granulosa, trophoblast and endometrial cells, and macaque embryos in response to 2,3,7,8-TCDD while being cultured *in vitro,* and the cellular mechanisms by which primate reproductive cells sustain toxic damage	
RJ Van Beneden	University of Maine	Examine the molecular mechanisms of tumorigenesis in clams exposed to 2,3,7,8-TCDD-containing sediments	
WM Weston	Thomas Jefferson University	Identify the regulatory elements associated with abnormal palate development in the mouse	
JP Whitlock	Stanford University	To understand the mechanisms by which mammalian cells adapt to environmental exposures and the mechanisms by which environmental exposures produce toxicity	NIEHS
LB Willett	Ohio State University, Wooster, OH	Cattle: methods to detect and monitor occurrence of potentially hazardous xenobiotics in their environment; methods to reduce or eliminate exposure; determine mechanisms by which xenobiotics are transported, bound, and mobilized; study target organ modification caused by xenobiotic chemicals	U. S. Department of Agriculture Cooperative State Research Service
MS Wolff	Mount Sinai School of Medicine of CUNY, New York, NY	Provide analyses for PAH and TCDD compounds that are environmentally important and that may contribute significantly to the assays to be done	NIEHS

NIEHS = National Institute of Environmental Health Sciences