CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of CDDs. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix C, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans for CDDs included in this chapter of the profile. Figures 2-2 and 2-3 provide an overview of the database of studies in experimental animals for 2,3,7,8-TCDD and other CDDs, respectively, included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to CDDs, but may not be inclusive of the entire body of literature.

Animal oral studies are presented in Table 2-2 and Figure 2-4 for 2,3,7,8-TCDD and Table 2-3 and Figure 2-5 for other CDDs. Animal dermal studies are presented in Tables 2-4 and 2-5; no inhalation data were identified for CDDs.

Levels of significant exposure (LSEs) 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. Effects have been classified into "less serious LOAELs" or "serious LOAELs (SLOAELs)." "Serious" effects (SLOAELs) are those that evoke failure in a biological system and can lead to morbidity or

mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of CDDs are indicated in Tables 2-2, 2-3, and 2-4 and Figures 2-4 and 2-5.

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

As illustrated in Figures 2-1, 2-2, and 2-3, the health effects of CDDs have been extensively evaluated in epidemiological and animal studies. Over 250 epidemiological studies have been identified (Figure 2-1), with developmental outcomes being the most frequently examined endpoint. Many of the epidemiological studies provided limited information on the exposure route and duration. Exposure likely involved multiple exposure routes, particularly inhalation and oral routes. Humans are exposed to a variety of CDD congeners; 2,3,7,8-TCDD is the predominant congener for a number of populations, including phenoxy herbicide workers and Seveso residents exposed to an accidental release of 2,3,7,8-TCDD. As presented in Figure 2-2, the toxicity of 2,3,7,8-TCDD has been investigated in over 350 animal studies. Most of these studies (approximately 75%) involved acute-duration oral exposure. The most well-studied health outcome was developmental toxicity, with approximately 100 more studies than the second most investigated endpoint, immune effects; other well-investigated endpoints include body weight, hepatic, and reproductive endpoints. A much smaller number of studies (approximately 60 studies) have examined the toxicity of 11 other CDD congeners: 2-MCDD, 2,3-DCDD, 2,7-DCDD, 2,3,7-TrCDD, 1,2,3,4-TCDD, 1,2,3,7,8-PeCDD, 1,2,4,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD. The most studied other CDD congener was HxCDD (administered as a single congener or as mixed HxCDD congeners) (22%), followed by 1,2,3,7,8-PeCDD (19%), 2,7-DCDD (14%), OCDD (12%), and 1,2,3,4,6,7,8-HpCDD (11%). As with

2,3,7,8-TCDD, the majority of the studies (79%) were acute-duration oral exposure studies. The most investigated endpoints include acute lethality, body weight, liver, and immune endpoints.

Toxic Equivalency Factors. The general population is not typically exposed to single CDD congeners; rather, they are environmentally exposed to mixtures of halogenated aromatic hydrocarbons, of which various CDDs are constituents. CDFs and PCBs frequently occur with CDDs in the environment. The toxic effects of CDDs, CDFs, and some non-ortho-substituted PCBs (collectively referred to as dioxin-like compounds or dioxins) share a common mechanism of action in that they are mediated through the aryl hydrocarbon receptor (AhR), resulting in similar adverse health outcomes. Although they share toxic endpoints, there are congener-specific differences in toxic potency. Experimental data evaluating the toxicity of mixtures of dioxin-like compounds provide strong evidence of additivity (van den Berg et al. 2006). To provide an estimate of the toxic potency of mixtures of these compounds while accounting for the toxic potency differences between them, a TEF approach was developed.

In the TEF approach for dioxin-like compounds, the relative effect potency of individual CDD, CDF, and PCB congeners for producing toxic or biological effects is estimated and expressed relative to that of the reference compound, 2,3,7,8-TCDD (TEF=1). The TEFs can be used, assuming additivity of the toxic response, for estimating the toxicity of an environmental mixture containing a known distribution of CDDs, CDFs, and/or PCBs. Given the assumption of additivity of the toxic responses, the total TEQ of a mixture is defined as the sum of the products of the concentration of each mixture component multiplied by its respective TEF. The resulting TEQ value is an estimate of the total 2,3,7,8-TCDD-like activity of the mixture (van den Berg et al. 2006).

An expert panel organized by the World Health Organization (WHO) initially developed TEFs for all 2,3,7,8-substituted CDDs and CDFs and several PCBs in 1993, and subsequent WHO expert panels updated these TEFs in 1998, 2005, and 2022. In the 2005 TEFs, PCB compounds were included if they met the following criteria: (1) they show a structural relationship to CDDs and CDFs; (2) they bind to the AhR; (3) they elicit AhR-mediated biochemical and toxic responses; and (4) they are persistent and accumulate in the food chain (van den Berg et al. 2006). For additional information on the development of the TEFs, see Haws et al. (2006), van den Berg et al. (2006), and DeVito et al. (2024). The 1998, 2005, and 2022 WHO TEFs are presented in Table 2-1; it is noted that most epidemiological studies reported in this toxicological profile used the 2005 WHO TEFs.

Table 2-1. Summary of World Health Organization (WHO) 1998, 2005, and 2022 Toxic Equivalency Factors (TEFs)

Compound	1998 TEF[a]	2005 TEF[a]	2022 TEF[a]
Chlorinated dibenzo-*p*-dioxins (CDDs)			
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PeCDD	1	1	0.4
1,2,3,4,7,8-HxCDD	0.1	0.1	0.09
1,2,3,6,7,8-HxCDD	0.1	0.1	0.07
1,2,3,7,8,9-HxCDD	0.1	0.1	0.05
1,2,3,4,6,7,8-HpCDD	0.01	0.01	0.05
OctaCDD	0.0001	0.0003	0.001
Chlorodibenzofurans (CDFs)			
2,3,7,8-TCDF	0.1	0.1	0.07
1,2,3,7,8-PeCDF	0.05	0.03	0.01
2,3,4,7,8-PeCDF	0.5	0.3	0.1
1,2,3,4,7,8-HxCDF	0.1	0.1	0.3
1,2,3,6,7,8-HxCDF	0.1	0.1	0.09
1,2,3,7,8,9-HxCDF	0.1	0.1	0.2
2,3,4,6,7,8-HxCDF	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.02
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.1
OctaCDF	0.0001	0.0003	0.002
Non-*ortho*-substituted polychlorinated biphenyls (PCBs)			
3,3′,4,4′-tetraCB (PCB 77)	0.0001	0.0001	0.0003
2,3,4,4′,5-tetraCB (PCB 81)	0.0001	0.0003	0.006
3,3′,4,4′,5-pentaCB (PCB 126)	0.1	0.1	0.05
3,3′,4,4′,5,5′-hexaCB (PCB 169)	0.01	0.03	0.005
Mono-*ortho*-substituted PCBs			
2,3,3′,4,4′-pentaCB (PCB 105)	0.0001	0.00003	0.00003
2,3, 4,4′,5-pentaCB (PCB 114)	0.0005	0.00003	0.00003
2,3′,4,4′,5-pentaCB (PCB 118)	0.0001	0.00003	0.00003
2′,3,4,4′,5-pentaCB (PCB 123)	0.0001	0.00003	0.00003
2,3,3′,4,4′,5-hexaCB (PCB 156)	0.0005	0.00003	0.00003
2,3,3′,4,4′,5′-hexaCB (PCB 157)	0.0005	0.00003	0.00003
2,3′,4,4′,5,5′-hexaCB (PCB 167)	0.000001	0.00003	0.00003
2,3,3′,4,4′,5,5′-heptaCB (PCB 189)	0.0001	0.00003	0.00003

[a]TEFs are relative to the toxicity of 2,3,7,8-TCDD.

Sources: DeVito et al. 2024; Van den Berg et al. 2006

Epidemiological Studies. The epidemiological database evaluating the toxicity of CDDs, CDFs, and/or PCBs is extensive. The database consists of occupational exposure studies, studies of communities living

near point sources, communities affected by accidental releases, and the general population exposed to background levels, primarily from CDDs, CDFs, and/or PCBs in the food supply. This profile will focus on the toxicity of CDDs and greater emphasis is placed on epidemiological studies with known exposure to CDDs, or a specific congener; for additional information on the toxicity of CDFs and PCBs, the reader is referred to the toxicological profiles on these compounds (ATSDR 2000, 2023).

With the exception of some occupational exposure and community exposure studies, exposure levels were not measured; most studies used serum lipid CDD, CDF, and/or PCB levels as a biomarker for exposure. Studies reported serum levels as individual congener levels; total CDD, CDF, and/or PCB levels; total CDD/CDF levels; TEFs for individual congeners; and total CDD, total CDD/CDF, or CDD/CDF/PCB TEQs. In many studies, serum 2,3,7,8-TCDD levels were measured a number of years after exposure termination. CDDs are highly persistent lipophilic compounds that 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 lipids 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. Some 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 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 living an area with CDD-contaminated soil.

As noted previously, epidemiological data come from a number of sources, and several cohorts have been followed for a number of years; brief descriptions of some of these cohorts are provided below.

Occupational Exposure. The first reported cases of industrial poisoning were in 1949 at a factory producing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) 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 (Moses et al. 1984). Approximately 228 workers (including production workers, laboratory personnel, and medical personnel) were affected. Between 1949 and 1968, three other explosive releases were reported: one 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 involved 106 workers at Philips-Duphar facility in Amsterdam, Netherlands (Holmstedt 1980); and the third was an explosion in a 2,4,5-TCP manufacturing facility in Coalite, England, involving 90 workers (May 1973). The accident at the Philips-Duphar facility involved both facility workers and cleanup workers (Holmstedt 1980). 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 2 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 10 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. Studies have also been conducted in residents living near a municipal incinerator or near a former pentachlorophenol (PCP) production facility in Taiwan.

The most widely studied release of 2,3,7,8-TCDD primarily involving residential exposures occurred in Seveso, Italy in 1976 (Mastroiacovo et al. 1988). The ICMESA factory produced trichlorophenol by hydrolysis of 1,2,4,5-tetrachlorobenzene 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 >1.3 kg of 2,3,7,8-TCDD was released into the atmosphere and that >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) $\mu g/m^2$. Dividing the populations into different zones based on soil levels has been criticized because it does not take into consideration actual exposure levels or 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 were 828–56,000 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 (5–577 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, the subjects 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 (1962–1970), 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-dichlorophenoxyacetic acid (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 War 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 <1 year, the military exposure was considered to be of intermediate duration if not stated otherwise in the original study. In addition to the studies of Vietnam War veterans and the Operation Ranch Hand cohort, a number of studies have been conducted in residents living in areas with heavy Agent Orange exposure. High levels of CDDs, particularly 2,3,7,8-TCDD, have been measured in soil and food from areas in Vietnam that were sprayed with Agent Orange. A study published in 2006 (Schecter et al. 2006) reported a median serum 2,3,7,8-TCDD level of 30.9 ppt lipid (range of 13.6–180 ppt lipid) in a small group of residents of Can Tho province. The medians in other Agent Orange sprayed areas ranged from 1.5 to 7.3 ppt lipid.

Animal Studies. The literature on the health effects of CDDs, especially 2,3,7,8-TCDD, following oral exposure is extensive; thus, it is not practical or realistic to cite all, or even most, of the 2,3,7,8-TCDD oral animal studies. Therefore, the discussion of 2,3,7,8-TCDD in this chapter emphasizes low dose studies that could help construct dose-response relationships and determine points of departure (PODs) for the various specific effects. As summarized in Figure 2-2, there are 350 papers evaluating the oral toxicity of 2,3,7,8-TCDD in animals cited in Chapter 2; however, not all of the studies are included in the LSE table and figure (Table 2-2 and Figure 2-4); for a particular endpoint, higher dose studies were excluded from the LSE table and figure. No exclusion criteria were used for the other CDD congeners due to the small number of studies for a specific congener.

Overview of Health Effects of CDDs. Although a large number of epidemiological studies have evaluated the toxicity of CDDs, the results are not consistent across studies. There are several contributing factors to this inconsistency including:

- Exposures to different mixes of CDD congeners
- Differences in CDD exposure levels
- Insensitive biomarker of exposure
- Exposure to low levels of CDDs
- Time elapsed between exposure and when health outcomes are assessed

Adverse health outcomes have been observed in animals for all health endpoints discussed in this chapter. Effects observed at the lowest doses include developmental toxicity, immunotoxicity, reproductive toxicity, and hepatotoxicity; cancer has also been observed.

- ***Developmental Effects***
  - *Epidemiological Studies.* Studies in highly exposed populations have found associations between 2,3,7,8-TCDD exposure and impaired development of the male reproductive system (decreased sperm concentrations and delayed puberty) when males were exposed as boys and between maternal 2,3,7,8-TCDD levels and neonatal thyroid-stimulating hormone (TSH) levels. Mixed results for neurodevelopmental effects have been observed in highly exposed populations. General population studies have not found consistent associations with birth outcome parameters or immune function.
  - *Animal Studies on 2,3,7,8-TCDD*. Oral exposure animal studies provide strong evidence of the developmental toxicity of 2,3,7,8-TCDD in several animal species (e.g., monkeys, rats, mice, hamsters). The observed effects include increases in fetal/newborn mortality; structural anomalies, such as cleft palate and hydronephrosis; decreased birth weight and growth; gastrointestinal hemorrhage; impaired development of the immune system; and

neurodevelopmental effects such as hyperactivity, altered social behaviors, and impaired learning.
  - *Animal Studies on Other CDD congeners.* A small number of oral animal studies evaluated the developmental toxicity of other CDD congeners. Observed effects include heart damage (2,7-DCDD), decreased thymus weight (1,2,3,7,8-PeCDD), and decreased growth (mixed HxCDD congeners). No developmental effects have been observed in the small number of studies evaluating 2-MCDD, 2,3-DCDD, 1,2,3,4-TCDD, or OCDD.

- ***Immunological Effects***
  - *Epidemiological Studies.* A small number of epidemiological studies evaluating immune competence have found suggestive, but inconsistent, evidence of immunotoxicity.
  - *Animal Studies on 2,3,7,8-TCDD.* A number of immune effects have been observed in animals following oral exposure to 2,3,7,8-TCDD. The observed effects include decreased thymus weight, thymic atrophy, and impaired immune function on tests of host resistance and response to antigens.
  - *Animal Studies on Other CDD congeners.* Decreases in thymus weights (1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD) and impaired immune function (2,7-DCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD) have also been observed in animals exposed to other CDD congeners.

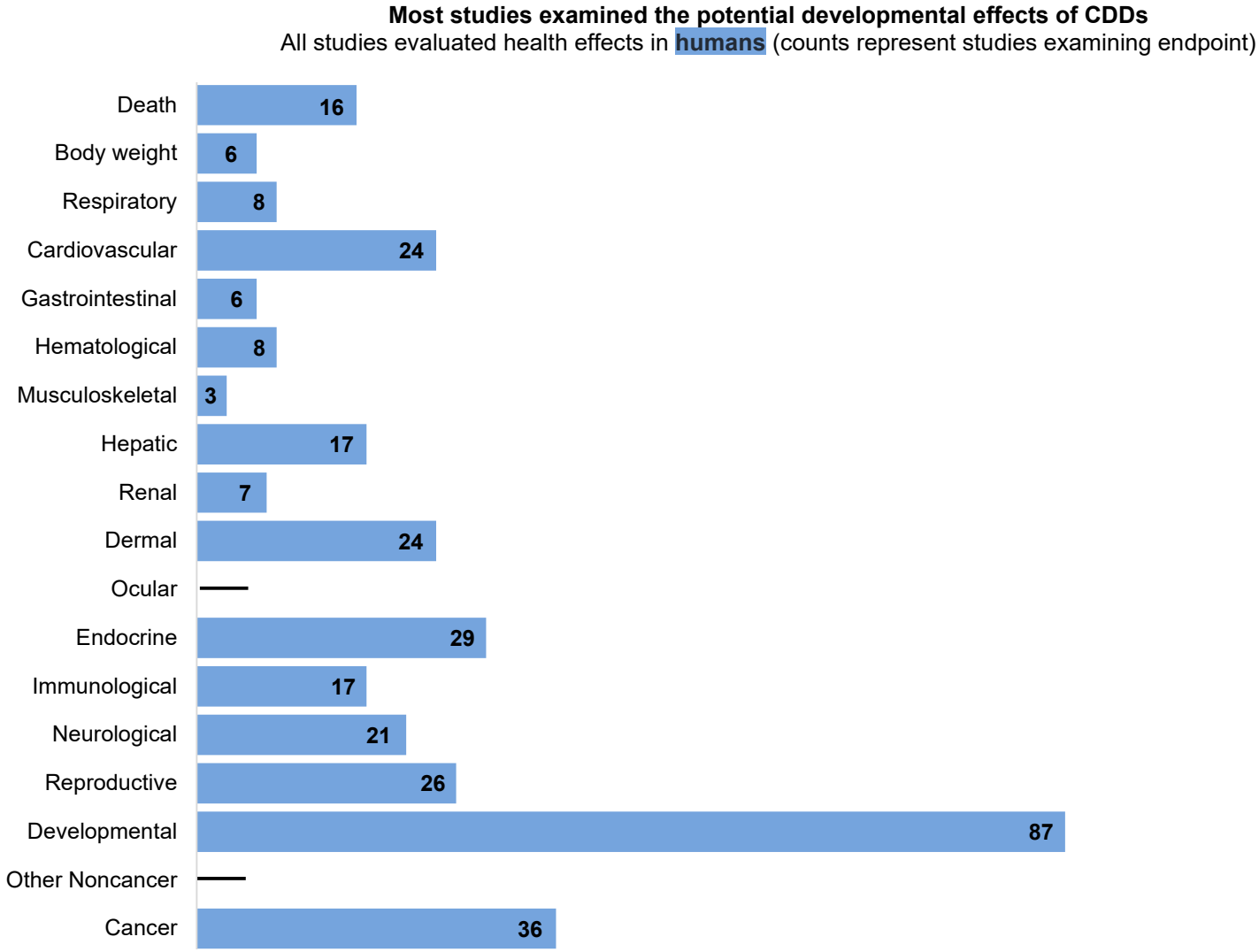
- ***Reproductive Effects***
  - *Epidemiological Studies.* Overall studies evaluating reproductive parameters in men have not found associations with CDD exposure. An increased time to pregnancy was observed in highly exposed women.
  - *Animals Studies on 2,3,7,8-TCDD.* Oral exposure studies in animals provide strong evidence of the reproductive toxicity of 2,3,7,8-TCDD. Effects include alterations in sperm parameters, decreased female fertility, and altered nursing behavior.
  - *Animal Studies on Other CDD Congeners.* The reproductive toxicity of other CDD congeners has not been evaluated.

- ***Hepatic Effects***
  - *Epidemiological Studies.* Inconsistent results of the hepatoxicity of CDDs in humans have been reported, with some studies reporting small alterations in serum liver enzyme lipid levels and others reporting no associations.
  - *Animal Studies on 2,3,7,8-TCDD.* Animal studies provide consistent strong evidence on the hepatoxicity of 2,3,7,8-TCDD. Observed liver effects include increases in liver weight, increases in serum alanine aminotransferase (ALT) levels, altered serum lipid levels, alterations in vitamin A storage, and histopathological alterations such as hepatocellular hypertrophy and necrosis and biliary hyperplasia.
  - *Animal Studies on Other CDD Congeners.* Hepatocellular damage has also been observed in animals exposed to 2,7-DCDD, a mixture of HxCDD congeners, and OCDD.

- ***Cancer Effects***
  - *Epidemiological Studies.* Studies of highly exposed populations have found associations between CDDs and lung cancer, soft tissue sarcomas, and non-Hodgkin lymphoma.
  - *Animal Studies on 2,3,7,8-TCDD.* Several types of tumors have been observed in animal studies including hepatocellular carcinoma, thyroid follicular cell adenoma, squamous cell carcinomas in the lung, hard palate, tongue, and oral mucosa.
  - *Animal Studies of Other CDD Congeners.* Hepatocellular carcinomas have been observed in mice exposed to a mixture of HxCDD congeners and to 2,7-DCDD.

- *Cancer Classifications.* HHS has classified 2,3,7,8-TCDD as known to be a human carcinogen. IARC has determined that 2,3,7,8-TCDD is carcinogenic to humans. EPA has categorized the mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD as a probable human carcinogen.

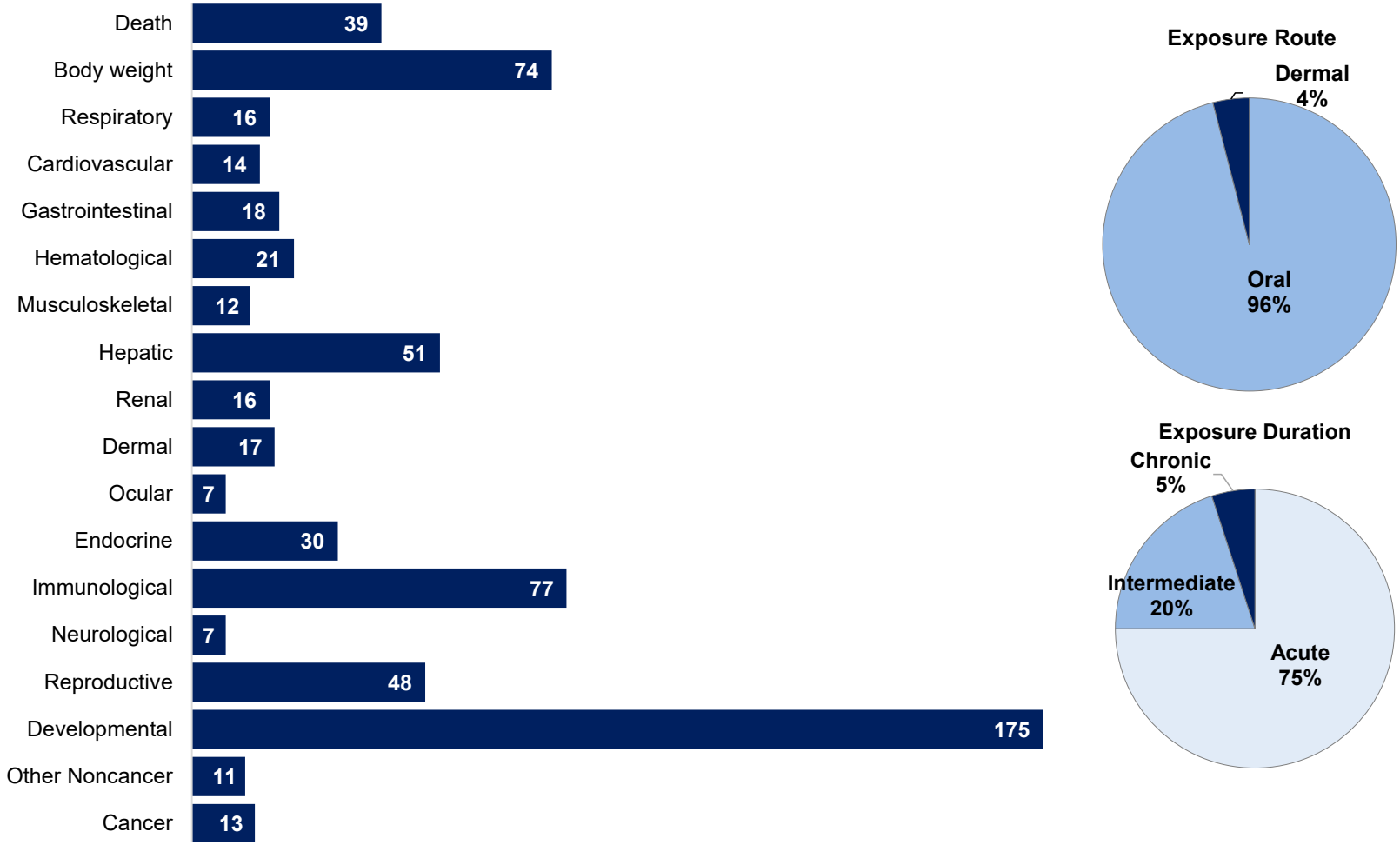
Figure 2-1. Overview of the Number of Studies Examining Chlorinated Dibenzo-*p*-Dioxins (CDDs) Human Health Effects*



*Includes studies discussed in Chapter 2. A total of 258 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

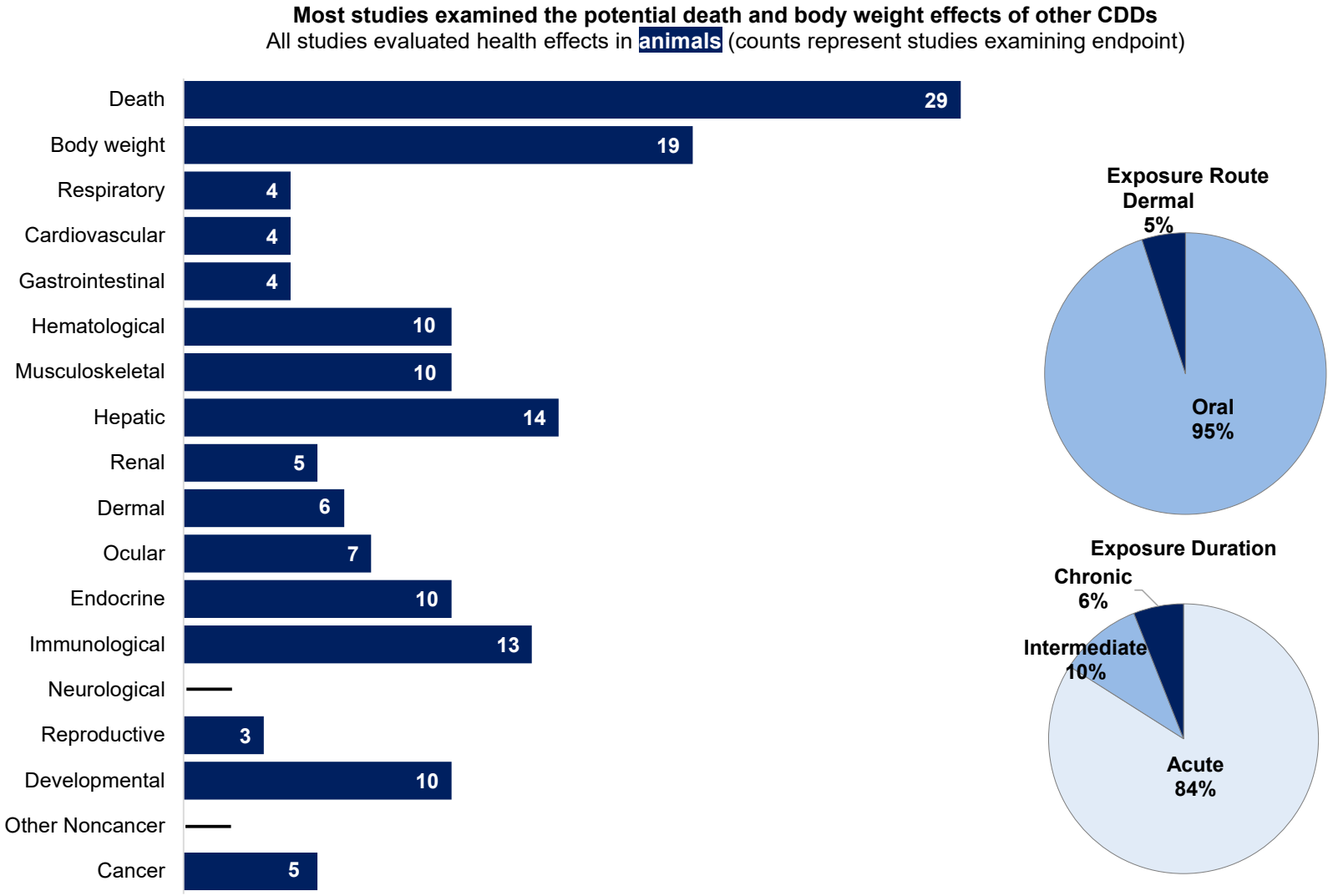
Figure 2-2. Overview of the Number of Animal Studies Examining 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) Health Effects*

Most studies examined the potential developmental effects of 2,3,7,8-TCDD
All studies evaluated health effects in animals (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 393 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Figure 2-3. Overview of the Number of Animal Studies Examining Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) Health Effects*



*Includes studies discussed in Chapter 2. A total of 62 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Guo et al. 2000									**2,3,7,8-TCDD**
1	Monkey (Cynomolgus) 4–7 F	GD 12 (GO)	0, 1, 2, 4	CS, OF	Develop			1	Early fetal loss
McConnell et al. 1978a									**2,3,7,8-TCDD**
2	Monkey (Rhesus) 3 F	Once (GO)	0, 70, 350	BW, GN, HP, BC, CS	Death			70	Increased mortality; 1/3 died
					Bd wt			70	28% lower terminal body weight
					Dermal		70		Nail loss and facial alopecia with acneiform lesions
					Ocular		70		Swelling and inflamed eyelids
					Immuno		70		Severe atrophy of the thymus
McNulty 1984									**2,3,7,8-TCDD**
3	Monkey (Rhesus) 3 F	GD 25, 30, 35, or 40 (GO)	0, 1	RX, DX, LE	Death			1	3/12 mothers died
					Develop			1	Increased occurrence of abortions
Moran et al. 2001									**2,3,7,8-TCDD**
4	Monkey (Cynomolgus) 10 F	Once (GO)	0, 1, 2, 4	RX, HP, BI	Repro	2		4	Decreased serum progesterone; histological evidence of anovulation
Scott et al. 2001									**2,3,7,8-TCDD**
5	Monkey (Cynomolgus) 11 F	Once (GO)	0, 1, 2, 4	HP	Repro		1		Squamous metaplasia in endocervix
Adamsson et al. 2008									**2,3,7,8-TCDD**
6	Rat (Sprague-Dawley) 6–8 F	GD 11 (GO)	0, 0.3, 1	DX, BI, BW, OW	Develop		0.3		Decreased testicular testosterone in 19-day male fetus

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Balk and Piper 1984									**2,3,7,8-TCDD**
7	Rat (Sprague-Dawley) 3-8 M	Once (GO)	0, 25	BI	Endocr		25		Decreased corticosterone levels on days 14 and 21 after dosing
Ball and Chhabra 1981									**2,3,7,8-TCDD**
8	Rat (Sprague-Dawley) 6 M	Once (GO)	0, 5, 100	BW, BI	Bd wt	5		100	25% decrease body weight
Bell et al. 2007a									**2,3,7,8-TCDD**
9	Rat (Wistar) 55–75 F	GD 15 (GO)	0, 0.05, 0.2, 1	RX, DX, HP, OW	Develop	0.2		1	Increased neonatal deaths during lactation (11% fewer pups/litter on PND 21); delayed puberty
Bestervelt et al. 1993									**2,3,7,8-TCDD**
10	Rat (Sprague-Dawley) 14–24 M	Once (GO)	0, 50	BI	Endocr		50		Increased serum ACTH; increased serum corticosterone on days 1 and 5 but decreased on days 10 and 14
Bjerke and Peterson 1994									**2,3,7,8-TCDD**
11	Rat (Holtzman) 10–12 F	GD 15 (GO)	0, 1.0	DX	Develop			1	Decreased percentage of pups born alive (30%), decreased pup body weight (12–14.5%), delayed preputial separation, decreased ventral prostate and seminal vesicle weights, decreased sperm production, feminization of sexual behavior
Bjerke et al. 1994a									**2,3,7,8-TCDD**
12	Rat (Holtzman) 10–12 F	GD 15 (GO)	0, 0.7	DX	Develop			0.7	Impaired development of reproductive system; decreased pup body weight (8–11%)

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bjerke et al. 1994b									**2,3,7,8-TCDD**
13	Rat (Holtzman) 10–12 F	GD 15 (GO)	0, 0.7	DX	Develop		0.7		Demasculinization and feminization of sexual behavior
Bookstaff et al. 1990									**2,3,7,8-TCDD**
14	Rat (Sprague-Dawley) 4–6 M	Once (GO)	0, 50, 100	BI	Repro		10		ED_{50} altered regulation of LH secretion
Boverhof et al. 2006									**2,3,7,8-TCDD**
15	Rat (Sprague-Dawley) 5 F	Once (GO)	0, 0.001, 0.01, 0.1, 1, 10, 30, 100	BW, OW, HP	Hepatic	10	30		Increased relative liver weight, hepatocellular hypertrophy
Boverhof et al. 2006									**2,3,7,8-TCDD**
16	Rat (Sprague-Dawley) 5 F	Once (GO)	0, 10	BW, BC, OW, HP	Hepatic		10		Minimal to moderate hepatocellular hypertrophy, increased relative liver weight; increased serum cholesterol, triglycerides, and FFA
Brown et al. 1998									**2,3,7,8-TCDD**
17	Rat (Sprague-Dawley) 8 F	GD 15 (GO)	0, 1	HP, DX	Develop			1	Alteration in mammary gland differentiation; increased number of chemically-induced mammary adenocarcinomas in pups; delayed vaginal opening, disruption of estrous cycle
Chaffin et al. 1996									**2,3,7,8-TCDD**
18	Rat (Holtzman) 9 F	GD 15 (GO)	0, 1	DX	Develop		1		Decreased serum estrogen levels in female offspring

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Christian et al. 1986									**2,3,7,8-TCDD**
19	Rat (Sprague-Dawley) 19 M	Once (GO)	0, 25, 37, 50, 75	BW, FI, HP, CS, LE	Bd wt			25	36–48% body weight loss
					Cardio	75			
					Gastro	75			
					Renal		25		Dilated convoluted tubules
Courtney et al. 1978									**2,3,7,8-TCDD**
20	Rat (Wistar) 4–6 F	Once (GO)	0, 100	BW, FI, WI	Bd wt			100	15–30% decreased weight
Crofton et al. 2005									**2,3,7,8-TCDD**
21	Rat (Long-Evans) 4–14 F	Once (GO)	0, 0.0001–10	BW, OF	Endocr		0.15		30% decrease in serum T4 (ED_{30})
De Heer et al. 1994a									**2,3,7,8-TCDD**
22	Rat (Wistar) 4 M	10 days (GO)	0, 1, 5, 25, 50, 150	BW, HP, OW	Immuno	1	5		Reduced relative thymus weight
De Heer et al. 1994b									**2,3,7,8-TCDD**
23	Rat (Wistar) 3 M	Once (GO)	0, 25	BW, OW, HP	Immuno		25		Reversible thymic atrophy starting on day 13
De Heer et al. 1994b									**2,3,7,8-TCDD**
24	Rat (Wistar) 4 M	4 days (GO)	0, 1, 5, 25	BI, OW	Immuno		1		Reduced number of immature CD4CD8 double positive thymocytes; decreased absolute and relative thymus weight
Dienhart et al. 2000									**2,3,7,8-TCDD**
25	Rat (Holtzman) 4–5 F	GD 15 (GO)	0, 1	DX, HP	Develop		1		Altered vaginal morphogenesis in pups

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Fan et al. 1996									**2,3,7,8-TCDD**
26	Rat (Sprague-Dawley) 3–10 M	Once (GO)	0, 1, 3, 10, 20, 30, 40, 90	IX, BW	Immuno	3	10		Impaired delayed-type hypersensitivity reaction
Fenton et al. 2002									**2,3,7,8-TCDD**
27	Rat (Long-Evans) 10 F	GD 15 (GO)	0, 1	RX, DX, HP	Develop		1		Delayed development of mammary gland in pups
Fenton et al. 2002									**2,3,7,8-TCDD**
28	Rat (Long-Evans) 5 F	GD 15, GD 20, PND 1, PND 3, PND 5, or PND 10 (GO)	0, 1	BC	Develop		1		Decreased serum TSH levels in 25- and 60-day offspring; decreased serum T4 levels in 60-day old offspring
Fernandez et al. 2010									**2,3,7,8-TCDD**
29	Rat Dark-Agouti NS F	Once GD 18 (GO)	0, 0.7	DX, BI	Develop		0.7		Delayed myelination in brain areas
Filgo et al. 2016									**2,3,7,8-TCDD**
30	Rat (Sprague-Dawley) 9 F	GDs 15 and 18 (G)	0, 0.5	DX	Develop		0.5		Delayed mammary gland development in male and female offspring
Finnila et al. 2010									**2,3,7,8-TCDD**
31	Rat (Sprague-Dawley) NS F	GD 11 (GO)	0, 1	DX, OF, HP	Develop		1		Reduced bone strength in offspring

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Flaws et al. 1997									**2,3,7,8-TCDD**
32	Rat (Holtzman) 8–48 F	GD 11, 15, or 18 (GO)	0, 1	DX	Develop		1		Cleft clitoris and vaginal thread in female offspring
Fletcher et al. 2001									**2,3,7,8-TCDD**
33	Rat (Sprague-Dawley) 5 M	Once (GO)	0, 0.12, 0.66, 3.5, 19, 100	LE, BW, OW, OF	Bd wt	3.5	19	100	SLOAEL: Decreased mean body weight gain (55%) LOAEL: Decreased mean body weight gain (10%)
					Hepatic		0.12		Increased relative liver weight; decreased hepatic vitamin A content
					Immuno	0.12	0.66		Decreased relative thymus weights
					Other noncancer		0.12		Decreased vitamin A content
Gehrs et al. 1997a									**2,3,7,8-TCDD**
34	Rat (Fischer-344) 6–7 F	GD 14 (GO)	0, 1, 3	DX	Develop		1		Alterations in lymphocyte phenotypes
Gehrs et al. 1997b									**2,3,7,8-TCDD**
35	Rat (Fischer-344) 13 F	GD 14 (GO)	0, 3	DX	Develop		3		Alterations in lymphocyte phenotypes
Gehrs et al. 1997b									**2,3,7,8-TCDD**
36	Rat (Fischer-344) 5 F	GD 14 (GO)	0, 1	DX	Develop		1		Alterations in lymphocyte phenotypes and decreased DTH response

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Giavini et al. 1983									**2,3,7,8-TCDD**
37	Rat (CRCD) 15 F	2 weeks (GO)	0, 0.125, 0.5, 2	RX, DX	Bd wt	0.125	0.5		Reduced maternal weight gain (12.8%)
					Neuro	0.5	2		Decreased activity in dams
					Repro	0.5		2	Increased preimplantation loss and decreased corpora lutea
					Develop	0.5		2	Increased incidence of cystic kidneys; increased fetal mortality
Gray and Ostby 1995									**2,3,7,8-TCDD**
38	Rat (Holtzman) 8 F	GD 15 (GO)	0, 1	DX, OF, HP	Develop			1	Decreased neonatal survival; malformations of external genitalia, decreased anogenital distance in female offspring
Gray and Ostby 1995									**2,3,7,8-TCDD**
39	Rat (Long-Evans) 8 F	GD 8 (GO)	0, 1	DX, HP	Develop			1	Malformations of external genitalia, decreased fertility, shortened reproductive lifespan
Gray and Ostby 1995									**2,3,7,8-TCDD**
40	Rat (Long-Evans) 8 F	GD 15 (GO)	0, 1	DX, HP	Develop			1	Decreased pup survival and body weight gain (19%); delayed age of vaginal opening, decreased urethral-vaginal distance, and vaginal thread
Gray et al. 1995									**2,3,7,8-TCDD**
41	Rat (Long-Evans) 8 F	GD 8 or 15 (GO)	0, 1	DX	Develop			1	Impaired development of reproductive system

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Gray et al. 1997a									**2,3,7,8-TCDD**
42	Rat (Long-Evans) 12 F	GD 15 (GO)	0, 0.05, 0.20, 0.80	DX	Develop	0.05	0.2		Urogenital morphological alterations, presence of vaginal thread, and cleft phallus
							0.05		Reduction in ejaculated sperm count
Gray et al. 1997b									**2,3,7,8-TCDD**
43	Rat (Long-Evans) 12 F	GD 15 (GO)	0, 0.05, 0.20, 0.80	DX	Develop		0.5	0.8	LOAEL: Delayed puberty in male offspring SLOAEL: Decreased pup survival from PND 3 to 22, decreased pup body weight (8–10%), decreased epididymal sperm numbers
Haavisto et al. 2001									**2,3,7,8-TCDD**
44	Rat (Han/Wistar) 2–8 F	GD 13.5 (GO)	0, 0,05, 0.1, 0.5, 1.0	DX	Develop	0.1	0.5		Decreased plasma testosterone levels in males
Haavisto et al. 2006									**2,3,7,8-TCDD**
45	Rat (Sprague-Dawley) 10 F	GD 13 (GO)	0, 0.04, 0.2, 1	DX, BI, HP	Develop	1			
Håkansson et al. 1989									**2,3,7,8-TCDD**
46	Rat (Sprague-Dawley) NS M	Once (GO)	0, 6.25, 12.5, 25, 50, 100	BI	Hepatic		6.25		Altered vitamin A storage
Hamm et al. 2000									**2,3,7,8-TCDD**
47	Rat (Long-Evans) 14 F	GD 15 (GO)	0, 1	DX, OW, HP	Develop		1		Altered development of seminal vesicles in offspring
Hanberg et al. 1989									**2,3,7,8-TCDD**
48	Rat (Sprague-Dawley) NS	Once (GO)	0, 0.12–100	BW, OW, LE	Immuno		26		ED_{50} for thymic atrophy

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Hattori et al. 2014									**2,3,7,8-TCDD**
49	Rat (NR) 4–6 F	GD 15 (GO)	0, 1	DX	Develop			1	20% decreased birth weight
Heimler et al. 1998									**2,3,7,8-TCDD**
50	Rat (Holtzman) F	GD 15 (GO)	0, 1	DX	Develop		1		Decreased number of antral and preantral ovarian follicles
Hermansky et al. 1988									**2,3,7,8-TCDD**
51	Rat (Sprague-Dawley) 6 F	3 days (GO)	0, 40	HP, CS, BI	Cardio		40		Decrease heart rate and decreased mean blood pressure
Hoegberg et al. 2003									**2,3,7,8-TCDD**
52	Rat (Sprague-Dawley) 6 M	Once (GO)	0, 0.1, 1.0, 10, 100	CS, BW, GN, OW, OF	Hepatic	1	10		Decreased hepatic retinyl esters and all trans retinoic acid
Hsu et al. 2018									**2,3,7,8-TCDD**
53	Rat (Sprague-Dawley) NR F	GD 14, GD 21, PND 7, PND 22 (GO)	0, 0.2	DX	Develop		0.2		Increased systolic blood pressure and mean arterial blood pressure in adult male offspring
Hsu et al. 2020									**2,3,7,8-TCDD**
54	Rat (Sprague-Dawley) NR F	GD 14, GD 21, PND 7, PND 22 (G)	0, 0.2	DX	Develop		0.2		Increased systolic blood pressure in adult male offspring
Hurst et al. 2002									**2,3,7,8-TCDD**
55	Rat (Long-Evans) NS F	GD 15 (GO)	0, 1	DX, GN, HP, BI	Develop		1		Altered morphogenesis of female reproductive tract

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Huuskonen et al. 1994									**2,3,7,8-TCDD**
56	Rat (Long-Evans) 9–17 F	GD 8 (GO)	1, 5	DX, OF, HP	Develop	1		5	Cleft palate, thymic atrophy, decreased number of live fetuses
Huuskonen et al. 1994									**2,3,7,8-TCDD**
57	Rat (Han/Wistar) 9–17 F	GD 8 (GO)	1, 10	DX, OF, HP	Develop	1		10	Hydronephrosis, thymic atrophy, gastrointestinal hemorrhage, decreased number of live fetuses
Huuskonen et al. 1994									**2,3,7,8-TCDD**
58	Rat (Han/Wistar) 9–17 F	GD 12 (GO)	1, 10	DX, OF, HP	Develop	1		10	Hydronephrosis, gastrointestinal hemorrhages, decreased number of live fetuses
Ikeda et al. 2002									**2,3,7,8-TCDD**
59	Rat (Sprague-Dawley) 3–6 F	GD 15 (GO)	0, 0.8, 1.6	DX	Develop			0.8	Decreased litter size on PND 2 and fetal survival on GD 20
Ikeda et al. 2005a									**2,3,7,8-TCDD**
60	Rat (Holtzman) 9 F	GD 15 (GO)	0, 0.2, 0.8	DX, BI	Develop		0.2		Demasculinization of male pups
Ishimura et al. 2002									**2,3,7,8-TCDD**
61	Rat (Holtzman) 3–6 F	GD 15 (GO)	0, 0.8, 1.6	DX	Develop	0.8		1.6	Increased number of dead fetuses
Kakeyama et al. 2003									**2,3,7,8-TCDD**
62	Rat (Long-Evans) 7–9 F	GD 15 (G)	0, 0.2, 0.8	DX, NX	Develop	0.2	0.8		Altered adult male sexual behavior after perinatal exposure
Kakeyama et al. 2007									**2,3,7,8-TCDD**
63	Rat (Long-Evans) 5–6 F	GD 15 (GO)	0, 0.2, 0.8	DX	Develop		0.2		Anxiety-like behavior and impaired performance on test of memory

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kakeyama et al. 2008									**2,3,7,8-TCDD**
64	Rat (Long-Evans) 16–18 F	GD 15 (GO)	0, 0.2, 0.8	RX, DX, HP, BW	Develop		0.2		Premature maturation of hypothalamic-pituitary axis, gonads and genitals
Kakeyama et al. 2014									**2,3,7,8-TCDD**
65	Rat (Long-Evans) 6 F	GD 15 (G)	0, 0.2, 0.8	DX	Develop		0.2		Anxiety-like behavior and impaired learning (not observed at 0.8 µg/kg); decreased body weight gain (10-15%) in adult offspring at 0.8 ug/kg/day
Kattainen et al. 2001									**2,3,7,8-TCDD**
66	Rat Line C 4–8 F	GD 15 (GO)	0, 0.03, 0.1, 0.3, 1	BW, DX	Develop		0.03		Decreased size of molars
Kelling et al. 1985									**2,3,7,8-TCDD**
67	Rat (Fischer-344) 20–24 M	Once (GO)	0, 100	BW, OW, WI, HP, LE	Death			100	95% died
Kelling et al. 1987									**2,3,7,8-TCDD**
68	Rat (Sprague-Dawley) 6–14 M	Once (GO)	0, 6.25, 25, 100	CS	Cardio		6.25		Increased basal tension of the left atria
Kransler et al. 2007									**2,3,7,8-TCDD**
69	Rat (Holtzman) 7–15 F	GD 10 (GO)	0, 1.5, 3, 6, 18	CS, DX, BW, OW, HE, BI	Bd wt	3	6	18	LOAEL: 13% reduced final maternal body weight SLOAEL: 30% reduced final maternal body weight
					Hemato	18			
					Hepatic	18			
					Develop			1.5	Intestinal hemorrhaging in fetuses

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kransler et al. 2009									**2,3,7,8-TCDD**
70	Rat (Holtzman) 11–18 F	GD 10 (GO)	0, 1.5, 6	OW, BW, DX, BI, HP, OF	Develop			1.5	Decreased viability on GD 20 and PND 7; lung immaturity and hypoplasia
Lewis et al. 2001									**2,3,7,8-TCDD**
71	Rat (Holtzman) 9–12 F	GD 15 (GO)	0, 1	DX, HP, BI, BW	Develop		1		Impaired mammary gland differentiation in offspring
Li et al. 1995a									**2,3,7,8-TCDD**
72	Rat (Sprague-Dawley) 5–10 F	Once (GO)	0.3, 1, 3, 10, 30, 60	OF, HP	Repro	3	10		Increased LH and FSH levels, altered ovulation
Li et al. 1995b									**2,3,7,8-TCDD**
73	Rat (Sprague-Dawley) 5–10 F	Once (GO)	0, 10	OF, HP	Repro		10		Irregular estrous cycle and ovulation
Lu et al. 2010									**2,3,7,8-TCDD**
74	Rat (Sprague-Dawley) 5 M	Once (GO)	0, 10	BW, BC, UR, OW, HP	Bd wt		10		Decreased mean body weight (11%)
					Hepatic		10		Increased relative liver weights, intermediate hepatocellular swelling and vacuolization; increased serum cholesterol and decreased serum triglycerides
Lu et al. 2009									**2,3,7,8-TCDD**
75	Rat (Sprague Dawley) 5 M	12 days (GO)	0, 10	BW, BC, OW, HP	Bd wt			10	Lower terminal body weight (28%)
					Renal		10		Increased serum creatinine and BUN; proximal tubular epithelial damage

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Mably et al. 1992a									**2,3,7,8-TCDD**
76	Rat (Holtzman) 5 F	GD 15 (GO)	0, 0.064, 0.16, 0.40, 1.0	BW, BI, OF, DX	Develop	0.064	0.16	1	SLOAEL: Decreased live birth index LOAEL: Delayed testis descent and decreased anogenital distance
Mably et al. 1992b									**2,3,7,8-TCDD**
77	Rat (Holtzman) NS	GD 15 (GO)	0, 0.064, 0.16, 0.40, 1.0	BI, OF, DX	Develop		0.064		Decreased masculine sexual behavior in male offspring
Mably et al. 1992c									**2,3,7,8-TCDD**
78	Rat (Holtzman) NS F	GD 15 (GO)	0, 0.064, 0.16, 0.40, 1.0	BI, RX, DX	Develop		0.064		Reduced sperm production in offspring at all ages
Mai et al. 2020									**2,3,7,8-TCDD**
79	Rat (Wistar) 10 F	GD 15 (GO)	0, 0.5, 1, 2	DX	Develop			0.5	Pre- and post-implantation losses in unexposed females mated to exposed F1 males, decreased sperm motility and increased abnormal sperm, degenerative changes in testes, and histological alterations in seminiferous tubules
Markowski et al. 2002									**2,3,7,8-TCDD**
80	Rat (Holtzman) 7–13 F	GD 15 (GO)	0, 0.06, 0.18, 0.54	DX, NX	Develop	0.06	0.18		Impaired performance on operant behavior test
Miettinen et al. 2002									**2,3,7,8-TCDD**
81	Rat (Line C) 5–7 F	GD 11 (GO)	0, 1	DX, BW	Develop			1	Decreased viability of neonates and arrested molar development

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Miettinen et al. 2005									**2,3,7,8-TCDD**
82	Rat (Line C) 3–5 F	GD 15 (GO)	0, 0.03, 0.1, 0.3, 1	DX, BW	Develop	0.3	1		Morphological and mechanical alterations in pup's bone
Miettinen et al. 2006									**2,3,7,8-TCDD**
83	Rat (Line C) NS F	GD 15 (GO)	0, 0.03, 0.1, 0.3, 1	DX, BW, HP	Develop		0.03	1	LOAEL: Enhanced caries susceptibility in pups SLOAEL: Increased pup perinatal mortality
Mitsui et al. 2006									**2,3,7,8-TCDD**
84	Rat (Wistar) NR F	GD 15 (GO)	0, 1	DX	Develop		1		Decreased body weight (7–8%) and impaired performance on test of contextual fear conditioning (males only)
Nayyar et al. 2002									**2,3,7,8-TCDD**
85	Rat (Sprague-Dawley) NS F	GD 15 (GO)	0, 0.25, 0.5, 1	DX, BI	Develop	0.5		1	Reduced pup weight on PNDs 3, 5, and 10 (10.5–19%)
Nguyen et al. 2013a									**2,3,7,8-TCDD**
86	Rat (Wistar) 5 F	GD 15 (G)	0, 1.0	DX	Develop		1		Increased activity and decreased social activity
Nishijo et al. 2007									**2,3,7,8-TCDD**
87	Rat (Wistar) 8–9 F	GDs 9–19 (GO)	0, 0.1	CS, DX	Develop		0.1		Decreased fetal body weight on GD 19 (10%)
							0.1		Delayed avoidance learning; reduced motor activity

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Nishimura et al. 2003									**2,3,7,8-TCDD**
88	Rat (Holtzman) 6 F	GD 15 (GO)	0, 0.2, 0.8	IX, DX, BI, HP	Develop		0.2	0.8	LOAEL: Decreased serum T4 on PND 21 (male pups only) SLOAEL: Decreased litter size; decreased serum T4 on PND 21, increased T4 on PND 49 (male pups only), increased T3 on PND 21 (female pups only), increased TSH on PND 21 and 49; thyroid hyperplasia
Nishimura et al. 2005b									**2,3,7,8-TCDD**
89	Rat (Holtzman) 12 F	GD 15 (GO)	0, 1	DX, HP, BI	Develop		1		Decreased serum T4 and increased TSH; thyroid hyperplasia
Nishimura et al. 2006									**2,3,7,8-TCDD**
90	Rat (Holtzman) 6 F	GD 15 (GO)	0, 1	DX, BI	Develop			1	Hydronephrosis, decreased pup body weight (11.7–13.4%)
Ohsako et al. 2001									**2,3,7,8-TCDD**
91	Rat (Holtzman) 6 F	GD 15 (GO)	0, 0.0125, 0.05, 0.2, 0.8	OW, RX, BC, DX, BI, HP	Develop	0.0125	0.05		Reduced anogenital distance on PND 120
Ohsako et al. 2002									**2,3,7,8-TCDD**
92	Rat (Sprague-Dawley) 5 F	GD 15 (GO)	0, 1	DX, BI	Develop		1		Decreased relative epididymal and cauda epididymal organ weights; reduced anogenital distance, decreased cauda sperm reserve
Ohsako et al. 2002									**2,3,7,8-TCDD**
93	Rat (Sprague-Dawley) 5 F	GD 18 (GO)	0, 1	DX, BI	Develop		1		Reduced anogenital distance

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Petroff et al. 2000									**2,3,7,8-TCDD**
94	Rat (Sprague-Dawley) 6–10 F	Once (GO)	0, 10	BW, RX, HP, BC	Repro		10		Reduced ovarian weight and ova shed
Potter et al. 1986									**2,3,7,8-TCDD**
95	Rat (Sprague-Dawley) 12 M	Once (GO)	0, 6.25, 12.5, 25, 50, 100	BW, FI, OW, HP, OF	Endocr		6.25		Decreased serum T4 and increased serum TSH
Raasmaja et al. 1996									**2,3,7,8-TCDD**
96	Rat (Long-Evans) 12 M	Once (GO)	0, 10, 20, 40	BW, OF	Endocr		10		Decreased serum T4 and decreased deiodination in peripheral tissues
Roth et al. 1988									**2,3,7,8-TCDD**
97	Rat (Sprague-Dawley) NS M	Once (GO)	0, 0.032, 0.32, 3.2, 10.6, 32.0	BW, BI	Endocr	0.032	0.32		Decreased serum T4 and T3 levels
Salisbury and Marcinkiewicz 2002									**2,3,7,8-TCDD**
98	Rat (Sprague-Dawley) 4–5 F	GD 15 (GO)	0, 1, 2.5	DX, BI, HP, BW	Develop			1	Reduced pup weight (8-15% at various time points) and number of days in estrous
Sanabria et al. 2016									**2,3,7,8-TCDD**
99	Rat (Wistar) 7–10 F	GD 15 (GO)	0, 0.1, 0.5, 1.0	DX	Develop	0.5	1		Decreased serum testosterone in F1 males
Schwetz et al. 1973									**2,3,7,8-TCDD**
100	Rat (Sherman) 5–10 M, F	Once (GO)	8, 16, 32, 63	CS, LE	Death			45 F	LD_{50}
								22 M	LD_{50}

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Seefeld et al. 1984a									**2,3,7,8-TCDD**
101	Rat (Sprague-Dawley) 13–37 M	Once (GO)	0, 5, 15, 25, 50	BW, FI, CS	Death			25	25% mortality
					Bd wt	5	15	25	LOAEL: 15% decreased body weight 15 days post exposure SLOAEL: Body weight loss (terminal body weight 49% lower than controls)
					Neuro		15		Decreased motor activity
Seefeld et al. 1984b									**2,3,7,8-TCDD**
102	Rat (Sprague-Dawley) 20 M	Once (GO)	0, 15	BW, FI, CS	Bd wt			15	60% decreased weight gain
Seo et al. 1995									**2,3,7,8-TCDD**
103	Rat (Sprague-Dawley) 15 F	GDs 10–16 (GO)	0.025, 0.1	DX, BI	Develop	0.025	0.1		Decreased T4 levels
Seo et al. 1999									**2,3,7,8-TCDD**
104	Rat (Sprague-Dawley) 28 F	GDs 10–16 (GO)	0, 0.1	DX, NX	Develop		0.1		Impaired visual reversal learning
Simanainen et al. 2002									**2,3,7,8-TCDD**
105	Rat (Long-Evans) 9–11 F	Once (GO)	0, 0.03–100	BW, OW, OF	Musc/skel		22		ED_{50} for incisor tooth defects
					Immuno		2.3		Decreased relative thymus weight (ED_{50})
Simanainen et al. 2002									**2,3,7,8-TCDD**
106	Rat Hans/Wistar 9–11 F	Once (GO)	0, 0.03–100	BW, OW, OF	Musc/skel		57		ED_{50} for incisor tooth defects

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Simanainen et al. 2004b									**2,3,7,8-TCDD**
107	Rat (Line C) 5–8 F	GD 15 (GO)	0, 0.03, 0.1, 0.3, 1	RX, DX, BI, OW	Develop	0.3	1		Decreased daily sperm production and cauda epididymal sperm count; decreased anogenital distance
Sommer et al. 1996									**2,3,7,8-TCDD**
108	Rat (Holtzman) 26–30 F	GD 15 (GO)	0, 1.0	DX	Develop			1	Increased offspring mortality, decreased male pup body weight (3–14%), delayed puberty, decreased daily sperm production, decreased absolute and relative ventral prostate weight, and epididymal sperm numbers
Sparschu et al. 1971									**2,3,7,8-TCDD**
109	Rat (Sprague-Dawley) 10–31 F	10 days, GDs 6–15 (GO)	0, 0.03, 0.125, 0.5, 2.0, 8.0	DX	Bd wt	0.5		2	Dam body weight on GD 20 was 22% lower than controls
					Develop	0.03	0.125		Intestinal hemorrhage in fetuses
Takeda et al. 2020									**2,3,7,8-TCDD**
110	Rat (Wistar) 109–111 F	GD 15 (GO)	0, 1	DX	Repro		1		Altered nursing behavior, decreased serum prolactin, decreased milk ejection volume
					Develop			1	Total litter loss, decreased litter size, decreased pup body weight (8–22% at various time points), decreased short-term memory in adult offspring

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Taura et al. 2014									**2,3,7,8-TCDD**
111	Rat (Wistar) NR F	GD 15 (GO)	0, 1	DX	Develop		1		Demasculinization of male sexual behavior
Theobald et al. 1991									**2,3,7,8-TCDD**
112	Rat (Sprague-Dawley) 6–9 M	Once (GO)	0, 19, 25, 33, 44, 58, 76, 100	HP, BI	Gastro		19		Increased weight of antral mucosa
Tomasini et al. 2012									**2,3,7,8-TCDD**
113	Rat (Dark-Agouti) NR F	GD 18 (GO)	0, 0.7	DX	Develop			0.7	Decreased total litter size and decreased male offspring weight on PND 60 (20%)
Viluksela et al. 2004									**2,3,7,8-TCDD**
114	Rat (Sprague-Dawley) 5 M	Once (GO)	0, 60	LE, BW, OF	Endocr		60		Decreased serum T4
Viluksela et al. 2004									**2,3,7,8-TCDD**
115	Rat (Sprague-Dawley) 5 M	Once (GO)	0, 0.1, 1, 5, 15, 30, 60	LE, BW, OF	Endocr	1	5		Decreased serum T4, decreased peripheral and thyroid gland deiodinase activity
Weissberg and Zinkl 1973									**2,3,7,8-TCDD**
116	Rat (CD) 4 F	10–14 days (GO)	0, 10	BC	Hemato		10		Increase in packed cell volume, erythrocytes, neutrophils; decrease in mean corpuscular hemoglobin and platelet count
Yang et al. 1994									**2,3,7,8-TCDD**
117	Rat (Fischer-344) 3 B	14 days (GO)	0, 0.72	IX	Immuno		0.72 F		Suppression in virus-augmented NK cell activity

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Yonemoto et al. 2005									**2,3,7,8-TCDD**
118	Rat (Long-Evans) 22 F	GD 15 (G)	0, 0.0125, 0.05, 0.2, 0.8	DX	Develop		0.0125		Decreased male/female sex ratio; not observed at doses ≥0.2 µg/kg
Yu et al. 2019									**2,3,7,8-TCDD**
119	Rat (Sprague-Dawley) 10 F	GDs 8–14 (GO)	0, 0.1, 0.5	DX	Develop		0.1		Shortened vaginal opening time in F3 generation
Yu et al. 2020									**2,3,7,8-TCDD**
120	Rat (Sprague-Dawley) 6–8 F	GDs 8–14 (GO)	0, 0.1, 0.5	DX	Develop		0.1		Decreased number of primordial follicles and increased number of primary and secondary ovarian follicles and corpora lutea in F2 generation
Zhang et al. 2018a									**2,3,7,8-TCDD**
121	Rat (Sprague-Dawley) 14 F	GDs 8–14 (GO)	0, 0.1, 0.5	DX	Develop		0.1		Decreased number of primordial follicles and increased number of secondary ovarian follicles and corpora lutea in F1 generation
Zhang et al. 2018b									**2,3,7,8-TCDD**
122	Rat (Sprague-Dawley) 4 F	GDs 8–14 (GO)	0, 0.2, 0.8	DX	Develop		0.2		Delayed negative geotaxis and cliff avoidance reflexes
Ao et al. 2009									**2,3,7,8-TCDD**
123	Mouse (C57BL/6J) 5 F	Once (GO)	0, 1.0, 3.0, 10, 50	OW, IX	Immuno		1		Suppressed IL-5 production in response to OVA exposure
Aragon et al. 2008a									**2,3,7,8-TCDD**
124	Mouse (C57BL/6N) NS F	GD 14 (GO)	0, 6	DX, HP, BI	Develop		6		Cardiac hypertrophy and mild hydronephrosis in offspring

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Aragon et al. 2008b									**2,3,7,8-TCDD**
125	Mouse (C57BL/6N) 23–24 F	GD 14.5 (GO)	0, 6	DX, OF, HP	Develop		6		Increased susceptibility of offspring to renal fibrosis and hypertension in adulthood
Blaylock et al. 1992									**2,3,7,8-TCDD**
126	Mouse (C57BL/6) 5F	GDs 6–14 (GO)	0, 1.5, 3.0	DX, OF, OW	Develop		1.5		Thymic atrophy and delayed thymocyte maturation
Boverhof et al. 2005									**2,3,7,8-TCDD**
127	Mouse (C57BL/6) 5 F	Once (GO)	0, 0.001, 0.01, 0.1, 1, 10, 100, 300	BW, OW, HP	Hepatic	0.1	1		Mild to moderate cytoplasmic vacuolization
Boverhof et al. 2006									**2,3,7,8-TCDD**
128	Mouse (C57BL/6) 5 F	Once (GO)	0, 0.001, 0.01, 0.1, 1, 10, 100, 300	BW, OW, HP	Hepatic	0.01	0.1		Cytoplasmic vacuolization
Burleson et al. 1996									**2,3,7,8-TCDD**
129	Mouse (B6C3F1) 20 F	Once (GO)	0, 0.001, 0.005, 0.01, 0.05, 0.1	IX, OW	Immuno	0.005[c]	0.01		Decreased influenza virus host resistance
Chen et al. 2013									**2,3,7,8-TCDD**
130	Mouse (BALB/c) 4–5 F	Once (GO)	0, 20	BW, OW, IX	Immuno		20		Decreased interferon-gamma, IL-2, IL-4, IL-5, and IL-10 levels and OVA-specific IgG1 and IgM levels in response to OVA exposure
Couture-Haws et al. 1991									**2,3,7,8-TCDD**
131	Mouse (C57BL/6N) 11–14 F	PND 1 (GO)	0, 6, 9, 12	DX	Develop		6		Hydronephrosis

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Couture-Haws et al. 1991									**2,3,7,8-TCDD**
132	Mouse (C57BL/6N) 10–13 F	PND 4 (GO)	0, 6, 9, 12	DX	Develop		6		Hydronephrosis
de Gannes et al. 2021									**2,3,7,8-TCDD**
133	Mouse (C57BL/6J) NR F	GD 0.5, GD 7.5, PND 10 (GO)	0, 1	DX	Develop		1		Increased systolic blood pressure and arterial pressure (females only) in response to angiotensin pathological stress in adult offspring
Endo et al. 2012									**2,3,7,8-TCDD**
134	Mouse (C57BL/6) 8 F	GD 12.5 (G)	0, 0.6, 3.0	DX	Develop		0.6		Impaired attainment of rapid behavioral shifts, compulsive repetitive behavior (0.06 µg/kg only), and low competitive dominance in adult offspring (0.6 µg/kg only)
Fader et al. 2018									**2,3,7,8-TCDD**
135	Mouse (C57BL/6) 5 M	Once (GO)	0, 30	HP	Musc/skel		30		Increased trabecular bone mass (increased bone volume fraction, thickness, bone marrow density and decreased spacing); increased cortical outer perimeter, area, and mineral content
Fletcher et al. 2001									**2,3,7,8-TCDD**
136	Mouse (C57BL/6) 5 M	Once (GO)	0, 1.6, 8, 40, 200, 1,000	LE, BW, OW, OF	Bd wt	8	40	200	SLOAEL: Decreased mean body weight gain (37%) LOAEL: Decreased mean body weight gain (13%)

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	8			
					Immuno	40			
					Other noncancer		1.6		Decreased vitamin A content
Frawley et al. 2014									**2,3,7,8-TCDD**
137	Mouse (B6C3F1/N) 14 F	Once (GO)	0, 0.1, 0.3, 0.5, 1, 3	BW, OW, HE, IX	Bd wt	3			
					Immuno		0.1		Decreased antibody plaque forming response to sRBCs
Greig 1984; Greig et al. 1987									**2,3,7,8-TCDD**
138	Mouse (A2G-hr/+) NS B	Once (GO)	0, 75	HP, CS, BI	Dermal		75		Skin thickening
Haijima et al. 2010									**2,3,7,8-TCDD**
139	Mouse (C57BL/6J) 9 F	GD 12.5 (GO)	0, 3	DX	Develop		3		Deficits in fear memory in adult male offspring
Holladay et al. 1991									**2,3,7,8-TCDD**
140	Mouse (B6C3F1) 5 F	GDs 6–14 (GO)	0, 1.5, 3.0	OW, IX, DX	Develop		1.5		Immunosuppression in pups, thymic atrophy, abnormal fetal thymocyte-maturation
Holsapple et al. 1986									**2,3,7,8-TCDD**
141	Mouse (B6C3F1) 9 F	14 days (GO)	0, 1	OW, GN, HP, HE, BI, BC, IX	Resp	1			
					Hemato	1			
					Hepatic		1		Hydropic degeneration, increased liver weight induced microsomal enzymes
					Renal	1			
					Immuno		1		Suppressed antibody response

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Holsapple et al. 1986									**2,3,7,8-TCDD**
142	Mouse (B6C3F1) 5 F	Once (GO)	0, 0.05, 0.1, 0.5, 1.0, 2.0	BI	Immuno	0.5	1		Suppressed antibody response
Inouye et al. 2005									**2,3,7,8-TCDD**
143	Mouse (C57BL/6N) 5–6 F	Once (GO)	0, 0.3, 1.0, 3.0	BW, OW, IX	Immuno		0.3		Reduced splenocyte production of IL-5 in response to OVA exposure
Ito et al. 2002									**2,3,7,8-TCDD**
144	Mouse (C57BL/6N) 5–6 F	Once (GO)	0, 0.2, 1, 5, 20	BW, OW, IX	Immuno	0.2	1		Decreased splenocytes and production of ovalbumin-specific IgG1 and IL-5 in response to OVA exposure
Jin et al. 2010									**2,3,7,8-TCDD**
145	Mouse (C57BL/6) 10 F	PNDs 1–4 (GO)	0, 1	DX	Develop			1	Decreased pup body weights (4, 19, or 10% on PNDs 7, 21, and 30), relative and absolute testis and epididymal weights, anogenital distance, epididymal sperm counts, and testicular testosterone levels
Keller et al. 2007									**2,3,7,8-TCDD**
146	Mouse (C57BL/6N) NS F	GD 13 (GO)	0, 0.01, 0.1, 1	DX, BW, OW	Develop	0.1	1		Altered molar and mandible shape
Keller et al. 2008									**2,3,7,8-TCDD**
147	Mouse (C3H/HeJ) NS F	GD 13 (GO)	0, 0.01, 0.1, 1	DX	Develop		0.01		Altered mandible shape

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kelling et al. 1985									**2,3,7,8-TCDD**
148	Mouse (C57BL/6) 21–22 M	Once (GO)	0, 360	BW, FI, WI, HP, LE	Death			360	69% died
Kinoshita et al. 2006									**2,3,7,8-TCDD**
149	Mouse (C57BL/6J) 5 F	Once (GO)	0, 0.1, 1.0, 5.0, 20	IX, HP	Immuno		1		Impaired oral tolerance
Li et al. 2006									**2,3,7,8-TCDD**
150	Mouse (NIH) 10 F	GDs 1–3, 4–8, or 1–8 (GO)	0, 0.002, 0.05, 0.1	RX	Repro	0.002		0.05	Preimplantation loss
Luebke et al. 1999									**2,3,7,8-TCDD**
151	Mouse (B6C3F1) 7 F	Once (GO)	0, 0.1, 1, 10, 30	IX	Immuno		1		Impaired response to *Trichinella spiralis* infection
Luebke et al. 1999									**2,3,7,8-TCDD**
152	Mouse (B6C3F1) 7 F	Once (GO)	0, 0.1, 1, 10	IX	Immuno	0.1	1		Impaired response to *Trichinella spiralis* infection
Luster et al. 1980									**2,3,7,8-TCDD**
153	Mouse (B6C3F1) NS F	GD 14, LDs 1, 7, and 14, (GO)	0, 1.0, 5.0, 15.0	BW, OW, HE	Develop	1			
Matulka et al. 1997									**2,3,7,8-TCDD**
154	Mouse (B6C3F1) 5 F	14 days (GO)	0, 0.3, 1.0, 3.0	IX	Immuno	0.3	1		Impaired response to sRBCs

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Miettinen et al. 2004									**2,3,7,8-TCDD**
155	Mouse (EGFR+/-) NS F	GD 10 (GO)	0, 1.5, 4.4, 19.1, 29.6, 30.1, 42, 55.6, 106	DX, HP	Develop	1.5	4.4		Hydronephrosis
Moore et al. 1973									**2,3,7,8-TCDD**
156	Mouse (C57BL/6) 14–27 F	GDs 10–13 (GO)	0, 1, 3	DX	Develop		1	3	LOAEL: Hydronephrosis SLOAEL: Cleft palate
Moore et al. 1973									**2,3,7,8-TCDD**
157	Mouse (C57Bl/6) 5–14 F	GD 10 (GO)	0, 1	DX	Develop		1		Hydronephrosis
Moore et al. 1973									**2,3,7,8-TCDD**
158	Mouse (C57B1/6) 3–9 F	Once at parturition (GO)	0, 1, 3, 10	DX	Develop		1		Hydronephrosis
Neubert and Dillmann 1972									**2,3,7,8-TCDD**
159	Mouse (NMRI) 10–12 F	GDs 6–15 (GO)	0, 0.3, 3.0, 4.5, 9.0	DX	Develop	0.3		3	Cleft palate
Pohjanvirta et al. 2012									**2,3,7,8-TCDD**
160	Mouse (C57BL/6Kuo) 6–12 M, 5–15 F	Once (GO)	M: 0, 125, 250, 500; F: 0, 250, 500; F: 0, 250, 500, 1,000, 2,000, 2,500, 5,000	LE, CS, BW	Death			500 M	100% mortality; LD_{50} of 305 ug/kg

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Pohjanvirta et al. 2012									**2,3,7,8-TCDD**
161	Mouse (C57BL/6NTac) 3–4 M, 3–4 F	Once (GO)	0, 120, 240, 480	LE, CS, BW	Death			240 M	75% mortality
					Bd wt	480 F		120 M	Weight loss (1.5–95% whereas controls gained weight)
Pohjanvirta et al. 2012									**2,3,7,8-TCDD**
162	Mouse (C57BL/6JBOMTac) 4–6 M, 6 F	Once (GO)	M: 0, 70, 140, 280 F: 0, 300, 900	LE, CS, BW	Death			900 F	100% mortality
								280 M	100% mortality
Safe and Luebke 2016									**2,3,7,8-TCDD**
163	Mouse (C57BL/6) 4 F	GD 12 (GO)	0, 0.5	DX	Develop		0.5		Ototoxicity (shift in auditory brainstem response)
Sha et al. 2021									**2,3,7,8-TCDD**
164	Mouse (C57BL/6J) 15 F	GDs 0.5 and 12.5, PND 7.5 (G)	0, 0.1, 10	DX	Develop		0.1	10	LOAEL: Hyperactive-like behaviors
									SLOAEL: Pup mortality
Silkworth et al. 1989b									**2,3,7,8-TCDD**
165	Mouse (C57BL/6J) 12–15 F	GDs 6–15 (GO)	0, 0.5, 1, 2, 4	BW, IX, DX	Bd wt	4			
					Immuno	0.5	1		Decreased relative thymus weight
					Develop		0.5		Hydronephrosis
Silkworth et al. 1989b									**2,3,7,8-TCDD**
166	Mouse (DBA/6J) 14–15 F	GDs 6–15 (GO)	0, 0.5, 1, 4, 8	BW, IX, DX	Bd wt	8			
					Develop		0.5		Hydronephrosis

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Smialowicz et al. 1997									**2,3,7,8-TCDD**
167	Mouse (B6C3F1) 8 F	Once (GO)	0, 0.1, 1.0, 10.0	BW, OW, IX	Immuno	0.1	1		Impaired response to sRBC; decreased relative spleen and thymus weights
Smith et al. 1976									**2,3,7,8-TCDD**
168	Mouse (CF-1) 14–41 F	10 days, GDs 6–15 (GO)	0, 001, 0.01, 1.0, 3.0	BW, CS, DX	Bd wt	3			
					Develop	0.1		1	Cleft palate
Sobolewski et al. 2014									**2,3,7,8-TCDD**
169	Mouse (C57BL/6) 7–11 F	GD 7, GD 14, and PND 2 (GO)	0, 0.25	DX	Develop		0.25		Delayed habituation, reduction in exploration of novel objects, slight memory deficits, altered response rates
Thackaberry et al. 2005a									**2,3,7,8-TCDD**
170	Mouse (C57BL/6N) 4–21 F	GD 14.5 (GO)	0, 1.5, 3, 6, 12, 24	OW, DX, BW, BI	Develop	1.5	3		Reduced relative fetal heart weight
Vorderstrasse et al. 2003									**2,3,7,8-TCDD**
171	Mouse (C57BL/6) 7–12 F	Once (GO)	0, 1, 2.5, 5, 7.5, 10	IX	Immuno		1		Decreased IgG2a and increased IgA levels in response to influenza virus
Vorderstrasse et al. 2004									**2,3,7,8-TCDD**
172	Mouse (C57BL/6J) 6–8 F	GDs 0, 7, and 14 (GO)	0, 1	BC, HP	Repro		1		Suppression of mammary gland differentiation; decreased serum progesterone and estradiol levels on GD 17

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
White et al. 1986									**2,3,7,8-TCDD**
173	Mouse (B6C3F1) 6–8 F	14 days (GO)	0, 0.01, 0.05, 0.1, 0.5, 1.0, 2.0	BC, BI	Immuno		0.01		Suppressed serum total hemolytic complement activity
Yang et al. 2005									**2,3,7,8-TCDD**
174	Mouse C57BL/6 5 M	Once (GO)	0, 40	OF	Hepatic		40		Decreased hepatic all-trans retinol and all-trans retinoic acid
Yang et al. 2005									**2,3,7,8-TCDD**
175	Mouse C57BL/6 5 M	14 days (GO)	0, 0.1	OF	Hepatic		0.1		Decreased hepatic all-trans retinol and all-trans retinoic acid
Zinkl et al. 1973									**2,3,7,8-TCDD**
176	Mouse (CD-1) 3–4 F	Once (GO)	0, 1, 10, 50	HE	Hemato		1		Reversible decreases in leukocyte and lymphocyte counts
Fletcher et al. 2001									**2,3,7,8-TCDD**
177	Hamster (Golden Syrian) 5 M	Once (GO)	0, 1.6, 8, 40, 200, 1,000	LE, BW, OW, OF	Bd wt	8	40	200	LOAEL: Decreased mean body weight (15%) SLOAEL: Decreased mean body weight (20%)
					Hepatic		1.6		Decreased vitamin A content
Hanberg et al. 1989									**2,3,7,8-TCDD**
178	Hamster (Golden Syrian) NS	Once (GO)	0, 1.6–1,000	BW, OW, LE	Hepatic		14		ED_{50} for liver enlargement
					Immuno		48		ED_{50} for thymic atrophy
Kransler et al. 2007									**2,3,7,8-TCDD**
179	Hamster (Golden Syrian) 9–12 F	Once GD 9 (GO)	0, 1.5, 3, 6, 18	CS, BI, DX, BW, OW, HE	Bd wt	18			
					Hemato		1.5		2-Fold increase in leukocytes
					Develop		1.5		Decreased thymus weight, kidney congestion

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Olson and McGarrigle 1992									**2,3,7,8-TCDD**
180	Hamster (Golden Syrian) NS F	GD 7 or 9 (GO)	0, 1.5, 3.6, 18	DX, OW, GN, HE	Develop		1.5	18	LOAEL: Hydronephrosis SLOAEL: Fetal mortality (58%)
Olson et al. 1980a									**2,3,7,8-TCDD**
181	Hamster (Golden Syrian) 4–5 B	Once (GO)	0, 5, 25, 100, 250, 500, 750, 2,000, 3,000	BW, HP, CS, BI, LE	Immuno	250	500		Thymic atrophy
Yellon et al. 2000									**2,3,7,8-TCDD**
182	Hamster (Siberian) 16–34 B	Once (GO)	0, 0.1, 2, 100	LE, CS, RX, DX, IX, BI	Death			2	Increased mortality during 20-week observation period (30%)
					Repro	0.1	2		Increased time to pregnancy
Giavini et al. 1982									**2,3,7,8-TCDD**
183	Rabbit (New Zealand) 10–15 F	GDs 6–15 (GO)	0, 0.1, 0.25, 0.5, 1.0	BW, GN, HP, CS, DX, LE	Bd wt	0.1		0.25	44% decreased weight gain in dams
					Develop		0.1		Skeletal anomalies (extra ribs)
Fletcher et al. 2001									**2,3,7,8-TCDD**
184	Guinea pig (Hartley) 5 M	Once (GO)	0, 0.012, 0.047, 0.18, 0.66, 2.5	LE, BW, OW, OF	Death			2.5	Death of 3/5 animals
					Bd wt	0.18		0.66	Decreased mean body weight gain (22%)
					Hepatic	0.047	0.18		Decreased vitamin A content
					Immuno	2.5			
Hanberg et al. 1989									**2,3,7,8-TCDD**
185	Guinea pig (Hartley) NS	Once (GO)	0, 0.012–2.5	BW, OW, LE	Immuno		0.8		ED_{50} for thymic atrophy

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Hochstein et al. 1988									**2,3,7,8-TCDD**
186	Mink 4 M	Once (GO)	0, 2.5, 5, 7.5	BW, OW, GN, BC, CS	Bd wt		2.5	5	LOAEL: 11% weight loss SLOAEL: 27% weight loss
					Gastro	2.5		5	Gastrointestinal ulcerations
					Hemato	7.5			
					Hepatic	2.5			
					Renal	2.5			
					Neuro	2.5			
Olson and McGarrigle 1992									**2,3,7,8-TCDD**
187	Guinea pig (Hartley) 5 F	GD 14 (GO)	0, 0.15, 1.5	DX	Develop	0.15		1.5	Fetal mortality, increased resorption, decreased spleen and thymus weight
Turner and Collins 1983									**2,3,7,8-TCDD**
188	Guinea pig (Hartley) 1 M, 4–6 F	Once (G)	0, 0.1, 0.5, 2.5, 12.5, 20.0	GN, HP, CS, LE	Hepatic		0.1		Focal necrosis
INTERMEDIATE EXPOSURE									
Allen et al. 1977									**2,3,7,8-TCDD**
189	Monkey (Rhesus) 8 F	9 months (F)	0.011	BW, GN, HP, BC, LE	Death			0.011	5/8 died
					Bd wt		0.011		12% weight loss
					Resp			0.011	Lung hemorrhage
					Cardio			0.011	Hemorrhage in epicardium, myocardium, and endocardium
					Gastro			0.011	Hypertrophy, hyperplasia, and metaplasia of gastric epithelium
					Hemato			0.011	Pancytopenia, bone marrow atrophy; lack of lymphoid germinal centers in spleen

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Musc/skel			0.011	Hemorrhage in muscles from the extremities
					Hepatic			0.011	Epithelial biliary hyperplasia; focal hemorrhages
					Renal		0.011		Tubular epithelial hyperplasia; petechial hemorrhages in urinary bladder
					Dermal		0.011		Facial alopecia, squamous metaplasia, hyperkeratoses; subcutaneous edema; petechial hemorrhaging
					Ocular		0.011		Periorbital edema
					Immuno		0.011		Lymph nodes atrophy
					Neuro			0.011	Hemorrhages in meninges
McNulty 1984									**2,3,7,8-TCDD**
190	Monkey (Rhesus) 2–4 F	3 weeks, 3 days/week (GO)	0, 0.02, 0.1, 0.6	BW, CS, DX, LE	Death			0.6	2/2 died
					Bd wt	0.02		0.1	Weight loss in mothers
					Resp	0.02	0.1		Epistaxis
					Gastro	0.02	0.1		Metaplasia of gastric mucosa
					Hemato	0.02		0.1	Anemia, bone marrow hypoplasia
					Hepatic		0.1		Biliary hyperplasia
					Dermal	0.02	0.1		Hair loss, periorbital edema, hyperkeratosis, squamous metaplasia of sebaceous glands
					Ocular	0.02	0.1		Thickening and reddening of eyelids

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Ahmed 2011									**2,3,7,8-TCDD**
191	Rat (Wistar) 6 F	GD 1–LD 30 (GO)	0, 0.2, 0.4	CS, DX	Develop		0.2		Decreased offspring T4, T3, and growth hormone levels and increased TSH levels; decreased cerebellar neurotransmitter levels on PNDs 10–30
Bell et al. 2007b									**2,3,7,8-TCDD**
192	Rat (Wistar) 65–75 F	12 weeks premating and during gestation and lactation periods (F)	0, 0.0024, 0.008, 0.046	CS, NX, BW, RX, OW, DX, HP	Repro Develop	0.046	 0.0024		 Delayed preputial separation
Chen et al. 2009									**2,3,7,8-TCDD**
193	Rat (Sprague-Dawley) 8 F	29 weeks 1 time/week (GO)	0, 0.02, 0.05, 0. 125	BW, OW, BI	Bd wt Musc/skel	0.05 0.125	0.125		12.2% reduced final body weight
Dhanabalan et al. 2010									**2,3,7,8-TCDD**
194	Rat (Wistar/NIN) 6 M	15 days (GO)	0, 0.1	BW, OW, BC, RX	Repro		0.1		Decreased epididymal sperm count, mobility, and viability and decreased serum testosterone levels
Dhanabalan et al. 2011									**2,3,7,8-TCDD**
195	Rat (Wistar/NIN) 6 M	15 days (GO)	0, 0.1	BW, OW, BC, RX	Repro		0.1		Decreased testicular daily sperm production and epididymal sperm motility, viability, and count; decreased serum testosterone levels

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
El-Tawil and Elsaieed 2005									**2,3,7,8-TCDD**
196	Rat (Sprague-Dawley) 10 M	60 days (GO)	0, 0.05, 0.1, 0.2	CS, BW, OW, RX, HP	Repro		0.05		Decreased epididymal sperm count and motility; increased sperm mortality and abnormalities; decreased reproductive organ weights
Erdemli et al. 2020									**2,3,7,8-TCDD**
197	Rat (Wistar) 10 M	1 month (GO)	0, 1	BC, HP	Renal		1		Glomerular and proximal and distal tubular epithelial damage
Gül et al. 2018									**2,3,7,8-TCDD**
198	Rat (Wistar) 10–15 F	16 weeks, 1 time/week (GO)	0, 0.14	BW, HP	Bd wt			0.14	56% decrease in total weight gain
					Repro		0.14		Decreased number of ovarian follicles at the post-primordial phase and corpus luteum
Harrill et al. 2015									**2,3,7,8-TCDD**
199	Rat (Sprague-Dawley) 10 F	4 weeks 4–5 days/week (GO)	0, 0.003, 0.022, 0.1, 0.3, 1	BW, HE, BC, OW, HP	Resp	1			
					Cardio	1			
					Hemato	0.022	0.1		Increased RBC and decreased MCV
					Hepatic	0.003	0.022		Increased relative liver weight and hepatocytic hypertrophy
					Renal	1			
					Immuno	0.1	0.3		Decreased relative thymus weight and thymic atrophy
					Repro	0.3			

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Harrill et al. 2016									**2,3,7,8-TCDD**
200	Rat (Sprague-Dawley) 10 F	4–5 days/week, 4 weeks (GO)	0, 0.003, 0.022, 0.1, 0.3, 1	BW, BC, HE, OW, HP	Hepatic	0.003	0.022		Hepatic hypertrophy, increased relative liver weight, increased serum cholesterol levels
					Immuno	0.022	0.1		Decreased relative thymus weight
Ikeda et al. 2005b									**2,3,7,8-TCDD**
201	Rat (Holtzman) 12 F	9 weeks, 1 day/week (GO)	0, 0.02	DX, RX, BW, OW	Develop		0.02		Reduced male/female ratio in F2 offspring on PND 2; decreased pup body weight and ventral prostate weight
İlhan et al. 2015									**2,3,7,8-TCDD**
202	Rat (Sprague-Dawley) 6 M	4 weeks (GO)	0, 0.5	OF	Cardio		0.5		Increased systolic blood pressure
Jablonska et al. 2010									**2,3,7,8-TCDD**
203	Rat Lewis-Furth 24 F	GDs 14 and 21 PNDs 7 and 14 PNDs 21–240 (GO)	0, 0.007	DX, BI	Develop		0.007		Accelerated onset of acyclicity in female rats
Latchoumycandane et al. 2002									**2,3,7,8-TCDD**
204	Rat (Wistar) 24 M	45 days (GO)	0, 0.001, 0.01, 0.1	BI, HP	Repro		0.001		Reduced epididymal sperm count
Li and Rozman 1995									**2,3,7,8-TCDD**
205	Rat (Sprague-Dawley) 6–7 M	10 weeks, 1 day/week (GO)	0, 0.003, 0.03, 0.16, 0.5, 1, 1.6	BI, BW, OW, LE	Death			1.6	57% mortality; mean time to death was 54 days
					Bd wt	0.03		0.16	38% decrease in body weight gain

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Endocr	0.003	0.03		Almost 50% reduction in total serum T4
Ma et al. 2010									**2,3,7,8-TCDD**
206	Rat (Sprague-Dawley) 32 M	29 weeks 1 time/week (GO)	0, 0.02, 0.05, 0.125	CS, BI, RX, BW, BC	Bd wt	0.05	0.125		14% reduced final body weight
					Repro	0.02	0.05		Reduced sperm counts; reduced serum testosterone
Murray et al. 1979									**2,3,7,8-TCDD**
207	Rat (Sprague-Dawley) 10–16 M, 20–32 F	3 generations (F)	0, 0.001, 0.01, 0.1	OW, GN, HP, DX	Repro	0.01		0.1	Decreased fertility in F0
					Develop			0.001	Decreased postnatal survival in F1 pups
NTP 1982b									**2,3,7,8-TCDD**
208	Rat (Osborne-Mendel) 10 M, 10 F	13 weeks, 2 days/week (GO)	0, 0.07, 0.14, 0.28, 0.56, 1.12	BW, HP, CS, LE	Bd wt	0.28 M	1.12 F	0.56 M	LOAEL: 16% lower body weight than controls at week 6 SLOAEL: 20% lower body weights than controls at week 6
					Resp	0.56 F			
NTP 2006									**2,3,7,8-TCDD**
209	Rat (Sprague-Dawley) 81–82 F	5 days/week 14 weeks (GO)	0, 0.002, 0.0071, 0.016, 0.032, 0.071	LE, CS, BW, BI, OW, GN, HP	Bd wt	0.071			
					Resp	0.071			
					Gastro	0.071			
					Hepatic	0.002	0.0071		30% increase in absolute liver weight
					Endocr	0.0071	0.016		Decreased FT4 and TT4; thyroid follicular cells hypertrophy
					Immuno	0.0071	0.016		Thymic atrophy
					Repro	0.071			

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 2006									**2,3,7,8-TCDD**
210	Rat (Sprague-Dawley) 81–82 F	5 days/week 31 weeks (GO)	0, 0.002, 0.0071, 0.016, 0.032, 0.071	LE, CS, BW, BI, OW, GN, HP	Bd wt	0.071			
					Resp	0.071			
					Gastro		0.071		Squamous hyperplasia of forestomach
					Hepatic	0.002	0.0071		Hepatocyte pigmentation; increased relative and absolute liver weight
					Endocr	0.0071	0.016		Decreased serum FT4 and TT4
					Immuno	0.016	0.032		Thymic atrophy
					Repro	0.071			
					Other noncancer	0.032	0.071		Vacuolization of acinar cell in pancreas
Sarihan et al. 2015									**2,3,7,8-TCDD**
211	Rat (Sprague-Dawley) 7 M	45 days (GO)	0, 0.3	BW, OW, HP, OF	Cardio			0.3	Decreased blood pressure, heart rate, oxygen saturation, arrythmias, long QT intervals, mild and moderate cardiac lesions
Sewall et al. 1995									**2,3,7,8-TCDD**
212	Rat (Sprague-Dawley) 6–9 F	30 weeks, 1 time/ 2 weeks (GO)	0, 0.0001, 0.00035, 0.001, 0.0035, 0.011, 0.036, 0.125	BI	Endocr	0.011	0.036		Reduction in serum T4

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Van Birgelen et al. 1995									**2,3,7,8-TCDD**
213	Rat (Sprague-Dawley) 8 F	13 weeks (F)	0, 0.014, 0.026, 0.047, 0.320, 1.02	BI, BW, OW, FI, BI, BC	Bd wt	0.026	0.047	1.02	LOAEL: 10% reduction in body weight gain SLOAEL: 72% reduction in body weight gain
					Hepatic		0.014		Reduction in hepatic retinol
					Renal		0.047		Increased relative kidney weight
					Endocr	0.026	0.047		Reduction in total serum T4
					Immuno		0.014		Decreased absolute and relative thymus weight
Viluksela et al. 1994									**2,3,7,8-TCDD**
214	Rat (Sprague-Dawley) 20 M	13 weeks, 10 doses (GO)	0, 0.8	BW, HE, LE, OW	Bd wt			0.8	30% decrease in body weight gain
					Hemato		0.8		Decrease in platelet count
					Hepatic		0.8		Increased relative liver weight and liver EROD activity; decreased liver PEPCK activity
					Immuno		0.8		Decreased absolute and relative thymus weight
Vos et al. 1973									**2,3,7,8-TCDD**
215	Rat (CD) 10 F	6 weeks, 1 day/week (GO)	0, 0.028, 0.14, 0.71	BW, HP, BC	Hemato	0.71			
					Immuno	0.14	0.71		Decreased absolute and relative thymus weight and slight cortical atrophy
Zinkl et al. 1973									**2,3,7,8-TCDD**
216	Rat (CD) 3–4 F	30 days (GO)	0, 0.1, 1.0, 10	BC, HE	Hemato		0.1		Thrombocytopenia
					Hepatic	0.1	1		Increased serum cholesterol

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
DeVito et al. 1994									**2,3,7,8-TCDD**
217	Mouse (B6C3F1) 5 F	13 weeks, 5 days/week (F)	0, 0.0011, 0.0032, 0.011, 0.032, 0.11	BI, BW, OW	Immuno	0.11			
Fader et al. 2015									**2,3,7,8-TCDD**
218	Mouse C57BL/6 8 F	28 days, once every 4 days (seven doses) (GO)	0, 0.0003, 0.003, 0.008, 0.03, 0.08, 0.3, 0.8, 10, 3, 8	BW, BC, OW, HP	Bd wt	8			
					Gastro	8			
					Hepatic	0.3	0.8		Increased relative liver weight, minimal centriacinar microvesicular vacuolization (indicative of hepatic steatosis), increased macrophage infiltration
Fader et al. 2015									**2,3,7,8-TCDD**
219	Mouse C57BL/6 8–16 M/F	28 days, once every 4 days (seven total exposures) (GO)	0, 8	IX	Immuno		8		Altered immune cell population in intestinal lamina propria
Fader et al. 2017a, 2017b									**2,3,7,8-TCDD**
220	Mouse C57BL/6 8 M	28 days, once every 4 days (seven doses) (GO)	0, 0.0003, 0.003, 0.008, 0.03, 0.08, 0.3, 0.8, 10, 3, 8	BW, BC, OW, HP	Bd wt	3		8	Decreased terminal body weight (27%)
					Gastro	3	8		Increased gastroduodenal and colonic para-cellular permeability, decreased gut motility
					Hepatic	0.03	0.08		Increased relative liver weight

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Fader et al. 2018									**2,3,7,8-TCDD**
221	Mouse (C57BL/6) 8 M, 8 F	28 days, once every 4 days (7 doses) (GO)	0, 8	BC, HP	Musc/skel		8		Increased trabecular bone mass (bone mineral density and content, thickness, and bone volume fraction), decreased trabecular spacing and number, and decreased bone marrow adiposity in both sexes; decreased osteoclasts in females and increased osteoblasts in males
Fader et al. 2018									**2,3,7,8-TCDD**
222	Mouse (C57BL/6) 8 M	28 days, once every 4 days (7 doses) (GO)	0, 0.003, 0.008, 0.03, 0.08, 0.3, 0.8, 3, 8	HP	Musc/skel	0.08	0.3		Decreased trabecular spacing
Herlin et al. 2013									**2,3,7,8-TCDD**
223	Mouse (C57BL/6J) 6 M, 6 F	10 weeks 1 time/week (GO)	0, 2.9; one time loading dose of 40 µg/kg followed by nine doses of 18 µg/kg (total dose over study of 200 µg/kg)	BW, BC, OW, HP	Bd wt Musc/skel	2.9	 2.9		Increased trabecular bone mass (increased bone volume fraction, bone mineral deposits, decreased spacing) and decreased cortical bone thickness in both sexes; imbalance of serum bone remodeling markers and mechanically weaker bones in females

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Hogaboam et al. 2008									**2,3,7,8-TCDD**
224	Mouse (C57BL/6) NS F	GDs 0, 7, and 14 LD 2 (GO)	0, 0.17	DX, IX, BI	Develop		0.17		Altered immune function (decreased virus specific CD8+ T cells and increased neutrophils and interferon-gamma levels in BALF) in response to influenza infection in adult offspring
Ishihara et al. 2007									**2,3,7,8-TCDD**
225	Mouse (ICR) 40 M	5 weeks (GO)	0, 0.0001, 0.1	DX	Repro	0.0001	0.1		Decreased male/female ratio in PND 0 pups
Ishihara et al. 2010									**2,3,7,8-TCDD**
226	Mouse (ICR) 49–59 M	5 weeks 1 time/week (GO)	0, 0.1	DX	Repro		0.1		Decreased F1 male/female ratio in embryos
Kopf et al. 2010									**2,3,7,8-TCDD**
227	Mouse C57BL/6 12–14 M	5 weeks, 5 days/week (F)	0, 0.13	BW, OW, OF	Bd wt	0.13			
					Cardio		0.13		Increased mean arterial pressure
					Hepatic		0.13		Increased absolute liver weight
Maranghi et al. 2013									**2,3,7,8-TCDD**
228	Mouse (BALB/c) 10–15 F	28 days (F)	0, 0.09	CS, BW, FI, BC, OW, GN, HP	Bd wt	0.09			
					Hepatic		0.09		Increased necrotic hepatocytes (incidences of pyknotic nuclei in hepatocytes) and tissue congestion
					Endocr		0.09		Thyroid follicular cell hypertrophy (increased follicular epithelium area to number of nuclei ratio)

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Immuno		0.09		Increased incidences of lymphocyte apoptosis in the thymus, follicular hyperplasia with germinal center development in the spleen
					Repro		0.09		Increased serum testosterone levels and testosterone/estradiol ratio
Ono et al. 2010									**2,3,7,8-TCDD**
229	Mouse (Hos:HR-1) 10 NR	54 days (GO)	0, 0.0003, 0.001	CS	Dermal	0.001			
Rasinger et al. 2018									**2,3,7,8-TCDD**
230	Mouse (BALB/c) 10 F	28 days (F)	0, 0.0009	CS, BW, FI, BC, OW, GN, HP	Bd wt	0.0009			
					Hepatic		0.0009		Lymphocytic inflammation in liver
					Immuno	0.0009			
Smialowicz et al. 2008									**2,3,7,8-TCDD**
231	Mouse (B6C3F1) 8–15 F	13 weeks, 5 days/week (GO)	0, 0.0011, 0.011, 0.11, 0.32	BW, OW, IX	Bd wt	0.0011	0.011		
					Immuno		0.0011		Decreased antibody response to sRBC
Sugita-Konishi et al. 2003									**2,3,7,8-TCDD**
232	Mouse (C57BL/6NCji) 8 F	LDs 0–17 (W)	0, 0.001, 0.011	DX, IX, BC, BI	Develop	0.001	0.011		Impaired clearance of bacteria from pups' spleen
Thigpen et al. 1975									**2,3,7,8-TCDD**
233	Mouse (C57BL/6Jfh) 60 M	4 weeks, 1 day/week (GO)	0, 0.07, 0.14, 0.71	BW, CS	Immuno	0.07	0.14		Impaired response to bacterial infection

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Thomas and Hinsdill 1979									**2,3,7,8-TCDD**
234	Mouse (Swiss-Webster) 10 F	4 weeks prior to mating and during gestation and lactation (F)	0, 0.13, 0.325, 0.65, 1.3, 2.6	DX, LE	Dermal	0.65	1.3		Alopecia, edema in dams
					Develop			1.3	Decreased pup survival
							0.325		Thymus atrophy; impaired response to sRBC
Umbreit et al. 1987									**2,3,7,8-TCDD**
235	Mouse (C57B/6) 10 F	25 weeks, 3 days/week (GO)	0, 1.3	CS, LE	Death			1.3	70% died
Vecchi et al. 1983									**2,3,7,8-TCDD**
236	Mouse (C57BL/6, DBA/2) NS M	5-8 weeks, 1 day/week (GO)	0, 0.07, 0.3	IX	Immuno		0.07		Decreased response to sRBC
Vorderstrasse et al. 2006									**2,3,7,8-TCDD**
237	Mouse (C57BL/6J) NS F	GDs 0, 7, and 14; LD 2 (GO)	0, 0.04, 0.1, 0.5	DX, IX, BI	Develop			0.5	Reduced pup survival
						0.04	0.1		Suppressed CD8+ T cell response to infection in offspring
Vos et al. 1973									**2,3,7,8-TCDD**
238	Mouse (B6D2F1) 5–7 M	4 weeks, 1 day/week (GO)	0, 0.028, 0.14, 0.71, 3.6	BW, GN, HP	Bd wt	0.71	3.6		17% reduced weight gain
					Immuno	0.14	0.71		Suppressed response in graft versus host test
Yang et al. 2005									**2,3,7,8-TCDD**
239	Mouse C57BL/6 5 M	28 or 42 days (GO)	0, 0.1	OF	Hepatic		0.1		Decreased hepatic all-trans retinol and all-trans retinoic acid

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Yin et al. 2012									**2,3,7,8-TCDD**
240	Mouse (NS) 8 M	7 weeks (GO)	0, 0.1	BC, HP	Repro		0.1		Decreased testicular FSH and LH levels and serum testosterone levels; decreased testicular spermatozoa levels; necrosis of spermatocytes and spermatogonia
DeCaprio et al. 1986									**2,3,7,8-TCDD**
241	Guinea pig (Hartley) 10 M, 10 F	90 days (F)	0, 0.0001, 0.0007, 0.005, 0.03	BW, OW, HP, BI, LE	Bd wt	0.0007	0.005		12–15% reduced weight gain
					Hemato	0.005			
					Hepatic	0.0007	0.005		Hepatocellular inclusions, hypertriglyceridemia
					Immuno	0.0007 M	0.005 M		Decreased absolute and relative thymus weight
Hochstein et al. 2001									**2,3,7,8-TCDD**
242	Mink Standard dark 12 F	132 days *ad libitum* (F)	0.00003, 0.0008, 0.003, 0.007, 0.07	BC, DX, HP, BI, HE, BW, OW, CS	Develop			0.003	Reduced kit survival in first 3 weeks
Vos et al. 1973									**2,3,7,8-TCDD**
243	Guinea pig (Hartley) 10 F	8 weeks, 1 day/week (GO)	0, 0.001, 0.006, 0.03, 0.14	BW, GN, LE	Hemato		0.001		Decreased lymphocytes
					Immuno		0.006		Impaired delayed hypersensitivity response to tuberculin and decreased thymus weight

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
CHRONIC EXPOSURE									
Bowman et al. 1989a, 1989b; Hong et al. 1989; Schantz and Bowman 1989; Schantz et al. 1986, 1992									**2,3,7,8-TCDD**
244	Monkey (Rhesus) 8 F	Up to 3.5–4 years (F)	0, 0.00012, 0.00064	CS, BW, RX, DX, IX	Repro	0.00012		0.00064	Decreased reproductive success
					Develop		0.00012[d]		Increased close, social contact between mothers and infants, impaired learning, and altered peer group social behavior and self-directed behaviors
Rier et al. 2001a									**2,3,7,8-TCDD**
245	Monkey (Rhesus) 8 F	3.5–4 years (F)	0, 0.00012, 0.00064	IX	Immuno		0.00012[d]		Impaired response to T-mitogen
Kociba et al. 1978									**2,3,7,8-TCDD**
246	Rat (Sprague-Dawley) 50 M, 50 F	2 years (F)	0, 0.001, 0.01, 0.1	BW, OW, FI, GN, HP, CS, BI	Death			0.1 F	Increased cumulative mortality
					Resp	0.001 F	0.01 F		Focal alveolar hyperplasia
					Cardio	0.01		0.1	Myocardial degeneration in females and periarteritis
					Gastro	0.1			
					Hemato	0.01	0.1		Decreased erythrocytes
					Musc/skel	0.1			
					Hepatic	0.001	0.01		Atrophy of hepatic cords, cytoplasmic vacuolization, fatty metamorphosis, hepatic necrosis and inflammation, bile duct hyperplasia, fibrosis, and periportal inflammation
					Renal	0.1			

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Endocr	0.01	0.1		Adrenal gland hyperplastic nodules, hematocysts, and cortical necrosis and hemorrhage; thyroid gland follicular cysts (males only), and pancreatic fibrosis (females only)
					Neuro	0.01 F		0.1 F	Hemorrhage in brain
					Repro	0.1			
					Cancer			0.1	CEL: hepatocellular carcinoma (females), squamous cell carcinoma in lung (females) squamous cell carcinoma of hard palate or nasal turbinates (males and females)
Murray et al. 1979									**2,3,7,8-TCDD**
247	Rat (Sprague-Dawley) 20 M, 20 F	12 months prior to mating (F)	0, 0.1	RX	Repro			0.1 F	Increased resorption in females mated with unexposed males
NTP 1982b									**2,3,7,8-TCDD**
248	Rat (Osborne-Mendel) 50–75 M, 50–75 F	104 weeks, 2 days/week (GO)	0, 0.0014, 0.0071, 0.071	BW, HP, GN, CS, LE	Bd wt	0.0071	0.071		12–19%% lower body weight than controls
					Resp	0.071			
					Cardio	0.071			
					Gastro	0.071			
					Hemato	0.071			
					Musc/skel	0.071			

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	0.0071	0.071		Toxic hepatitis (lipidosis, hydropic hepatocellular degeneration, proliferation of periportal bile ductules, mild fibrosis)
					Renal	0.071			
					Dermal	0.071			
					Ocular	0.071			
					Endocr	0.071			
					Immuno	0.071			
					Neuro	0.071			
					Repro	0.071			
					Cancer			0.071 F	CEL: increased incidence of neoplastic nodules in liver or hepatocellular carcinoma
								0.0071 M	CEL: increased incidence of thyroid follicular cell adenoma or carcinoma
NTP 2006									**2,3,7,8-TCDD**
249	Rat (Sprague-Dawley) 81–82 F	5 days/week 105 weeks (GO)	0, 0.002, 0.0071, 0.016, 0.032, 0.071	LE, CS, BW, BI, OW, GN, HP	Bd wt	0.032	0.071		16% reduced final body weight
					Resp		0.002		Bronchiolar metaplasia of alveolar epithelium
					Cardio	0.002	0.0071		Cardiomyopathy
					Gastro	0.032	0.071		Squamous hyperplasia of forestomach and squamous hyperplasia of gingival mucosa
					Hepatic		0.002		Hepatocyte hypertrophy and inflammation

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal	0.016	0.032		Hyperplasia of transitional renal epithelium
					Dermal	0.071			
					Ocular	0.071			
					Endocr	0.0071	0.016		Hyperplasia of adrenal gland cortex; thyroid follicular cell hypertrophy, increased T4 levels
					Immuno	0.002	0.0071		Thymic atrophy
					Neuro	0.071			
					Repro	0.0071	0.016		Dilation of clitoral gland ducts
					Cancer			0.071	CEL: liver, lung, and oral mucosa malignant tumors
Della Porta et al. 1987									**2,3,7,8-TCDD**
250	Mouse (B6C3) 43–50 M, 42–49 F	52 weeks, 1 day/week (GO)	0, 0.36, 0.72	BW, HP, GN, LE	Death			0.36	Increased mortality
					Bd wt			0.36	33% decreased weight gain
					Dermal		0.36		Dermatitis
					Cancer			0.36	CEL: hepatocellular adenoma or carcinoma
NTP 1982b									**2,3,7,8-TCDD**
251	Mouse (B6C3F1) 50–75 M, 50–75 F	104 weeks, 2 day/week (GO)	M: 0, 0.0014, 0.0071, 0.071; F: 0, 0.006, 0.03, 0.3	BW, GN, HP, CS, LE	Bd wt	0.3			
					Resp	0.3			
					Cardio	0.3			
					Gastro	0.3			
					Hemato	0.3			
					Musc/skel	0.3			

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	0.0071	0.071		Toxic hepatitis (lipidosis, bile duct hyperplasia, pericellular fibrosis)
					Renal	0.0071	0.071		Lymphocytic inflammatory infiltration in kidneys
					Dermal	0.3			
					Ocular	0.3			
					Endocr	0.3			
					Immuno	0.3			
					Neuro	0.3			
					Repro	0.3			
					Cancer			0.3 F	CEL: Thyroid follicular cell adenoma and histiocytic lymphomas
								0.071 M	CEL: Hepatocellular adenoma or carcinoma
Oughton et al. 1995									**2,3,7,8-TCDD**
252	Mouse (C57BL/6N) 10–14 F	14–15 months, 1 day/week (GO)	0, 0.03	BW, HE, OW, BI, BC	Bd wt	0.03			
					Hemato	0.03			
					Immuno		0.03		Decreased percentage of splenic memory T cells, increased percentage of splenic naïve T helper cells

Table 2-2. Levels of Significant Exposure to 2,3,7,8-Tetrachloroibenzo-*p*-Dioxin – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Toth et al. 1979									**2,3,7,8-TCDD**
253	Mouse (Swiss) 45 M	1 year, 1 day/week (GO)	0, 0.001, 0.1, 1.0	BW, GN, HP, CS, LE	Death			1	Decreased survival (34% decreased life span)
					Dermal		0.001		Skin lesions and generalized amyloidosis
					Cancer			0.1	CEL: hepatocellular carcinoma

[a]Doses adjusted for intermittent exposure.
[b]The number corresponds to entries in Figure 2-4; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-4. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[c]Used to derive a provisional acute-duration oral minimal risk level (MRL) of 0.0002 µg/kg/day ($2x10^{-4}$ µg/kg/day) for 2,3,7,8-TCDD based on a NOAEL of 0.005 µg/kg/day and divided by a total uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability) and a modifying factor of 0.7 (to adjust for the higher bioavailability of 2,3,7,8-TCDD from an oil gavage vehicle than from food).
[d]Used to derive a provisional chronic-duration oral minimal risk level (MRL) of $4x10^{-7}$ µg/kg/day for 2,3,7,8-TCDD based on a LOAEL of 0.00012 µg/kg/day for neurodevelopmental and immunological effects in the mothers (Bowman et al. 1989a, 1989b; Hong et al. 1989; Rier et al. 2001a; Schantz et al. 1986, 1992; Schantz and Bowman 1989) and divided by a total uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans, and 10 for human variability).

ACTH = adrenocorticotropin hormone; B = both males and females; BALF = bronchioalveolar fluid; BC = serum (blood) chemistry; Bd wt or BW = body weight; BI = biochemical changes; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DTH = delayed-type hypersensitivity; DX = developmental toxicity; ED_{30} = effective dose that produces a 30% response; ED_{50} = median effective dose; Endocr = endocrine; EROD = 7-ethoxy-resorufin-O-deethylase; (F) = feed; F = female(s); FFA = free fatty acid; FI = food intake; FSH = follicle-stimulating hormone; FT4 = free thyroxine; (G) = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; (GO) = gavage in oil; HE = hematology; Hemato = hematological; HP = histopathology; Ig = immonuglobulin; IL = interleukin; Immuno = immunological; IX = immune function; LD = lactation day; LD_{50} = median lethal dose; LE = lethality; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCV = mean corpuscular volume; Musc/skel = musculoskeletal; Neuro = neurological; NK = natural killer; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OVA = ovalbumin; OW = organ weight; PEPCK = phosphoenolpyruvate carboxykinase; PND = postnatal day; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive function; sRBC = sheep red blood cell; SLOAEL = serious lowest-observed-adverse-effect level; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; TT4 = total thyroxine; UR = urinalysis; WI = water intake

Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

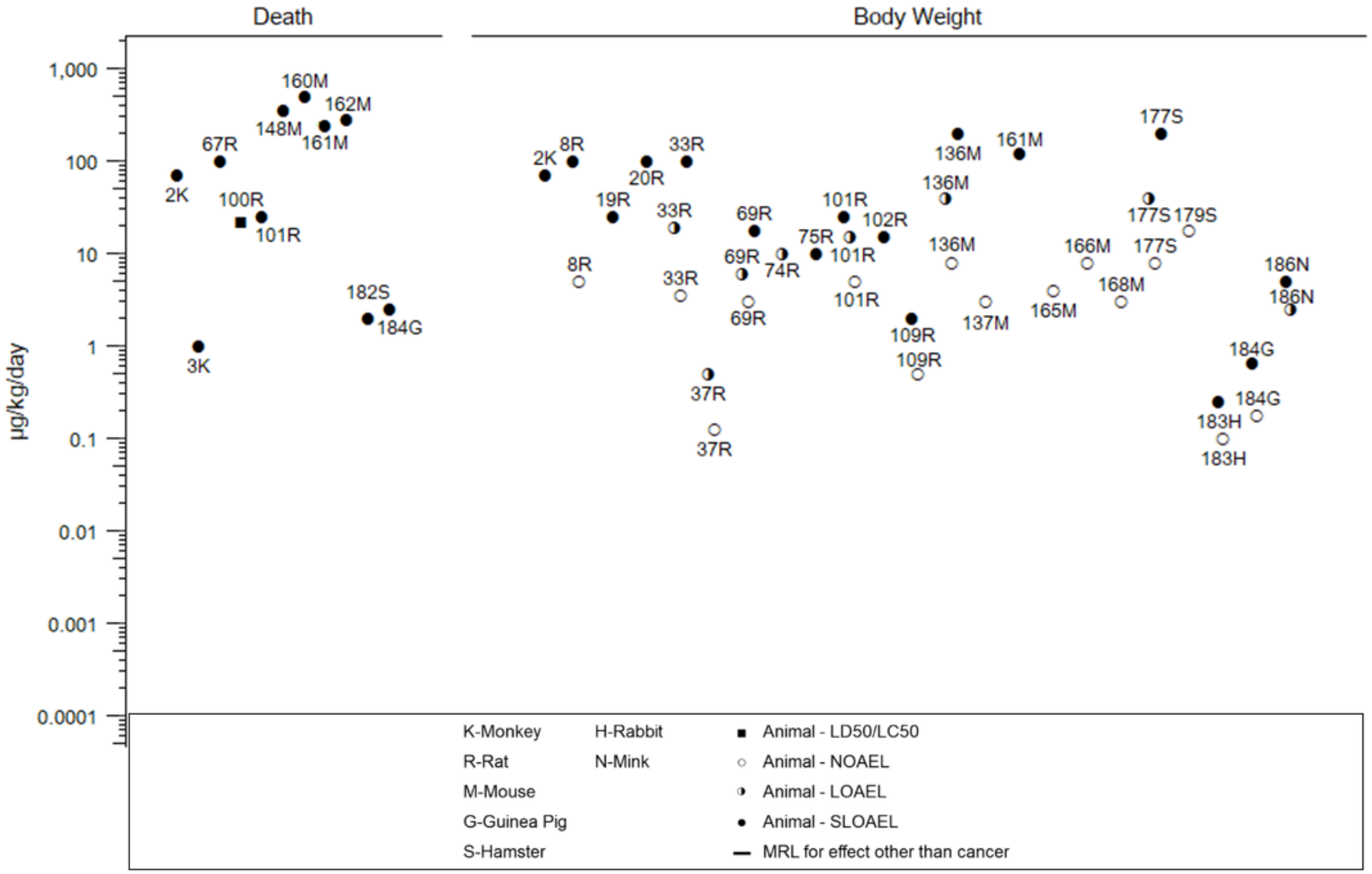


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

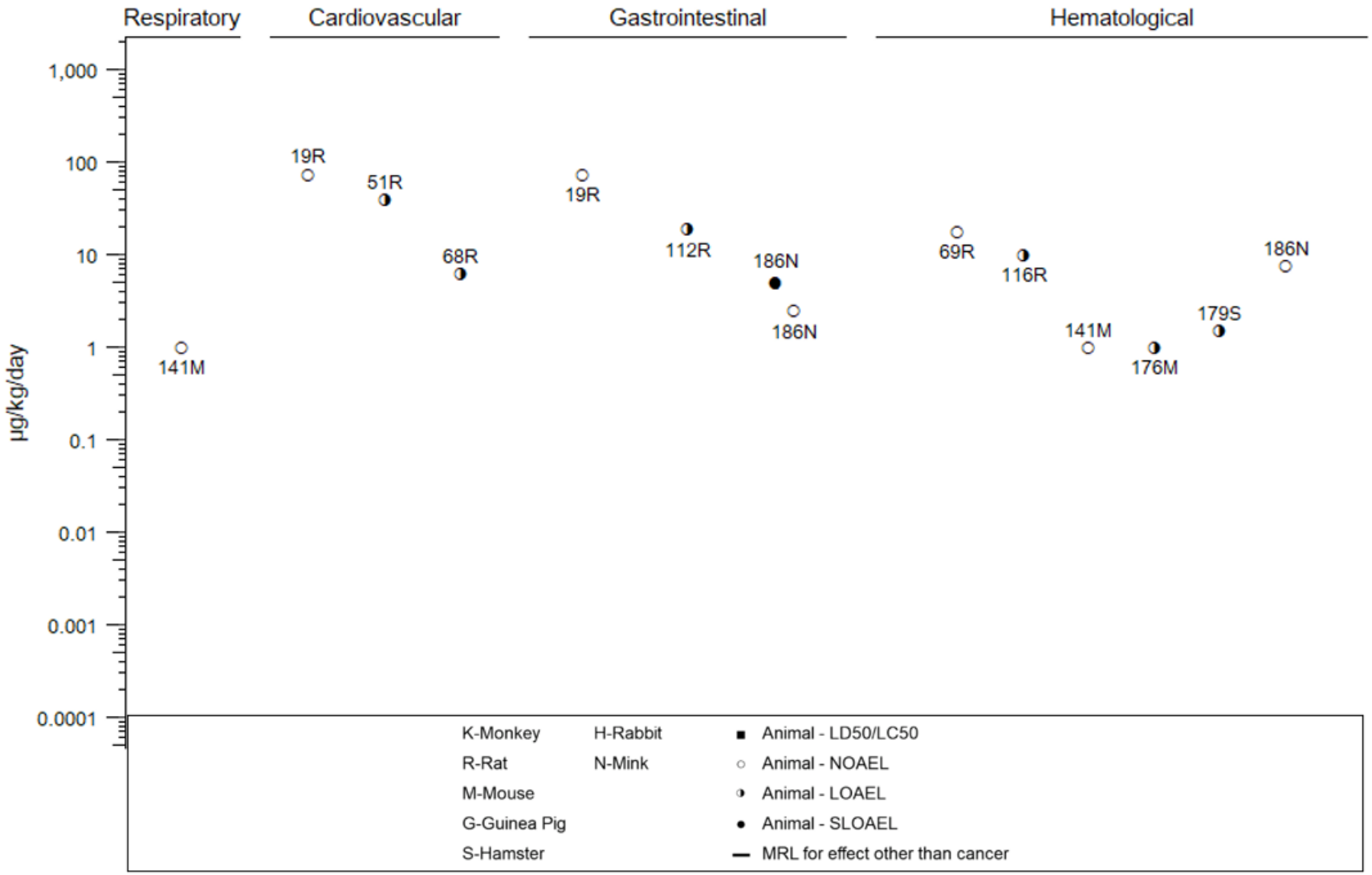


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

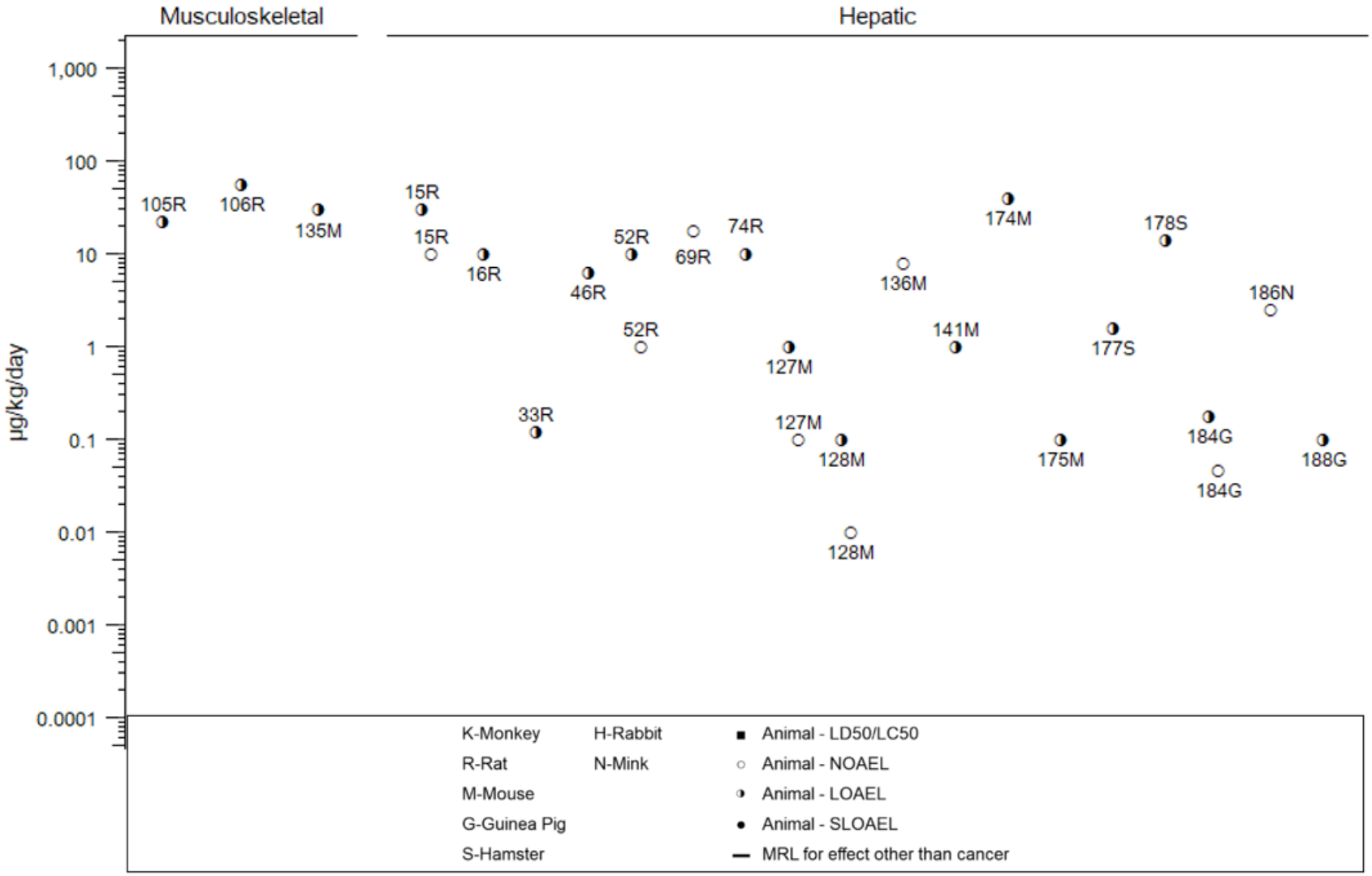


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

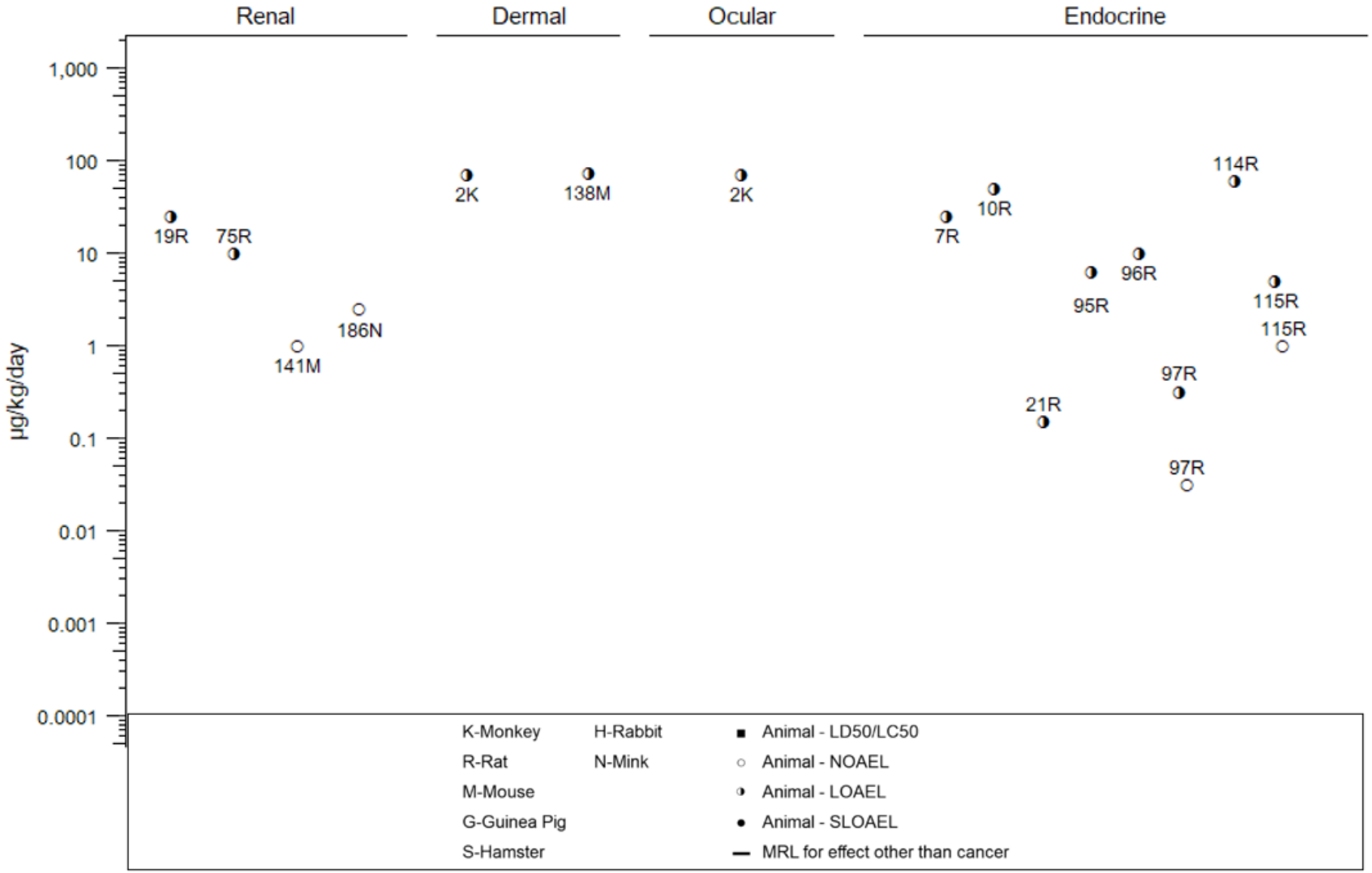


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

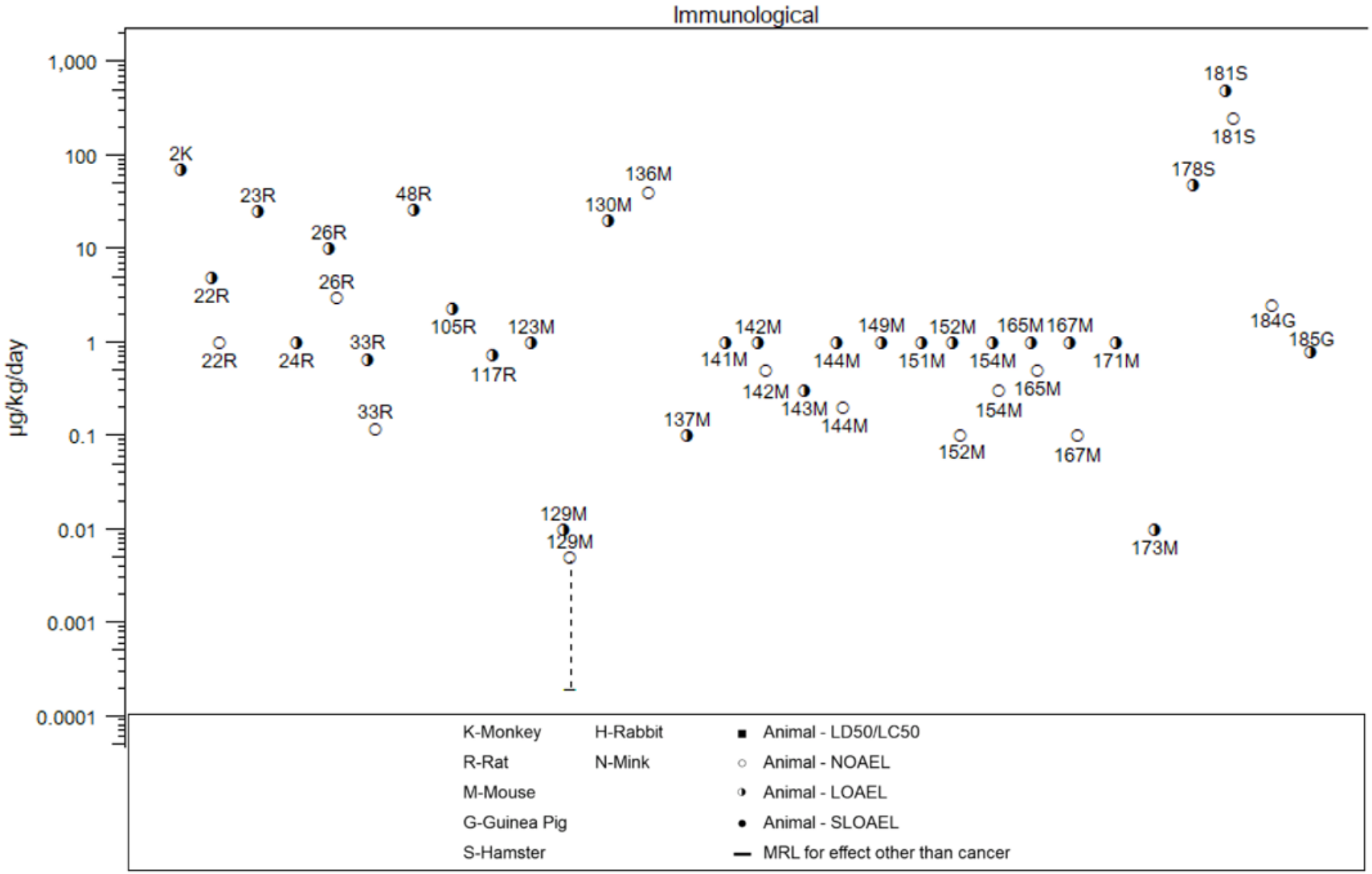


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

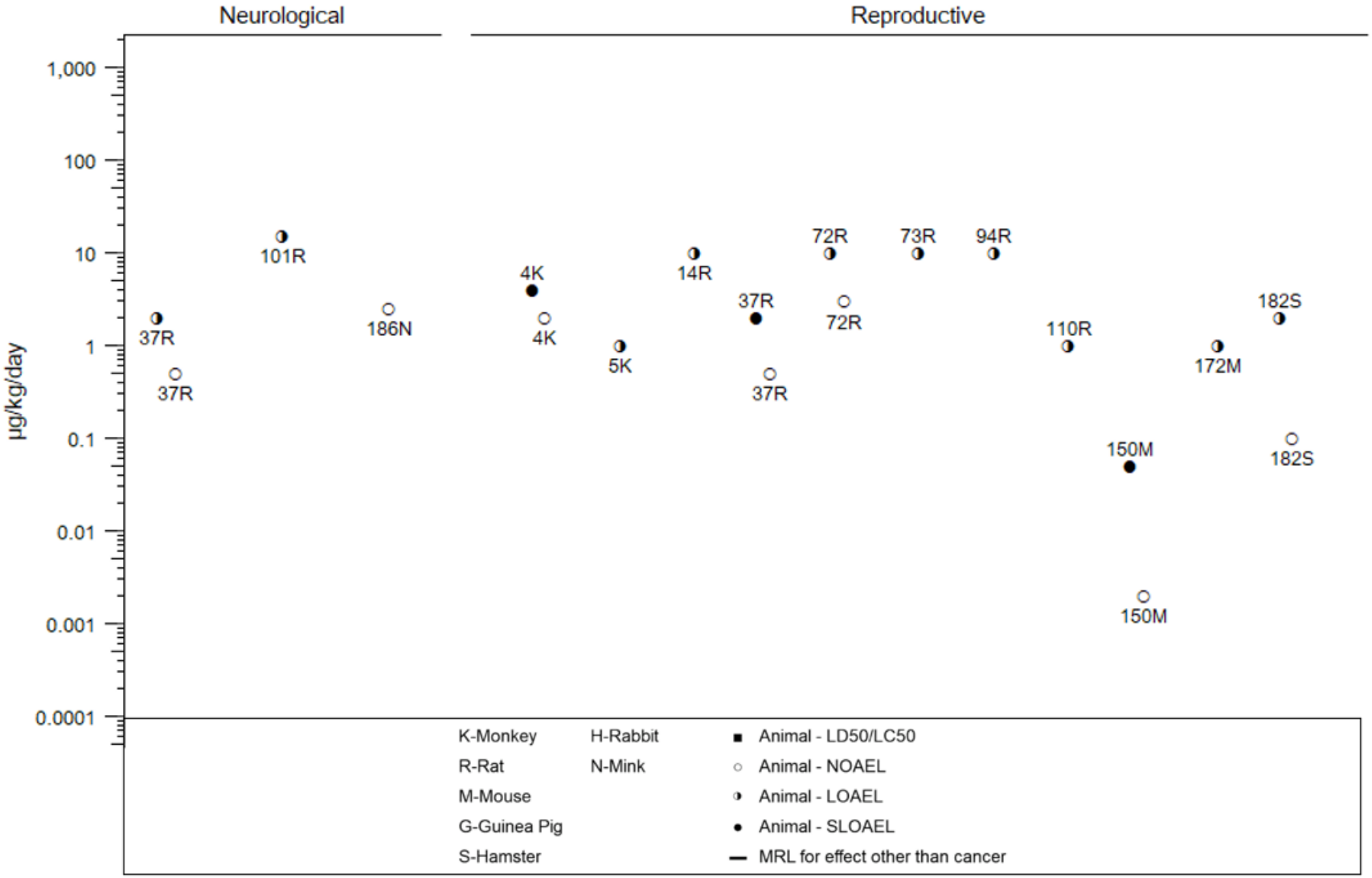


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

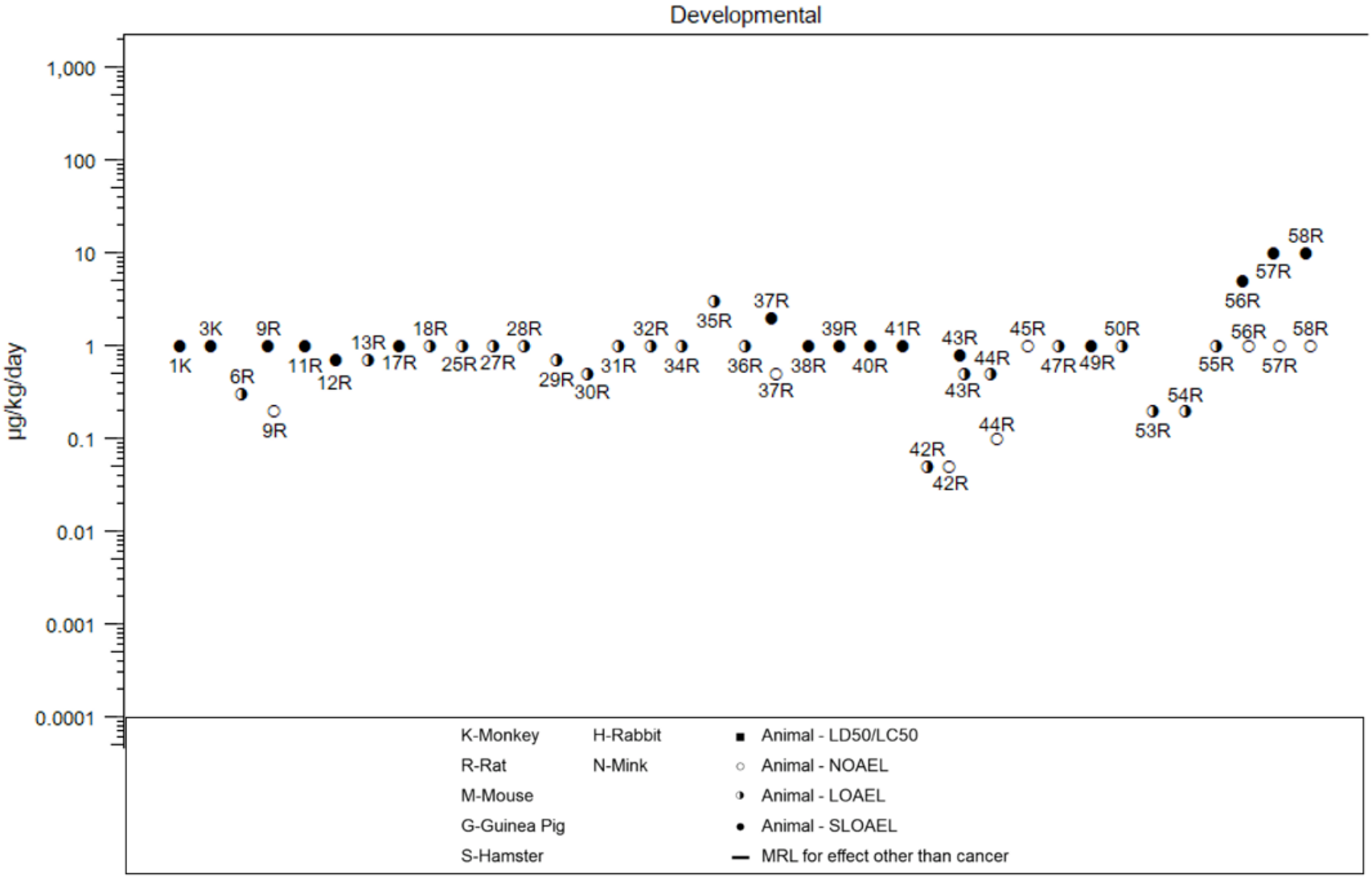


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

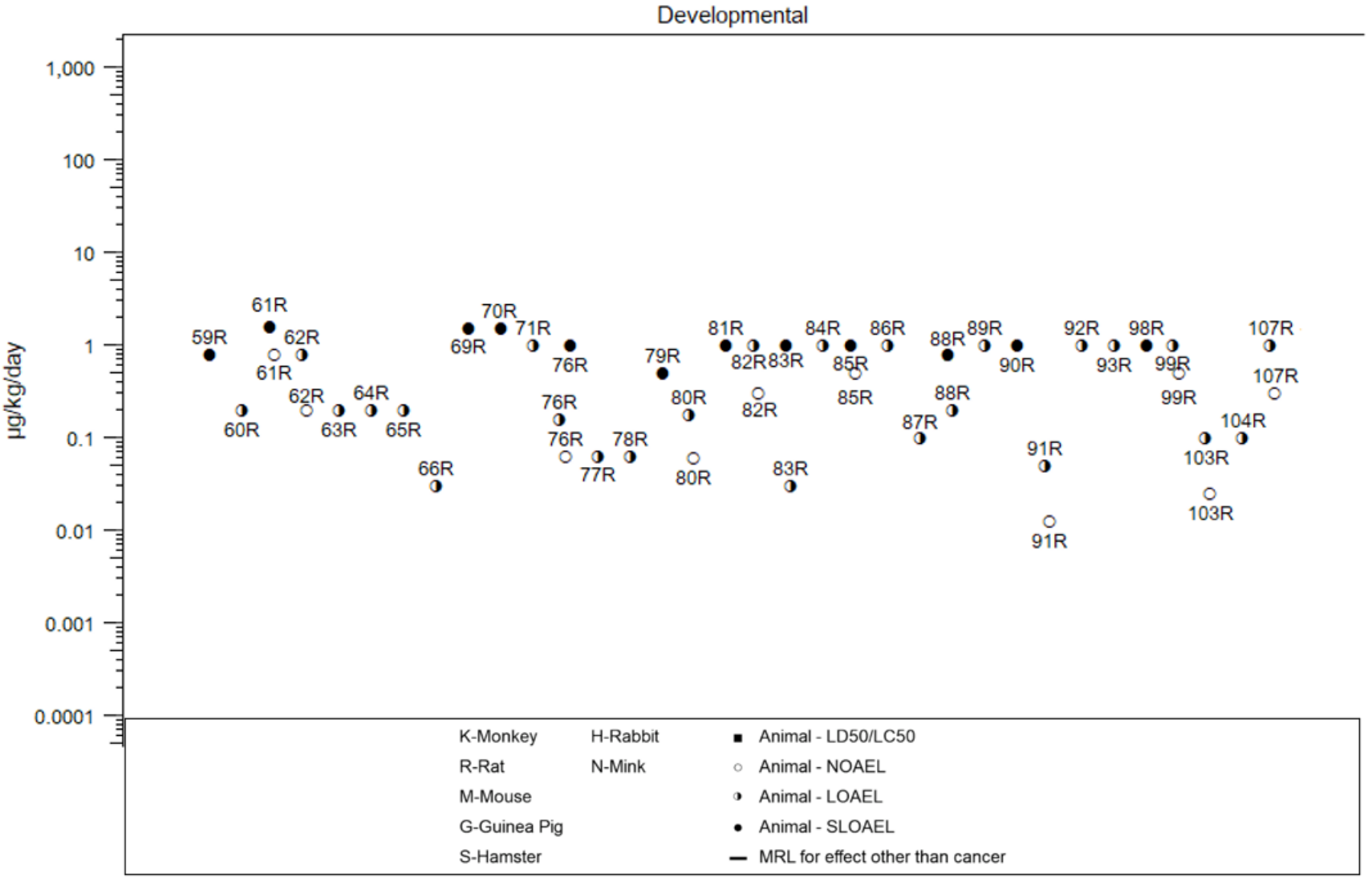


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

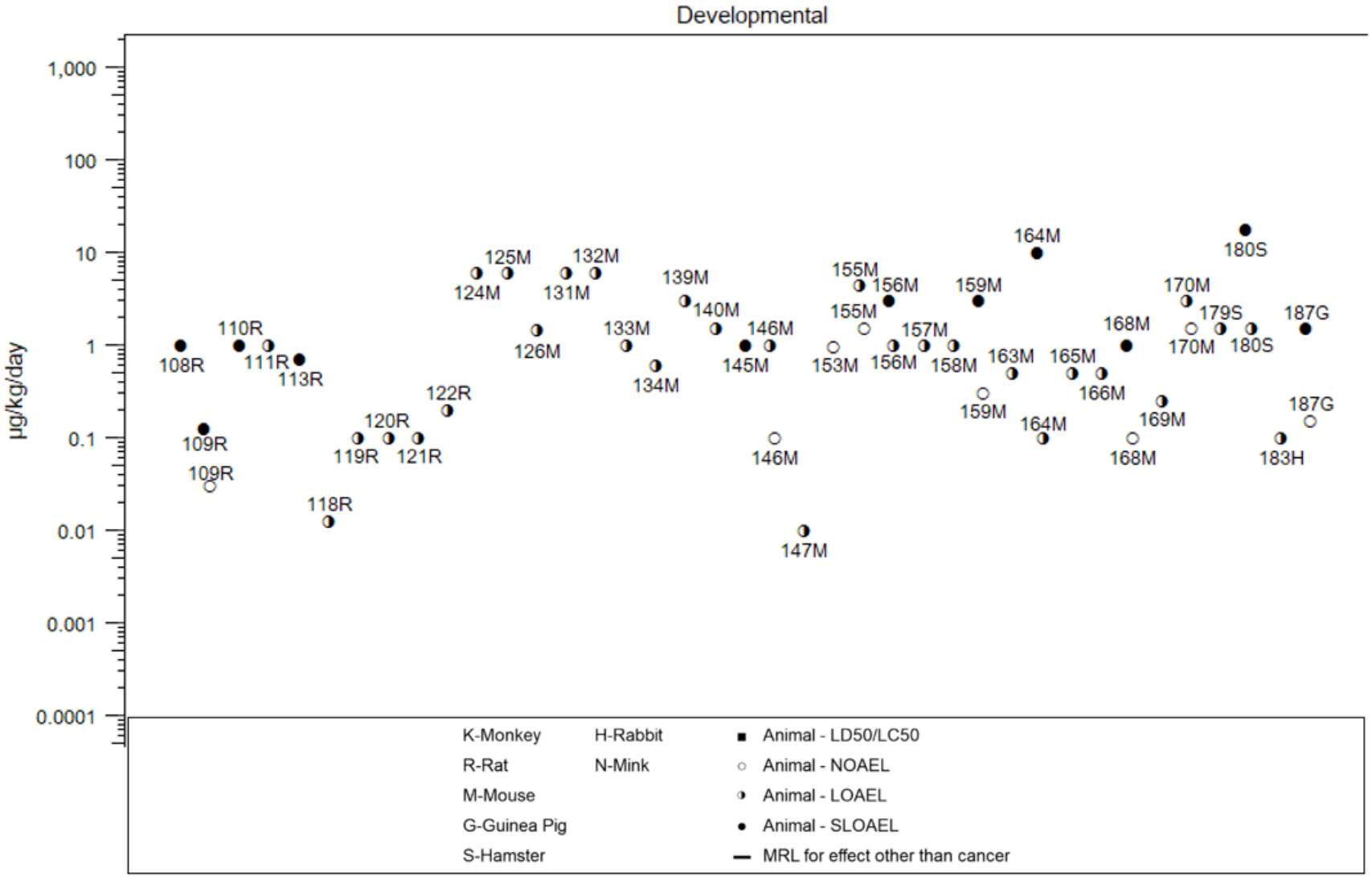


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Acute (≤14 days)

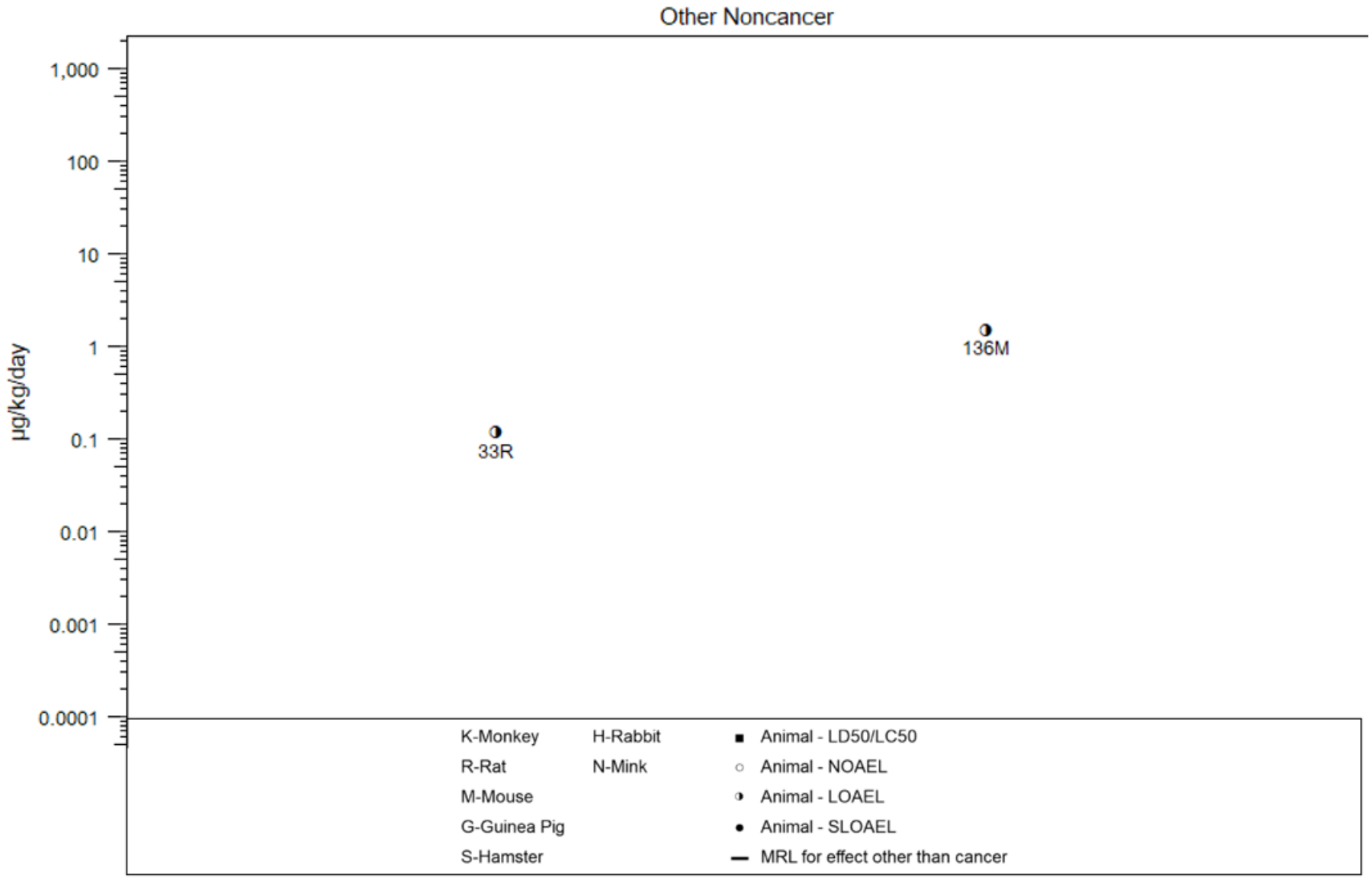


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Intermediate (15–364 days)

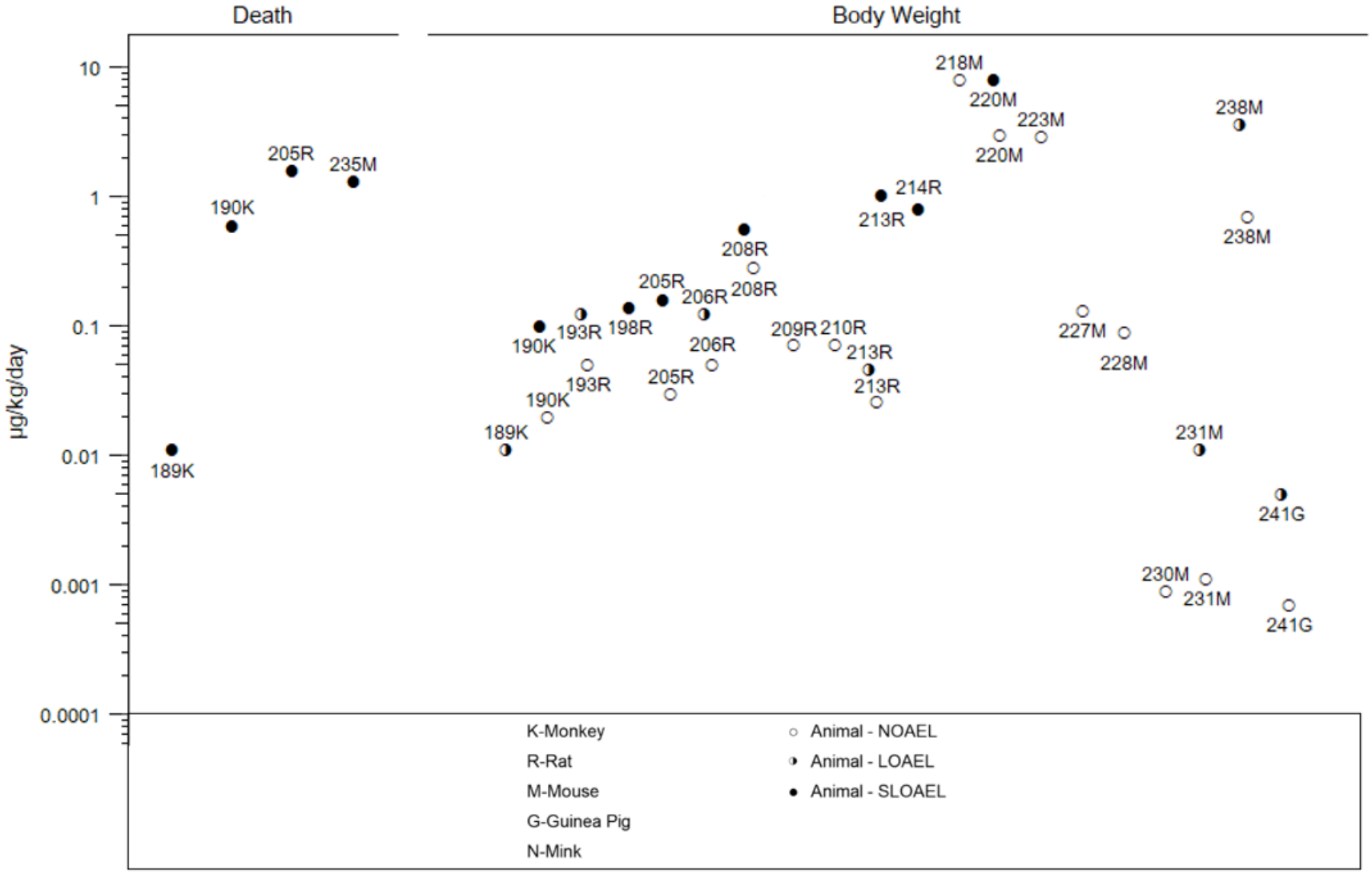


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Intermediate (15–364 days)

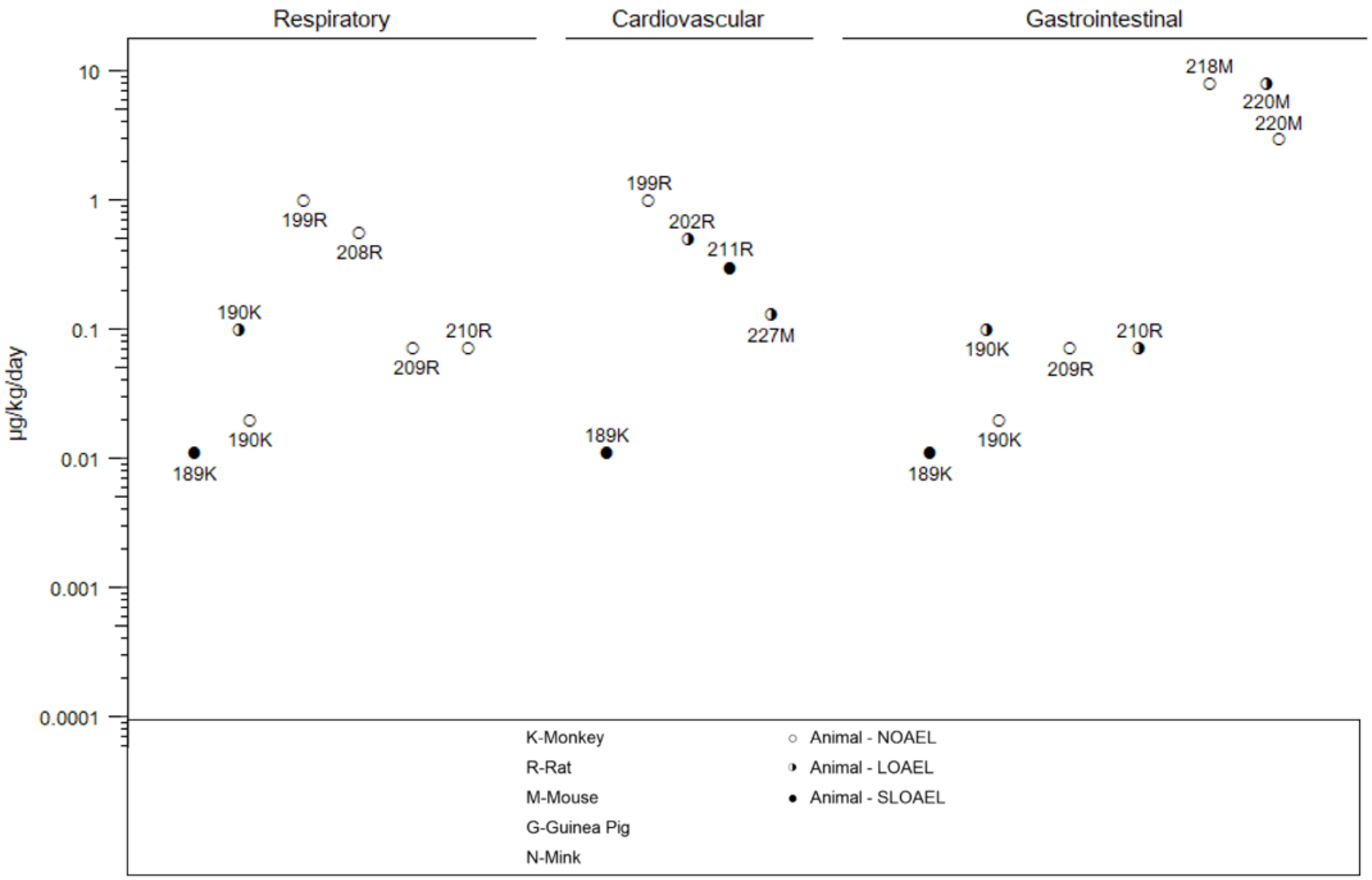


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Intermediate (15–364 days)

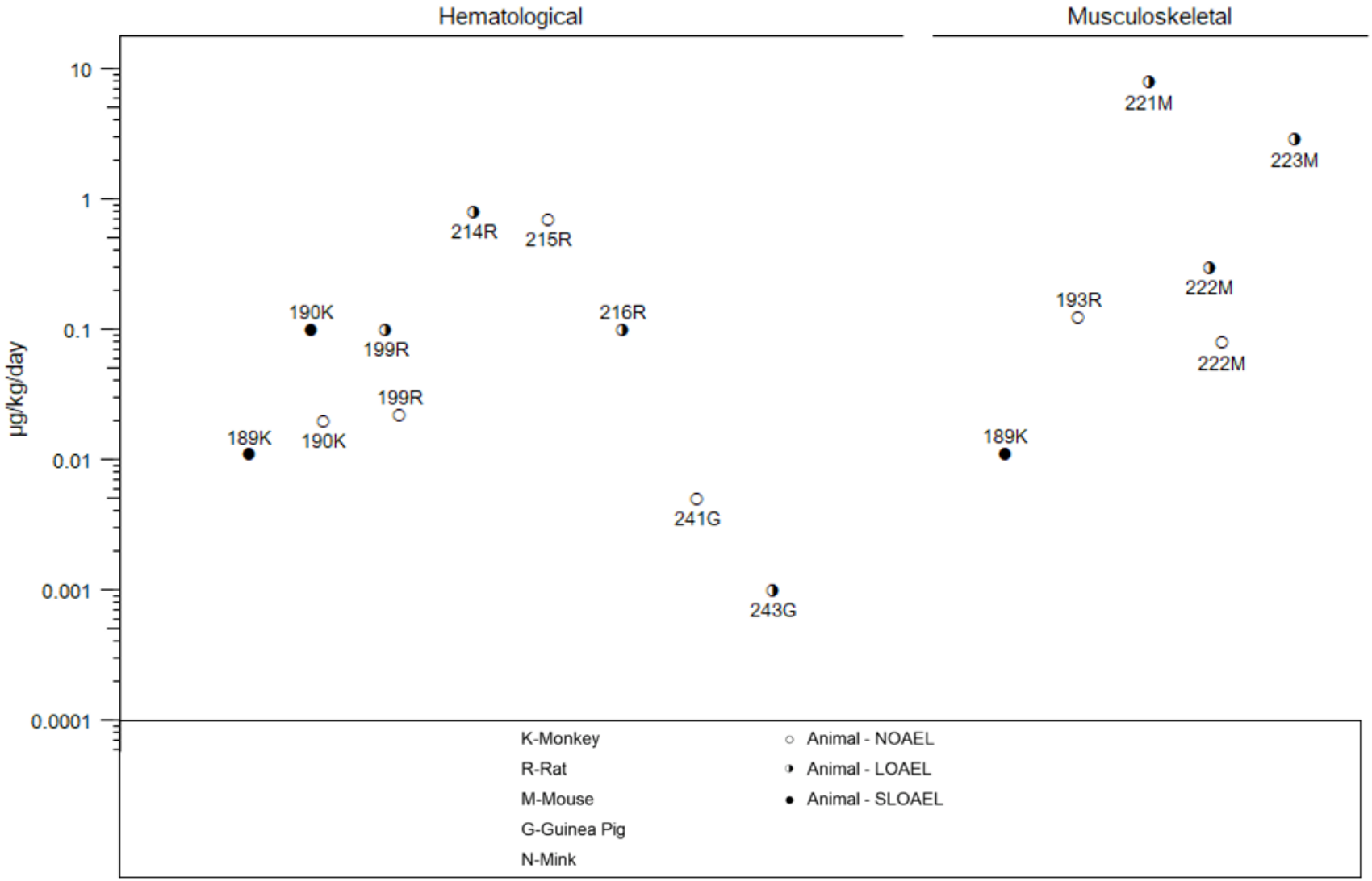


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Intermediate (15–364 days)

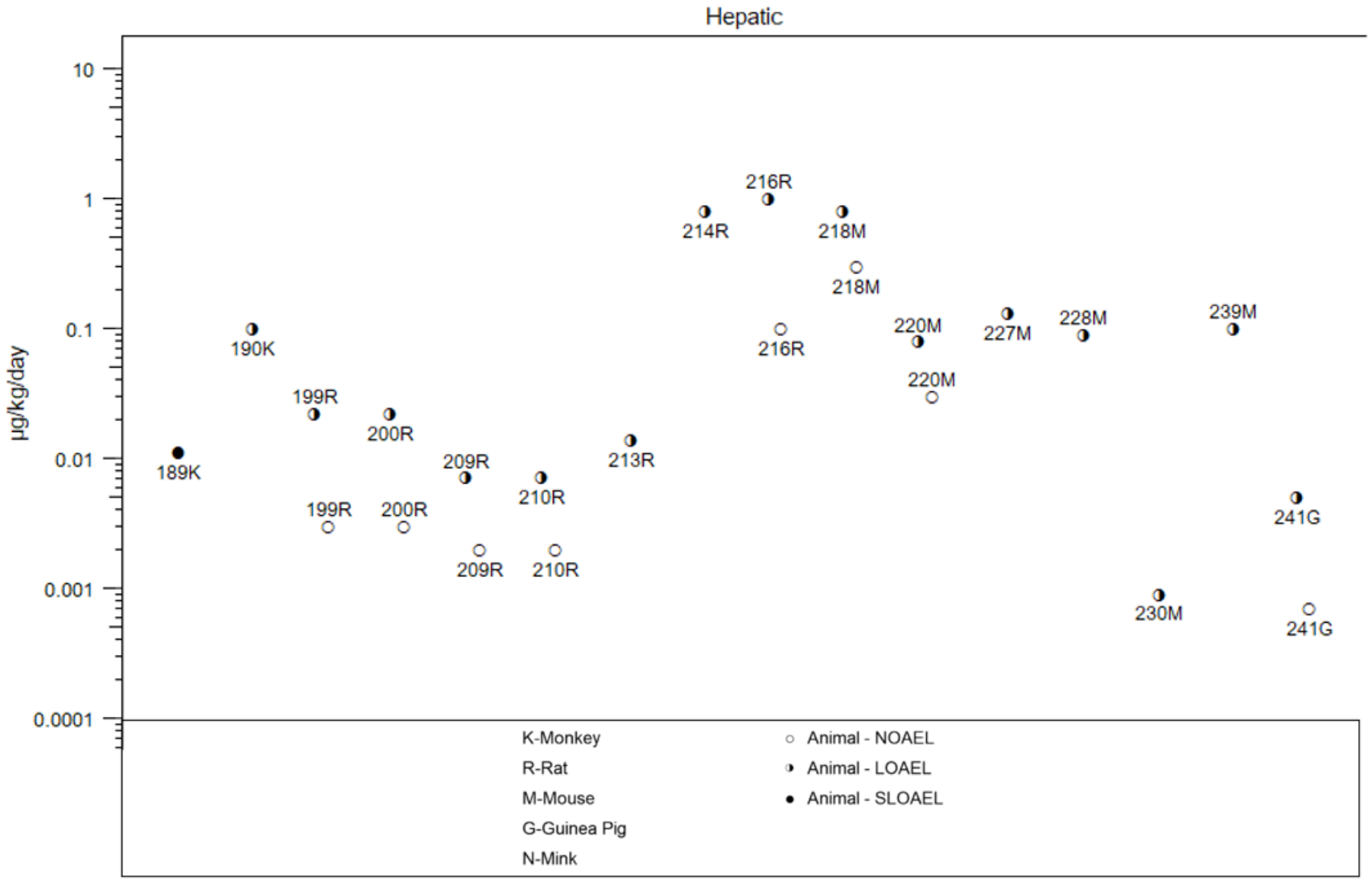


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Intermediate (15–364 days)

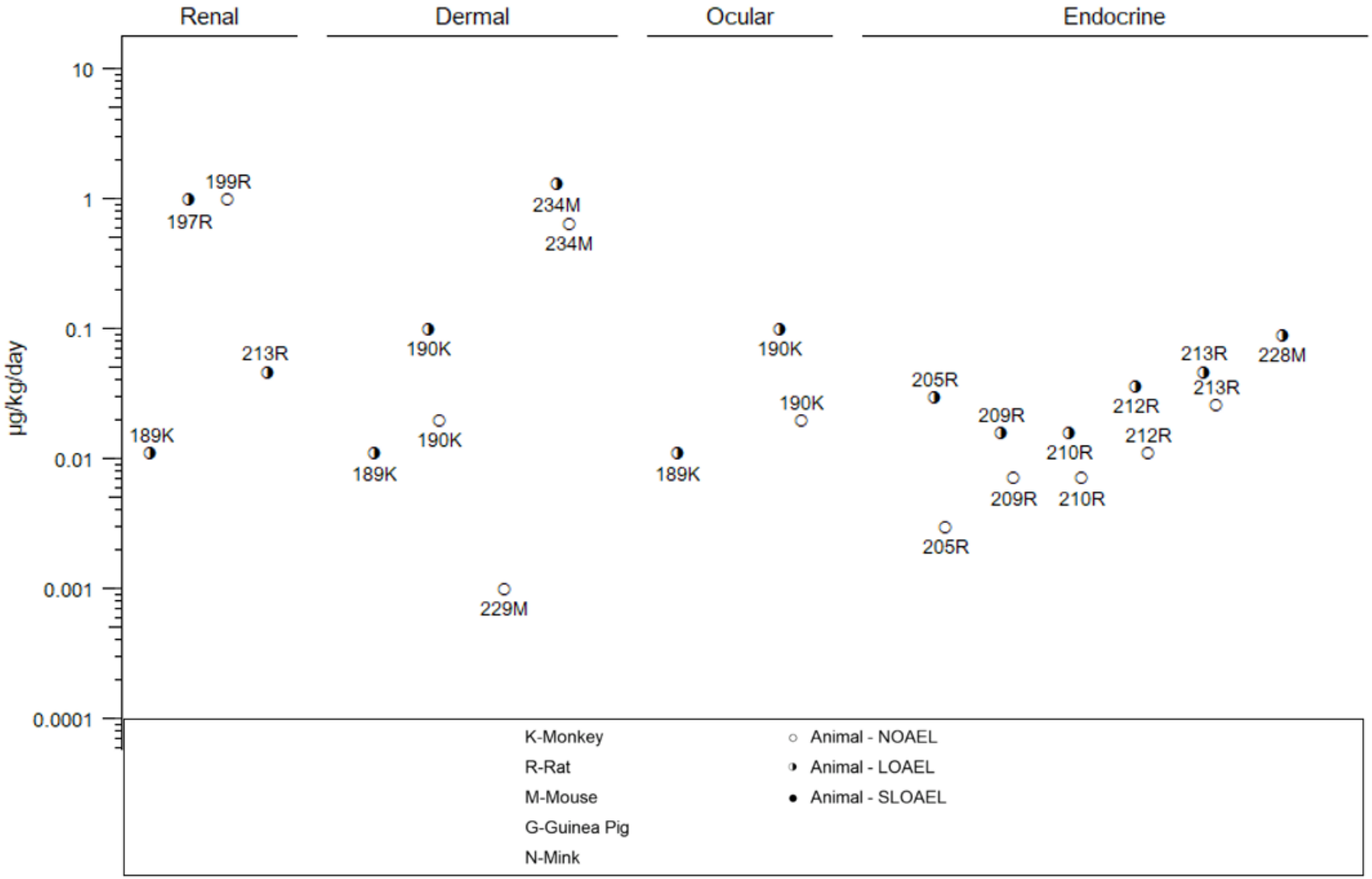


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Intermediate (15–364 days)

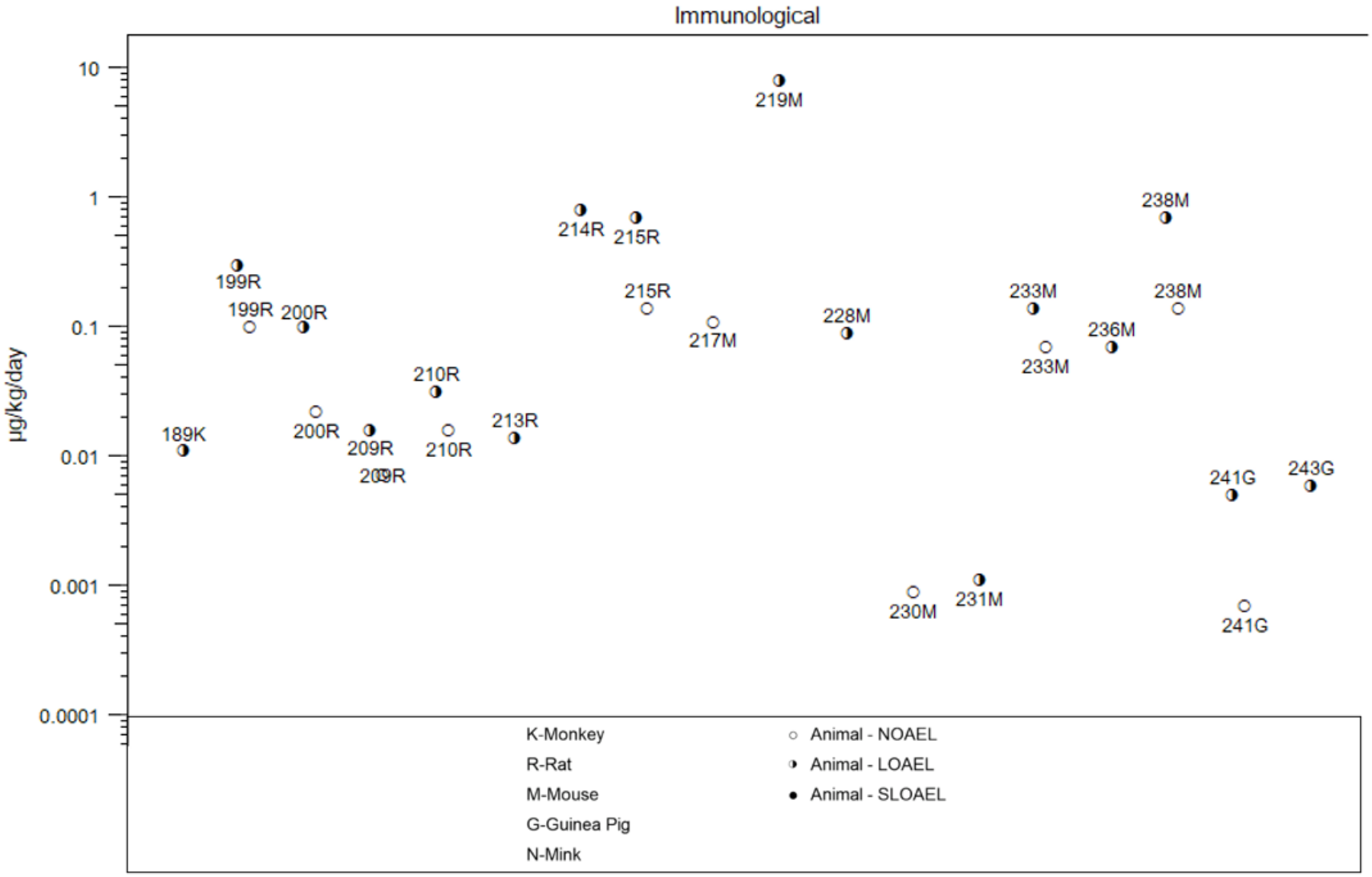


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Intermediate (15–364 days)

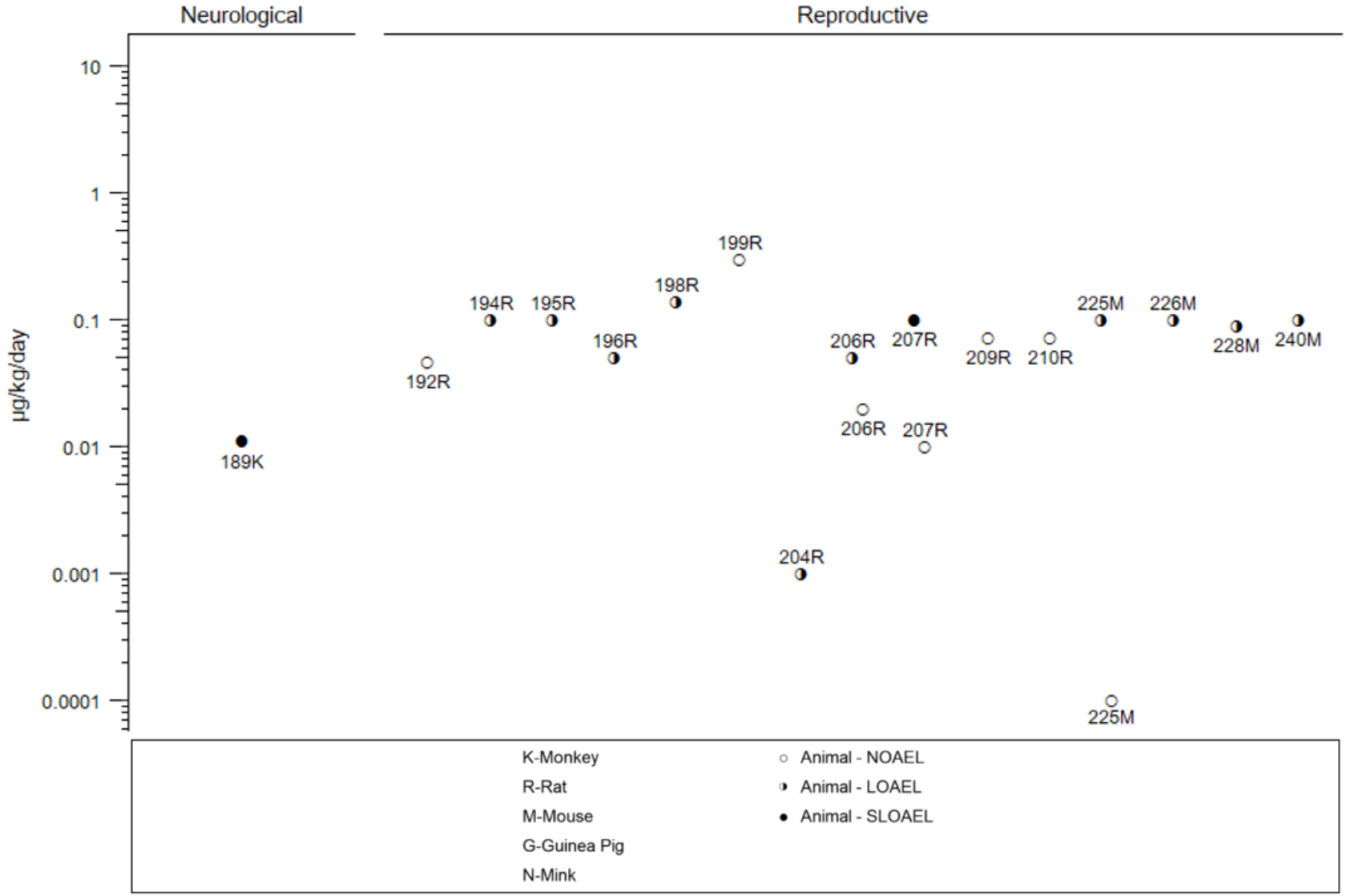


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Intermediate (15–364 days)

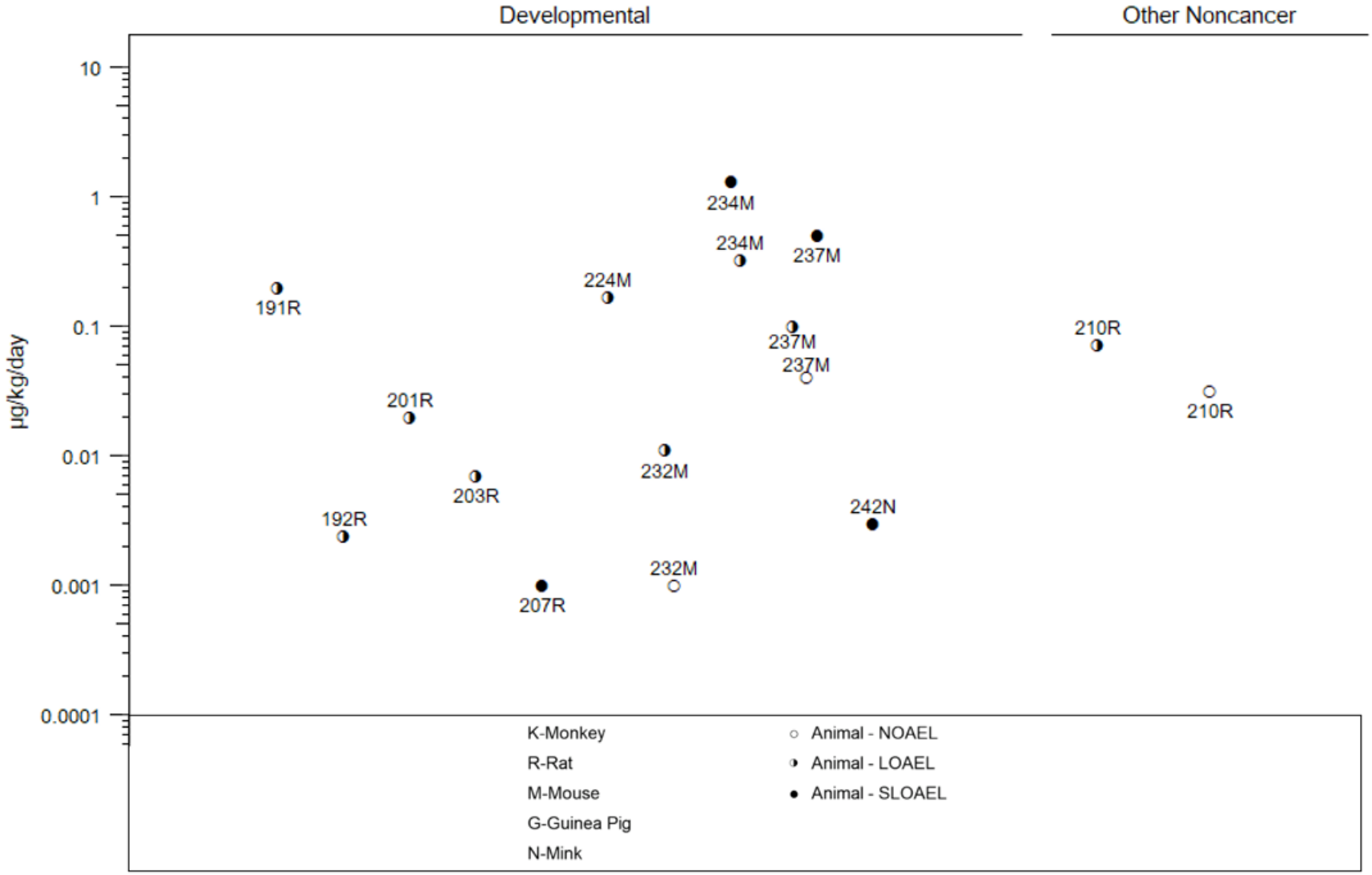


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Chronic (≥365 days)

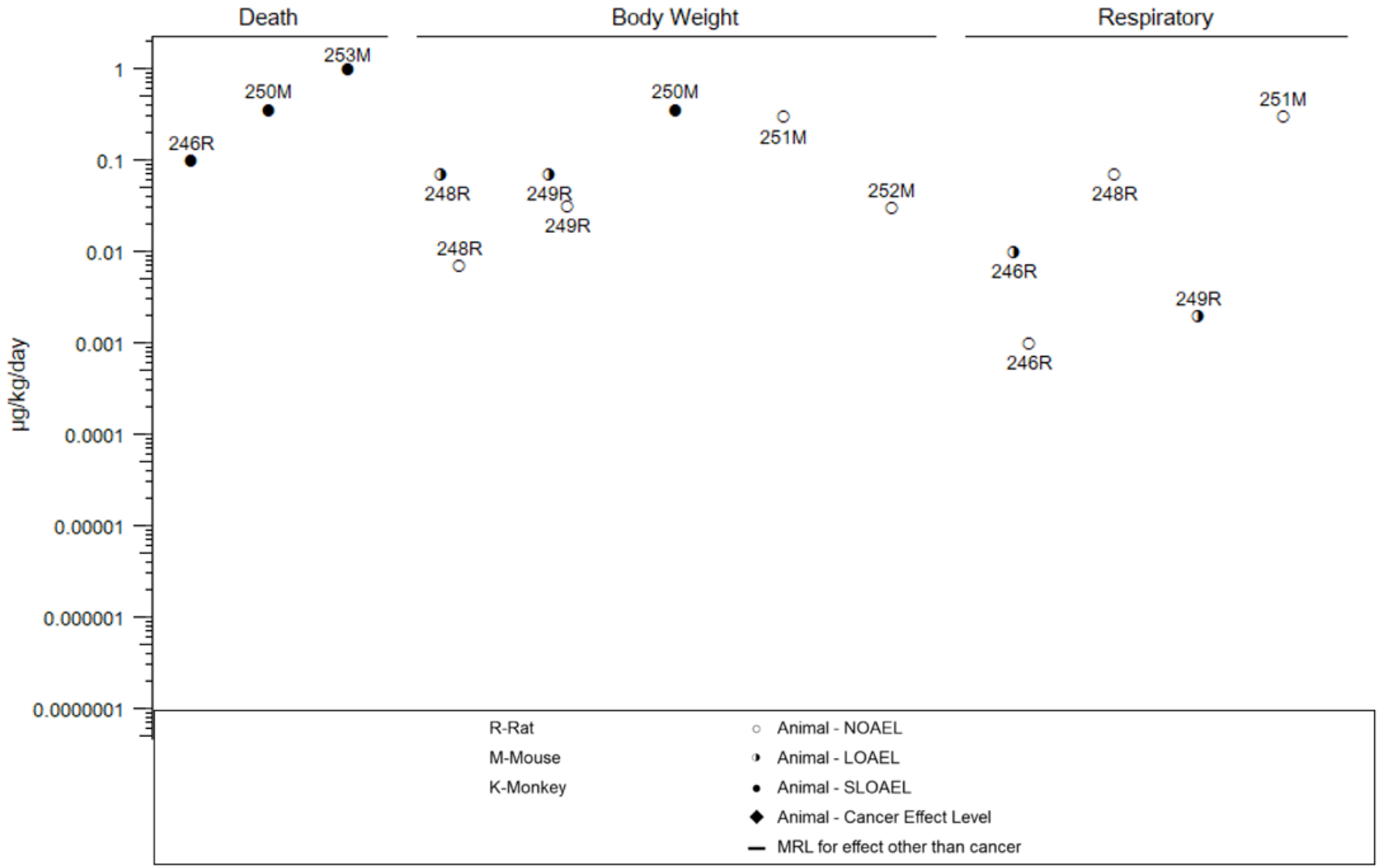


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Chronic (≥365 days)

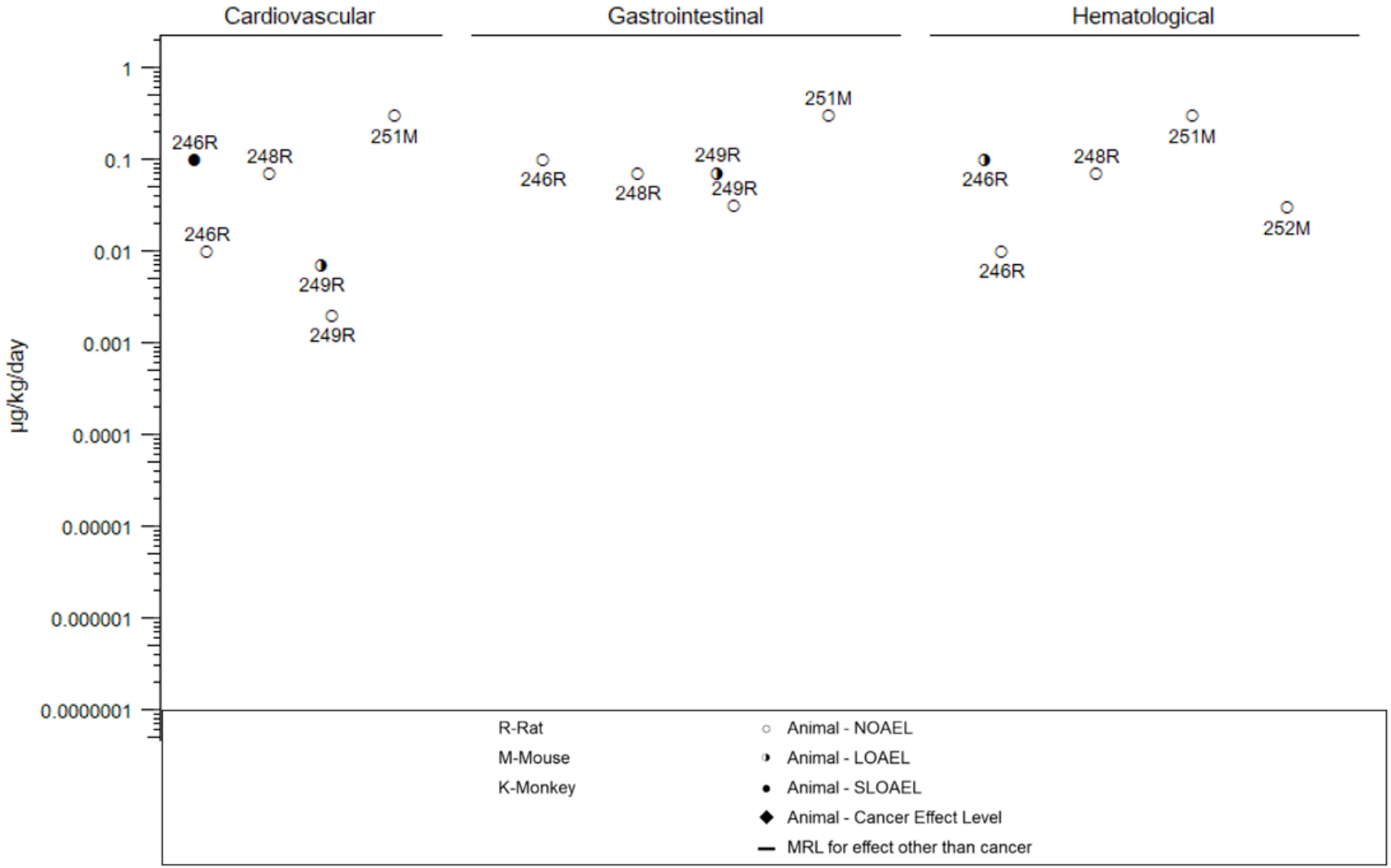


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Chronic (≥365 days)

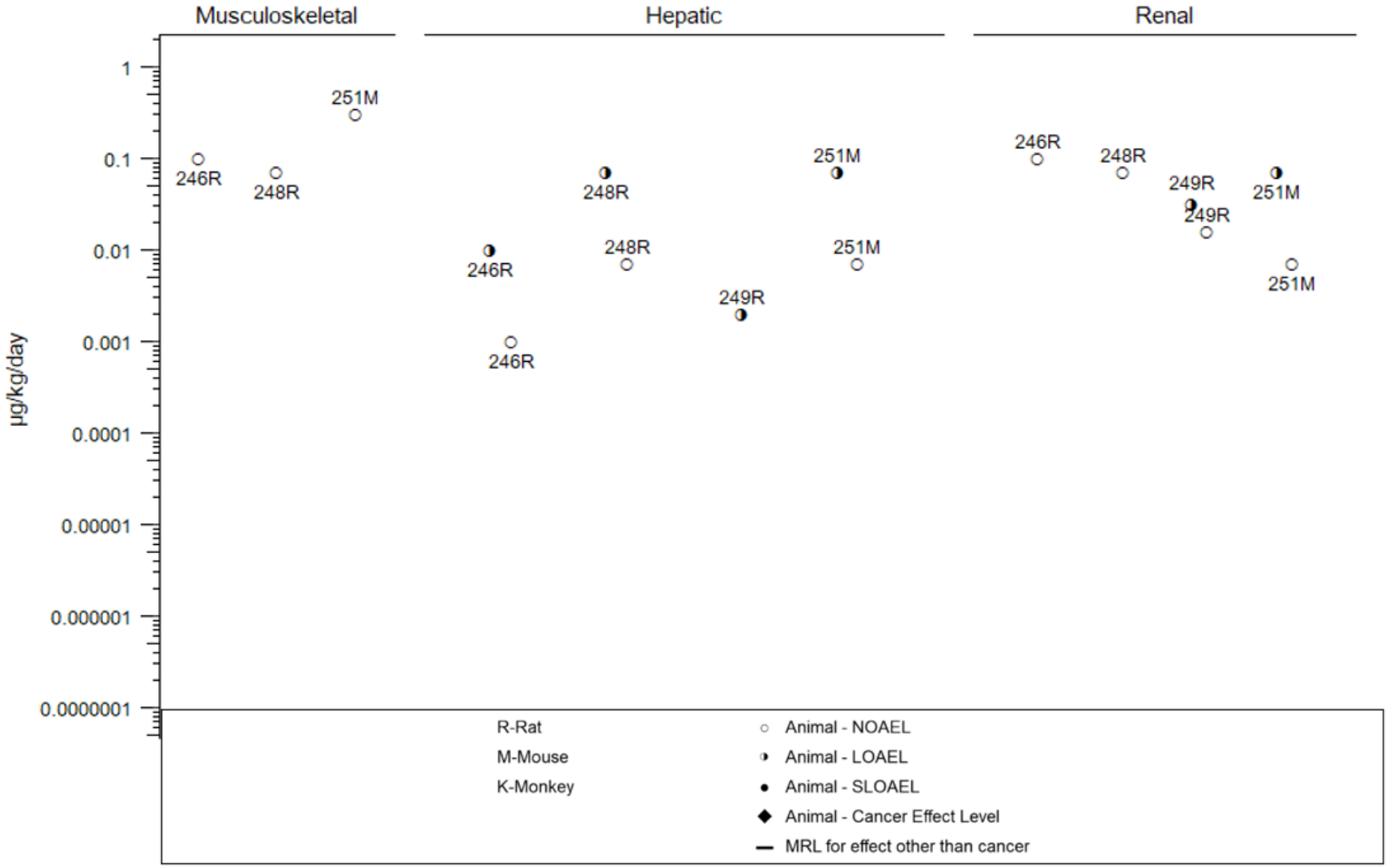


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Chronic (≥365 days)

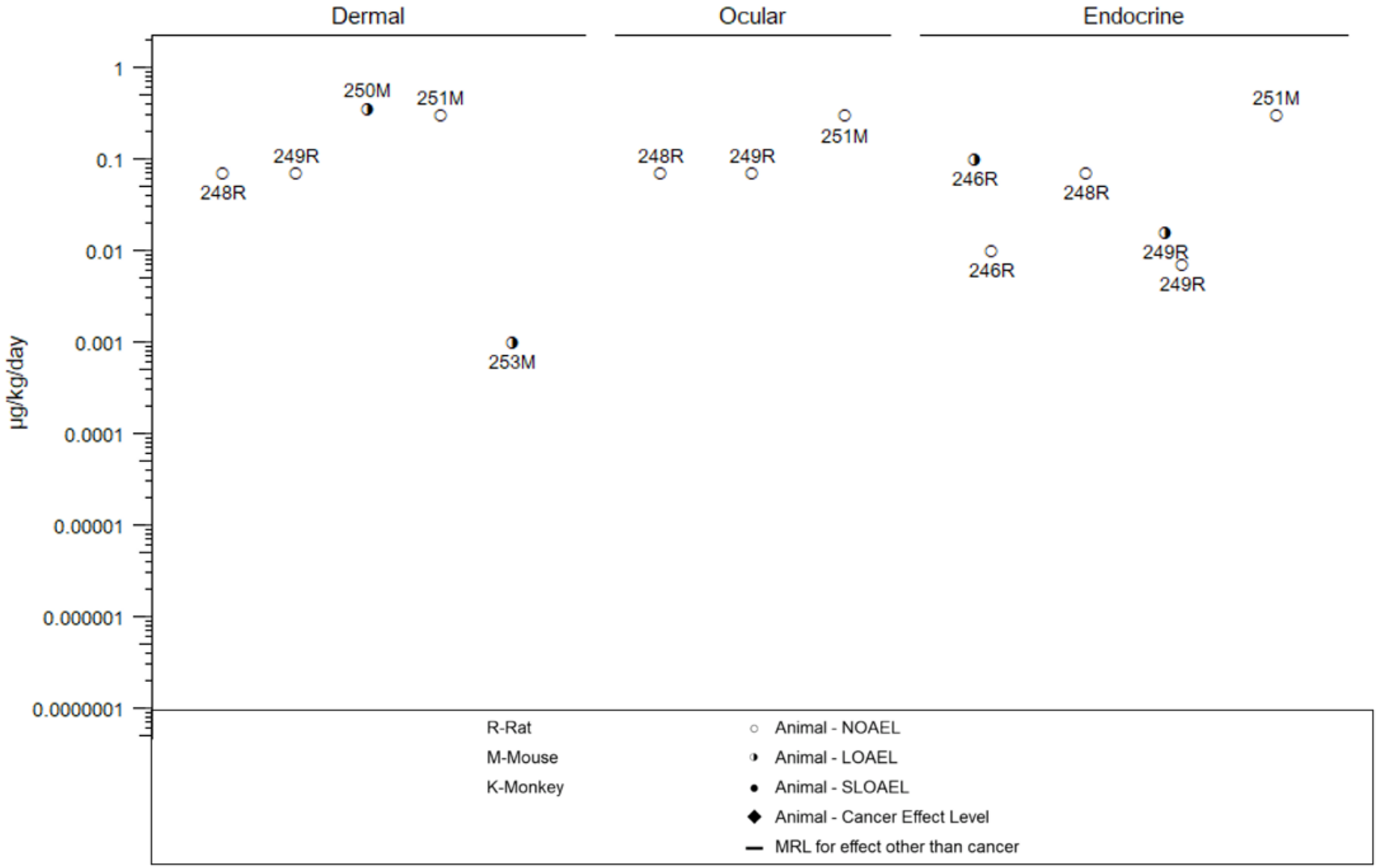


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Chronic (≥365 days)

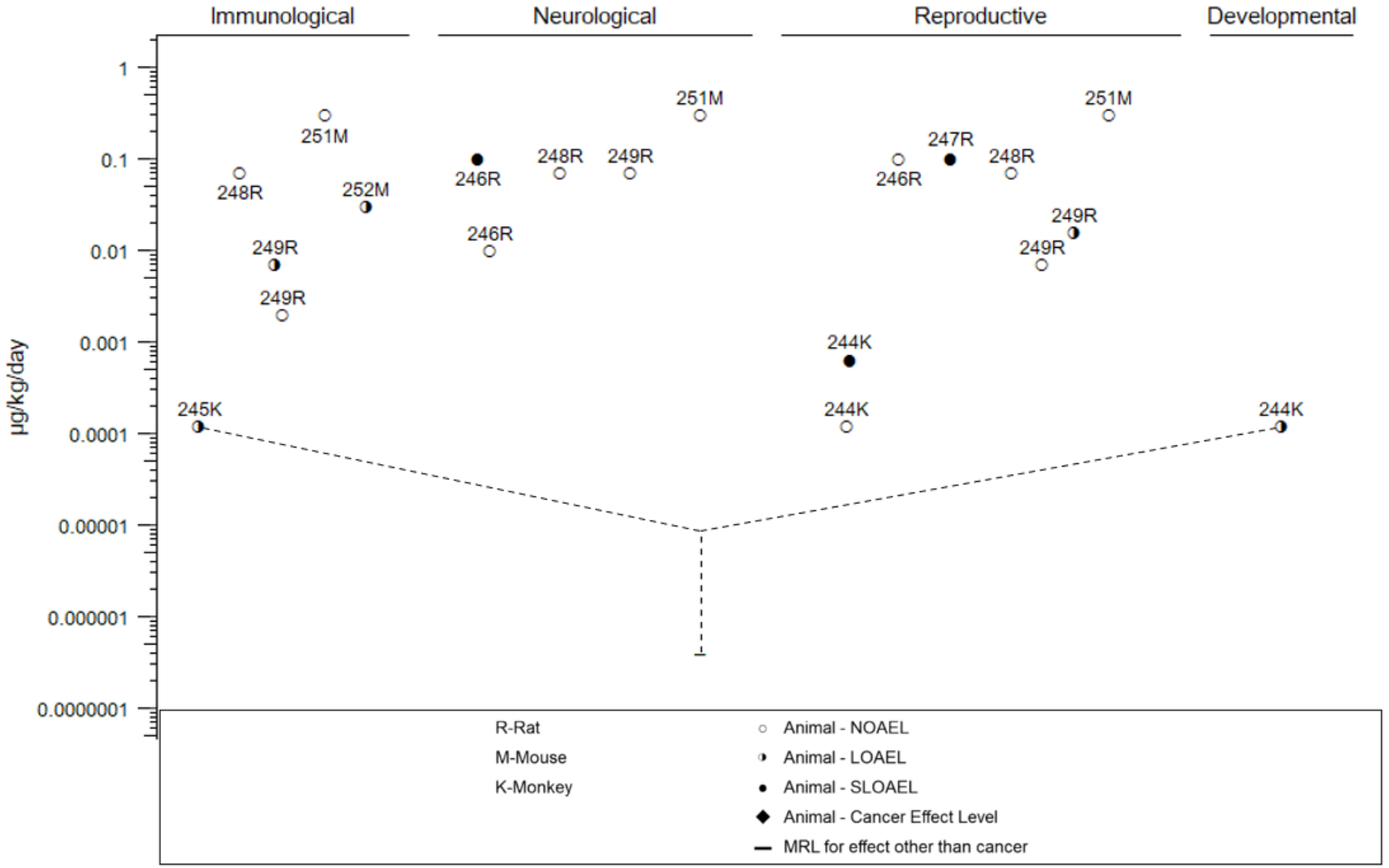


Figure 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Oral
Chronic (≥365 days)

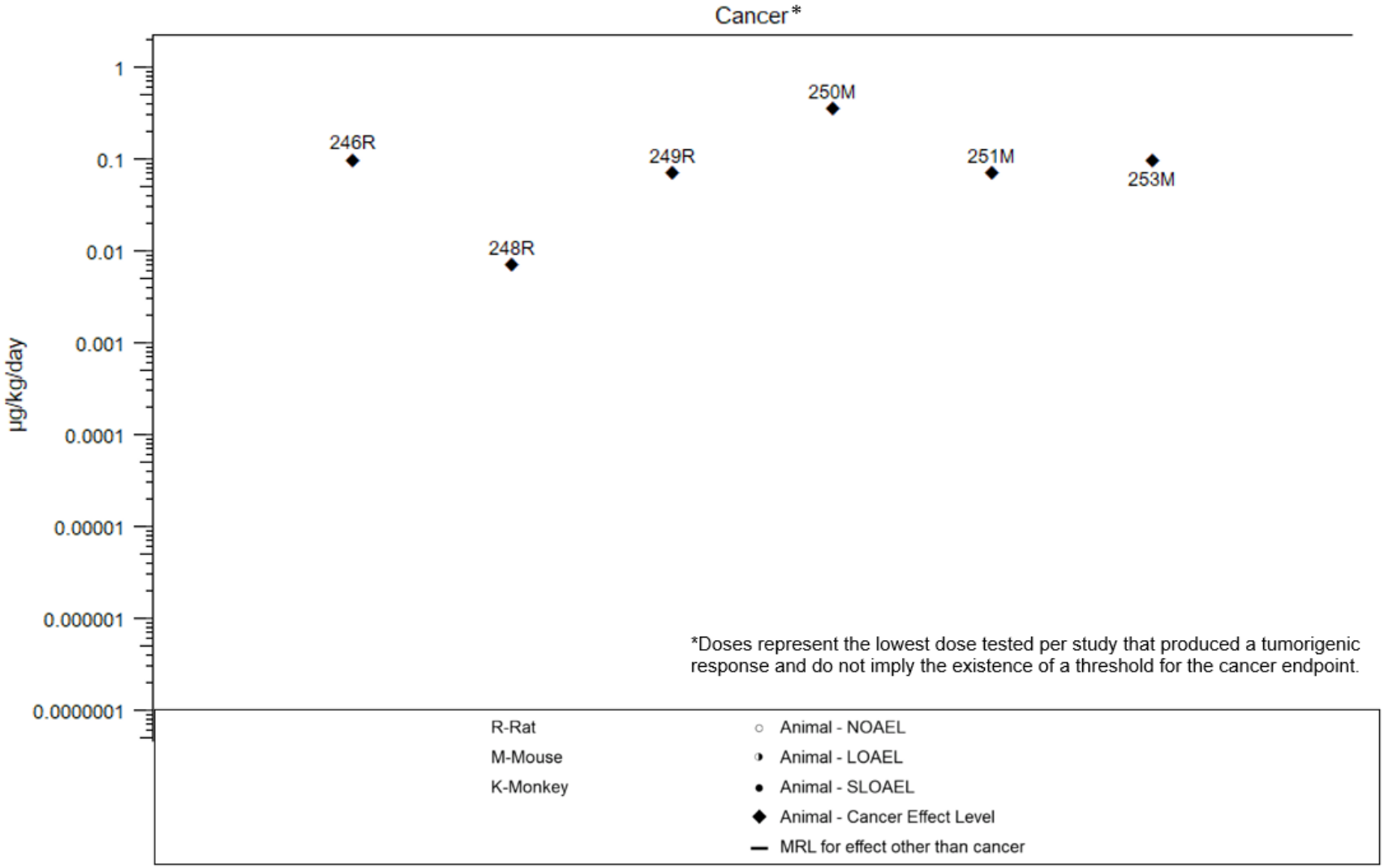


Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Couture et al. 1988									**OCDD**
1	Rat (Fischer-344) 5 M	2 weeks, 5 days/week (GO)	0, 36	BC, BI	Hemato	36			
					Hepatic	36			
Crofton et al. 2005									**1,2,3,7,8-PeCDD**
2	Rat (Long-Evans) 4–14 F	Once (GO)	0, 0.003–10	BW, OF	Bd wt	10			
					Endocr		1.51		30% decrease in serum T4
Khera and Ruddick 1973									**2,3-DCDD**
3	Rat (Wistar) 11–12 F	GDs 6–15 (GO)	0, 1,000, 2,000	DX	Develop	2,000			
Khera and Ruddick 1973									**1,2,3,4-TCDD**
4	Rat (Wistar) 10–15 F	GDs 6–15 (GO)	0, 50, 100, 200, 400, 800	DX	Develop	800			
Khera and Ruddick 1973									**2,7-DCDD**
5	Rat (Wistar) 13–15 F	GDs 6–15 (GO)	0, 250, 500, 1,000, 2,000	DX, RX	Develop	2,000			
Khera and Ruddick 1973									**2-MCDD**
6	Rat (Wistar) 11–12 F	GDs 6–15 (GO)	0, 1,000, 2,000	DX	Develop	2,000			
Madsen and Larsen 1989									**1,2,3,7,8-PeCDD**
7	Rat (Wistar) 8–10 F	GD 16 (G)	0, 0.5, 2, 10	DX	Develop		0.5		Decreased thymus weight

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NCI/NTP 1980									**1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD mixture**
8	Rat (Osborne-Mendel) 4 M, 4 F	Once (GO)	500, 10,000	CS, LE	Death			800 F	LD_{50}
								1,800 M	LD_{50}
Rozman et al. 2005									**1,2,3,4,6,7,8-HpCDD**
9	Rat (Sprague-Dawley) 30–36 F	Once (GO)	0, 1,000, 2,800, 3,100, 3,400, 3,800, 4,100		Cancer			3,400	CEL: lung cancer
Schwetz et al. 1973									**2,7-DCDD**
10	Rat (Sprague-Dawley) 7 F	GDs 6–15 (GO)	100,000	DX	Develop	100,000			
Schwetz et al. 1973									**HxCDD, unspecified mixture**
11	Rat (Sprague-Dawley) 10 F	GDs 6–15 (GO)	0.1, 1.0, 10, 100	DX	Bd wt	1		10	39% decreased maternal weight gain
					Develop	0.1	1		Subcutaneous edema
Schwetz et al. 1973									**OCDD**
12	Rat (Sprague-Dawley) 10 F	GDs 6–15 (GO)	100,000, 500,000	DX, RX	Develop	100,000	500,000		Subcutaneous edema
Simanainen et al. 2002									**1,2,3,7,8-PeCDD**
13	Rat (Long-Evans) 9–11 F	Once (GO)	0, 0.1–300	BW, OW, OF	Bd wt			14	Decreased body weight (ED_{50})
					Musc/skel		24		ED_{50} for incisor tooth defects
					Endocr		3.6		Decreased serum T4 (ED_{50})

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Immuno		7.2		Decreased relative thymus weight (ED_{50})
Simanainen et al. 2002									**1,2,3,4,7,8-HxCDD**
14	Rat (Long-Evans) 9–11 F	Once (GO)	0, 0.3-300	BW, OW, OF	Bd wt			140	Decreased body weight (ED_{50})
					Musc/skel		130		ED_{50} for incisor tooth defects
					Endocr		21		Decreased serum T4 (ED_{50})
					Immuno		37		Decreased relative thymus weight (ED_{50})
Simanainen et al. 2002									**1,2,3,4,6,7,8-HpCDD**
15	Rat (Long-Evans) 9–11 F	Once (GO)	0, 0.3–3,000	BW, OW, OF	Bd wt			980	Decreased body weight (ED_{50})
					Musc/skel		630		ED_{50} for incisor tooth defects
					Endocr		47		Decreased serum T4 (ED_{50})
					Immuno		150		Decreased relative thymus weight (ED_{50})
Simanainen et al. 2002									**1,2,3,7,8-PeCDD**
16	Rat Hans/Wistar 9–11 F	Once (GO)	0, 0.1–300	BW, OW, OF	Bd wt			32	Decreased body weight (ED_{50})
					Musc/skel		27		ED_{50} for incisor tooth defects
					Endocr		1.4		Decreased serum T4 (ED_{50})
					Immuno		10		Decreased relative thymus weight (ED_{50})
Simanainen et al. 2002									**1,2,3,4,7,8-HxCDD**
17	Rat Hans/Wistar 9–11 F	Once (GO)	0, 0.3–300	BW, OW, OF	Bd wt			390	Decreased body weight (ED_{50})
					Musc/skel		64		ED_{50} for incisor tooth defects
					Endocr		5.1		Decreased serum T4 (ED_{50})
					Immuno		14		Decreased relative thymus weight (ED_{50})

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Simanainen et al. 2002									**1,2,3,4,6,7,8-HpCDD**
18	Rat Hans/Wistar 9–11 F	Once (GO)	0, 0.3–3,000	BW, OW, OF	Bd wt			2,500	Decreased body weight (ED_{50})
					Musc/skel		760		ED_{50} for incisor tooth defects
					Endocr		99		Decreased serum T4 (ED_{50})
					Immuno		610		Decreased relative thymus weight (ED_{50})
Stahl et al. 1992									**1,2,3,7,8-PeCDD**
19	Rat (Sprague-Dawley) 5–10 M	Once (GO)	0, 100, 150, 200, 300	BW, LE	Death			206	LD_{50}
Stahl et al. 1992									**1,2,3,4,7,8-HxCDD**
20	Rat (Sprague-Dawley) 5–10 NS	1 day, 2 times/day (GO)	0, 700, 1,000, 1,400	BW, LE	Death			887	LD_{50}
Stahl et al. 1992									**1,2,3,4,6,7,8-HpCDD**
21	Rat (Sprague-Dawley) 5–10 NS	1 day, 4 times/day (GO)	0, 300, 5,000, 8,000	BW, LE	Death			6,325	LD_{50}
Ao et al. 2009									**1,2,3,7,8-PeCDD**
22	Mouse (C57BL/6J) 5 F	Once (GO)	0, 1.0, 3.0, 10, 50	OW, IX	Immuno		1		Suppressed IL-5 production in response to OVA exposure
Courtney 1976									**OCDD**
23	Mouse (CD-1) 6 F	GDs 7–16 (GO)	0, 5, 20	BW, OW, DX	Develop	20			

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Courtney 1976									**1,2,3,4-TCDD**
24	Mouse (CD-1) 4–15 F	GDs 7–16 (GO)	0, 100, 250, 500, 1,000	BW, OW, DX	Develop	1,000			
Holsapple et al. 1986									**OCDD**
25	Mouse (B6C3F1) 5–9 F	14 days, 1 time/day (GO)	0, 1, 10	BI	Immuno	10			
Holsapple et al. 1986									**2,7-DCDD**
26	Mouse (B6C3F1) 5–9 F	14 days (GO)	0, 0.1, 1, 10	BI	Hepatic	10			
					Immuno		0.1		Suppressed antibody response to sRBC
Kerkvliet and Brauner 1987									**1,2,3,4,6,7,8-HpCDD**
27	Mouse (C57B1/6) 3–12 B	Once (GO)	0, 20, 100, 500	IX	Immuno		20		Decreased splenic antibody response to sRBC
McConnell et al. 1978b									**1,2,3,7,8-PeCDD**
28	Mouse (C57BL/6) 6–9 M	Once (GO)	NS	BW, GN, HP, CS, LE	Death			337.5	LD_{50}
McConnell et al. 1978b									**1,2,3,4,7,8-HxCDD**
29	Mouse (C57BL/6) 6–9 M	Once (GO)	NS	BW, GN, HP, CS, LE	Death			825	LD_{50}
McConnell et al. 1978b									**1,2,3,6,7,8-HxCDD**
30	Mouse (C57BL/6) 6–9 M	Once (GO)	NS	BW, GN, HP, CS, LE	Death			1,250	LD_{50}

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NCI/NTP 1980									**1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD mixture**
31	Mouse (B6C3F1) 4 M, 4 F	Once (GO)	500–10,000	LE	Death			500 F	LD_{50}
								750 M	LD_{50}
White et al. 1986									**1,2,3,6,7,8-HxCDD**
32	Mouse (B6C3F1) 6–8 F	14 days (GO)	0, 0.1, 1.0, 10	BC, CS, BI	Immuno	0.1	1		Suppressed serum complement activity
McConnell et al. 1978b									**1,2,3,4,7,8-HxCDD**
33	Guinea pig (Hartley) 6–9 M	Once (GO)	NS	BW, GN, HP, CS, LE	Death			72.5	LD_{50}
McConnell et al. 1978b									**1,2,3,7,8-PeCDD**
34	Guinea pig (Hartley) 6–9 M	Once (GO)	NS	BW, GN, HP, CS, LE	Death			3.1	LD_{50}
McConnell et al. 1978b									**2,3,7-TrCDD**
35	Guinea pig (Hartley) 6–9 M	Once (GO)	NS	BW, GN, HP, CS, LE	Death			29,444	LD_{50}
McConnell et al. 1978b									**1,2,3,6,7,8-HxCDD**
36	Guinea pig (Hartley) 6–9 M	Once (GO)	NS	BW, GN, HP, CS, LE	Death			70	LD_{50}
McConnell et al. 1978b									**1,2,4,7,8-PeCDD**
37	Guinea pig (Hartley) 6–9 M	Once (GO)	NS	BW, GN, HP, CS, LE	Death			1,125	LD_{50}

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
INTERMEDIATE EXPOSURE									
Couture et al. 1988									**OCDD**
38	Rat (Fischer-344) 5 M	4–13 weeks, 5 days/week (GO)	0, 36	BC, BI	Hemato		36		Increased lymphocytes, decreased MCH, MCV, HGB
					Hepatic		36		Cytoplasmic vacuolization
NCI/NTP 1980									**1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD mixture**
39	Rat 10 M, 10 F	13 weeks, 1 day/week (GO)	0, 0.36, 0.71, 1.4, 7.1, 14	BW, CS, HP	Bd wt	0.36	0.71		13–18% decreased body weight gain
					Hemato	1.4	7.1		Splenic hyperplasia
					Hepatic	1.4	7.1 M		Moderate hepatotoxicity
Viluksela et al. 1994									**1,2,3,4,6,7,8-HpCDD**
40	Rat (Sprague-Dawley) 20 M	13 weeks, 10 doses (GO)	0, 0.3, 4, 24, 73, 110	BW, LE, HE, OW, BI, BC	Death			110	50% mortality; first death on day 31
					Bd wt	24	73	110	LOAEL: 13% decrease in body weight gain SLOAEL: 48% decrease in body weight gain
					Hemato	24	73		Decrease in platelet count
					Hepatic	0.3	4		Increased relative liver weight and EROD activity
					Endocr	4	24		Decrease in serum total T4
					Immuno	0.3	4		Decrease in absolute and relative thymus weight

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Viluksela et al. 1998a, 1998b									**1,2,3,7,8-PeCDD**
41	Rat (Sprague-Dawley) 20 M, 20 F	13 weeks, 10 doses (GO)	M: 0, 3.8; F: 0, 2.6	BW, LE, HE, BC, BI, CS	Death			2.6 F	15/20 died during treatment period; first death on day 16
					Bd wt		2.6 F		Body weight reduced by 18% relative to controls at the end of dosing period
								3.8 M	Body weight reduced by 27% relative to controls at the end of dosing period
					Hemato		2.6 F		Decreased hematocrit; reduced platelet count
					Dermal		2.6 F		Occasional hair loss; sores in ears, nose, neck, tail, and feet
					Endocr		3.8 M		69% decrease in serum T4
Viluksela et al. 1998a, 1998b									**1,2,3,4,7,8-HxCDD**
42	Rat (Sprague-Dawley) 20 M, 20 F	13 weeks, 10 doses (GO)	M: 0, 15.4; F: 0, 10.3	BW, LE, HE, BI, BC, CS	Death			10.3 F	5/20 died during treatment period; first death on day 61
					Bd wt	10.3 F			
								15.4 M	Body weight reduced by 24% relative to controls at the end of dosing period
					Hemato		10.3 F		Decreased hematocrit; reduced platelet count
					Dermal		10.3 F		Occasional hair loss; sores in ears, nose, neck, tail, and feet
					Endocr		15.4 M		69% decrease in serum T4

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NCI/NTP 1980									**1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD mixture**
43	Mouse (B6C3F1) 10 M, 10 F	13 weeks, 1 day/week (GO)	0, 0.18, 0.36, 0.71, 1.4, 7.1	BW, HP, CS	Bd wt		0.18		13–17% decreased weight gain
					Hepatic	0.71	1.4		Mild hepatotoxicity
CHRONIC EXPOSURE									
NCI/NTP 1979									**2,7-DCDD**
44	Rat (Osborne-Mendel) 35 M, 35 F	110 weeks, 7 days/week (F)	0, 250,000, 500,000	BW, GN, HP, CS	Bd wt		250,000		17% decreased body weight gain
					Resp	500,000			
					Cardio	500,000			
					Gastro	500,000			
					Hemato	500,000			
					Musc/skel	500,000			
					Hepatic		250,000		Fatty changes
					Renal	500,000			
					Dermal	500,000			
NCI/NTP 1980									**1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD mixture**
45	Rat (Osborne-Mendel) 50–75 M	104 weeks, 2 days/week (GO)	0, 0.18, 0.34, 0.7	BW, OW, GN, HP, CS	Bd wt			0.18	38% decreased weight gain
					Resp		0.18		Adenomatous hyperplasia of the lungs
					Cardio	0.7			
					Gastro	0.7			
					Hemato	0.7			
					Musc/skel	0.7			
					Hepatic		0.18		Toxic hepatitis (lipidosis, mild fibrosis, bile duct hyperplasia)
					Renal	0.7			

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Dermal	0.7			
					Cancer			0.34	CEL: hepatocellular carcinoma or liver neoplastic nodules
NCI/NTP 1979									**2,7-DCDD**
46	Mouse (B6C3F1) 50 M, 50 F	90 weeks, 7 days/week (F)	0, 650,000, 1,300,000	BW, GN, HP, CS	Bd wt		650,000		16% decreased body weight gain
					Resp	1,300,000			
					Cardio	1,300,000			
					Gastro	1,300,000			
					Hemato	1,300,000			
					Musc/skel	1,300,000			
					Hepatic	650,000 F	1,300,000 F		Focal necrosis
					Renal	1,300,000			
					Dermal	1,300,000			
					Cancer			650,000 M	CEL: hepatocellular carcinoma or adenoma, lymphoma, leukemia, hemangiosarcomas
NCI/NTP 1980									**1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD mixture**
47	Mouse (B6C3F1) 50–75 M	104 weeks, 2 days/week (GO)	M: 0, 0.18, 0.34, 0.7; F: 0, 0.34, 0.7, 1.4	BW, OW, GN, HP, CS	Bd wt	1.4			
					Resp	1.4			
					Cardio	1.4			
					Gastro	1.4			
					Hemato	1.4			
					Musc/skel	1.4			

Table 2-3. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral (µg/kg/day)[a]

Figure key[b]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic		0.7		Toxic hepatitis (degenerative hepatocellular changes and/or necrosis associated with mild fibrosis)
					Renal	1.4			
					Dermal	1.4			
					Cancer			0.7	CEL: hepatocellular carcinomas and adenomas

[a]Doses adjusted for intermittent exposure.
[b]The number corresponds to entries in Figure 2-5; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-5. Where such differences exist, only the levels of effect for the most sensitive sex are presented.

B = both males and females; BC = serum (blood) chemistry; Bd wt or BW = body weight; BI = biochemical changes; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; DCDD = dichlorodibenzo-*p*-dioxin; Develop = developmental; DX = developmental toxicity; ED_{50} = median effective dose; Endocr = endocrine; EROD = 7-ethoxy-resorufin-O-deethylase; (F) = feed; F = female(s); (G) = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; (GO) = gavage in oil; HE = hematology; Hemato = hematological; HGB = hemoglobin; HP = histopathology; HpCDD = heptachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; IL = interleukin; Immuno = immunological; IX = immune function; LD_{50} = median lethal dose; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCDD = monochlorodibenzo-*p*-dioxin; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OCDD = octachlorodibenzo-*p*-dioxin; OF = organ function; OVA = ovalbumin; OW = organ weight; PeCDD = pentachlorodibenzo-*p*-dioxin; Repro = reproductive; Resp = respiratory; RX = reproductive function; sRBC = sheep red blood cell; SLOAEL = serious lowest-observed-adverse-effect level; T4 = thyroxine; TCDD = tetrachlorodibenzo-*p*-dioxin;

Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Acute (≤14 days)

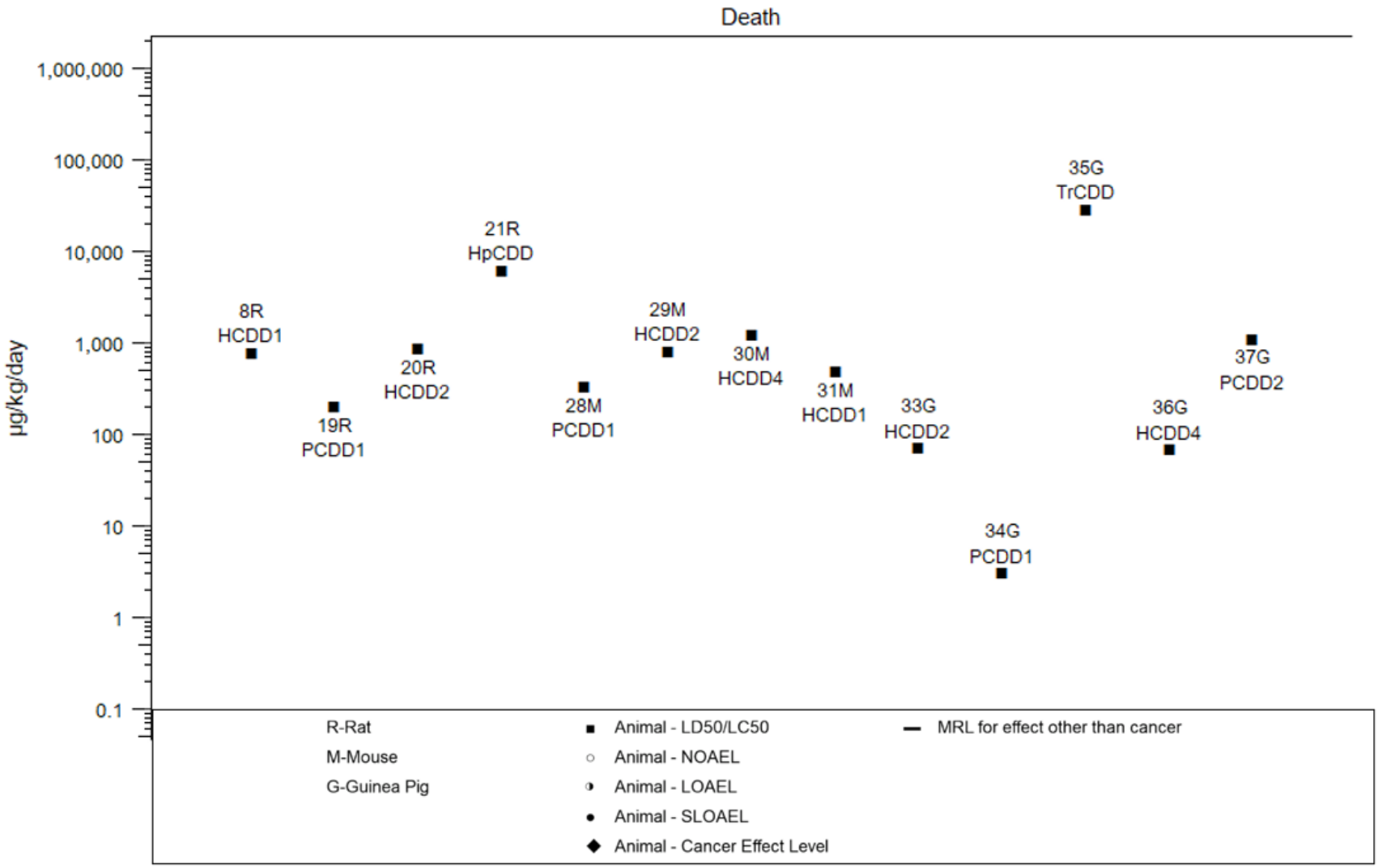


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Acute (≤14 days)

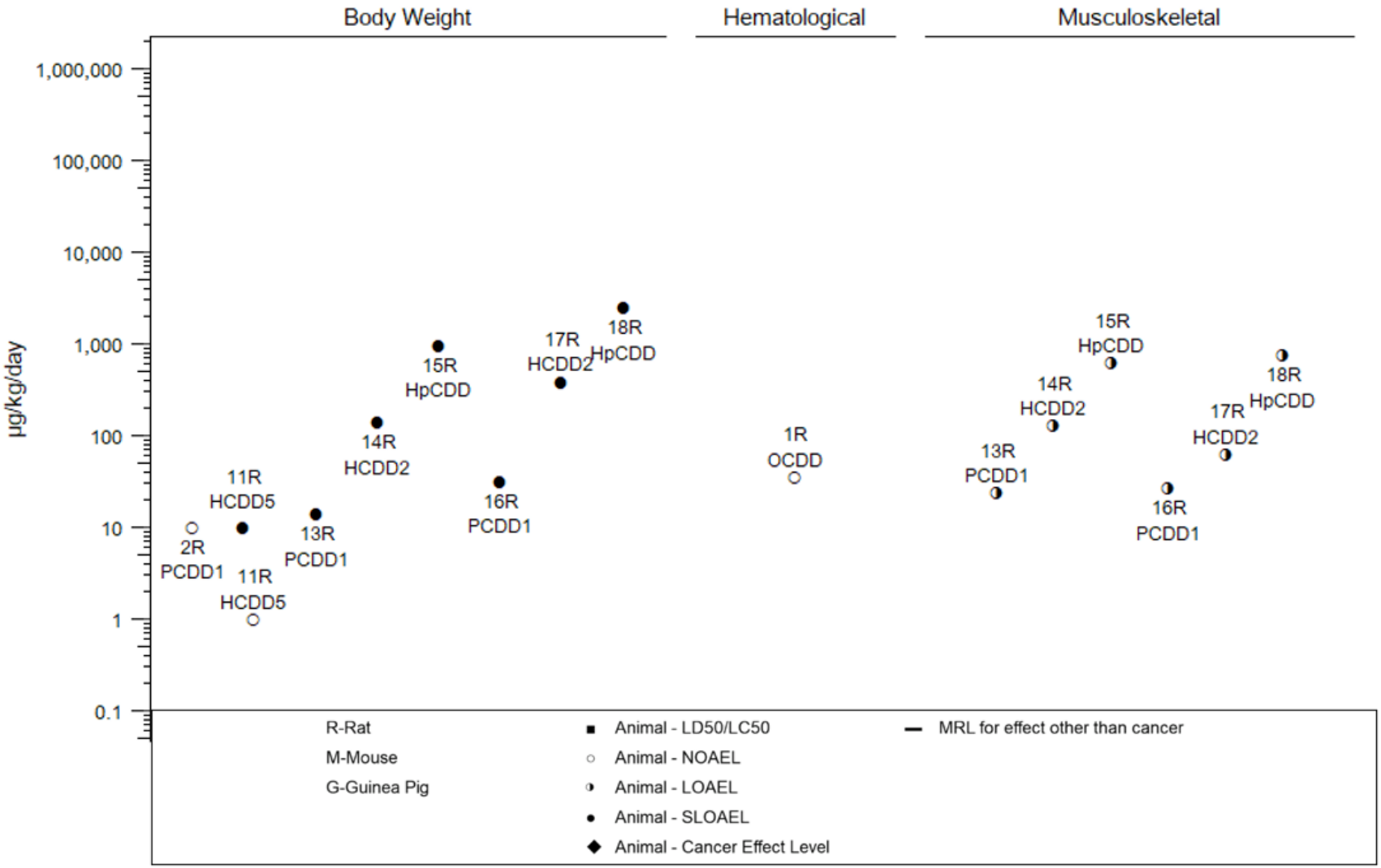


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Acute (≤14 days)

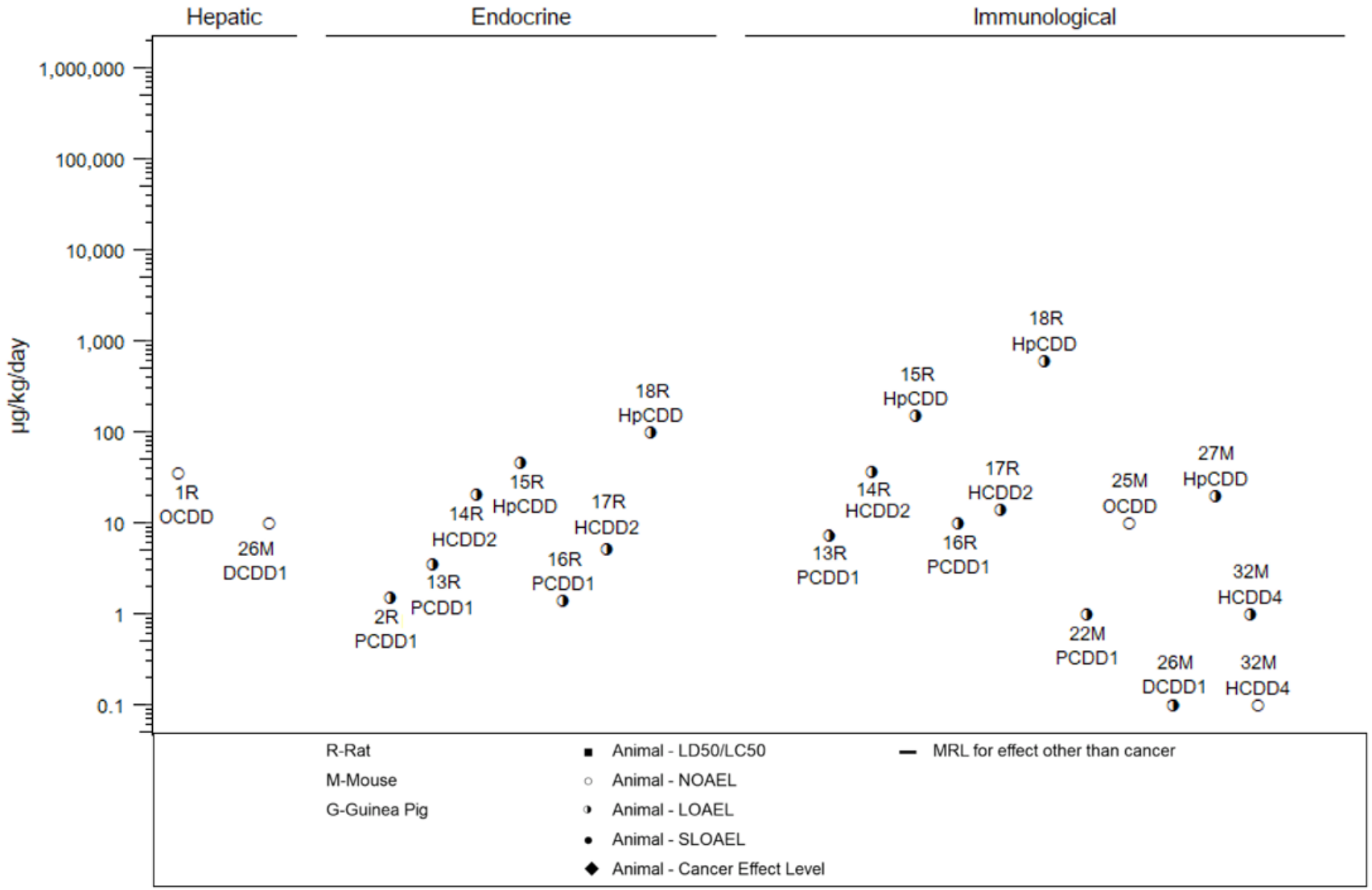


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Acute (≤14 days)

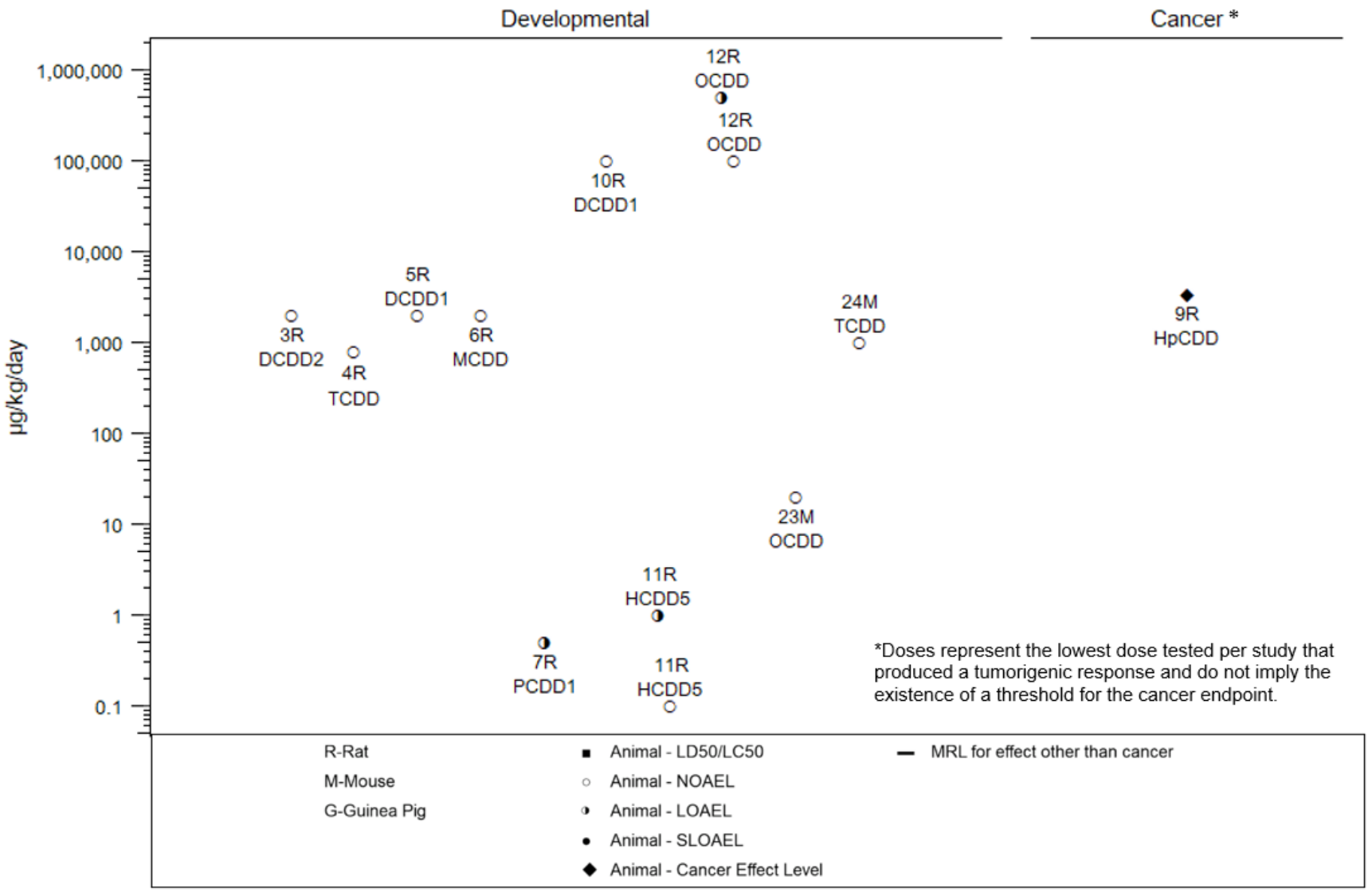


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Intermediate (15–364 days)

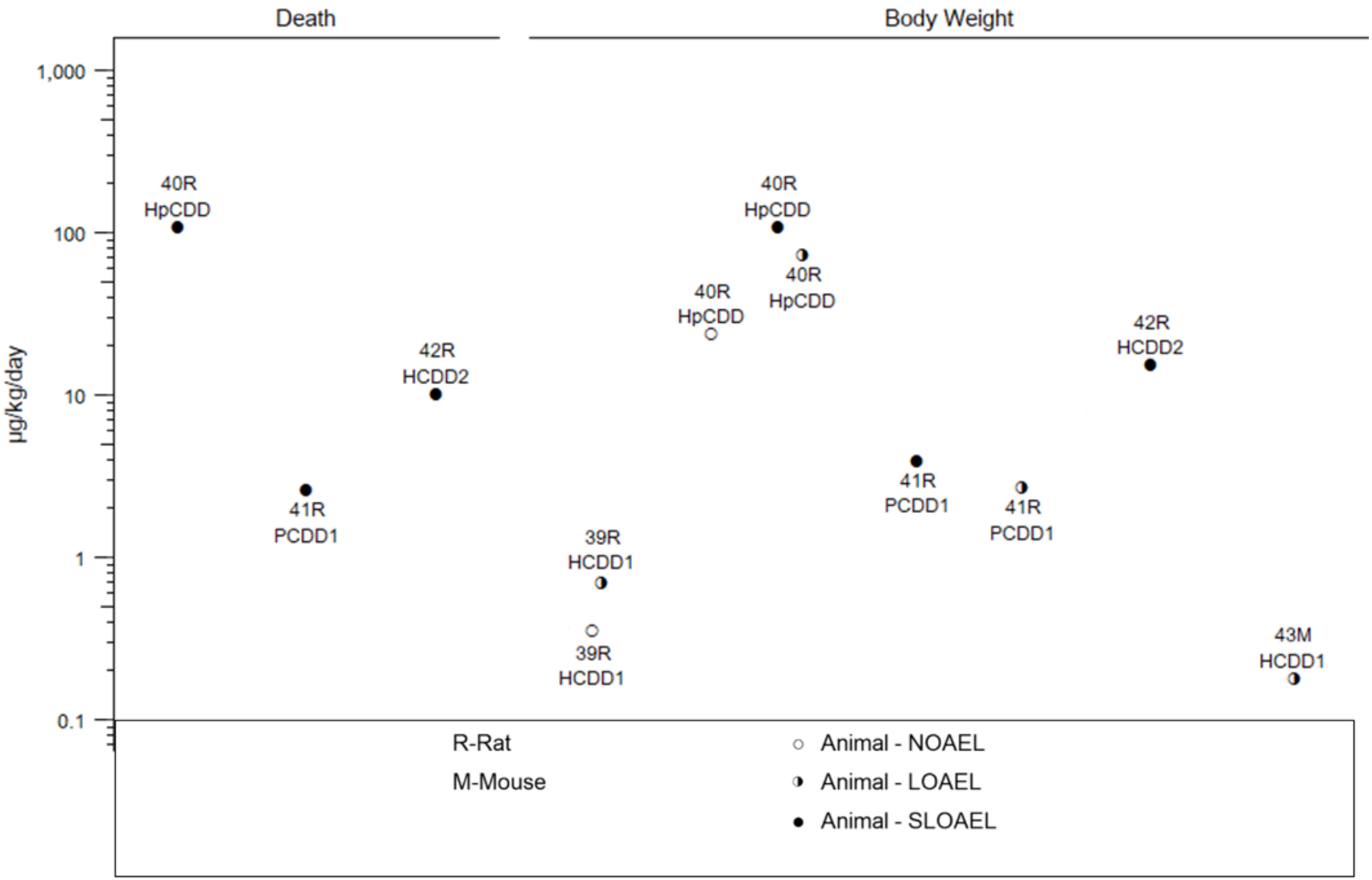


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Intermediate (15–364 days)

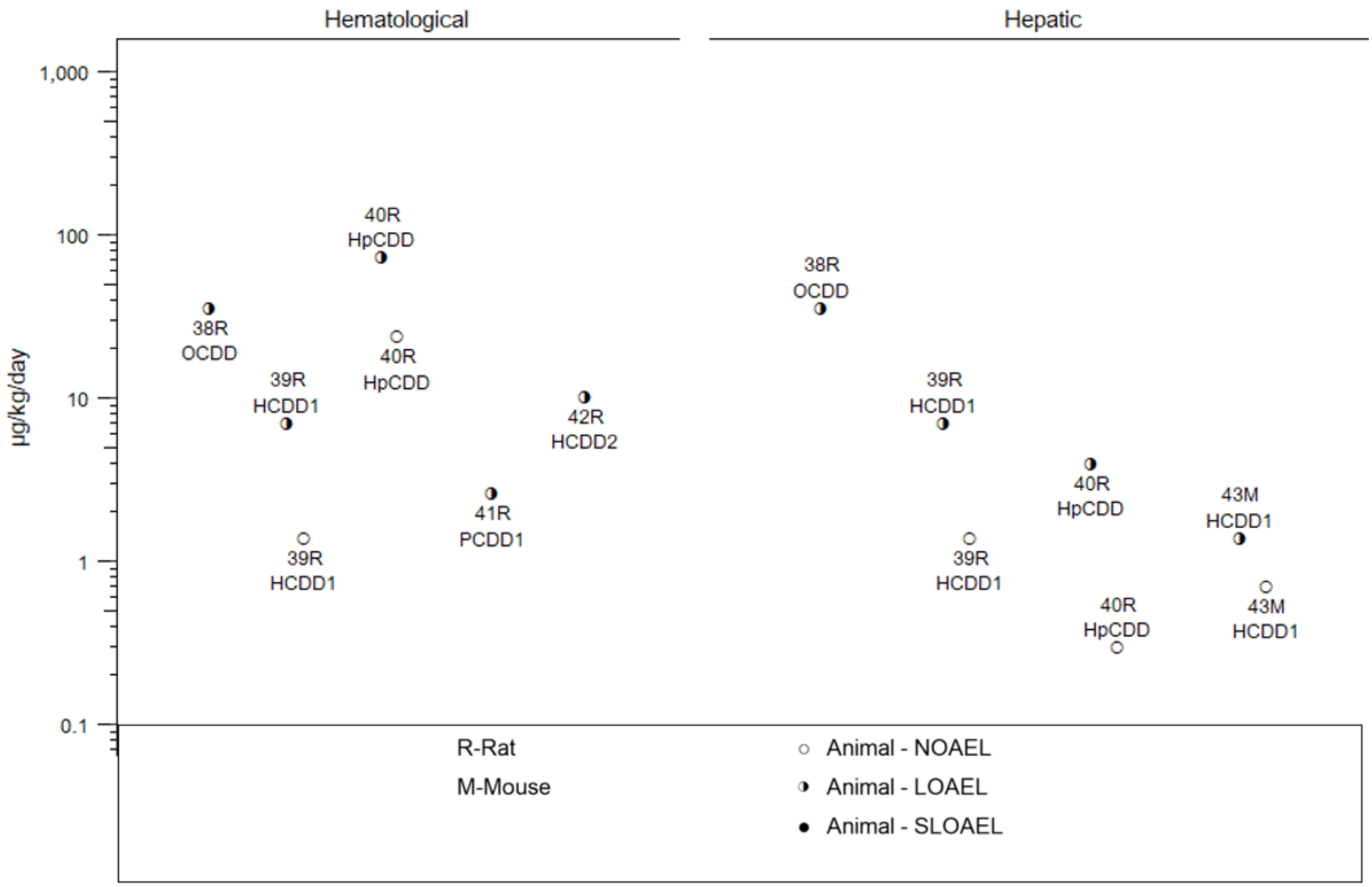


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Intermediate (15–364 days)

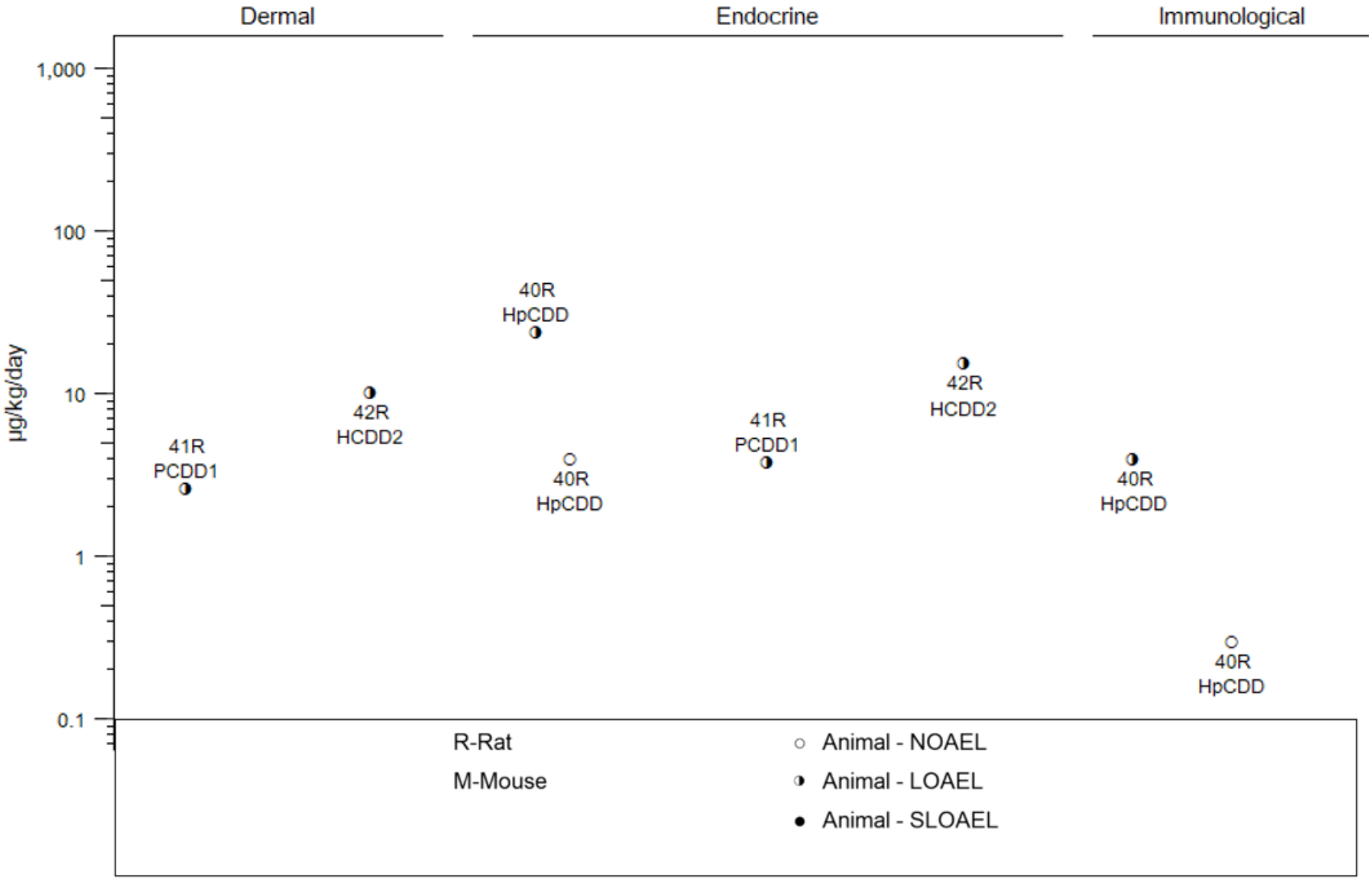


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Chronic (≥365 days)

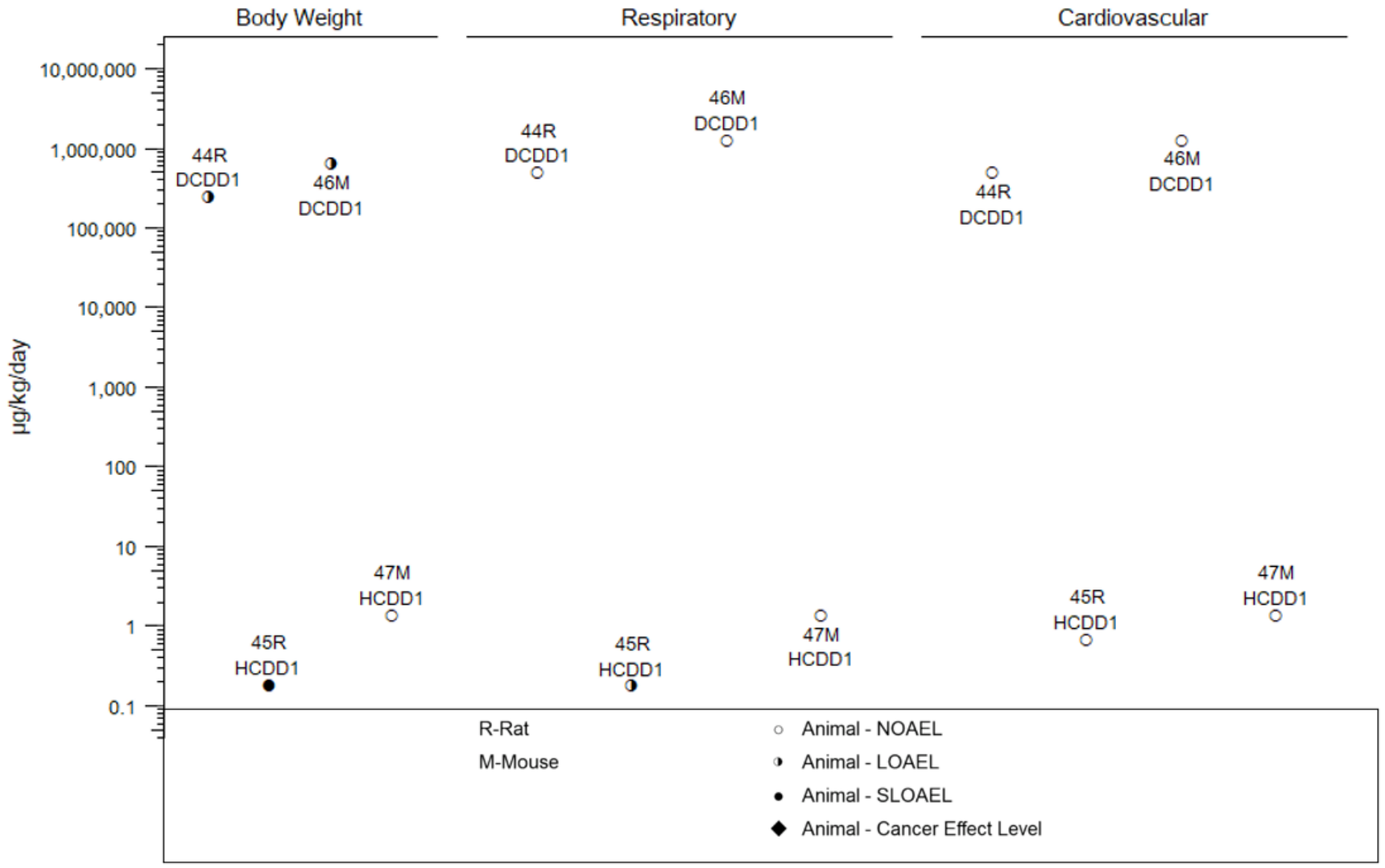


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Chronic (≥365 days)

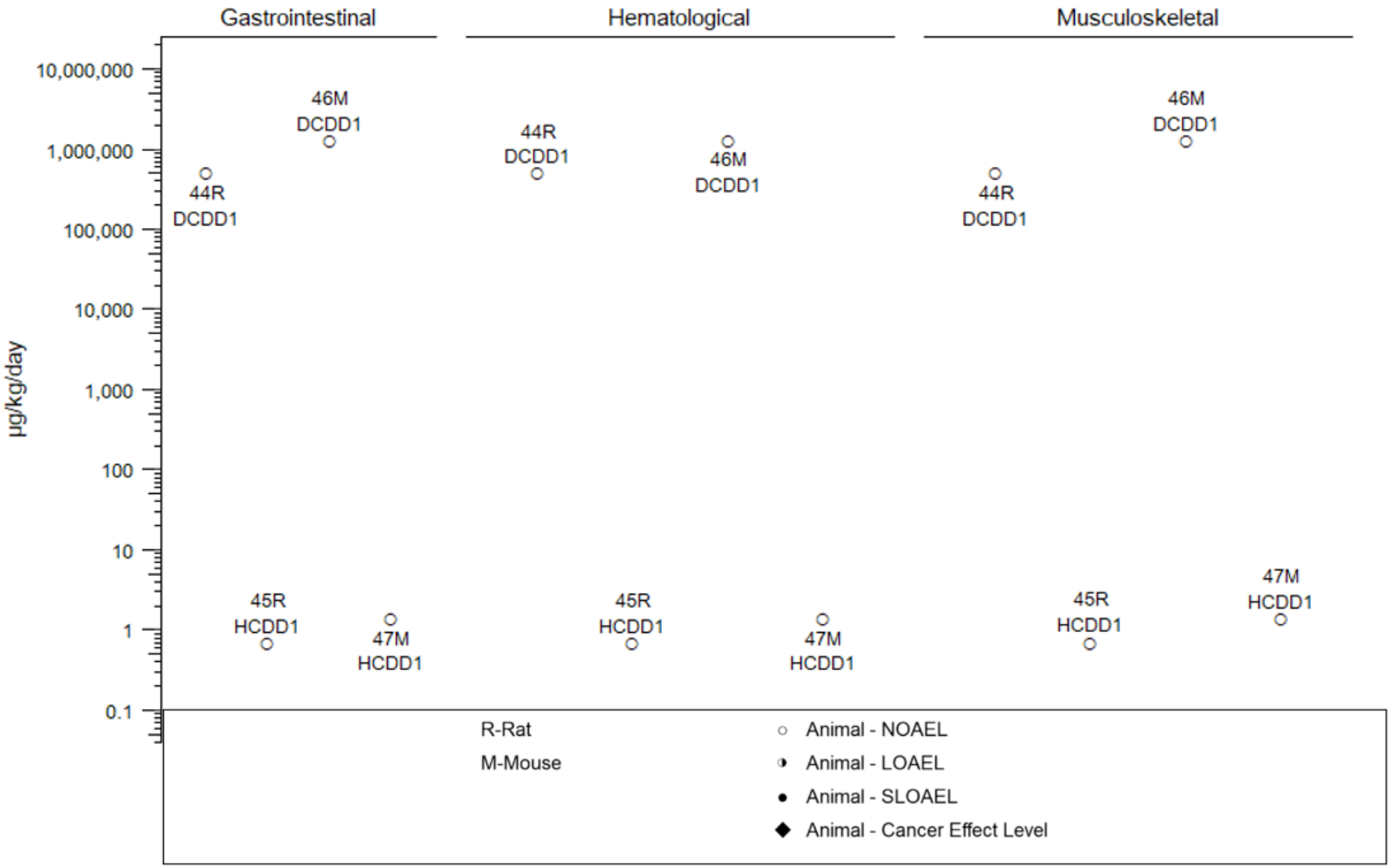


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Chronic (≥365 days)

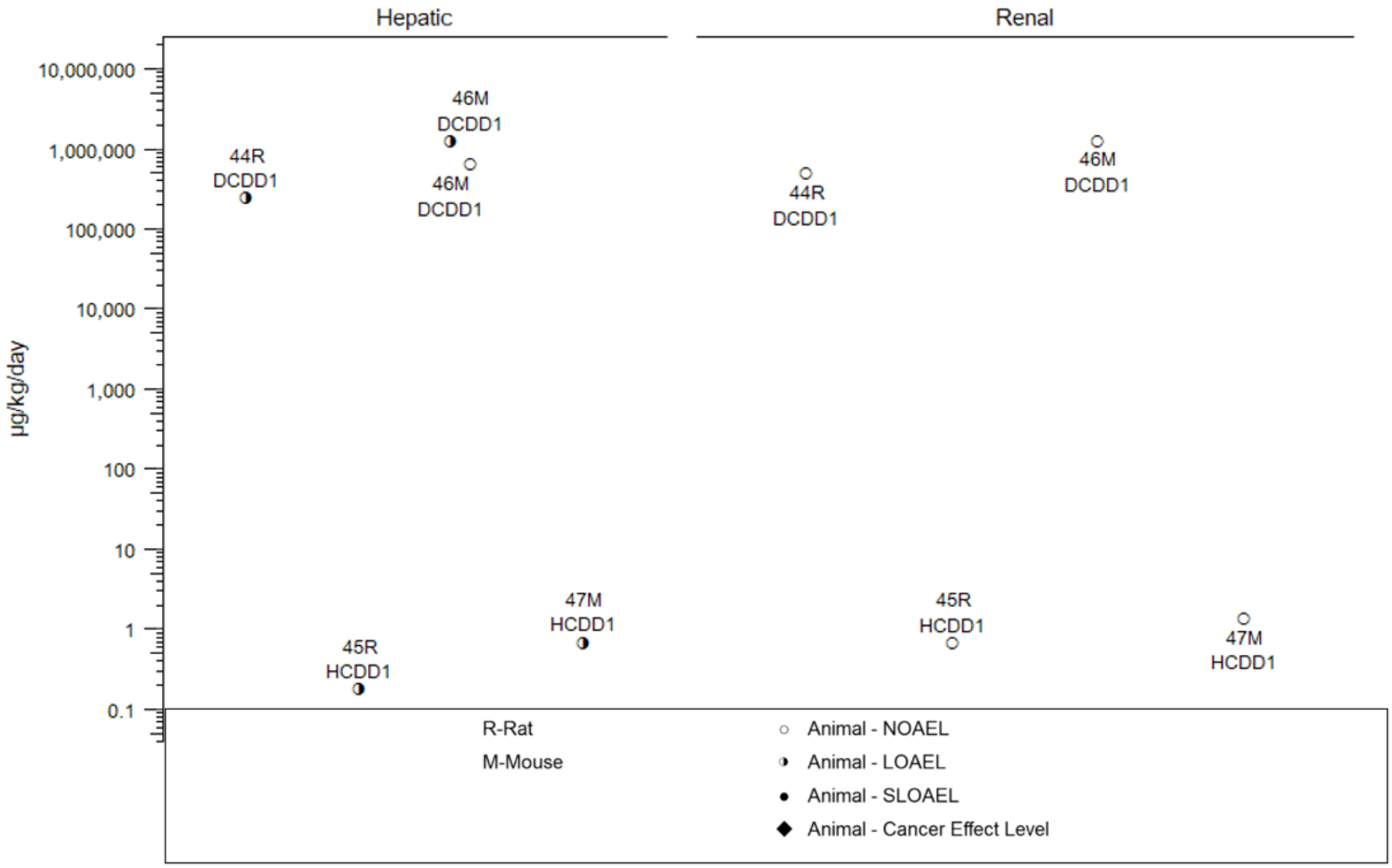


Figure 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Oral
Chronic (≥365 days)

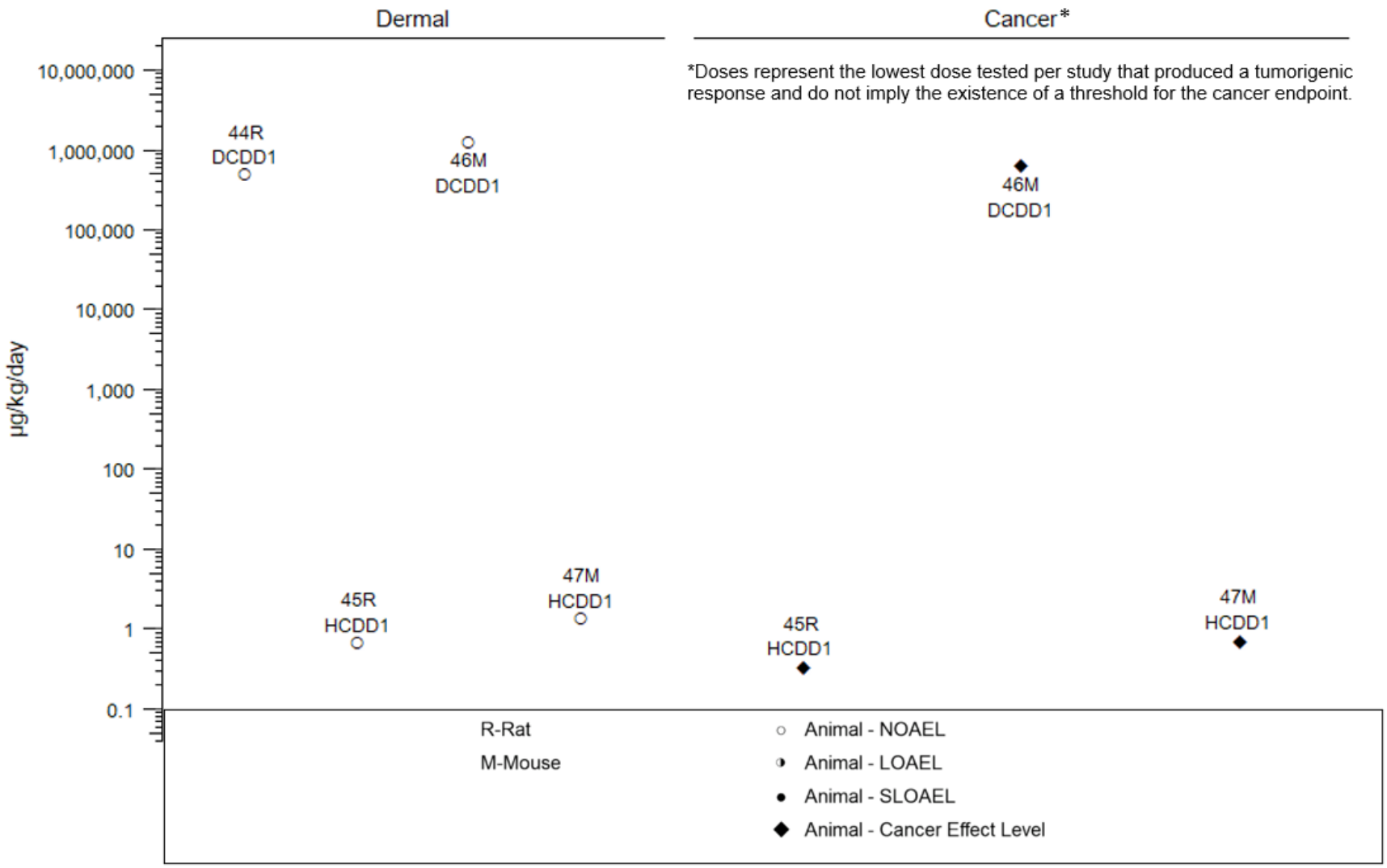


Table 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Puhvel and Sakamoto 1988								**2,3,7,8-TCDD**
Mouse (HRS/J) 5 F	2 weeks, 3 days/week	0, 0.01, 0.1 µg	HP, CS, BI	Dermal		0.01		LOAEL: Epidermal hyperkeratosis and hyperplasia and involution of sebaceous glands in newborns LOAEL: Epidermal hyperkeratosis and hyperplasia and involution of sebaceous glands in adults
Schwetz et al. 1973								**2,3,7,8-TCDD**
Rabbit (NS) NS B	Once	31.6, 63, 126, 252, 500 µg/kg	CS, LE	Death			275	LD50
Schwetz et al. 1973								**2,3,7,8-TCDD**
Rabbit (NS) NS	Once	2,000 µg	CS	Ocular		2,000		Transient inflammation of conjunctiva
INTERMEDIATE EXPOSURE								
Berry et al. 1978, 1979								**2,3,7,8-TCDD**
Mouse (CD-1) 30 F	30 weeks, 2 days/week	0.1 µg	HP	Dermal		0.1		Acne-like lesion
Hebert et al. 1990								**2,3,7,8-TCDD**
Mouse (HRS/J hairless) 20 F	20 weeks, 2 days/week	0, 0.0025, 0.005, 0.010 µg	BW, OW, HP, CS	Bd wt		0.01		16% decreased body weight gain
				Hepatic		0.0025		Increased relative liver weight
				Immuno	0.005	0.01		Decreased thymus/ body weight ratio in non-initiated mice
				Cancer			0.0025	Increased number of skin squamous cell papilloma and hyperproliferative nodules

Table 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Hebert et al. 1990								**2,3,7,8-TCDD**
Mouse (HRS/J) 20 F	20 weeks, 2 days/week	0, 0.010 µg	BW, OW, HP	Hepatic		0.01		Hypertrophy
NTP 1982a								**2,3,7,8-TCDD**
Mouse (Swiss-Webster) 10 M, 10 F	13 weeks, 3 days/week	0, 0.005, 0.01, 0.05, 0.1, 0.625, 1.25, 2.5, 5, 10 µg	BW, GN, HP, CS, LE	Death			0.625	50% died in both sexes
				Resp	0.01	0.05		Bronchiolar adenomatoid changes with hyperplasia
				Hepatic		0.005 M		Fatty degeneration
Poland et al. 1982								**2,3,7,8-TCDD**
Mouse (HRS/J hairless) 20 F	20 weeks, 2 days/week	0, 0.00375, 0.0075, 0.015, 0.030 µg	HP, CS	Cancer			0.00375	Skin papilloma following initiation
Poland et al. 1984								**2,3,7,8-TCDD**
Mouse (Hybrid) NS B	4 weeks, 1 day/week	0.3 µg	HP, CS	Dermal		0.3		Epidermal hyperplasia, hyperkeratosis and keratinized cyst formation in hairless mutants
Poland et al. 1984								**2,3,7,8-TCDD**
Mouse (DBA/2J) NS B	4 weeks, 1 day/week	1.0 µg	HP, CS	Dermal		1		Epidermal hyperplasia, hyperkeratosis and keratinized cyst formation in hairless mutants
Puhvel et al. 1982								**2,3,7,8-TCDD**
Mouse (HRS/J, Skh:HR-1) 3 F	4 weeks, 3 days/week	0, 0.1 µg	BW, HP, CS	Hepatic		0.1		Increased microsomal enzyme-activity
				Dermal		0.1		Hyperkeratosis absence of sebaceous glands

Table 2-4. Levels of Significant Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
CHRONIC EXPOSURE								
NTP 1982a								**2,3,7,8-TCDD**
Mouse (Swiss-Webster) 30–45 M	99–104 weeks, 5 days/week	0, 0.001 µg (M), 0.005 µg (F)	BW, OW, GN, HP, CS, LE	Death			0.001	Decreased probability of survival
				Bd wt	0.005			
				Resp	0.005			
				Cardio	0.005			
				Gastro	0.005			
				Hemato	0.005			
				Hepatic	0.005			
					0.001			
				Renal	0.005			
				Dermal	0.005			
				Repro	0.005			
				Cancer			0.005	CEL: fibrosarcoma without initiation

B = both males and females; Bd wt or BW = body weight; BI = biochemical changes; CEL = cancer effect level; Cardio = cardiovascular; CS = clinical signs; F = female(s); Gastro = gastrointestinal; GN = gross necropsy; Hemato = hematological; HP = histopathology; Immuno = immunological; LD_{50} = median lethal dose; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Repro = reproductive; Resp = respiratory

Table 2-5. Levels of Significant Exposure to Other Chlorinated Dibenzo-*p*-Dioxins (CDDs) – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Schwetz et al. 1973								**HxCDD, unspecified mixture**
Rabbit (NS) NS	Once	2,000 µg	CS	Ocular		2,000		Transient inflammation of conjunctiva
Schwetz et al. 1973								**OCDD**
Rabbit (NS) NS	Once	2,000 µg	CS	Ocular		2,000		Transient inflammation of conjunctiva
Schwetz et al. 1973								**2,7-DCDD**
Rabbit (NS) NS	Once	2,000 µg	CS	Ocular		2,000		Transient inflammation of conjunctiva

CS = clinical signs; DCDD = dichlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified; OCDD = octachlorodibenzo-*p*-dioxin

2.2 DEATH

Overview. Epidemiological studies evaluating possible associations between dioxin exposure and cause-specific deaths are discussed in subsequent sections of Chapter 2; this section reviews studies examining all-cause mortality. Studies have evaluated all-cause mortality in several populations, including workers at phenoxy herbicide or chlorophenol manufacturing facilities, workers exposed to 2,3,7,8-TCDD as a result of an accident, the Seveso population, and Vietnam veterans. Most studies have not found increases in all-cause mortality.

Oral exposure studies have estimated LD_{50} (lethal dose, kill for 50% of dosed animals during a certain time interval) values in several species (and strains) of animals exposed to 2,3,7,8-TCDD and several other congeners. The oral LD_{50} values for 2,3,7,8-TCDD differ between species and strains, and range from 0.6 µg/kg in Hartley guinea pigs to >3,000 µg/kg in DBA/2J mice and 5,051 µg/kg in Syrian hamsters. In all species tested, a pronounced wasting syndrome was the major contributor to death. Increases in mortality or decreased survival have also been reported in animals following intermediate- or chronic-duration oral exposure to 2,3,7,8-TCDD. Dermal exposure studies with 2,3,7,8-TCDD have also reported increased mortality following acute-duration exposure in rats (LD_{50} value of 275 µg/kg) and intermediate- and chronic-duration exposures in mice.

LD_{50} values have also been estimated in rats, mice, and guinea pigs exposed to several different CDD congeners. Studies in Sprague-Dawley rats allow for a comparison of LD_{50} values for other CDD congeners; the LD_{50} values decreased as the number of chlorine atoms increased with 1,2,3,7,8-PeCDD being the most lethal and OCDD being the least lethal. A comparison of LD_{50} values provides evidence that 2,3,7,8-TCDD is the most lethal of all the congeners tested and OCDD was the least lethal as tested animals survived very high doses. For example, the LD_{50} values in Sprague-Dawley rats were 43 µg/kg for 2,3,7,8-TCDD, 206 µg/kg for 1,2,3,7,8-PeCDD, 887 µg/kg for 1,2,3,4,7,8-HxCDD, 6,325 µg/kg for 1,2,3,4,6,7,8-HpCDD, and >1,000,000 ($1x10^6$) µg/kg for OCDD. Studies in guinea pigs suggest that the 2,3,7,8-substituted congeners were more lethal; for example, the LD_{50} values were 3.1 µg/kg for 1,2,3,7,8-PeCDD and 1,125 µg/kg for 1,2,4,7,8-PeCDD. In an intermediate-duration oral study, the serious LOAELs for death were lowest for 1,2,3,7,8-PeCDD followed by 1,2,3,4,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD.

Epidemiological Studies. 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. Several studies reported increased mortality following dioxin exposure linked to specific health effects; these are discussed in subsequent sections of this chapter. No significant increases in the number of all-cause deaths were observed in workers at phenoxy herbicide or chlorophenol manufacturing facilities (Collins et al. 2016; Cook et al. 1986, 1987; Fingerhut et al. 1991; McBride et al. 2009, 2018; 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 (Ketchum and Michalek 2005; Wolfe et al. 1985). In a study of chemical manufacturing workers, an increase in the risk of all-cause mortality was observed in male workers, but not in female workers (Manuwald et al. 2012). The median cumulative job exposure to 2,3,7,8-TCDD was higher in males (77.4 ppt) than in females (19.5 ppt).

2,3,7,8-TCDD—Animal Studies. Numerous studies provided doses associated with death following exposure to 2,3,7,8-TCDD in animals. LD_{50} values varied not only across species, but also among different strains of the same species. A summary of the LD_{50} values following a single oral dose of 2,3,7,8-TCDD is presented in Table 2-6; these data are not presented in the LSE table or figure (Table 2-2 and Figure 2-4). These results suggest that guinea pigs were the most sensitive species, while hamsters were the most resistant (up to 5,000 times greater lethal doses). The animals died following a latency period of several days (mean values varied from 9 to 43 days). In almost all laboratory animals, a pronounced wasting syndrome appears to be a major contributor to lethality.

Table 2-6. LD_{50} Values in Laboratory Animals Following a Single Oral Dose of 2,3,7,8-TCDD

Species (strain)	LD_{50} (µg/kg)	Reference
Sherman rat	22 (M) 45 (F)	Schwetz et al. 1973
Sprague-Dawley rat	43 (M)	Stahl et al. 1992
Long-Evans rat	60 (M) 100 (F)	Fan and Rozman 1995
Fischer 344 rat	164–340 (M)	Walden and Schiller 1985
Osborne-Mendel rat	165 (M) 125 (F)	NTP 1982b
C57BL mouse	146 (M)	Smith et al. 1981

Table 2-6. LD_{50} Values in Laboratory Animals Following a Single Oral Dose of 2,3,7,8-TCDD

Species (strain)	LD_{50} (µg/kg)	Reference
DBA/2J mouse	>3,000 (M)	Weber et al. 1995
New Zealand rabbit	115	Schwetz et al. 1973
Hartley guinea pig	0.6 (M) 2.1 (F)	Schwetz et al. 1973
Hartley guinea pig	1.75 (M)	McConnell et al. 1984
Hartley guinea pig	2.5 (F)	Silkworth et al. 1982
Syrian hamster	1,157 (M and F)	Olson et al. 1980a
Syrian hamster	5,051 (M)	Henck et al. 1981
Mink	4.2 (M)	Hochstein et al. 1988

2,3,7,8-TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; F = females; LD_{50} = dose calculated to cause death in 50% of animals; M = males

Increases in mortality or decreased survival have been also observed in repeated exposure studies. Increased incidences of early deaths were observed in rats exposed to 1–3 µg/kg/day 2,3,7,8-TCDD for 3–13 weeks (Li and Rozman 1995; NTP 1982b; Van Miller et al. 1977) and in mice exposed to 1.3 µg/kg/day for 25 weeks (Umbreit et al. 1987). As with acute lethality studies, deaths occurred at lower doses in guinea pigs (0.03 µg/kg/day) (DeCaprio et al. 1986) than in rats or mice. Studies in monkeys reported deaths at 0.6 µg/kg/day in pregnant monkeys exposed for 3 weeks (McNulty 1984) and in monkeys exposed to 0.011 µg/kg/day for 9 months (Allen et al. 1977). In chronic-duration studies, increased cumulative mortality was observed in female rats exposed to 0.1 µg/kg/day for 2 years (Kociba et al. 1978) and decreased survival was observed in mice exposed to 0.1 or 1.0 µg/kg/day for 1 year (Della Porta et al. 1987; Toth et al. 1979). No alterations in survival were observed in 2-year studies of male and female rats exposed to 0.071 µg/kg/day or female mice exposed to 0.3 µg/kg/day (NTP 1982b). In all species, severe weight loss and body fat depletion were experienced prior to death, but other overt toxic signs were not typically observed.

Information regarding mortality following dermal exposure to 2,3,7,8-TCDD in animals is limited. A dermal LD_{50} value of 275 µg/kg was estimated in rabbits (Schwetz et al. 1973). Deaths occurred within 12–22 days, but the cause of death was not specifically indicated. Increased mortality was observed in mice exposed 3 days/week to 2,3,7,8-TCDD at 0.6255 µg for 13 weeks (NTP 1982a) and decreased survival was observed in male and female mice exposed to 0.001 µg or 0.005 µg, respectively, for 2 years (NTP 1982a). Increased mortality was observed in male ICR mice exposed twice weekly to 0.125 µg

2,3,7,8-TCDD for 20 weeks (Chang et al. 2005). No increase in lethality was reported in HRS/J hairless mice dermally exposed to 0.0025 µg, 2 days/week for 20 weeks (Hebert et al. 1990).

Other CDD Congeners—Animal Studies. Several studies have evaluated the acute lethality of other CDD congeners in rats, mice, and guinea pigs. The results of these studies are presented in Table 2-7; these data are not summarized in the LSE table or figure (Table 2-3 or Figure 2-5). The LD_{50} values for other CDD congeners increased with the degree of chlorination for PeCDD, HxCDD, and HpCDD. No deaths were observed in rats or mice exposed to at least 1,000,000 µg/kg 2,7-DCDD or OCDD.

Table 2-7. LD_{50} Values in Laboratory Animals Following a Single Oral Dose of Other CDD Congeners

Congener	Species	LD_{50} (µg/kg)	Reference
1,2,3,7,8-PeCDD	Sprague-Dawley rat	206 (M)	Stahl et al. 1992
1,2,3,7,8,9-HxCDD and 1,2,3,6,7,8-HxCDD mixture	Osborne-Mendel rat	1,800 (M) 800 (F)	NCI/NTP 1980
HxCDD (mixture of isomers)	Sprague-Dawley rat	>10,000 (M)	Schwetz et al. 1978
1,2,3,4,7,8-HxCDD	Sprague-Dawley rat	887(M)	Stahl et al. 1992
1,2,3,4,6,7,8-HpCDD	Sprague-Dawley rat	6,325 (M)	Stahl et al. 1992
OCDD	Sprague-Dawley rat	>1,000,000 (F)	Schwetz et al. 1978
2,7-DCDD	Swiss Webster mouse	>2,000,000 (M)	Schwetz et al. 1978
2,3,7-TrCDD	C57BL/6 mouse	>3,000 (M)	McConnell et al. 1978b
1,2,4,7,8-PeCDD	C57BL/6 mouse	>5,000 (M)	McConnell et al. 1978b
1,2,3,7,8-PeCDD	C57BL/6 mouse	337.5 (M)	McConnell et al. 1978b
1,2,3,7,8,9-HxCDD and 1,2,3,6,7,8-HxCDD mixture	B6C3F1 mouse	750 (M) 500 (F)	NCI/NTP 1980
1,2,3,6,7,8-HxCDD	C57BL/6 mouse	1,250 (M)	McConnell et al. 1978b
1,2,3,7,8,9-HxCDD	C57BL/6 mouse	>1,440 (M)	McConnell et al. 1978b
1,2,3,4,7,8-HxCDD	C57BL/6 mouse	825 (M)	McConnell et al. 1978b
OCDD	Swiss Webster mouse	>4,000,000 (M)	Schwetz et al. 1978
2,8-DCDD	Hartley guinea pig	>300,000 (M)	McConnell et al. 1978b
2,3,7-TrCDD	Hartley guinea pig	29,444 (M)	McConnell et al. 1978b
1,2,4,7,8-PeCDD	Hartley guinea pig	1,125 (M)	McConnell et al. 1978b
1,2,3,7,8-PeCDD	Hartley guinea pig	3.1 (M)	McConnell et al. 1978b
1,2,3,6,7,8-HxCDD	Hartley guinea pig	70–100 (M)	McConnell et al. 1978b
1,2,3,7,8,9-HxCDD	Hartley guinea pig	60–100 (M)	McConnell et al. 1978b

Table 2-7. LD_{50} Values in Laboratory Animals Following a Single Oral Dose of Other CDD Congeners

Congener	Species	LD_{50} (µg/kg)	Reference
1,2,3,4,7,8-HxCDD	Hartley guinea pig	72.5 (M)	McConnell et al. 1978b
1,2,3,4,7,8-HpCDD	Hartley guinea pig	>600 (M)	McConnell et al. 1978b

DCDD = dichlorodibenzo-*p*-dioxin; F = females; HpCDD = heptachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; LD_{50} = dose calculated to cause death in 50% of animals; M = males; OCDD = octachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin; TCDD = tetrachlorodibenzo-*p*-dioxin; TrCDD = trichlorodibenzo-*p*-dioxin

A series of studies conducted by Viluksela et al. (1994, 1998a) allow for a comparison of the lethality of three CDD congeners in Sprague-Dawley rats administered 10 doses of CDDs in a 13-week period. Increases in mortality were observed at 2.6 µg/kg/day 1,2,3,7,8-PeCDD (75%), 10.3 µg/kg/day 1,2,3,4,7,8-HxCDD (25%), and 110 µg/kg/day (50%) 1,2,3,4,6,7,8-HpCDD. The main causes of death were wasting syndrome, hemorrhage, and anemia (Viluksela et al. 1994, 1998a). No effects on survival were observed following chronic dietary exposure of Osborne-Mendel rats and B6C3F1 mice to $5x10^5$ and $1.3x10^6$ µg/kg/day of 2,7-DCDD, respectively (NCI/NTP 1979), 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 and 0.7 µg/kg/day, respectively (NCI/NTP 1980).

2.3 BODY WEIGHT

Overview. There are limited epidemiological studies evaluating associations between CDD exposure and body weight effects. Weight loss has been reported in a couple of cases of exposure to high levels of exposure and a general population study found an association between serum OCDD levels and body mass index (BMI).

In contrast, a large number of animal studies have reported decreases in body weight following oral or dermal exposure to 2,3,7,8-TCDD or oral exposure to several other CDD congeners. Body weight effects have been consistently observed in animal oral exposure studies in all species evaluated. At high doses, a wasting syndrome characterized as weight loss or lack of weight gain have been observed in monkeys, rats, mice, and mink; this is typically observed at lethal doses. Exposure to lower doses of 2,3,7,8-TCDD results in decreases in body weight gain or terminal body weights. A species comparison of the dose associated with a 50% decrease in body weight gain following a single dose exposure to 2,3,7,8-TCDD found that Hartley guinea pigs were the most sensitive followed by Sprague-Dawley rats, C57BL/6 mice,

and Golden Syrian hamsters. In long-term studies, body weight effects were observed at ≥0.047 µg/kg/day in rats, ≥2.8 µg/kg/day in mice, and 0.005 µg/kg/day in guinea pigs following intermediate-duration oral exposure and at ≥0.071 µg/kg/day in rats and 0.36 µg/kg/day in mice following chronic-duration oral exposure. Body weight effects have also been observed in repeated-exposure dermal studies at doses of 0.1 and 0.005 µg/kg/day following intermediate- and chronic-duration exposure, respectively.

Body weight effects have also been observed following acute-, intermediate-, or chronic-duration oral exposure to other CDD congeners. The lowest LOAELs were 0.18 µg/kg/day for a mixture of HxCDD congeners in Osborne-Mendel rats following intermediate- or chronic-duration exposure and 2.6 µg/kg/day for 1,2,3,7,8-PeCDD in Sprague-Dawley rats following intermediate-duration exposure.

Epidemiological Studies. 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-duration 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.

In a prospective study of boys (8–9 years of age at enrollment) living in Chapevsk, Russia, serum CDD/CDF/PCB TEQ levels were inversely associated with BMI at age 11–12 years (Burns et al. 2011) and age 19 years (Burns et al. 2020). Inverse associations between serum TEQs and height-adjusted fat and fat-free mass indices were also found at age 19 years (Burns et al. 2020). The median serum TEQ was 21.1 pg/g lipid (Burns et al. 2011, 2020). In a study utilizing National Health and Nutrition Examination Survey (NHANES) 1999–2002 data, associations between serum OCDD levels and BMI and waist circumference were observed (Elobeid et al. 2010).

2,3,7,8-TCDD—Animal studies. A characteristic effect of exposure to high doses of 2,3,7,8-TCDD in animals is wasting syndrome. Numerous studies have reported weight loss or a lack of weight gain in rats following a single, lethal 2,3,7,8-TCDD exposure. For example, these effects have been observed in rats (Christian et al. 1986; Kelling et al. 1985; Seefeld and Peterson 1984; Seefeld et al. 1984a; Walden and Schiller 1985), mice (Kelling et al. 1985), monkeys (McConnell et al. 1978a), and mink (Hochstein et al. 1988). Several studies have investigated the basis for this significant decrease in body weight gain. The initial decrease in body weight gain or weight loss appears to be associated with hypophagia rather than malabsorption (Kelling et al. 1985; Moore et al. 1985; Seefeld and Peterson 1984). At sublethal

2,3,7,8-TCDD doses, there appears to be a reduction in the regulation level for body weight; long term, the rats can maintain body weight but at a subnormal body weight (Seefeld and Peterson 1984).

Decreases in body weight gain or terminal body weights of at least 10% have been observed in rats administered a single oral dose of 2,3,7,8-TCDD. LOAEL values were >10 µg/kg in Sprague-Dawley rats (Boverhof et al. 2006; Fletcher et al. 2001; Lu et al. 2010; Moore et al. 1985; Seefeld et al. 1984a; Thunberg et al. 1979); no alteration in body weight gain was observed in Long-Evans rats administered 40 µg/kg (Raasmaja et al. 1996). Decreases in body weight gain or terminal body weights were infrequently reported in mice exposed to a single, nonlethal dose of 2,3,7,8-TCDD. Decreases ≥10% have been reported in C57BL/6 mice at ≥40 µg/kg (Fletcher et al. 2001; Pohjanvirta et al. 2012) and in DBA/2 mice at 1,500 µg/kg (Weber et al. 1995). No alterations in body weight were observed in B6C3F1 mice at ≤10 µg/kg (Diliberto et al. 1995; Frawley et al. 2014) or BALB/c mice at 20 µg/kg (Chen et al. 2013). A 15% decrease in body weight was observed in Golden Syrian hamsters at 40 µg/kg (Fletcher et al. 2001). Greater than 20% decreases in body weight gain or terminal body weights following acute-duration oral exposure have been observed in rats at doses ≥0.66 µg/kg (Boverhof et al. 2006; Fletcher et al. 2001; Roth et al. 1988; Seefeld et al. 1984b; Viluksela et al. 2004), monkeys at 70 µg/kg/day (McConnell et al. 1978a), mice at 200 µg/kg (Fletcher et al. 2001), hamsters at 200 µg/kg (Fletcher et al. 2001), and mink at 5 µg/kg/day (Hochstein et al. 1988). A species comparison of the dose resulting in a 50% reduction in body weight gain following administration of a single dose of 2,3,7,8-TCDD was conducted by Hanberg et al. (1989). The median effective dose (ED_{50}) values were 1.8 µg/kg in Hartley guinea pigs, 89 µg/kg in Sprague-Dawley rats, 890 µg/kg in C57BL/6 mice, and 1,000 µg/kg in Golden Syrian hamsters.

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. Decreased body weight was observed in Osborne-Mendel rats exposed for 13 weeks to 0.56 µg/kg/day (NTP 1982b), Sprague-Dawley rats treated with ≥0.047 µg/kg/day for 10–29 weeks (Chen et al. 2009; Li and Rozman 1995; Ma et al. 2010; NTP 2006; Van Birgelen et al. 1995; Viluksela et al. 1994), Wistar rats administered 0.14 µg/kg/day (Gül et al. 2018), in guinea pigs exposed to 0.005 µg/kg/day in the feed (DeCaprio et al. 1986), and mice exposed to ≥2.8 µg/kg/day (Fader et al. 2017a, 2017b; Thigpen et al. 1975; Vos et al. 1973). Weight loss was recorded in Rhesus monkeys exposed to 0.011 µg/kg/day for 9 months (Allen et al. 1977). In contrast to these findings, a study of C57BL/6J mice exposed to a high-fat diet (60% calorie intake from saturated fat) and administered via gavage 1 µg/kg 2,3,7,8-TCDD once a week (0.14 µg/kg/day) for 32 weeks found significant increases in body weight gain (Brulport et al. 2017).

In chronic-duration experiments with 2,3,7,8-TCDD, decreased body weight gain was reported in Osborne-Mendel and Sprague-Dawley rats exposed via gavage to 0.071 µg/kg/day (NTP 1982b, 2006) and in B6C3 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/kg/day 2,3,7,8-TCDD (Oughton et al. 1995).

Decreased maternal weight gain has been reported in Rhesus monkeys at 0.1 µg/kg/day (McNulty 1984), Holtzman rats at 6 µg/kg/day (Kransler et al. 2007), CRCD rats at 0.5 µg/kg (Giavini et al. 1983), Sprague-Dawley rats at 0.5 µg/kg (Sparschu et al. 1971b), CD-1 mice at 100 µg/kg/day (Courtney 1976), and New Zealand rabbits at 0.25 µg/kg/day (Giavini et al. 1982).

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-duration exposure to 0.005 µg 2,3,7,8-TCDD 3 days/week (NTP 1982a).

Other CDD Congeners—Animal Studies. A small number of studies have evaluated the effect of other CDD congeners on body weight in animals; these data are summarized in Table 2-8. The data suggest that the degree of chlorination affects the toxicity, with the most toxic other CDD congener being PeCDD, and that toxicity decreases with increasing number of carbons for the higher chlorinated compounds. Simanainen et al. (2002) conducted a comparison of the ED_{50} (50% reduction in body weight measured 8 days post-exposure) for several CDDs and in two rat strains. In both rat strains, the ED_{50} values for 1,2,3,4,7,8-HxCDD were approximately 10 times higher than for 1,2,3,7,8-PeCDD and 6–7 times lower than 1,2,3,4,6,7,8-HpCDD. As a comparison, the ED_{50} values for 2,3,7,8-TCDD were 6.3 µg/kg in Long-Evans rats and 19 µg/kg in Han/Wistar rats (Simanainen et al. 2002). A decrease in maternal body weight gain of 39% was observed in Sprague-Dawley rats exposed to 10 µg/kg/day HxCDD mixture on gestation days (GDs) 6–15 (Schwetz et al. 1973).

Table 2-8. Alterations in Body Weight in Animals Orally Exposed to Other CDD Congeners

CDD congener	Duration	Species	Lowest LOAEL (µg/kg/day)	Reference
10–19% Decrease in body weight gain or terminal body weights				
2,7-DCDD	14 days	CD-1 mouse	>1,000	Courtney 1976
	110 weeks	Osborne-Mendel rat	250,000	NCI/NTP 1979
	90 weeks	B6C3F1 mouse	650,000	NCI/NTP 1979
1,2,3,7,8-PeCDD	13 weeks	Sprague-Dawley rat	2.6	Viluksela et al. 1998a, 1998b
HxCDD mixture	13 weeks	Osborne-Mendel rat	0.71	NCI/NTP 1980
	13 weeks	B6C3F1 mouse	0.18	NCI/NTP 1980
OCDD	14 days	CD-1 mouse	>1,000	Courtney 1976
≥20% Decrease in body weight gain or terminal body weight				
1,2,3,7,8-PeCDD	Once	Long-Evans rat Hans/Wistar rat	14 32	Simanainen et al. 2002
	13 weeks	Sprague-Dawley rat	3.8	Viluksela et al. 1998a, 1998b
1,2,3,4,7,8-HxCDD	Once	Long-Evans rat Hans/Wistar rat	140 390	Simanainen et al. 2002
	13 weeks	Sprague-Dawley rat	15.4	Viluksela et al. 1998a, 1998b
HxCDD mixture	104 weeks	Osborne-Mendel rat	0.18	NCI/NTP 1980
1,2,3,4,6,7,8-HpCDD	Once	Long-Evans rat Hans/Wistar rat	980 2,500	Simanainen et al. 2002
	13 weeks	Sprague-Dawley rat	110	Viluksela et al. 1994

CDD = chlorinated dibenzo-*p*-dioxin; DCDD = dichlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; LOAEL = lowest-observed-adverse-effect level; OCDD = octachlorodibenzo-*p*-dioxin; PeCDD = pentachlorodibenzo-*p*-dioxin

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 B6C3F1 mice exposed to $2.5x10^5$ and $6.5x10^5$ µg/kg/day of 2,7-DCDD, respectively, in the feed (NCI/NTP 1979).

2.4 RESPIRATORY

Overview. There are limited data on CDD-induced respiratory effects. Several occupational exposure, Seveso cohort, and Vietnam War veteran studies have examined respiratory tract effects. Symptoms of respiratory tract irritation were observed in workers exposed to 2,3,7,8-TCDD as a result of an industrial

accident. Conflicting results for lung function have been reported in studies of workers and Vietnam War veterans.

A small number of animal studies have examined potential respiratory effects in animals orally exposed to 2,3,7,8-TCDD. Lung damage has been reported in monkeys and rats following intermediate- or chronic-duration oral exposure. Lung lesions have been observed in rats chronically exposed to a mixture of HxCDD congeners but were not observed in similarly exposed mice. No respiratory effects were observed in rats or mice chronically exposed to 2,7-DCDD.

Epidemiological Studies. 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 1972). 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). Similarly, inverse associations have been found between CDD/CDF TEQs intake (estimated from dietary intake and air monitoring data) and forced vital capacity (FVC) or forced expiratory volume in 1 second (FEV_1) in workers at an automobile foundry facility (Zhang et al. 2020). When workers were grouped by smoking status, the inverse associations were observed in the smokers and nonsmokers. Calvert et al. (1991) found no significant differences in ventilatory function between a group of workers employed 15 years earlier in the production of sodium trichlorophenol (NaTCP), 2,4,5-T ester, or hexachlorophene and 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 in 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 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 study authors (USAF 1991), the differences in the mean level between high- and low-exposure subjects were not clinically important. The study 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. In contrast, a study of Korean Vietnam veterans exposed to Agent Orange found higher incidences of diseases of the respiratory tract among veterans with higher Agent Orange exposure (Yi et al. 2014). Specific diseases included pneumonia not due to influenza, chronic bronchitis, bronchiectasis, and asthma.

A follow-up of the cohort involved in the Seveso accident reported a significant increase in deaths (four deaths) from chronic obstructive pulmonary disease in males from zone A and in females from zone B (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 in Section 2.5, Cardiovascular, Pesatori et al. (1998) stated that stress related to the disaster experience among this cohort 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.

2,3,7,8-TCDD—Animal Studies. 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.

One study evaluated potential respiratory effects following acute-duration oral exposure and found no histological alterations in B6C3F1 mice exposed to 1 μg/kg/day for 14 days (Holsapple et al. 1986). Respiratory tract damage has been observed in longer-term studies. Epistaxis (bleeding from the nose) was reported in Rhesus monkeys exposed via gavage to 0.1 μg/kg/day, 3 days/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); five of eight monkeys died at this dose level.

Bronchiolar metaplasia of the alveolar epithelium was observed in the lungs of female Harlan Sprague-Dawley rats administered gavage doses ≥0.002 μg/kg/day 2,3,7,8-TCDD for 2 years; histiocytic infiltration was observed at ≥0.016 μg/kg/day (NTP 2006). Bronchiolar metaplasia was also observed in

a 0.071 µg/kg/day stop-exposure group (30-week exposure followed by vehicle administration until the end of the 2-year study), but the incidence was significantly lower than the 0.071 µg/kg/day continuous exposure group (NTP 2006). No significant increases in respiratory tract lesions were observed in rats exposed to ≤0.071 µg/kg/day for 14 or 31 weeks (NTP 2006). Tritscher et al. (2000) also reported alveolar epithelial metaplasia in female Sprague-Dawley rats administered 2,3,7,8-TCDD biweekly for 60 weeks (average daily dose of 0.125 µg/kg/day) (Tritscher et al. 2000); exposure for 14 or 30 weeks did not result in lung lesions. A third chronic-duration study in female Sprague-Dawley rats reported focal alveolar hyperplasia at 0.01 µg/kg/day 2,3,7,8-TCDD in the feed (Kociba et al. 1978). At 0.1 µg/kg/day, lung effects included pulmonary edema, focal interstitial inflammation and fibrosis, and squamous metaplasia (Kociba et al. 1978); the investigators noted that the observed effects were more extensive in females, as compared to the males. In contrast, no respiratory effects were observed in Osborne-Mendel rats chronically administered 0.071 µg/kg/day for 2 years (NTP 1982b) or in B6C3F1 mice exposed to 0.3 µg/kg/day 2 days/week for 2 years (NTP 1982b).

Dermal exposure of Swiss Webster mice to 0.05 µg 2,3,7,8-TCDD 3 days/week for 13 weeks resulted in bronchiolar adenomatoid changes; the NOAEL was 0.01 µg (NTP 1982a). No respiratory effects were observed in Swiss Webster mice following chronic-duration exposure to 0.005 µg (NTP 1982a).

Other CDD Congeners—Animal Studies. No respiratory effects were found in rats and mice chronically exposed by diet to $5x10^5$ and $1.3x10^6$ µg/kg/day of 2,7-DCDD, respectively (NCI/NTP 1979). 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 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.

2.5 CARDIOVASCULAR

Overview. Cardiovascular outcomes have been evaluated in several populations including workers, Vietnam War veterans, Seveso cohort, communities living in areas with contaminated soil, and the general population. These studies have found inconsistent results. Several studies of phenoxy herbicide production workers or applicators have reported increased mortality from cardiovascular disease, particularly ischemic heart disease. However, many of these studies did not control for potential

confounding variables such as smoking. Other studies of workers have not found associations with cardiovascular deaths or the incidence of several cardiovascular outcomes. Studies in the Seveso cohort have found increases in deaths from cardiovascular deaths; however, several investigators attributed this to post-accident stress. Inconsistent results have been observed in studies evaluating possible associations between CDD exposure and cerebrovascular disease, hypertension, and arteriosclerosis and vascular function.

Animal studies have reported cardiovascular effects in animals orally exposed to 2,3,7,8-TCDD. A small number of animal studies have evaluated cardiovascular function; studies have found alterations in blood pressure; however, the direction of the change was not consistent. Chronic-duration oral exposure to ≥0.071 µg/kg/day 2,3,7,8-TCDD resulted in cardiomyopathy and arteritis in rats. No histopathological alterations were observed in the hearts of rats and mice exposed following chronic-duration oral exposure to 2,7-DCDD or a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD.

Epidemiological Studies. Possible associations between CDD exposure and cardiovascular disease have been examined in several populations including production workers and applicators, Vietnam veterans with Agent Orange exposure, Seveso residents, residents in communities with contaminated soil, and the general population. A summary of the epidemiological studies is presented in Table 2-9.

Several occupational exposure studies evaluating mortality causes found increased risk of deaths due to cardiovascular disease. 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. Increased risks for cardiovascular disease and ischemic heart disease mortality were found in workers with extrapolated serum lipid 2,3,7,8-TCDD levels ≥348 pg/g lipid (current 2,3,7,8-TCDD levels were used to estimate 2,3,7,8-TCDD levels at the end of exposure). Additionally, 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 cardiovascular 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 workers in 36 cohorts from 12 countries exposed to phenoxyacid herbicides and chlorophenols from 1939 to 1992 detected an increased risk for death from cardiovascular disease, especially ischemic heart disease, among the exposed workers (Vena et al. 1998). Risks did not

Table 2-9. Cardiovascular Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Occupational			
Calvert et al. 1998 Cross-sectional study of former workers (n=281) at two 2,4,5-T production facilities in New Jersey and Missouri and unexposed workers (n=260)	Current 2,3,7,8-TCDD level: 220 pg/g lipid Half-life extrapolated 2,3,7,8-TCDD level to estimate TCDD concentration at the time of exposure cessation: 1,900 pg/g lipid	Myocardial infarction	↔
		Angina	↔
		Arrhythmia	↔
		Hypertension	↔
		Abnormal arterial flow	↔
Flesch-Janys et al. 1995 Retrospective cohort study of 1,189 male workers in a phenoxy herbicide, chlorophenols, and other pesticide facility in Germany	Estimated 2,3,7,8-TCDD levels using blood/adipose samples from 190 workers and work histories 4th quintile: 49.3–156.7 pg/g lipid 10th decile: 344.7–3,890.2 pg/g lipid	Cardiovascular disease deaths	↑, 4th quintile
		Ischemic heart disease deaths	↑, 10th decile
Moses et al. 1984 Cross-sectional study of 226 workers at a 2,4,5-T production facility	Chloracne used as a surrogate for exposure	Myocardial infarction	↔
		Angina	↔
Pelclova et al. 2007 2004 follow-up examination of 15 workers exposed more than 35 years earlier to TCDD in an industrial setting in herbicide production plant; 14 controls	1996 mean plasma level was 256 pg 2,3,7,8-TCDD/g lipid (range=14–760 pg/g lipid); estimated range at the time of exposure (1965–1968) was 3,300–74,000 pg 2,3,7,8-TCDD/g lipids	Impaired skin microvascular reactivity; presence of endothelial dysfunction	↑
Steenland et al. 1999 A 6-year extended follow-up of the large NIOSH cohort of workers (n=5,132) from 12 factories exposed during 1960s–1983	Blood samples from workers (n=253) suggested estimated mean serum level of 2,000 ppt in lipids at the time of exposure; exposure categories were created based on points obtained by attributed job-exposure matrix	Ischemic heart disease deaths	↔
Suskind and Hertzberg 1984 Cross-sectional study of 204 exposed and 163 unexposed workers at a 2,4,5-T manufacturing facility in West Virginia	Not measured	Coronary artery disease	↔
		Hypertension	↔
		Angina	↔

Table 2-9. Cardiovascular Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Mannetje et al. 2005 Cross-sectional study of a total of 813 producers and 699 sprayers classified as exposed to dioxin and phenoxy herbicides in a New Zealand study	Job codes were used for exposure evaluation	Ischemic heart disease deaths	↔
		Cardiovascular disease deaths	↔
Vena et al. 1998 Cross-sectional study of 21,863 workers in the IARC International cohort study of phenoxy herbicide and chlorophenol production workers and sprayers	Exposure to 2,3,7,8-TCDD and higher CDDs estimated from blood levels, job records, and levels in workplace environment	All circulatory disease deaths	↑
		Ischemic heart disease deaths	↑
		Cerebrovascular disease deaths	↔
Vietnam War Veterans and Operation Ranch Hand Veterans			
Kang et al. 2006 Cross-sectional study of 1,499 members of the U.S. Army Chemical Corp involved in handling and spraying Agent Orange during the Vietnam War and 1,428 non-Vietnam veterans	Mean serum 2,3,7,8-TCDD levels in Vietnam veteran group: 4.3 ng/g lipid	Heart disease among herbicide sprayers	↑
		Hypertension among herbicide sprayers	↑
Ketchum and Michalek 2005 Cross-sectional study of 1,262 deceased Operation Ranch Hand veterans	Job history used as a surrogate for exposure	Atherosclerotic heart disease deaths among ground crew	↑
		Cardiomyopathy deaths among ground crew	↔
		Cerebrovascular disease deaths among ground crew	↔
		Hypertensive disease deaths among ground crew	↔

Table 2-9. Cardiovascular Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Kim et al. 2012 Cross-sectional study of Korean men undergoing coronary angiograms due acute coronary syndrome divided into two groups: veterans (n=121) exposed to Agent Orange and a group (n=130) with no exposure to 2,3,7,8-TCDD	Self-reported exposure	Hypertension (comparison between two groups)	↑
		Severity of coronary lesions (comparison between two groups)	↔
		Major adverse cardiovascular events (comparison between two groups)	↔
Kim et al. 2014 Cross-sectional study of two groups of patients undergoing coronary angiograms; 1,245 Korean veterans exposed to Agent Orange in the Vietnam war and 506 patients with no history of exposure to 2,3,7,8-TCDD	Self-reported exposure	Hypertension (comparison between two groups)	↑
		Myocardial infarction (comparison between two groups)	↑
		Coronary artery lesions (comparison between two groups)	↑
USAF 1991 Cross-sectional report of 866 Operation Ranch Hand personnel and a comparison group of 1,198	Not measured	Essential hypertension	↔
		Arrhythmias	↔
Wolfe et al. 1985 Retrospective study of 1,278 Operation Ranch Hand personnel	Not reported	Blood pressure	↔
		EKG	↔
Yi et al. 2013 Group of 114,562 Korean veterans of the Vietnam War exposed to Agent Orange	Exposure based on military record	Circulatory diseases	↑
		Hypertension	↔
		Myocardial infarction	↑
		Angina pectoris	↔
		Heart failure	↔
		Arrhythmia	↔
		Cerebral hemorrhage	↔

Table 2-9. Cardiovascular Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Cerebral infarction	↑
		Arteriosclerosis	↔
Yi et al. 2014 Group of 111,726 Korean veterans of the Vietnam War exposed to Agent Orange	Self-reported exposure	Hypertension	↔
		Ischemic heart diseases	↑
		Stroke	↑
		Cerebral infarction	↑
		Arteriosclerosis	↔
Seveso, Italy			
Bertazzi et al. 1989a Retrospective cohort study of 30,703 residents living in the Seveso area at the time of the accident	Not measured	Chronic ischemic heart disease deaths	↑, men ↔, women
		Acute myocardial infarction deaths	↔
		Cerebrovascular disease deaths	↔
Bertazzi et al. 1989b Retrospective cohort study of 1,559 deaths of residents of Seveso	Not measured	Acute myocardial infarction deaths	↑, men ↔, women
Pesatori et al. 1998 Retrospective cohort study 15-year follow-up of the Seveso cohort (n=3,987 deaths)	Soil contamination levels (not reported) in three zones used as a biomarker of exposure	Hypertension deaths	↔, men ↑, women
		Ischemic heart disease deaths	↔, men and women
		Myocardial infarction deaths	↔, men and women
		Chronic ischemic heart disease deaths	↑, men ↔, women
		Cerebrovascular disease deaths	↔, men and women
Communities with contaminated soil			
Chang et al. 2010b Cross-sectional study of 1,490 residents living near a deserted PCP factory in Taiwan	Blood CDD/CDF TEQ levels not reported	Systolic blood pressure	↔
		Diastolic blood pressure	↑

Table 2-9. Cardiovascular Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Chang et al. 2011a Cross-sectional study of 914 residents living near a deserted PCP factory in Taiwan	Mean serum CDD/CDF level: 18.3 pg TEQ/g lipid	Cardiovascular disease	↑
General population			
Donat-Vargas et al. 2020 Cross-sectional study of male participants (n=1844) in the Aragon Worker's Health Study in Spain	Total dioxin (no additional information provided) levels consumed in the diet: 519 pg/day for the 1st quartile and 809 pg/day in the 4th quartile	Coronary artery calcium score (indicator of subclinical atherosclerosis)	↔
Lind et al. 2012 Participants (n=1,016) in the Prospective Investigation of the Vasculature in Uppsala Seniors study in Sweden; participants were 70 years of age	Serum median OCDD level: 2.6 pg/mL	Carotid artery plaques	↑
		Carotid artery intima media thickness	↔
		Carotid artery intima media complex	↔
Nakamoto et al. 2013 Cross-sectional study of 1,063 men and 1,201 women in Japan	Median serum total CDDs/CDFs: 9.8 pg TEQ/g lipid	Hypertension	↑, 4th quartile

↑ = association; ↔ = no association; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; EKG = electrocardiogram; IARC = International Agency for Research on Cancer; NIOSH = National Institute for Occupational Safety and Health; OCDD = octachlorodibenzo-*p*-dioxin; PCP = pentachlorophenol; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalency

differ across latency categories or by year of first exposure but increased slightly by duration of exposure except for those with ≥20 years of exposure. Vena et al. (1998) indicated, 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. A 6-year follow-up study was conducted on the original National Institute for Occupational Safety and Health (NIOSH) cohort of workers exposed to 2,3,7,8-TCDD in occupational settings during production of phenoxy herbicide and chlorophenol (Steenland et al. 1999). No associations between blood CDD levels and ischemic heart disease deaths were found. However, internal analyses using Cox regression found statistically significant exposure-response trends. Only a small subset of workers in the original cohort had dioxin serum levels analyzed. The mean TCDD serum level was back-estimated to be 2,000 pg/g lipid at the time of exposure (1960s–1983). A study of phenoxy herbicides producers and sprayers in New Zealand study did not find increases in the risk of deaths from ischemic heart disease and all cardiovascular diseases (Mannetje et al. 2005). Studies evaluating alterations in the incidence of cardiovascular disease have not found associations between 2,3,7,8-TCDD occupational exposure and the incidence of myocardial infarction (Calvert et al. 1998; Moses et al. 1984) or angina (Calvert et al. 1998; Moses et al. 1984; Suskind and Hertzberg 1984).

In the 10-year period following the Seveso accident, there was an association between the risk of death from chronic ischemic heart disease in men, which was predominantly due to the increased risk during the first 5-year period (Bertazzi et al. 1989a). When the residents were divided into contamination zones, there were associations with the risk of death from chronic heart disease in zones A and R, but not in zone B, for the first 5-year period and only in zone R for the 10-year period (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. A 5-year follow-up found increased risk of chronic ischemic heart disease in males, deaths from chronic rheumatic heart disease in females, and deaths from hypertensive vascular disease in females, all from zone A, the most severely affected area (Pesatori et al. 1998). 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 indicated that the disaster experience with its burden of psychosocial stressors may have played a major role in the increased deaths found. An association between serum CDD/CDF levels and cardiovascular disease was found in a cross-sectional study of residents living near a deserted PCP factory (Chang et al. 2011a). Increased risk of cardiovascular disease has been inconsistently reported in studies of Vietnam

War veterans. Increases in the risk of myocardial infarctions (Kim et al. 2014; Yi et al. 2013), cerebrovascular infarction (Yi et al. 2014), ischemic heart disease (Yi et al. 2014), and atherosclerotic heart disease (Ketchum and Michalek 2005) have been reported in some studies, whereas other studies have not reported cardiovascular disease risk increases (Ketchum and Michalek 2005; Kim et al. 2012).

Studies evaluating possible associations between CDD exposure and cerebrovascular disease have not found associations in workers (Vena et al. 1998), Vietnam War veterans (Ketchum and Michalek 2005), or Seveso residents (Bertazzi et al. 1989a; Pesatori et al. 1998). An increased risk of cerebral infarction was observed in two studies of Vietnam War veterans (Yi et al. 2013, 2014); one of the studies also found an increased risk of stroke (Yi et al. 2014).

A number of studies have evaluated the potential association between CDDs exposure and hypertension. Two studies of workers involved in the production of 2,4,5-T did not find increased risks of hypertension (Calvert et al. 1998; Suskind and Hertzberg 1984). Increases in the risk of hypertension were found in studies of Vietnam War veterans involved in herbicide spraying (Kang et al. 2006) or self-reporting exposure to Agent Orange (Kim et al. 2012, 2014). However, other studies of Operation Ranch Hand personnel (Ketchum and Michalek 2005; USAF 1991; Wolfe et al. 1985) or other veterans (Yi et al. 2013, 2014) did not find associations with hypertension. An increased risk of hypertension deaths was found among female Seveso residents, but not among males (Pesatori et al. 1998). Chang et al. (2010b) found an association between serum CDD/CDF TEQ levels and diastolic blood pressure in residents living near a deserted PCP facility; no association was found for systolic blood pressure.

A small number of studies have evaluated arteriosclerosis and vascular function. Ketchum and Michalek (2005) reported increased risk of atherosclerotic heart disease deaths among Operation Ranch Hand ground crew personnel. In contrast, Yi and associates did not find increased risks of arteriosclerosis among Vietnam War veterans (Yi et al. 2013, 2014). Pelclova et al. (2007) reported impaired vascular function, as measured by skin microvascular reactivity, in workers previously exposed to high levels of 2,3,7,8-TCDD in an herbicide production facility. A general population study found an association between serum OCDD levels and carotid artery plaques, but no association with carotid artery intima media thickness or complex (Lind et al. 2012). Another general population study (Donat-Vargas et al. 2020) found no association between total dioxin levels in the diet and coronary artery calcium score, which is an indicator of subclinical atherosclerosis.

2,3,7,8-TCDD—Animal Studies. Cardiovascular effects, including impaired cardiovascular function and histopathological alterations, have been detected in animals following acute-, intermediate-, and chronic-duration oral exposure to 2,3,7,8-TCDD.

Several studies have evaluated potential alterations in blood pressure. Three daily oral doses of 40 µg/kg/day 2,3,7,8-TCDD resulted in depressed mean arterial blood pressure (34%), measured 6 days post-exposure, in Sprague-Dawley rats (Hermansky et al. 1988). Decreased mean arterial blood pressure (31%) was also observed in Sprague-Dawley rats administered via gavage 0.28 µg/kg/day 2,3,7,8-TCDD for 45 days (Sarihan et al. 2015). In contrast, a time-course study in C57BL/6 mice administered 0.18 µg/kg 2,3,7,8-TCDD via capsule, 5 days/week for 35 days reported increased mean arterial blood pressure (approximately 20%) (Kopf et al. 2010). The increased blood pressure began on day 15 and plateaued at 25 days. Gavage administration of 0.5 µg/kg/day for 28 days resulted in a significant increase in systolic blood pressure (25%) in Sprague-Dawley rats (İlhan et al. 2015). The limited number of studies precludes assessing whether the inconsistent results are due to differences in dose-response or duration of exposure.

Other studies of cardiovascular function reported an increased sensitivity to the inotropic (left atrium) and chronotropic (right atrium) effects of isoproterenol in Sprague-Dawley rats administered a single dose of 100 µg/kg 2,3,7,8-TCDD (Kelling et al. 1987). Electrocardiograms revealed atrial fibrillation, ST depression, T wave and P wave negativity, QTS prolongation, bundle branch block, and biphasic P waves in Sprague-Dawley rats administered 0.28 µg/kg/day for 45 days (Sarihan et al. 2015). In C57BL/6 mice, 0.18 µg/kg 2,3,7,8-TCDD administered 5 days/week for 35 days resulted in increased acetylcholine-dependent vasorelaxation of the aortic rings (Kopf et al. 2010).

Longer-term oral exposure to lethal doses resulted in histopathological lesions. Hemorrhages in the epicardial, myocardial, and endocardial tissues were observed in monkeys that died after exposure to diets providing 0.011 µg/kg/day of 2,3,7,8-TCDD for 9 months (Allen et al. 1977). Myocardial degenerative changes and periarteritis were reported in Sprague-Dawley rats exposed to a diet providing 0.1 µg/kg/day of 2,3,7,8-TCDD for 2 years (Kociba et al. 1978); no histological alterations were observed at the highest nonlethal dose (0.01 µg/kg/day). In contrast, minimal to mild cardiomyopathy was observed in another chronic-duration exposure study in which female Harlan Sprague-Dawley rats were gavaged with 0.0071 µg/kg/day for 2 years (Jokinen et al. 2003; NTP 2006). The investigators noted that the cardiomyopathy, which was characterized as multiple foci of myocardial degeneration scattered within the ventricular walls, was similar to lesions observed in aging rats. A significant increase in the incidence

of cardiomyopathy was also observed in rats administered 0.071 µg/kg/day for 30 weeks and allowed to recover for the remainder of the 2-year study; however, the incidence was significantly lower than in the rats administered 0.071 µg/kg/day for 2 years (NTP 2006). Additionally, significant increases in the incidence of arteritis (characterized as circumferential fibrinoid necrosis of the tunica media, proliferation of adventitial connective tissue with adventitial thickening, and infiltration of the adventitia) were observed in the arteries in the mesentery and pancreas in rats exposed to 0.071 µg/kg (Jokinen et al. 2003).

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

Other CDD Congeners—Animal Studies. No histopathological lesions were observed in the hearts of rats and mice chronically exposed in the diet to $5x10^5$ and $1.3x10^6$ µg/kg/day 2,7-DCDD, respectively (NCI/NTP 1979a), or by gavage for 104 weeks 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).

2.6 GASTROINTESTINAL

Overview. A small number of epidemiological studies have examined gastrointestinal outcomes. Inconsistent results for associations between CDD exposure and the incidence of ulcers have been reported in studies of workers and Vietnam War veterans potentially exposed to Agent Orange.

Gastrointestinal lesions have been observed in the stomachs and small intestines of several animal species orally exposed to 2,3,7,8-TCDD. Administration of a single lethal oral dose of 2,3,7,8-TCDD resulted in gastrointestinal tract ulceration, ileitis, and hyperplasia. Repeated oral exposure to nonlethal doses resulted in gastric mucosal metaplasia and gastric ulcers in monkeys and forestomach hyperplasia in mice. Gingival mucosal lesions have also been observed in rats following gavage administration of 2,3,7,8-TCDD. Chronic-duration oral studies have not found gastrointestinal lesions in rats or mice exposed to 2,7-DCDD or a mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD congeners.

Epidemiological Studies. A small number of epidemiological studies evaluated gastrointestinal effects resulting from occupational exposure or exposure to Agent Orange during the Vietnam War; the results of these studies are summarized in Table 2-10.

Table 2-10. Gastrointestinal Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Occupational			
Bond et al. 1983 Cross-sectional study of workers exposed at a 2,4,5-T production facility (n=87) or workers involved in a chloracne incident in an area of trichlorophenol production (n=22); medical surveillance results were compared to unexposed workers	Not measured	Ulcer	↑
		Digestive system diseases	↑
Calvert et al. 1992 Cross-sectional study of former workers (n=281) at two 2,4,5-T production facilities in New Jersey and Missouri and unexposed workers (n=260)	Current 2,3,7,8-TCDD level: 220 pg/g lipid Half-life extrapolated 2,3,7,8-TCDD level to estimate TCDD concentration at the time of exposure cessation: 1,900 pg/g lipid	Ulcer	↔
		Gastritis	↔
		Gastrointestinal hemorrhage	↔
Suskind and Hertzberg 1984 Cross-sectional study of 204 exposed and 163 unexposed workers at a 2,4,5-T manufacturing facility in West Virginia	Not measured	Ulcer	↑
Vietnam War veterans and Operation Ranch Hand veterans			
USAF 1991 Cross-sectional report of 866 Operation Ranch Hand personnel and a comparison group of 1,198	Not measured	Ulcer	↔
Yi et al. 2013 Group of 114,562 Korean veterans of the Vietnam War exposed to Agent Orange	Exposure based on military record	Gastritis	↔
		Peptic ulcer	↔
		Enterocolitis	↔

Table 2-10. Gastrointestinal Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Yi et al. 2014 Group of 111,726 Korean veterans of the Vietnam War exposed to Agent Orange	Self-reported exposure	Gastritis and duodenitis	↔
		Peptic ulcer	↑
		Ulcerative colitis	↔
		Crohn's disease	↔

↑ = association; ↔ = no association; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; CDD = chlorinated dibenzo-*p*-dioxin; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Two occupational exposure studies of workers exposed to substances contaminated with 2,3,7,8-TCDD ≥15 years prior reported an increase in ulcers (Bond et al. 1983; Suskind and Hertzberg 1984); no alterations in ulcer prevalence were observed in a third occupational exposure study (Calvert et al. 1992). Ulcers were also reported in two studies of Korean Vietnam Veterans self-reporting exposure to Agent Orange (Yi et al. 2013, 2014). However, in the Yi et al. (2013) study, the association was not increased when Agent Orange exposure was based on battalion/company level proximity. No alterations in ulcer prevalence were observed in Operation Ranch Hand personnel (USAF 1991). No consistent alterations in other gastrointestinal diseases were found (Calvert et al. 1992; Yi et al. 2013, 2014).

2,3,7,8-TCDD—Animal Studies. Major 2,3,7,8-TCDD-induced effects in various animal species include the wasting syndrome and hypophagia that occur after a single near-lethal dose or after repeated dosing (discussed in Section 2.3, Body Weight). Studies of effects on the gastrointestinal system have been carried out to investigate the mechanism of this starvation-like syndrome. 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 mucosa content and concentration of gastrin (29 and 22 µg/kg, respectively) and somatostatin (24 and 19 µg/kg, respectively) were 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.

Several studies have reported histological alterations in the gastrointestinal tract. Observed effects in animals receiving a single lethal oral dose of 2,3,7,8-TCDD include epithelial hyperplasia in the stomach of Rhesus monkeys exposed to 70 µg/kg (McConnell et al. 1978a), gastrointestinal tract ulceration and bloody stools in minks at 5 µg/kg (Hochstein et al. 1988), and moderate to severe ileitis (characterized by hyperplasia of the mucosal epithelium with hemorrhaging and necrosis) and peritonitis in hamsters at ≥1,000 µg/kg 2,3,7,8-TCDD (Olson et al. 1980a); hypertrophy, hyperplasia, and metaplasia were observed in Rhesus monkeys exposed to 0.011 µg/kg/day for 9 months (Allen et al. 1977).

Gastrointestinal lesions were also observed in animals following repeated oral exposure to nonlethal doses: gastric mucosal metaplasia in Rhesus monkeys at 0.1 µg/kg/day for 3 weeks (McNulty 1984) and minimal to mild squamous hyperplasia of the forestomach in female Harlan Sprague-Dawley rats at 0.071 µg/kg/day 2,3,7,8-TCDD for 2 years (NTP 2006). No gastrointestinal effects were observed in Sprague-Dawley or Osborne-Mendel rats exposed to 0.1 or 0.071 µg/kg/day, respectively, for 2 years (Kociba et al. 1978; NTP 1982b) or in B6C3F1 mice exposed to 0.3 µg/kg/day (NTP 1982b).

No histopathological changes were observed in the gastrointestinal tract of Swiss Webster mice dermally exposed to 0.005 µg 2,3,7,8-TCDD 3 days/week for 99–104 weeks (NTP 1982a).

NTP (2006) also reported significant increases in the incidence of gingival squamous hyperplasia of the oral mucosa in female rats exposed to all tested doses of 2,3,7,8-TCDD (≥0.002 µg/kg). The lesion was characterized as a focal lesion occurring in the gingival oral mucosa adjacent to molars; the ends of hair shafts and/or inflammation were often present in the same area as the hyperplasia.

Other CDD congeners—Animal Studies. Gastrointestinal lesions were not observed following exposure of rats and mice to $5x10^5$ and $1.3x10^6$ 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).

2.7 HEMATOLOGICAL

Overview. A small number of epidemiological studies have evaluated hematological endpoints; in general, most of the studies did not find alterations in hematological parameters. In studies that did find effects, the magnitudes of the alteration were small and not likely to be clinically significant.

Hematological effects, such as alterations (increases and decreases) in erythrocyte and leukocyte levels, have been reported in 2,3,7,8-TCDD oral exposure animal studies; however, the results are not consistent across studies. These nonspecific changes were probably due to the broad systemic toxicity of 2,3,7,8-TCDD rather than a direct effect on the hematological system. Hematological effects have been observed at lethal doses of 1,2,3,7,8-PeCDD and 1,2,3,4,7,8-HxCDD and splenic hyperplasia was observed in mice exposed to 2,7,-DCDD. These data were not considered adequate to establish a relationship between exposure to other CDD congeners and hematological toxicity.

Epidemiological Studies. A small number of epidemiological studies evaluated potential hematological effects resulting from exposure to 2,3,7,8-TCDD. 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, WBCs, 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 alterations in hematological parameters were observed in children living near a municipal waste incinerator in China (Xu et al. 2019a). 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 WBC and platelet levels.

2,3,7,8-TCDD—Animal Studies. A number of hematological effects have been observed in animals following oral exposure to 2,3,7,8-TCDD, although effects have not been consistently observed across studies. Increased levels of erythrocytes have been observed in CD rats exposed to 10 µg/kg/day for 14 days (Weissberg and Zinkl 1973) and Sprague-Dawley rats exposed to 0.1 µg/kg/day for 4 weeks (Harrill et al. 2015); a 2-year study in Sprague-Dawley rats reported decreased erythrocyte levels at 0.1 µg/kg/day (Kociba et al. 1978). No alterations in erythrocyte levels were observed in C57BL/6N mice exposed to 0.03 µg/kg/day for 14–15 months (Oughton et al. 1995) or guinea pigs exposed to 0.03 µg/kg/day for 90 days (DeCaprio et al. 1986). Total and differential leukocyte levels have also been affected by oral exposure to 2,3,7,8-TCDD. Increased total leukocyte levels have been observed in Golden Syrian hamsters exposed to 1.5 µg/kg during pregnancy (Kransler et al. 2007), but not in Hartley guinea pigs or Holtzman rats similarly exposed to 1.5 or 18 µg/kg, respectively (Kransler et al. 2007). No alterations in total leukocyte levels were observed in CD rats administered 0.71 µg/kg/day for 6 weeks

(Vos et al. 1973), C57BL/6N mice at 0.03 µg/kg/day for 14–15 months (Oughton et al. 1995), or guinea pigs administered 0.03 µg/kg/day for 90 days (DeCaprio et al. 1986). Zinkl et al. (1973) reported a decrease in leukocyte levels in CD-1 mice administered a single dose of 1 µg/kg. Decreased lymphocyte levels were observed in CD-1 mice administered 1 µg/kg once (Zinkl et al. 1973) and in Hartley guinea pigs administered 0.001 µg/kg/day for 8 weeks (Vos et al. 1973). In contrast, increased lymphocyte levels, as well as neutrophil and monocyte levels, were observed in CD rats exposed to 10 µg/kg for 10–14 days (Weissberg and Zinkl 1973). Other hematological effects that have been observed include decreased platelet counts in rats at ≥0.1 µg/kg/day (Viluksela et al. 1994; Weissberg and Zinkl 1973; Zinkl et al. 1973) and a decrease in the vitamin-K-dependent blood coagulation factor VII in rats administered a single dose of 96 µg/kg (Bouwman et al. 1999). Bone marrow hypoplasia was observed in Rhesus monkeys at ≥0.011 µg/kg/day (Allen et al. 1977; McNulty 1984) and CD rats at 10 µg/kg/day for 10–14 days (Weissberg and Zinkl 1973); no bone marrow alterations were observed following chronic-duration exposure of Osborne-Mendel rats or B6C3F1 mice exposed to 0.071 or 0.3 µg/kg/day, respectively (NTP 1982b). No hematological alterations were observed in Swiss Webster mice dermally exposed to 0.005 µg 2,3,7,8-TCDD (NTP 1982a) for a chronic duration.

Other CDD Congeners—Animal Studies. 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 were 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-PeCDD/kg/day or 10.3 µg 1,2,3,4,7,8-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 B6C3F1 mice chronically exposed to $5x10^5$ and $1.3x10^6$ µg/kg/day of 2,7-DCDD, respectively, in feed (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, 2 days/week for 104 weeks by gavage (NCI/NTP 1980).

2.8 MUSCULOSKELETAL

Overview. A very small number of epidemiological studies have evaluated the possible association between CDD exposure and musculoskeletal effects. General population studies examined possible associations between CDD congeners and bone mineral density and walking speed, and a study of the Seveso cohort examined dental defects.

Potential musculoskeletal effects have been poorly studied in animals. Increases in bone mass have been observed in two intermediate-duration oral studies of 2,3,7,8-TCDD in mice. Tooth defects have been observed in rats exposed to 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, or 1,2,3,4,6,7,8-HpCDD. Chronic-duration oral exposure studies with 2,3,7,8-TCDD, 2,7-DCDD, or a mixture of HxCDD congeners have not found histological alterations in muscles or bones.

Epidemiological Studies. There is limited information on the effect of CDD exposure in humans on the musculoskeletal system. Some information 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.

Cho et al. (2011) examined the possible association between 1,2,3,4,6,7,8-HpCDD and OCDD levels and bone mineral density using NHANES (1999–2004) data. No associations were found for either congener in men or women less than 50 years of age or older than 50 years. In another study utilizing NHANES (1999–2000 and 2001–2002) data, Xu et al. (2019b) examined possible associations between CDD body burden and walking speed. No associations were found for 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, or OCDD.

Potential dental defects were examined in 48 Seveso residents and 65 controls (Alaluusua et al. 2004); mean serum 2,3,7,8-TCDD levels were 130, 383, and 1,830 ppt, for residents in zones R, B, and A, respectively, and 15 ppt in controls. Ninety-three percent (25 of 27) of children who were <5 years old at the time of the incident in 1976 had developmental enamel defects as adults. For the 38 children who were >5 years old, only 2 developed enamel defects. The data suggest that a window of susceptibility exists in early childhood for the effect to manifest itself later in life. Hypodontia was found in 6 of

48 exposed individuals and 3 of 65 controls. In contrast, dental caries and periodontal disease did not increase with exposure. The incidence of all dental effects for the exposed groups was 10% (zone R), 45% (zone B), and 60% (zone A). The reference group had a 26% incidence.

2,3,7,8-TCDD—Animal Studies. The musculoskeletal system does not appear to be a major target of toxicity in animals exposed to 2,3,7,8-TCDD. Focal areas of hemorrhaging and edema were observed in the musculoskeletal system of severely debilitated monkeys following dietary exposure to 0.011 µg/kg/day of 2,3,7,8-TCDD for 9 months (Allen et al. 1977). No histological alterations were observed in the musculoskeletal system in Sprague-Dawley rats exposed to 0.1 µg/kg/day in the diet for 2 years (Kociba et al. 1978) or in Osborne-Mendel rats and B6C3F1 mice chronically exposed 2 days a week by gavage to 0.071 or 0.3 µg/kg/day of 2,3,7,8-TCDD, respectively (NTP 1982b).

Increased trabecular bone mass (bone mineral density and content, thickness, and bone volume fraction) and decreased trabecular spacing were observed in juvenile C57BL/6 mice administered 3 and 0.30 µg/kg/day for 28-days (Fader et al. 2018). Administration of a single dose of 8 µg/kg/day, resulted in increased bone mineral fraction, increased trabecular thickness, decreased trabecular spacing, increased mineral content, and increased trabecular mineral density 7 days post exposure (Fader et al. 2018). The observed reductions in bone resorption biomarker and osteoclast surface to bone surface ratio are suggestive of impaired bone resorption. Similar results have been found in adult C57BL/6J mice administered 2.9 µg/kg/day 2,3,7,8-TCDD for 10 weeks (Herlin et al. 2013). The observed effects included increased trabecular bone mass (increased bone volume fraction, bone mineral deposits, and decreased spacing), decreased cortical bone thickness, imbalance of bone remodeling markers, and mechanically weaker bones.

Kiukkonen et al. (2002) examined the effect of intermediate-duration exposure to 2,3,7,8-TCDD on the lower incisor teeth in two strains of female rats (Han/Wistar and Long-Evans). Exposure to 0.12 or 1.2 µg/kg/day for 20 weeks resulted in discoloration, an opening of the pulp chamber to the lingual dental surface, and pulpal perforation to the lingual dental surface in both strains. Histological examination of the teeth showed larger-than-normal pulp chamber, pulpal cell death, and arrested dentin formation. The severity of the effects was dose-related, and no significant differences were found between the rat strains. ED_{50} values were estimated for incisor tooth defects in Han/Wistar and Long-Evans rats administered a single dose of 2,3,7,8-TCDD; the ED_{50} values were 57 and 22 µg/kg, respectively (Simanainen et al. 2002).

Other CDD Congeners—Animal Studies. Chronic-duration experiments with other congeners showed no musculoskeletal effects in Osborne-Mendel rats and B6C3F1 mice exposed in the diet to $5x10^5$ and $1.3x10^6$ 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).

Simanainen et al. (2002) estimated ED_{50} values of 27, 64, and 760 µg/kg for incisor tooth defects in Han/Wistar rats administered a single dose of 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, or 1,2,3,4,6,7,8-HpCDD, respectively. In Long-Evans rats, the ED_{50} values were 24, 130, or 630 µg/kg, respectively.

2.9 HEPATIC

Overview. The potential hepatotoxicity of CDDs has been investigated in studies of workers, Seveso residents, residents living in areas with contaminated soil, Vietnam War veterans, the general population, and a large number of studies in laboratory animals. Results of a small number of studies examining the association between CDD exposure and liver diseases are inconsistent. Inconsistent results have also been reported in studies examining serum liver enzymes and lipid levels, with some studies reporting increases and other studies finding no alterations. In studies reporting alterations, the magnitudes of the alteration were small.

Although the results in humans are inconsistent, the results from animal studies provide strong evidence 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. Liver effects have been observed in numerous oral exposure studies of 2,3,7,8-TCDD at all exposure durations and in all species tested. The observed effects include increases in relative liver weight, increases in serum ALT levels, alterations in serum lipid levels, decreases in liver vitamin A levels, and histopathological alterations. Acute-duration oral exposures to 2,3,7,8-TCDD resulted in hepatocellular hypertrophy and vacuolization at doses ≥0.1 µg/kg/day. In intermediate-duration studies, liver effects were observed at ≥0.016 µg/kg/day and included hepatocellular hypertrophy, vacuolization, necrosis, and inflammation; similar effects were observed following chronic-duration exposure with the lowest LOAELs of ≥0.002 µg/kg/day. Long-term oral studies of 2,3,7,8-TCDD also reported biliary hyperplasia at ≥0.01 µg/kg/day. Toxic hepatitis was observed in rats exposed to 250,000 µg/kg/day 2,7-DCDD or 0.18 µg/kg/day mixture of 1,2,3,6,7,8-HxCDD and

1,2,3,7,8,9-HxCDD; cytoplasmic fatty vacuolization was observed in rats exposed to 36 µg/kg/day OCDD.

Epidemiological Studies. Several epidemiological studies have evaluated associations between CDD exposure, primarily 2,3,7,8-TCDD, and hepatic effects in workers, Seveso residents, residents living in areas with contaminated soil, Vietnam War veterans, and the general population. A summary of these studies is presented in Table 2-11. Most of these studies evaluated possible associations with liver enzyme levels and dyslipidemias; three studies examined possible associations with liver disease.

A medical survey of workers at two sodium trichlorophenol production facilities found no evidence of an elevated risk of clinical liver disease (hepatitis, cirrhosis, or fatty liver) (Calvert et al. 1992). An examination of children in Seveso found no increases in the risk of liver enlargement or scleral jaundice (Caramaschi et al. 1981). An increased risk of fatty liver was found in a study of residents living near a closed PCP facility in Taiwan (Lee et al. 2006); the association was found among residents with serum CDD/CDF TEQs in the fourth quartile. Among Vietnam War veterans with self-reported exposure to Agent Orange, there was no association with chronic hepatitis and an association for liver cirrhosis (Yi et al. 2014).

Studies examining liver enzymes primarily examined serum/plasma ALT, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and alkaline phosphatase activities; some studies evaluated serum activity levels while other studies examined the risk of abnormal levels. The results of these studies of workers, Seveso residents, residents living in areas with contaminated soil, and the general population are inconsistent. Increases in serum ALT levels were observed in two studies (Hoffman et al. 1986; Neuberger et al. 1999) but not in other studies (Yorita Christensen et al. 2013; Lee et al. 2006; Mocarelli et al. 1986; Ott et al. 1994). A study of Seveso children found an increased risk of abnormal serum ALT levels (Caramaschi et al. 1981) and two studies of workers did not find associations (Calvert et al. 1992; Moses et al. 1984). Increased serum AST levels have been reported (Hoffman et al. 1986; Lee et al. 2006; Mocarelli et al. 1986; Neuberger et al. 1999). However, Ott et al. (1994) did not find alterations in serum AST levels among workers, and no studies found an increased risk of abnormal AST levels (Calvert et al. 1992; Caramaschi et al. 1981; Moses et al. 1984). Several studies of workers (Calvert et al. 1992; Moses et al. 1984) and Seveso children (Caramaschi et al. 1981; Mocarelli et al. 1986) reported increased risk of abnormal serum GGT levels; Neuberger et al. (1999) also reported elevated serum GGT levels in workers. However, no association between CDD exposure and serum GGT

Table 2-11. Hepatic Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Occupational			
Calvert et al. 1992 Cross-sectional study of former workers (n=281) at two 2,4,5-T production facilities in New Jersey and Missouri and unexposed workers (n=260)	Current 2,3,7,8-TCDD level: 220 pg/g lipid Half-life extrapolated 2,3,7,8-TCDD level to estimate TCDD concentration at the time of exposure cessation: 1,900 pg/g lipid	Hepatitis	↔
		Cirrhosis	↔
		Fatty liver	↔
		Abnormal serum ALT	↔
		Abnormal serum AST	↔
		Abnormal serum GGT	↑
Calvert et al. 1996 Cross-sectional study of former workers (n=281) at two 2,4,5-T production facilities in New Jersey and Missouri and unexposed workers (n=260)	Current median 2,3,7,8-TCDD level: 0.406 pg/g serum in workers and 0.0369 pg/g serum in referents	Abnormal total cholesterol	↔
		Abnormal HDL cholesterol	↑, among workers with serum 2,3,7,8-TCDD levels of 1.516–19.717 pg/g lipid
		Abnormal triglyceride	↔
Mannetje et al. 2018 Cross-sectional study in former employees (n=245) of a phenoxy herbicide production facility in New Zealand	Work history and 2007–2008 serum 2,3,7,8-TCDD levels ≥10 pg/g lipid	Abnormal serum total cholesterol	↔, highly exposed job
		Abnormal serum triglyceride	↑, highly exposed job
		Abnormal HDL cholesterol	↑, highly exposed job
		Abnormal LDL cholesterol	↔, highly exposed job
Moses et al. 1984 Cross-sectional study of 206 workers at a 2,4,5-T production facility in the United States	Comparisons between workers with and without chloracne	Abnormal serum γ-glutamyl transferase	↑
		Abnormal serum triglycerides	↔
		Abnormal serum ALT	↔
		Abnormal serum AST	↔
Neuberger et al. 1999 Cross-sectional study of 56 workers with chloracne involved in the production of 2,4,5-trichlorophenol and 2,4,5-T at a facility in Austria; a matched control group was also examined	Median serum 2,3,7,8-TCDD level: 280.0 pg TEQ/g lipid (workers)	Serum ALT (compared to controls)	↑
		Serum AST (compared to controls)	↑
		Serum GGT (compared to controls)	↑

Table 2-11. Hepatic Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Ott et al. 1994 Retrospective cohort study of 138 workers exposed to 2,3,7,8-TCDD due to an accident at a trichlorophenol facility in Germany	Current 2,3,7,8-TCDD serum levels: <1–553 ppt Back calculated 2,3,7,8-TCDD serum levels: 3.3–12,000 ppt	Serum AST	↔
		Serum ALT	↔
		Serum GGT	↔
		Serum alkaline phosphatase	↑, using back calculated TCDD levels or chloracne status as biomarker
Pazderova-Vejlupková et al. 1981 Case series of 55 workers at a 2,4,5-T production facility in Czechoslovakia with signs of 2,3,7,8-TCDD toxicity (95% of workers had chloracne)	Not measured	Serum cholesterol	↑
Pelclova et al. 2001 Case series of 13 workers at a trichlorophenoxyacetic acid production facility in Czechoslovakia exposed to high levels of 2,3,7,8-TCDD during an accident 30 years ago	Mean serum 2,3,7,8-TCDD level in 1996: 256 pg/g lipid	Plasma cholesterol	↑
		Plasma triglycerides	↑
		Plasma lipids	↑
Suskind and Hertzberg 1984 Cross-sectional study of 204 exposed and 163 unexposed workers at a 2,4,5-T manufacturing facility in West Virginia	Not measured; comparisons between exposed and unexposed workers	Plasma cholesterol	↔
		Plasma triglycerides	↔
		Plasma LDL cholesterol	↔
		Plasma HDL cholesterol	↔
Seveso, Italy			
Assennato et al. 1989 Retrospective cohort study of 193 Seveso residents (88% were children <15 years of age) with chloracne and 182 controls from a neighboring region	Not measured	Serum GGT	↔
		Serum triglycerides	↔
		Serum cholesterol	↔
		Serum HDL cholesterol	↔

Table 2-11. Hepatic Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Caramaschi et al. 1981 Retrospective cohort study of 164 children with chloracne living in Seveso at the time of the accident and 182 children from the same area without chloracne	Not measured	Liver enlargement and/or scleral jaundice	↔
		Abnormal serum GGT	↑
		Abnormal serum AST	↔
		Abnormal serum ALT	↑
		Abnormal serum cholesterol	↔
		Abnormal serum alkaline phosphatase	↔
Mocarelli et al. 1986 Retrospective cohort study of 6–10-year-old children in zone A (n=69), zone B (n=83), and zone R (n=241, served as control group)	Not measured	Serum ALT	↔
		Serum AST	↑, boys
		Serum GGT	↑, boys
		Serum alkaline phosphatase	↔
		Serum triglycerides	↔
		Serum cholesterol	↔
Vietnam War veterans and Operation Ranch Hand veterans			
Yi et al. 2014 Group of 111,726 Korean veterans of the Vietnam War exposed to Agent Orange	Self-reported exposure	Chronic hepatitis	↔
		Liver cirrhosis	↑
Communities living in areas with contaminated soil			
Hoffman et al. 1986 Cross-sectional study of 154 people living in Quail Run Mobile Home Park and exposed to 2,3,7,8-TCDD in soil and 155 control subjects	Years of residence in the park used as surrogate for exposure	Serum triglycerides	↔
		Serum total cholesterol	↔
		Serum AST	↑
		Serum ALT	↑
		Serum GGT	↔
		Serum alkaline phosphatase	↑

Table 2-11. Hepatic Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Lee et al. 2006 Cross-sectional study of 52 residents living in the vicinity of a closed PCP manufacturing facility in Taiwan and 33 residents in a nearby control facility	Serum CDD/CDF TEQs: 80.1 pg TEQ/g lipid in residents and 50.9 pg TEQ/g lipid in controls Serum CDD/CDF TEQs (pg TEQ/g lipid): • 1st quartile: <22.93 • 4th quartile: ≥78.42	Fatty liver	↑, 4th quartile
		Serum cholesterol	↔, 4th quartile
		Serum triglyceride	↔, 4th quartile
		Serum AST	↑, 4th quartile
		Serum ALT	↔, 4th quartile
General population			
Yorita Christensen et al. 2013 Cross-sectional study using NHANES (2003–2004) data	Quartiles concentrations not reported	Serum ALT	↔, 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, or OCDD
Nakamoto et al. 2013 Cross-sectional study of 1,063 men and 1,201 women in Japan	Median serum total CDDs/CDFs: 9.8 pg TEQ/g lipid	Hyperlipidemia	↑, 2nd quartile and trend

↑ = association; ↔ = no association; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; GGT = gamma-glutamyl transferase; HDL = high-density lipoprotein; HpCDD = heptachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; LDL = low-density lipoprotein; NHANES = National Health and Nutrition Examination Survey; OCDD = octachlorodibenzo-*p*-dioxin; PCP = pentachlorophenol; TCDD = tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalency

levels were observed in other studies of workers (Ott et al. 1994), Seveso children (Assennato et al. 1989), or residents living in an area with contaminated soil (Hoffman et al. 1986). Four studies evaluated possible associations with serum alkaline phosphatase levels; a study of workers (Ott et al. 1994) and residents living in an area with contaminated soil (Hoffman et al. 1986) found increased levels and two studies of Seveso children found no association (Caramaschi et al. 1981; Mocarelli et al. 1986).

As with the findings on serum liver enzyme levels, inconsistent results have been reported for serum lipids. Pelclova et al. (2001) reported increased serum triglycerides in workers, but other studies have not found increases in serum triglycerides (Assennato et al. 1989; Hoffman et al. 1986; Mocarelli et al. 1986; Suskind and Hertzberg 1984) or in the risk of abnormal triglycerides (Calvert et al. 1996; Moses et al. 1984). With the exception of a study of workers by Pelclova et al. (2001), studies of workers (Suskind and Hertzberg 1984), Seveso children (Assennato et al. 1989; Mocarelli et al. 1986), and communities living in areas with contaminated soil (Hoffman et al. 1986; Lee et al. 2006) did not find associations between CDD exposure and increased serum cholesterol levels. Calvert et al. (1996) found associations with an increased risk of abnormal total cholesterol levels and abnormal high-density lipoprotein (HDL) cholesterol in workers. However, HDL cholesterol and low-density lipoprotein (LDL) cholesterol levels were not elevated in studies of workers (Suskind and Hertzberg 1984) or Seveso children (Assennato et al. 1989).

2,3,7,8-TCDD—Animal Studies. Effects on the liver have been seen after acute-, intermediate-, and chronic-duration oral exposure and intermediate-duration dermal exposure to 2,3,7,8-TCDD. The observed effects include increases in liver weight, alterations in serum liver enzymes, alterations in liver and serum lipid levels, and histological alterations. Increased relative liver weights were observed in rats, mice, and hamsters at doses of ≥0.12, 3, and 14 µg/kg, respectively, following a single-dose oral exposure (Fletcher et al. 2001; Hanberg et al. 1989; Weber et al. 1995) and in rats and mice at ≥0.022 and 0.08 µg/kg/day, respectively (Fader et al. 2017b; Harrill et al. 2015), following intermediate-duration oral exposure.

As shown in Table 2-12, increases in serum ALT levels have been observed at doses ≥1 µg/kg; the magnitude of change is typically >250%. A number of studies have demonstrated alterations in serum lipid levels, in particular serum triglyceride and cholesterol levels. Inconsistent results have been found for serum triglyceride levels, with some studies reporting increases and others reporting decreases; however, this may be related to the amount of time lapse between dosing and sample collection. A study in rats reported increased serum triglyceride levels 24 hours post-exposure to 40 µg/kg 2,3,7,8-TCDD and

decreased levels 7 days post-exposure (Fletcher et al. 2005). Similarly, Boverhof et al. (2006) reported peak serum triglyceride levels 24 hours post-exposure, followed by a marked decrease in levels by 72 hours post-exposure. This time course could explain why Kakizuka et al. (2015) reported decreased serum triglyceride levels; rats were sacrificed 7 days post-exposure. Studies in rats have consistently found increased serum cholesterol levels following acute-duration oral exposure to 2,3,7,8-TCDD (see Table 2-12). A time-course study in rats administered a single dose of 40 µg/kg 2,3,7,8-TCDD demonstrated an initial decrease in serum cholesterol levels followed by increased levels 24 hours and 7 days post-exposure (Fletcher et al. 2005). Increases in free fatty acid levels have also been observed following single-dose administration (Boverhof et al. 2005, 2006; Kakizuka et al. 2015). Several studies have reported a depletion of vitamin A levels in the liver; decreases in hepatic retinoids and retinol levels have also been reported (see Table 2-12).

Table 2-12. Hepatic Clinical Chemistry in Rats and Mice Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose[a] (µg/kg)	Serum ALT	Serum triglycerides	Serum cholesterol	Other effects	Reference
Sprague-Dawley rat, once	10	↔	↑ (185%)	↑ (30%)	↑ FFA (87%)	Boverhof et al. 2006
Sprague-Dawley rat, once	40		↑ 24 hours (74%) ↓ 7 days (47%)	↑ (61%)	↓ hepatic retinoids	Fletcher et al. 2005
Sprague-Dawley rat, once	6.25				Altered vitamin A storage	Håkansson et al. 1989
Wistar rat, once	60		↓ (40%)	↑ (30%)	↔ hepatic cholesterol, ↔ FFA, ↑ serum bile acids	Kakizuka et al. 2015
Sprague-Dawley rat, 12 days	10	↔	↔	↔	↑ total bilirubin	Lu et al. 2010
Sprague-Dawley rat, once	10				↓ retinol storage	Thunberg et al. 1984
Fischer 344 rat, once	45		↑ (55%)	↑ (146%)		Walden and Schiller 1985
C57BL/6 mouse, once	30	↑ (260%)	↑ (40%)	↓ (28%)	↑ FFA (28%)	Boverhof et al. 2005, 2006
A2G-hr/+ mouse, once	75	↑ (412%)				Greig et al. 1987

Table 2-12. Hepatic Clinical Chemistry in Rats and Mice Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose[a] (µg/kg)	Serum ALT	Serum triglycerides	Serum cholesterol	Other effects	Reference
C57BL/6 mouse, 2 weeks (1 time/week)	2.9	↑ (629%)				Lamb et al. 2016
C57BL/6 mouse, once	125	↑ (300%, males)				Pohjanvirta et al. 2012
Sprague-Dawley rat, 13 weeks	0.01				↓ hepatic retinol	Van Birgelen et al. 1995
CD rat, 30 days	1			↑ (70%)		Zinkl et al. 1973

[a]Doses were duration-adjusted for continuous exposure.

↑ = association; ↓ = inverse association; ↔ = no association; ALT = alanine aminotransferase; FFA = free fatty acid

A variety of histopathological alterations have been observed in the liver following acute-, intermediate-, or chronic-duration oral exposure to 2,3,7,8-TCDD; see Table 2-13. Single-dose exposure to 10–40 µg/kg resulted in hypertrophy in rats. Other effects observed in acutely exposed rats include cytoplasmic vacuolization at 10 µg/kg and necrosis and inflammation at 40 µg/kg. In mice, the lowest LOAEL for histological alterations was 30 µg/kg; at this dose, cytoplasmic vacuolization and necrosis were observed. Fatty changes were observed at 75 µg/kg and inflammation was observed at 500 µg/kg. Guinea pigs were more sensitive than rats and mice, with necrosis occurring following a single dose of 0.1 µg/kg. Long-term oral exposure resulted in hypertrophy, necrosis, inflammation, and fatty changes in rats at doses ≥0.013 µg/kg/day and cytoplasmic vacuolization and necrosis in mice at ≥0.09 µg/kg/day. Biliary hyperplasia has been observed in monkeys following intermediate-duration exposure to ≥0.01 µg/kg/day and in rats following chronic-duration oral exposure to ≥0.01 µg/kg/day. Other hepatic lesions observed in rats exposed to 2,3,7,8-TCDD for 2 years included bile duct cysts at 0.016 µg/kg/day and cholangiofibrosis, portal fibrosis, and nodular hyperplasia at 0.032 µg/kg/day (NTP 2006). Another 2-year study found toxic hepatitis in rats and mice administered 0.02 µg/kg/day (NTP 1982b).

Table 2-13. Histopathological Alterations in the Liver of Experimental Animals Resulting From Oral Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Oral doses[a] (µg/kg/day) resulting in histopathological alterations							
Species, duration	Hypertrophy	Necrosis	Inflammation	Fatty changes	Cytoplasmic vacuolization	Hepatocytes with pyknotic nuclei	Biliary hyperplasia	Reference
Sprague-Dawley rat, once	10							Boverhof et al. 2006
Sprague-Dawley rat, once	10							Boverhof et al. 2006
Sprague-Dawley rat, once	25							Christian et al. 1986
Sprague-Dawley rat, once	40							Fletcher et al. 2005
Sprague-Dawley rat, 3 days		40	40					Hermansky et al. 1988
Sprague-Dawley rat, 12 days					10			Lu et al. 2010
C57BL/6 mouse, once					30			Boverhof et al. 2005
C57BL/6 mouse, once					1			Boverhof et al. 2005
C57BL/6 mouse, once					0.1			Boverhof et al. 2006
C57BL/6 mouse, once	30				30			Boverhof et al. 2006
A2G-jr/+ mouse, once		75		75				Greig 1984, 1987
C57BL/6 mouse, once					30			Kopec et al. 2010
C57BL/6 mouse, once		30			30			Kopec et al. 2008

Table 2-13. Histopathological Alterations in the Liver of Experimental Animals Resulting From Oral Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Oral doses[a] (µg/kg/day) resulting in histopathological alterations							
Species, duration	Hypertrophy	Necrosis	Inflammation	Fatty changes	Cytoplasmic vacuolization	Hepatocytes with pyknotic nuclei	Biliary hyperplasia	Reference
C57BL/6 mouse, once		500	500		500			Pohjanvirta et al. 2012
Hartley guinea pig, once		0.1						Turner and Collins 1983
Rhesus monkey, 9 months							0.011	Allen et al. 1977
Rhesus monkey, 3 weeks, 3 days/week							0.1	McNulty 1984
Sprague-Dawley rat, 4 weeks (19 doses)	0.022							Harrill et al. 2015
Sprague-Dawley rat, 14 weeks, 5 days/week	0.016							NTP 2006
Sprague-Dawley rat, 31 weeks	0.016			0.071				NTP 2006
C57BL/6 mouse, 28 days					0.8			Fader et al. 2015
C57BL/6 mouse, 28 days (seven doses)		0.8			0.3			Fader et al. 2017b
BALB/c mouse, 28 days						0.09		Maranghi et al. 2013
BALB/c mouse, 28 days			0.0009					Rasinger et al. 2018
Sprague-Dawley rat, 2 years		0.01	0.01	0.01			0.01	Kociba et al. 1978

Table 2-13. Histopathological Alterations in the Liver of Experimental Animals Resulting From Oral Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Oral doses[a] (µg/kg/day) resulting in histopathological alterations							
Species, duration	Hypertrophy	Necrosis	Inflammation	Fatty changes	Cytoplasmic vacuolization	Hepatocytes with pyknotic nuclei	Biliary hyperplasia	Reference
Sprague-Dawley rat, 2 years	0.002	0.002	0.002				0.016	NTP 2006

[a]Doses were adjusted for continuous exposure.

The National Toxicology Program (NTP) 2-year study in female rats grouped all non-neoplastic liver changes together (termed toxic hepatopathy) in order to evaluate the incidence and severity dose-response (NTP 2006). The incidences of toxic hepatopathy increased with dose; 15, 57, 85, and 100% at 0.0071, 0.016, 0.032, and 0.071 µg/kg/day, respectively. The respective severity scores were 1.3, 1.2, 1.8, and 3.5 (a severity score of 1 was considered minimal and 4 considered marked). The NOAEL of toxic hepatopathy was 0.002 µg/kg/day; note that there were significant increases in specific types of lesions at this dose level. The NTP (2006) study also demonstrated duration-dependent increases in the severity of effects and the pattern of hepatotoxicity. Hepatocellular hypertrophy and diffuse fatty changes were observed at 14 weeks; hepatocellular hypertrophy, diffuse fatty changes, and inflammation were observed after 31 weeks of exposure; hepatocellular hypertrophy, diffuse fatty changes, inflammation, and bile duct hyperplasia were observed after 53 weeks of exposure; and hepatocellular hypertrophy, diffuse fatty changes, inflammation, bile duct hyperplasia, bile duct cysts, necrosis, cholangiofibrosis, portal fibrosis, and nodular hyperplasia were observed after 2 years of exposure.

In dermal exposure studies, liver hypertrophy was observed in mice administered 0.01 µg 2 times/week for 20 weeks (Hebert et al. 1990) and fatty changes were observed in male mice administered 0.005 µg 3 times/week for 13 weeks (NTP 1982a). No hepatic effects were observed in male mice chronically exposed to 0.001 µg 3 times/week for 2 years.

Other CDD Congeners—Animal Studies. A small number of studies have evaluated the hepatotoxicity of other CDD congeners. In acute-duration oral exposure studies, no histological alterations were observed in mice exposed to 10 µg/kg/day 2,7-DCDD for 14 days (Holsapple et al. 1986), 1,000 µg/kg 1,2,3,4-TCDD once (Courtney 1976), or 20 µg/kg/day OCDD for 10 days (Courtney 1976). Liver effects were reported following longer-term oral exposure. Toxic hepatitis (characterized as centrilobular fatty metamorphosis and/or necrosis) was observed in rats and mice exposed to 250,000 or 1,300,000 µg/kg/day, respectively, 2,7-DCDD for 110 weeks (NCI/NTP 1979a). Toxic hepatitis (characterized as degenerative hepatocellular changes, mild fibrosis, and bile duct hyperplasia) was also observed in rats and mice exposed to 0.18 or 0.7 µg/kg/day mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD 2 days/week for 2 years (NCI/NTP 1980). Intermediate-duration exposure to 36 µg/kg/day OCDD resulted in cytoplasmic fatty vacuolization (Couture et al. 1988) in rats; no liver alterations were observed in rats exposed for 2 weeks (Couture et al. 1988).

2.10 RENAL

Overview. A few epidemiological studies evaluated potential renal effects with mixed results. Renal effects have been reported in animals following oral exposure to 2,3,7,8-TCDD or 1,2,3,4,6,7,8-HpCDD. Evidence of impaired renal function (increases in serum creatinine and urea nitrogen levels) and histological alterations have been reported in rats, mice, and monkeys orally exposed to 2,3,7,8-TCDD. The lowest LOAELs for renal effects were 10 µg/kg/day for increased serum creatinine and urea nitrogen levels and proximal tubular damage in rats following acute-duration exposure, 0.01 µg/kg/day for tubular epithelial hyperplasia in monkeys following intermediate-duration exposure, and 0.032 µg/kg/day for transitional epithelial hyperplasia in rats following chronic-duration exposure. Renal lesions were also observed in rats administered 1,2,3,4,6,7,8-HpCDD over a lifetime but not in rats exposed to 2,7-DCDD or a mixture of HxCDD congeners for 2 years.

Epidemiological Studies. 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 microscopic 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). A study of a community near a production facility with serum dioxin levels found an association between high dioxin levels (CDD/CDF TEQ ≥20 pg TEQ/g lipid) and chronic kidney disease (Huang et al. 2016). 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). Using NHANES 1999–2004 data, Everett and Thompson (2016) found an association between serum 1,2,3,6,7,8-HxCDD levels of ≥0.299 pg/g lipid and the risk of nephropathy among adults.

2,3,7,8-TCDD—Animal Studies. 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-duration exposure to 2,3,7,8-TCDD caused dilation of convoluted tubules and Bowman's spaces at 10 µg/kg/day or 25 µg/kg in Sprague-Dawley rats (Christian et al. 1986; Lu et al. 2009). 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) and Wistar rats exposed to

1 µg/kg/day for 1 month (Erdemli et al. 2020). Increased serum creatinine and urea levels were also observed in rats exposed to 10 µg/kg/day for 12 days (Lu et al. 2009) or 1 µg/kg/day for 1 month (Erdemli et al. 2020). No renal effects were observed in rats exposed to ≤0.071 µg/kg/day for 14 or 31 weeks (NTP 2006). Chronic-duration exposure to 0.032 or 0.071 µg/kg/day 2,3,7,8-TCDD for 2 years resulted in increases in the incidence of transitional epithelial hyperplasia in the kidneys of female Sprague-Dawley rats (NTP 2006). Mild nephropathy was also observed in rats exposed to 0.071 µg/kg/day for 2 years or for 30 weeks followed by a 16.5-month recovery period (NTP 2006); the incidence in the stop-exposure group was significantly lower than the continuous exposure group. Chronic-duration exposure of B6C3F1 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. 1978).

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/week for 99–104 weeks (NTP 1982a).

Other CDD Congeners—Animal Studies. An increase in the prevalence of non-malignant kidney lesions were observed in female Sprague-Dawley rats administered 4 µg/kg/day 1,2,3,4,6,7,8-HpCDD over a lifetime; the lesions were described as glomerulonephritis, nephritis, nephropathy, hydronephrosis, and proteinuria; however, the incidences for specific lesions were not reported (Rozman et al. 2005). Studies with other congeners reported no renal effects following chronic-duration exposure to 0.34 or 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 $5x10^5$ and $1.3x10^6$ µg/kg/day of 2,7-DCDD in the feed in rats and mice, respectively (NCI/NTP 1979a).

2.11 DERMAL

Overview. Epidemiological and animal studies provide evidence that the skin is a target tissue following exposure to high doses of CDDs. 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. Chloracne may persist 20–30 years postexposure. Interindividual differences in susceptibility do exist and may be linked to

genetic polymorphism. Other dermal conditions reported include hypertrichosis, hyperpigmentation, and solar elastosis.

Dermal effects have been observed in animals following oral exposure to 2,3,7,8-TCDD or other congeners. The most commonly reported effects include hair loss and dermatitis in monkeys and mice exposed to 2,3,7,8-TCDD and hair loss in rats exposed to 1,2,3,7,8-PeCDD or 1,2,3,4,7,8-HxCDD. Dermal exposure to 2,3,7,8-TCDD can result in damage to sebaceous glands in mice.

Epidemiological Studies. The most 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-duration 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. 1989; Moses and Prioleau 1985; Poland et al. 1971) and after industrial accidents in several locations throughout the world (Goldman 1972; 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 study 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 that 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). 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, vesiculobullous 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 (within 3–6 months of exposure) and 15 late cases (within 7–10 months of exposure) of chloracne were seen, and in zone B, 9 delayed cases were observed. In all zones, 50 early-appearing 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 six children with type 4 chloracne and levels of 828, 1,690, and 7,420 ppt were found in three 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) (Needham et al. 1991). 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.

The results of a further examination of Operation Ranch Hand veterans were published (Burton et al. 1998). The cohort consisted of 930 exposed subjects and 1,200 comparison individuals who served in Southeast Asia (SEA) during the same period, but who were not involved with spraying herbicides. The study authors examined the associations between serum dioxin levels and: (1) chloracne; (2) occurrence of acne relative to the tour of duty in SEA; and (3) 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 ranges of initial dioxin levels in the low and high categories were 27.7–94.1 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 (OR) 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% confidence interval [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" PCBs were also detected, but it was estimated that in the workers, 2,3,7,8-TCDD contributed >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 TEQs in 1967 were 226–1,707 ppt, assuming a 10-year half-life and 1,173–9,366 ppt assuming a 5-year half-life. 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 eight 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 were 163–1,935 ppt (lipid basis), and by assuming a half-life of 7 years, the study authors estimated that the blood concentrations during the exposure were 545–9,894 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 two subjects with a disposition to hypersensitive skin reactions were not included in the analysis.

Two follow-up studies were located regarding dermal effects in humans following exposure to dioxins. The first follow-up was on a case-control study that originally included 159 cases of chloracne reported during the time period of 1969–1975 in TCDD-contaminated production of the herbicide, 2,4,5-T (Kogevinas et al. 1993, 1997). Only 50 survivors remained in 1996 and constituted the follow-up study cohort (Neuberger et al. 1999). Chloracne was found in 15 males and 1 female out of the surviving 50 cases originally diagnosed with chloracne. Similarly, a follow-up examination of 13 workers exposed 30 years ago (Jirasek et al. 1976) to TCDD in an industrial incident in an herbicide production plant was conducted (Pelclova et al. 2001). The current mean plasma level was 256 pg TCDD/g lipid (range: 14–760 pg/g lipid) in the follow-up study. Chloracne persisted in two individuals with their respective current TCDD levels of 760 and 420 pg/g lipids. In contrast, no chloracne was found in one individual with 600 pg TCDD/g lipids body burden.

Some insights regarding differences in individual susceptibility may be inferred from a genetic polymorphism study in CYP1A1 and GSTM1 in human populations exposed to PCB/CDF-contaminated

oil in Taiwan in 1979 (Tsai et al. 2006). About 2,000 people consumed the contaminated oil in the Yu-Cheng incident (see ATSDR 2023 for more details). Predominant dermal effects included chloracne, abnormal nails, hyperkeratosis, and skin allergies. In the genetic polymorphism study, 393 exposed and 181 control individuals were examined (Tsai et al. 2006). Among highly exposed individuals (>51 ppb PCB), combined CYP1A1-MspI mutant genotype and GSTM1-null genotype were linked to increased risk of chloracne (OR: 2.8). Among individuals with intermediate-duration exposures (≤51 ppb PCB), GSTM1-null genotype was linked to dermal allergies in both CYP1A1 genotypic groups.

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 et al. 1971; Suskind and Hertzberg 1984). In the cohort examined by Suskind and Hertzberg (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).

2,3,7,8-TCDD—Animal Studies. A number of changes in the skin have been observed in rodents and monkeys following oral exposure to 2,3,7,8-TCDD. In monkeys, skin lesions seen after a single oral dose or repeated dosing resemble the chloracne observed in humans. Nail loss and facial hair loss with acneiform lesions were observed in Rhesus monkeys following acute-duration 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 following intermediate-duration exposure to 0.011 µg/kg/day of 2,3,7,8-TCDD in the diet (Allen et al. 1977) or exposure to 0.1 µg/day, 3 days/week for 3 weeks (McNulty 1984). Skin thickening was observed in A2G-hr/+ mice exposed to a single dose of 75 µg/kg 2,3,7,8-TCDD (Greig 1984). A 10-week exposure to 1.3 µg/kg/day resulted in alopecia and edema in Swiss-Webster mouse dams (Thomas and Hinsdill 1979). No alteration in scratching behavior was observed in hairless mice administered via gavage 0.001 µg/kg/day TCDD for 54 days (Ono et al. 2010); however, dermal application of an external stimuli (distilled water or acetone/olive oil) resulted in increased scratching behavior at 0.0003 µg/kg/day. Chronic-duration exposure by gavage to

2,3,7,8-TCDD induced dermatitis in B6C3F1 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 B6C3F1 mice, dermatitis regressed after discontinuation of treatment (Della Porta et al. 1987). In contrast, no dermal effects were observed in Osborne-Mendel rats and B6C3F1 mice following chronic-duration exposure to 0.071 and 0.3 µg/kg/day of 2,3,7,8-TCDD, respectively, by gavage for 104 weeks (NTP 1982b).

The dermal toxicity to 2,3,7,8-TCDD following dermal exposure has been investigated. Epidermal hyperplasia and hyperkeratosis and involution of sebaceous glands were observed in newborn and adult hairless HRS/J mice dermally exposed 3 days/week for 2 weeks to 0.01 µg (newborns) or 0.1 µg (adults) (Puhvel and Sakamoto 1988); the reactions were similar in the adults and newborns. A similar exposure of haired HRS/J mice only resulted in involution of sebaceous glands (Puhvel and Sakamoto 1988). A 4-week exposure of HRS/J mice resulted in hyperkeratinization of the stratum corneum, epidermal hyperplasia, and an absence of sebaceous glands and follicles (Puhvel et al. 1982). 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/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/week for up to 104 weeks (NTP 1982a). Poland et al. (1984) evaluated the toxicity of 2,3,7,8-TCDD in several strains of mice. A once-a-week exposure of 0.3 µg 2,3,7,8-TCDD for 4 weeks resulted in sebaceous gland metaplasia, and epidermal hyperplasia, hyperkeratosis, and keratinized cyst formation in the hairless mutants of HRS/J, C57BL/6J, and C3H/HeN strains; no dermal lesions were observed in the haired mutants. Similar results were observed in hairless DBA/2J mice administered 1 µg 2,3,7,8-TCDD once a week for 4 weeks (Poland et al. 1984). There are a number of limitations in the reporting of the study, including lack of information on the number of animals tested and incidence data and the lack of a control group, which makes it difficult to compare across strains. Based on the severity scores, it appears that HRS/J mice may be more sensitive than the other strains.

Other CDD Congeners—Animal Studies. No dermal effects were found in Osborne-Mendel rats and B6C3F1 mice gavaged with 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, 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-duration exposure of Osborne-Mendel rats and B6C3F1 mice to $5x10^5$ and $1.3x10^6$ µg/kg/day of 2,7-DCDD, respectively, in the feed (NCI/NTP 1979a).

2.12 OCULAR

Overview. One epidemiological study reported eye irritation in workers with chloracne. Ocular effects (swelling and inflamed eye lids) have been reported in monkeys orally exposed to 2,3,7,8-TCDD. Ocular application of 2,3,7,8-TCDD, 2,7-DCDD, mixed HxCDD, or OCDD resulted in conjunctival inflammation in rabbits.

Epidemiological Studies. 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.

2,3,7,8-TCDD—Animal Studies. Ocular effects have been observed in Rhesus monkeys following acute- or intermediate-duration oral exposure to 2,3,7,8-TCDD. Swelling and inflamed eyelids were observed following a single-dose exposure of 70 µg/kg (McConnell et al. 1978a). Intermediate-duration exposure to 0.011 µg/kg/day in the diet or 0.1 µg/kg/day via gavage resulted in periorbital edema (Allen et al. 1977) and gavage administration of 0.1 µg/kg/day resulted in thickening and reddening of the eyelids (McNulty 1984). No ocular effects were observed in Osborne-Mendel or Sprague-Dawley rats following chronic-duration exposure to 0.071 µg/kg/day 2,3,7,8-TCDD by gavage for 104 weeks (NTP 1982b, 2006) or B6C3F1 mice administered 0.3 µg/kg/day for 2 years (NTP 1982b).

A single application of 2,000 µg 2,3,7,8-TCDD into the conjunctival sac of rabbits caused transient pain and conjunctival inflammation and delayed conjunctival chemosis (Schwetz et al. 1973); no corneal injury or iritis were observed.

Other CDD Congeners—Animal Studies. No ocular effects were found in Osborne-Mendel rats and B6C3F1 mice gavaged with 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, for 104 weeks (NCI/NTP 1980). Similarly, no effects were observed following chronic-duration exposure of Osborne-Mendel rats and B6C3F1 mice to $5x10^5$ and $1.3x10^6$ µg/kg/day of 2,7-DCDD, respectively, in the feed (NCI/NTP 1979a).

Transient pain and conjunctival inflammation, but no corneal injury or iritis, were observed in rabbits following a single application of 2,000 µg 2,7-DCDD, mixed HxCDD, or OCDD into the conjunctival sac of rabbits (Schwetz et al. 1973).

2.13 ENDOCRINE

Overview. Potential endocrine effects have been reported in studies of workers, Vietnam War veterans, Seveso cohort, communities living in areas with contaminated soil, and the general population. These studies have primarily focused on thyroid alterations and diabetes. Epidemiological studies have not found consistent alterations in thyroid hormone levels or thyroid disease. A number of studies have found associations between CDD exposure and an increased risk of diabetes.

CDDs were shown to alter endocrine parameters mostly in oral exposure rodent studies of 2,3,7,8-TCDD. One of the better characterized effects was a decrease in serum thyroxine (T4), caused apparently by CDD-induced T4 metabolism and excretion. A number of studies have evaluated thyroid hormone levels in animals orally exposed to 2,3,7,8-TCDD. Decreases in serum T4 levels have been observed in acute-duration studies at doses ≥5 µg/kg and in intermediate-duration studies at doses ≥0.016 µg/kg/day. Results for serum triiodothyronine (T3) levels are less consistent across studies, and TSH levels are increased following high-dose, acute-duration exposure but has not been observed at lower intermediate- or chronic-duration exposures. Some studies in rodents have also reported thyroid gland follicular cell hypertrophy. Decreases in serum T4 levels have also been observed following a single dose exposure to other congeners. A study comparing ED_{50} values across congeners found that 2,3,7,8-TCDD was the most potent, followed by 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD; the ED_{50} for 1,2,3,4,6,7,8-HpCDD was 3 orders of magnitude higher than for 2,3,7,8-TCDD.

Epidemiological Studies. Most epidemiological studies have not found consistent alterations in thyroid hormone levels or thyroid disease associated with 2,3,7,8-TCDD or CDD/CDF exposure; see Table 2-14 for study summaries. Elevated free or total serum T4 levels were observed in workers in highly exposed jobs (Mannetje et al. 2018), women who were premenarchal at the time of the Seveso accident (Chevrier et al. 2014); and one study found an inverse association between CDD/CDF/dioxin-like PCBs and free T4 in a study of anglers (Bloom et al. 2006). Other studies did not find alterations in serum T4 levels (Darnerud et al. 2010; Foster et al. 2005; Jennings et al. 1988; Lignell et al. 2016; Pavuk et al. 2003; Xu et al. 2019a; Zhang et al. 2010). Two studies found alterations in serum T3 levels; an association between CDD/CDF levels and free T3, but not total T3, was found in children living near a municipal waste incinerator (Xu et al. 2019a) and an inverse association between human milk CDD/CDF levels and total T3 was found in a general population study (Lignell et al. 2016). Occupational (Jennings et al. 1988), Seveso (Chevrier et al. 2014), or general population (Bloom et al. 2006; Darnerud et al. 2010) studies

Table 2-14. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Thyroid effects		
Occupational			
Jennings et al. 1988 Cross-sectional study of 18 workers at a 2,4,5-T production facility exposed to 2,3,7,8-TCDD as a result of an industrial accident and 15 workers not exposed	Not measured	T4	↔
		T3	↔
		TSH	↔
Mannetje et al. 2018 Cross-sectional study in former employees (n=245) of a phenoxy herbicide production facility in New Zealand	Work history and 2007–2008 serum 2,3,7,8-TCDD levels ≥10 pg/g lipid	Hypothyroid	↔, highly exposed job ↔, TCDD concentration
		Free T4	↑, highly exposed job ↔, TCDD concentration
		TSH	↔, highly exposed job ↔, TCDD concentration
Zober et al. 1994 Cohort morbidity study of 175 2,4,5-T production workers accidently exposed to 2,3,7,8-TCDD; referents were workers with no known 2,3,7,8-TCDD exposure	Geometric mean 2,3,7,8-TCDD levels back-calculated to the time of the accident: 148 ppt in workers without chloracne and 1,118 ppt in workers with severe chloracne	Thyroid diseases	↑, as compared to referents
Vietnam War veterans and Operation Ranch Hand veterans			
Pavuk et al. 2003 Cross-sectional study of U.S. Air Force veterans of Operation Ranch Hand (n=1,009) and veteran controls (n=1,429)	Groups: high (>94 ppt), low (>10 and <94 ppt), background (<10 ppt), controls (4.6 ppt)	Total T4	↔
		Free T4	↔
		T3 uptake	↔
		TSH	↑, high exposure
		Hyperthyroidism	↔
		Hypothyroidism	↔

Table 2-14. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Yi et al. 2014 Group of 111,726 Korean veterans of the Vietnam War exposed to Agent Orange	Self-reported exposure	Hypothyroidism	↑
		Nontoxic goiter	↑
		Hyperthyroidism	↔
		Thyroiditis	↔
		Autoimmune thyroiditis	↑
Seveso, Italy			
Chevrier et al. 2014 Prospective cohort study of participants in the Seveso Women's Health study (n=909 in 1976 and 260 in 1996); thyroid hormone levels measured in 1996 and 2008	Median serum 2,3,7,8-TCDD levels: 60.2 ppt in 1976 and 7.0 ppt in 1996	1996 total T4 levels 2008 total T4 levels	↑, 1976 TCDD ↔, 1996 TCDD
		1996 free T4 levels 2008 free T4 levels	↔, 1976 TCDD ↔, 1996 TCDD
		1996 free T3 levels 2008 free T3 levels	↔, 1976 TCDD ↔, 1996 TCDD
		1996 TSH levels 2008 TSH levels	↔, 1976 TCDD ↔, 1996 TCDD
Communities with contaminated soil			
Xu et al. 2019a Cross-sectional study of 10-year-old children (n=82) living near a municipal waste incinerator and children (n=49) living in an uncontaminated area in China	Mean blood CDD/CDF levels: 3.40 pg TEQ/g lipid for exposed group and 2.77 pg TEQ/g lipid for controls	Free T3	↑
		T3	↔
		Free T4	↔
		T4	↔
		TSH	↔
Zhang et al. 2010 Cross-sectional study of 25 pregnant women living in an e-waste area and 25 pregnant women living in an uncontaminated area in China	Median CDD/CDF cord blood levels: 0.041 pg TEQ/g lipid	Total T4	↔
		TSH	↔

Table 2-14. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
General population			
Bloom et al. 2006 Prospective study of 38 anglers participating in the New York Angler Cohort study	Median CDD/CDF/dioxin-like PCBs serum concentration: 5.963 pg TEQ/g	Total T4	↔
		Free T4	↓
		T3	↔
		TSH	↔
Darnerud et al. 2010 Prospective study of 180 mother-infant pairs living in Sweden	Median CDD/CDF human milk level: 9 pg TEQ/g lipid	Free T4	↔
		Total T3	↔
		TSH	↔
Foster et al. 2005 Cross-sectional examination; pregnant women (n=150) attending a prenatal diagnosis clinic	Mean serum lipid-adjusted dioxin-like activity TEQs: 0.34 pg/g	T4	↔
		TSH	↔
Lignell et al. 2016 Prospective study of 91 mother infant pairs living in Sweden; same population as Darnerud et al. (2010)	Median CDD/CDF human milk level: 9 pg TEQ/g lipid	Total T3	↓
		Free T4	↔
		TSH	↔
Diabetes			
Occupational			
Calvert et al. 1999 Cross-sectional study in workers (n=281 exposed and 260 controls) exposed >15 years before in the production of 2,4,5-trichlorophenol in the United States	Mean TCDD level in exposed: 220 pg/g lipid; in controls: 7 pg/g; the half-life extrapolated concentrations to the time exposure stopped averaged 1,900 pg/g	Diabetes	↔

Table 2-14. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Mannetje et al. 2018 Cross-sectional study in former employees (n=245) of a phenoxy herbicide production facility in New Zealand	Work history and 2007–2008 serum 2,3,7,8-TCDD levels ≥10 pg/g lipid	Diabetes	↑, highly exposed job ↔, TCDD concentration
		Glucose	↑, highly exposed job ↔, TCDD concentration
Pelcl et al. 2018 Cross-sectional study of eight former workers at a 2,4,5-T production facility and eight controls	Median 2,3,7,8-TCDD levels: 112 pg/g lipid in workers and 12 pg/g lipid in controls	Prevalence of diabetes	↑
Yamamoto et al. 2015a Cross-sectional study of 678 male workers at 36 municipal and private waste incineration plants in Japan	4th quartile CDDs levels: ≥8.98 pg TEQ/g lipid	Diabetes mellitus	↑, 4th quartile
Vietnam War veterans and Operation Ranch Hand veterans			
Henriksen et al. 1997 Cross-sectional study of Operation Ranch Hand veterans (n=989) and a comparison group of (1,276)	Median serum 2,3,7,8-TCDD levels: background 5.7 ppt, low 52.7 ppt, high 197.5 ppt, control ≤4.0 ppt	Risk of diabetes mellitus	↑, high exposure
Kang et al. 2006 Health survey of 1,499 Vietnam veterans and 1,428 non-Vietnam veterans assigned to chemical operations jobs conducted using a computer-assisted telephone interview system	Serum TCDD analyzed on subgroups; a self-reported history of spraying Agent Orange used to categorize exposed	Diabetes	↑
Longnecker and Michalek 2000 Cross-section study of 1,197 veterans in the Air Force Health Study who never had contact with dioxin-contaminated herbicides	4th quartile TCDD level: ≥5.2 pg/g lipid	Diabetes	↑

Table 2-14. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Michalek et al. 1999a Cross-sectional study of Air Force Ranch Hand veterans exposed to TCDD in Vietnam (1962–1971) and veteran controls not exposed; 1992 follow-up High Ranch Hand exposure Diabetics (n=43) Nondiabetics (n=205) Low Ranch Hand exposure Diabetics (n=36) Nondiabetics (n=211) Background Ranch Hand exposure Diabetics (n=32) Nondiabetics (n=344) Controls Diabetics (n=125) Nondiabetics (n=996)	Median current serum 2,3,7,8-TCDD in background, low-, and high-exposure groups: 5.7, 15, and 45.8 ppt, respectively	Fasting glucose	↔
		Insulin	↑, high exposure, non-diabetics
USAF 1991 Cross-sectional report of 866 Operation Ranch Hand personnel and a comparison group of 1,198	Not measured	Glucose intolerance	↑
		Risk of diabetes	↑
Yi et al. 2014 Group of 111,726 Korean veterans of the Vietnam War exposed to Agent Orange	Self-reported exposure	Diabetes	↑
Seveso, Italy			
Bertazzi et al. 2001 Retrospective cohort mortality study of Seveso residents (n=804 in zone A and n=5,941 in zone B); follow-up to the Bertazzi et al. (1993, 1997) studies	Not reported	Diabetes deaths	↔

Table 2-14. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Pesatori et al. 1998 Retrospective cohort study of the 15-year follow-up of the Seveso cohort (n=3,987 deaths)	Soil contamination levels in three zones used as a biomarker of exposure	Diabetes deaths	↔, males ↑, females
Warner et al. 2013 Retrospective cohort study of female residents of Seveso at the time of the accident	4th quartile serum 2,3,7,8-TCDD: >135 ppt	Diabetes	↔
Communities with contaminated soil			
Chang et al. 2010a Cross-sectional study of 1,234 people living near a former PCP production facility in Taiwan	Median CDD/CDF concentration: 20.5 pg TEQ/g lipid	Fasting blood glucose	↑
		Insulin resistance	↑
		Pancreatic β-cell function	↔
Chang et al. 2011b Cross-sectional study of 1,449 people living near a former PCP production facility in Taiwan	Median CDD/CDF concentration: 33.2 pg TEQ/g lipid	HOMA-IR	↑
		HOMA-β-cell	↔
Chang et al. 2016 Cross-sectional study of 2,876 people living near a former PCP production facility in Taiwan	Mean CDD/CDF concentration: 21.9–44.8 pg TEQ/g lipid	Blood glucose	↑
		HOMA-IR	↑
Cranmer et al. 2000 Cross-sectional study of 69 individuals living within 25 miles of a Superfund site	TCDD levels ranged from 2 to 94 ppt Lower serum levels 2–15 ppt (n=62); higher levels >15 ppt (n=7)	Plasma insulin concentrations after a 75-g glucose load	↑

Table 2-14. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Huang et al. 2015 Cross-sectional study of 2,898 adults living near a former PCP production facility in Taiwan	2nd tertile CDD/CDF serum level: 20–63 pg TEQ/g lipid	Diabetes	↑, 2nd tertile
General population			
Fierens et al. 2003 Volunteer-case study in Belgium; environmental exposure to CDDs, CDFs, PCBs+12 marker PCB (not TEQs)	Total TEQs (geometric mean): Cases (n=9) 64.2 pg/g Controls (n=248) 32.8 pg/g	Diabetes	↑, with higher dioxins

↑ = association; ↓ = inverse association; ↔ = no association; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; HOMA-IR = homeostatic model assessment of insulin resistance; HOMA-β-cell = homeostatic model assessment of pancreatic beta-cell function; PCB = polychlorinated biphenyl; PCP = pentachlorophenol; T3 = triiodothyronine; T4 = thyroxine; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalency; TSH = thyroid-stimulating hormone

have not found associations between CDDs and serum T3 levels. Apart from a study of Operation Ranch Hand veterans, which found increased serum TSH levels in a high-exposure group (Pavuk et al. 2003), no associations between CDDs and TSH levels have been found (Bloom et al. 2006; Chevrier et al. 2014; Darnerud et al. 2010; Foster et al. 2005; Jennings et al. 1988; Lignell et al. 2016; Mannetje et al. 2018; Xu et al. 2019a; Zhang et al. 2010).

Several studies have evaluated the possible associations between CDD exposure and thyroid diseases. A 35-year follow-up study of workers exposed to 2,3,7,8-TCDD during the BASF accident found an 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 and <1,000 ppt; the incidence did not differ between the groups. Among Korean Vietnam War veterans who self-reported exposure to Agent Orange, there was an increase in the prevalence of hypothyroidism, nontoxic goiter, and autoimmune thyroiditis, but no effect of hyperthyroidism or thyroiditis prevalence (Yi et al. 2014). Another study of Vietnam veterans did not find associations between serum 2,3,7,8-TCDD and the prevalence of hyperthyroidism or hypothyroidism (Pavuk et al. 2003).

Epidemiological studies have also evaluated possible associations between CDD exposure and the risk of diabetes; see Table 2-14 for study summaries. A number of studies have found associations between 2,3,7,8-TCDD or CDD blood levels and increased risk of diabetes among workers (Mannetje et al. 2018; Pelcl et al. 2018; Yamamoto et al. 2015a), Vietnam War veterans (Henriksen et al. 1997; Kang et al. 2006; Longnecker and Michalek 2000; USAF 1991; Yi et al. 2014), communities with contaminated soil (Huang et al. 2015), and the general population (Fierens et al. 2003). Two studies evaluating the Seveso cohort did not find an increased risk of diabetes or diabetes deaths (Bertazzi et al. 2001; Warner et al. 2013), although one study found an increased risk of diabetes deaths in women, but not in men (Pesatori et al. 1998).

2,3,7,8-TCDD—Animal Studies. Animal studies evaluating endocrine outcomes have primarily focused on the thyroid. A number of studies have reported significant decreases in serum T4 levels in rats following acute- or intermediate-duration exposure to 2,3,7,8-TCDD; a summary of these studies is presented in Table 2-15. The magnitude of the decrease was 21–65% and effective doses were as low as 5 µg/kg following a single dose (Viluksela et al. 2004) and 0.016 µg/kg/day following a 13-week exposure (NTP 2006). Results for serum T3 levels were less consistent across studies with some studies reporting 9–43% increases (Bastomsky 1977; Hermansky et al. 1988; Potter et al. 1986) and other studies

not finding significant alterations at doses associated with T4 level decreases (Fan and Rozman 1995; Raasmaja et al. 1996; Sewall et al. 1995; Van Birgelen et al. 1995; Viluksela et al. 2004). At higher doses, increases in TSH levels were observed in rats acutely exposed to 2,3,7,8-TCDD (Bastomsky 1977; Potter et al. 1986), but not at lower intermediate- or chronic-duration doses (NTP 2006). Bastomsky (1977) suggested that the decrease in T4 appeared to be the result of an increased biliary excretion of T4-glucuronide, and this was attributed to induction of uridine 5'-diphospho-glucuronosyltransferase (UDP-glucuronyltransferase) by 2,3,7,8-TCDD; UDP-glucuronyltransferase catalyzes glucuronidation of T4 and clearance. The increase in T3 was consistent with increased thyroid secretion from thyrotropin (TSH) stimulation. A small number of studies have evaluated potential histopathological alterations. Thyroid gland follicular cell hypertrophy was observed in female Sprague-Dawley rats exposed to 0.016 µg/kg/day for 14 weeks, 0.032 µg/kg/day for 31 weeks, 0.071 µg/kg/day for 53 weeks, or 0.032 µg/kg/day for 2 years (NTP 2006) and in BALB/c mice exposed to 0.09 µg/kg/day for 28 days (Maranghi et al. 2013). Thyroid gland follicular cysts were observed in male Sprague-Dawley rats exposed to 0.1 µg/kg/day for 2 years (Kociba et al. 1978). Other studies have not found histological alterations following acute-duration (Potter et al. 1986) or chronic-duration exposure (NTP 1982b).

Table 2-15. Results of Studies Evaluating Thyroid Outcomes in Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose (µg/kg)	T3	T4	TSH	Histopathology	Reference
Sprague-Dawley rat, once	25	↑ (43%)	↓ (48%)	↑ (356%)		Bastomsky 1977
Long-Evans rat, once	0.15		↓ (30%)			Crofton et al. 2005
Long-Evans rat, once	12	↔	↓ (44%)			Fan and Rozman 1995
Sprague-Dawley rat, 3 days	40	↑ (9%)	↓ (65%)			Hermansky et al. 1988
Sprague-Dawley rat, once	6.25	↑ (12%)	↓ (50%)	↑ (138%)	↔	Potter et al. 1986
Long-Evans rat, once	10	↔	↓ (58%)			Raasmaja et al. 1996
Sprague-Dawley rat, once	5	↔	↓ (40%)			Viluksela et al. 2004
Sprague-Dawley rat, 4 weeks	1				↔	Harrill et al. 2015

Table 2-15. Results of Studies Evaluating Thyroid Outcomes in Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose (µg/kg)	T3	T4	TSH	Histopathology	Reference
Sprague-Dawley rat, 10 weeks	0.03		↓ (50%)			Li and Rozman 1995
BALB/c mouse, 28 days	0.09				Follicular cell hypertrophy	Maranghi et al. 2013
Sprague-Dawley rat, 14 or 31 weeks	0.022		↓ (25–34%)	↔	Follicular cell hypertrophy after 14 weeks	NTP 2006
Sprague-Dawley rat, 30 weeks	0.036	↔	↓ (25%)			Sewall et al. 1995
Sprague-Dawley rat, 13 weeks	0.047	↔	↓ (21%)			Van Birgelen et al. 1995
Sprague-Dawley rat, 13 weeks	0.8	↔	↓ (47%)			Viluksela et al. 1994
Sprague-Dawley rat, 2 years	0.1				↔	Kociba et al. 1978
Osborne-Mendel rat, 2 years	0.071				↔	NTP 1982b
B6C3F1 mouse, 2 years	0.3				↔	NTP 1982b
Sprague-Dawley rat, 53 weeks	0.0071	↑ (14%)	↔	↔		NTP 2006
Sprague-Dawley rat, 2 years	0.032				Follicular cell hypertrophy	NTP 2006

↑ = association; ↓ = inverse association; ↔ = no association; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone

Adverse effects have also been observed in other endocrine tissues of laboratory animals orally exposed to 2,3,7,8-TCDD. NTP (2006) found significant increases in the incidence of hyperplasia of the adrenal cortex in female rats administered ≥0.016 µg/kg/day 2,3,7,8-TCDD 5 days/week for 2 years; atrophy was observed at the highest dose tested (0.071 µg/kg/day). The cortical atrophy was characterized by the loss of cortical epithelial cells within the zona fasciculata and zona reticularis with a subsequent reduction in

cortical thickness. Pitt et al. (2000) examined the effect of a single dose gavage exposure to 10 µg/kg 2,3,7,8-TCDD on pituitary-adrenal gland function in male Sprague-Dawley rats. Ten days after exposure, no significant alterations in pituitary or plasma adrenocorticotropin levels, pituitary weight, adrenal or plasma corticosterone levels, or adrenal gland weight were observed. A 46% decrease in the ratio of adrenocorticotropin to corticosterone levels was observed; although the alteration was not statistically significant due to the low statistical power of the study, the investigators noted that the change was biological significant. In *ex vivo* studies, Pitt et al. (2000) also found no significant alterations in corticotrophin-releasing-hormone-stimulated adrenocorticotropin secretion from the pituitary gland or adrenocorticotropin-stimulated corticosterone secretion from the adrenal gland.

A series of studies conducted by Blackwell et al. (1998) examined the potential association between 2,3,7,8-TCDD exposure and type II diabetes. There were no alterations in serum glucose levels in male C57BL/6J mice maintained on a diabetic diet (high fat, high simple carbohydrate diet) or a normal diet for 2 weeks prior to a single gavage administration of 1–60 µg/kg. Similarly, repeated exposure to 0.0015 or 0.15 µg/kg/day 2,3,7,8-TCDD for 4, 8, or 12 weeks or 0.0015–0.15 µg/kg/day for 16 weeks did not alter serum glucose levels in resting or fasting mice on either diet. A decrease in serum glucose levels was observed in male Sprague-Dawley rats 7 days after receiving a single dose of 40 µg/kg 2,3,7,8-TCDD (Fletcher et al. 2005b); at earlier time points (6 or 24 hours after dosing), no changes in serum glucose levels were found. No significant alterations in serum glucose or insulin levels were observed in female Sprague-Dawley rats administered an initial loading dose of 3.2 µg/kg 2,3,7,8-TCDD followed by a maintenance dose of 0.32 µg/kg every third day for 20 weeks (Croutch et al. 2005). However, decreases in serum insulin-like growth factor-I and hepatic phosphoenolpyruvate carboxykinase (PEPCK) protein levels were observed and suggest an early effect on energy metabolism; decreases in PEPCK activity and messenger ribonucleic acid (mRNA) levels were found, but the changes were not statistically significant at most time points.

Significant decreases in plasma glucose levels and liver glycogen content were observed in female Long-Evans rats administered a single dose of ≥5 µg/kg, in male Long-Evans rats significant decreases were observed at ≥10 µg/kg (Viluksela et al. 1999). When a pair-fed control group was used as the comparison group rather than *ad-libitum*-fed controls, the only significant difference in plasma glucose level was in males exposed to 50 µg/kg. PEPCK activity in the liver was significantly decreased in male rats exposed to ≥5 µg/kg and female rats at ≥10 µg/kg. In the pair-fed controls, PEPCK was significantly higher than the *ad libitum* controls and 50 µg/kg 2,3,7,8-TCDD exposed rats. Additionally, significant increases in plasma glucogenic amino acids were observed in females (males were not examined) at ≥10 µg/kg and

plasma ketogenic amino acids were increased at ≥5 µg/kg. As compared to the pair-fed control group only, the increases in plasma glucogenic and ketogenic amino acids were significant only in the 50 µg/kg group. The investigators noted that the lack of change in plasma urea levels suggested decreased utilization of amino acids for gluconeogenesis, which is likely due to the decreased activity of PEPCK. Viluksela et al. (1999) similarly exposed Han/Wistar rats and found significant decreases in female rats administered ≥500 µg/kg; no significant alterations were observed in the male rats. No alterations in liver glycogen content or plasma amino acid levels were observed; however, a decrease in PEPCK activity was observed in the males exposed to ≥50 µg/kg. Similar to the findings for 2,3,7,8-TCDD, no alterations in serum glucose or insulin levels were observed in female Sprague-Dawley rats administered an initial loading dose of 80 µg/kg HxCDD followed by maintenance doses of 8 µg/kg every 9 days for 20 weeks (Croutch et al. 2005), but decreases in PEPCK protein levels and nonsignificant decreases in PEPCK mRNA and activity levels and insulin growth factor-I levels were observed.

Intermediate-duration exposure to 0.071 µg/kg/day 2,3,7,8-TCDD (5 days/week) resulted in a significant increase in minimal acinar cytoplasmic vacuolization in the pancreas of female Harlan Sprague-Dawley rats (NTP 2006); the lesions were observed after 31 weeks of exposure, but not after 14 weeks. A 2-year exposure to ≥0.032 µg/kg/day resulted in significant increases in the incidence of acinar cytoplasmic vacuolization (NTP 2006; Nyska et al. 2004). At 0.071 µg/kg/day, there were also significant increases in the incidence of chronic active inflammation and acinar atrophy.

Other CDD Congeners—Animal Studies. A small number of studies have examined potential endocrine effects in laboratory animals. An ED_{30} for serum T4 levels (30% reduction in serum levels, as compared to controls) of 1.51 µg/kg/day (95% CI of 1.10–1.92 µg/kg/day) was estimated in female Long-Evans rats administered 0.003–10 µg/kg/day 1,2,3,7,8-PeCDD in corn oil for 4 days (Crofton et al. 2005). Simanainen et al. (2002) estimated the ED_{50} values for decreases in serum T4 levels in male Han/Wistar and Long-Evans rats receiving a single gavage dose of several CDD congeners. The ED_{50} values were 1.4, 4.1, and 99 µg/kg in Han/Wistar rats administered 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD, respectively. In Long-Evans rats, the ED_{50} values were 3.6, 21, and 47 µg/kg, respectively.

2.14 IMMUNOLOGICAL

Overview. The available data provide strong evidence that immunotoxicity is a sensitive target of CDD toxicity. Epidemiological studies of workers, Seveso cohort, Vietnam War veterans, communities living

in areas with contaminated soil, and the general population and experimental studies in monkeys, rats, mice, guinea pigs, and hamsters have evaluated immunological outcomes. The epidemiological studies provide suggestive evidence; however 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 limited number of studies evaluating immune competence in humans.

Studies in laboratory animals have reported effects on primary and secondary immune organs and adaptive immune function. Decreases in thymus weight and thymic atrophy are commonly reported in oral exposure studies, with respective LOAELs of ≥0.66 and ≥0.8 µg/kg for acute-duration exposure and ≥0.005 and 0.016 µg/kg/day for intermediate-duration exposure; the lowest LOAEL for thymic atrophy following chronic-duration exposure is 0.0071 µg/kg/day. The most well-studied alteration in immune function is impaired host resistance and impaired response to antigens. Impaired immune function has been observed at doses of 0.01 µg/kg following acute-duration oral exposure, 0.0011 µg/kg/day following intermediate-duration exposure, and 0.00012 µg/kg/day following chronic-duration exposure.

The immune system is also a sensitive target of toxicity for other CDD congeners. Decreases in thymus weights have been observed in animals orally exposed to 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, or 1,2,3,4,6,7,8-HpCDD. Impaired immune function has also been observed in animals orally exposed to 2,7-DCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD.

Epidemiological Studies. A number of epidemiological studies have evaluated the potential immunotoxicity of 2,3,7,8-TCDD and other CDD congeners; the results of these studies are summarized in Table 2-16. These studies have evaluated a number of immune endpoints including immunoglobulin (Ig) levels, complement and cytokine levels, lymphocyte levels and phenotypes, natural killer (NK) cell levels, and tests of immune function (antibody responses, disease resistance, delayed hypersensitivity, and hypersensitivity). Consistent results have not been observed across studies, which likely reflects differences in exposures, differences in the populations, and the tests used to assess immunotoxicity.

Table 2-16. Immunological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Occupational			
Jennings et al. 1988 Cross-sectional study of 18 workers at a 2,4,5-T production facility exposed to 2,3,7,8-TCDD released after an accident and 15 matched controls		Immunoglobulins: IgA, IgG, IgM, IgD, IgE	↔
		Total lymphocyte, T cell count, T-helper cells, T-suppressor cells	↔
		Natural killer cells	↑
		Lymphocyte proliferation test response to phytohemagglutinin A	↔
Jung et al. 1998 Cross-sectional study of 29 former workers at a German pesticide facility highly exposed to 2,3,7,8-TCDD and 28 external controls	Median serum 2,3,7,8-TCDD level: 217 pg/g lipid in exposed workers and 3.9 pg/g lipid in controls	Frequency of infectious diseases	↔
		Immunoglobulins: IgA, IgG, IgM	↔
		Tetanus antibodies 3 weeks after vaccination	↔
		Lymphocyte subgroups: activated T cells	↓
		Lymphocyte subgroups: B cells, activated B cells, T-helper cells, $CD3^+$ killer cells, natural killer cells	↔
		Lymphocyte proliferation test response to phytohemagglutinin, pokeweed mitogen, or tetanus toxoid	↔

Table 2-16. Immunological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Halperin et al. 1998 Cross-sectional study of 259 workers at two 2,4,5-T production facilities in the United States and 243 unexposed referents	Serum 2,3,7,8-TCDD levels: Workers: 3rd quintile: 52–125 pg/g lipid 5th quintile: 298–3,389 pg/g lipid Referents: 6.4 pg/g lipid (random sample of referents)	Immune markers: CD3, CD4, CCD4/CDW29, CD4/CD45, CD8/CD11B+ Lymphocytes, neutrophils IgG Complement Proliferation in response to phytohemagglutinin	↔
		CD26 (activated T cells)	↓, 3rd quintile
		Lymphocyte proliferation test response to mitogens (concanavalin and pokeweed)	↑, 5th quintile
Hosnijeh et al. 2011 Cross-sectional study of 45 workers at a chlorophenoxy herbicide facility in the Netherlands; 108 non-exposed workers (39 from same facility and 69 from a comparable facility) were also examined	Current serum TCDD levels: 3.3 ppt in exposed workers and 1.2 and 0.4 ppt in control groups	Immunoglobulins: IgG, IgA, IgM, IgD, IgE	↔
		Complement 3 or Complement 4	↔
Hosnijeh et al. 2012a, 2012b Cross-sectional study of 85 workers at a chlorophenoxy herbicide facility in the Netherlands; 47 workers were exposed to high levels of 2,3,7,8-TCDD and 38 were exposed to low levels	Current serum TCDD levels: 3.25 ppt in high-exposed workers and 1.07 ppt in low-exposed workers	Cytokines: IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, granulocyte-macrophage colony stimulating factor, tumor necrosis factor-α, epidermal growth factor, eotaxin, granulocyte colony stimulating factor, melanoma growth stimulating activity/growth related oncogene, interferon gamma-induced protein 10, monocyte chemotactic protein-1, macrophage derived chemokine, macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, soluble CD40 ligand	↔

Table 2-16. Immunological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Cytokines: fibroblast growth factor 2, fractalkine, transforming growth factor-α	↓
		Leukocytes	↔
		B cells	↓
		IgG/IgA+ memory B cells	↓
		T cells	↔
		CD4/CD8 ratio	↔
Neubert et al. 1993, 1995 Cross-sectional study of 12 workers in Germany exposed to CDDs/CDFs and 77 referents	Median serum TCDD level of 41.5 ppt and total CDD/CDFs of 133.3 TEQ ppt Referents divided into three groups, median serum TCDD levels 2, 5, and 11 ppt in the low-, medium-, and high-level subgroups; median CDD/CDF levels of 18, 28, and 49 TEQ ppt, respectively	$CD4^{+}CD45R0$	↑
		Lymphocyte proliferation in response to pokeweed mitogen, phytohemagglutinin, concanavalin A	↔
Ott et al. 1994 Retrospective cohort study of 138 workers exposed to 2,3,7,8-TCDD due to an accident at a trichlorophenol facility in Germany	Current 2,3,7,8-TCDD serum levels: <1–553 ppt Back calculated 2,3,7,8-TCDD serum levels: 3.3–12,000 ppt	IgA IgG	↑, current TCDD, back-calculated TCDD
		Complement C4	↑, current TCDD
		IgM Complement C3 Lymphocytes, natural killer cells, B-cells, T-cells, T-helper cells, T-suppressor cells, CD4/CD8 ratio	↔

Table 2-16. Immunological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Tonn et al. 1996 Cross-sectional study of 11 workers at a 2,4,5-trichlorophenol production facility in Germany and 10 matched controls	Mean serum 2,3,7,8-TCDD levels: 329.5 pg/g lipid in workers	Lymphocyte subsets	↔
		Lymphocyte proliferation in response to phytohemagglutinin and pokeweed	↔
		Response to human lymphocyte antigen-allogeneic lymphocytes and interleukin-2 boosted proliferation	↓
Zober et al. 1994 Cohort morbidity study of 175 2,4,5-T production workers accidently exposed to 2,3,7,8-TCDD; referents were workers with no known 2,3,7,8-TCDD exposure	Geometric mean 2,3,7,8-TCDD levels back calculated to the time of the accident: 148 ppt in workers without chloracne and 1,118 ppt in workers with severe chloracne	Infectious and parasitic disease	↑, severe chloracne subgroup ↑, TCDD levels >1,000 ppt
Seveso, Italy			
Baccarelli et al. 2004 Retrospective cohort study of 62 adults from zones A and B and 59 controls	Not reported	IgG	↓
		IgM, IgA, complement C3, complement #4	↔
Vietnam War veterans and Operation Ranch Hand veterans			
Kim et al. 2003 Cross-sectional study of Korean Vietnam War veterans; 24 veterans with service in Agent Orange sprayed areas with chronic illness, 27 veterans with service in Agent Orange sprayed areas without chronic illness, and 36 age-matched controls with no Vietnam War military service	Not measured	Total and differential leukocyte counts	↔
		IgE	↑, both veteran groups
		IgG1	↓, veterans with illness
		Interferon-γ	↓, veterans with illness
		IL-4	↑, both veteran groups
		Tumor necrosis factor-α, IL-10	↔

Table 2-16. Immunological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Michalek et al. 1999b Cross-sectional study 914 Operation Ranch Hand veterans (n=393 background exposure, n=261 low exposure, and n=260 high exposure) and 1,186 veterans not involved in spraying herbicides	Median current serum 2,3,7,8-TCDD levels: background exposure group 5.7 ppt; low-exposure group 52.8 ppt; high-exposure group 194.7 ppt; comparison group 4.0 ppt	$CD16^+CD56^+CD3^+$	↓, high-exposure group
		CD3, CD5, $CD4^+CD3+$, $CD8^+CD3^+$, CD20, $CD16^+CD56^+CD3^-$, CD25, $CD25^+CD3^+$	↔
		IgA, IgG, IgM	↔
USAF 1991 Cross-sectional study of 866 Operation Ranch Hand personnel and a comparison group of 1,198	Not reported	IgA	↑
		IgG, IgM	↔
Communities living in areas with contaminated soil			
Evans et al. 1988 Cross-sectional study; follow-up to the Hoffman et al. (1986) study examining subjects who had anergy or relative anergy on skin testing, 28/50 exposed and 15/27 unexposed subjects were re-evaluated	Not measured	Delayed hypersensitivity response	↔
		Total lymphocyte count, T-cell subset population	↔
		Lymphocyte proliferation response to phytohemagglutinin, concanavalin A, pokeweed mitogen, tetanus toxoid	↔
		IgG	↔
Hoffman et al. 1986 Cross-sectional study of 154 people living in Quail Run Mobile Home Park and exposed to 2,3,7,8-TCDD in soil and 155 control subjects	Years of residence in the park used as surrogate for exposure	Lymphocyte proliferation response to phytohemagglutinin, concanavalin A, pokeweed mitogen, tetanus toxoid	↔
		IgG	↔
		Delayed-type hypersensitivity skin test	↑
		Lymphocyte subsets: CD3, CD4, CD8, CD11	↔

Table 2-16. Immunological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Webb et al. 1989 Cross-sectional study of 41 individuals in Missouri exposed TCDD-contaminated soil by living in an area with contaminated soil, riding or caring for horses in contaminated stable arenas, or working in a hexachlorophene production facility or truck terminals where the grounds were sprayed with TCDD-contaminated waste oil	Adipose tissue 2,3,7,8-TCDD levels: 16 subjects had levels <20 ppt, 13 had levels 20–60 ppt, and 12 had levels >60 ppt	Delayed-type hypersensitivity skin test	↔
		IgG	↑
		IgA, IgM	↔
		Lymphocyte subsets: CD3, CD8	↑
		Lymphocyte subsets: CD4, CD14, CD18	↔
		Lymphocyte proliferation response to phytohemagglutinin, concanavalin A, pokeweed mitogen, tetanus toxoid	↔
General population			
Nakamoto et al. 2013 Cross-sectional study of 1,063 men and 1,201 women in Japan	Median serum total CDDs/CDFs: 9.8 pg TEQ/g lipid	Atopic dermatitis	↔, 4th quartile ↓, trend
		Allergic rhinitis	↔, 4th quartile ↔, trend

↑ = association; ↓ = inverse association; ↔ = no association; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; Ig = immunoglobin; IL = interleukin; TEQ = toxic equivalency

Four studies found associations between CDD exposure and serum IgG levels, with two studies finding positive associations (Ott et al. 1994; Webb et al. 1989) and two studies finding inverse associations (Baccarelli et al. 2004; Kim et al. 2003). Other studies found no association (Evans et al. 1988; Hoffman et al. 1986; Jennings et al. 1988; Jung et al. 1998; Halperin et al. 1998; Hosnijeh et al. 2011; Michalek et al. 1999b; USAF 1991). Similarly, studies by Ott et al. (1994) and USAF (1991) found associations between CDD exposure and serum IgA levels in workers and Ranch Hand veterans, respectively, but most studies did not find an association (Baccarelli et al. 2004; Jennings et al. 1988; Jung et al. 1998; Hosnijeh et al. 2011; Michalek et al. 1999b; Webb et al. 1989). In general, most studies looking at possible associations with other immunoglobulins have not found associations (see Table 2-16). Some studies have found associations between CDD exposure and levels of specific cytokines or complement (Hosnijeh et al. 2012a, 2012b; Kim et al. 2003; Ott et al. 1994); however, interpretation is limited by the small number of studies and differences in the cytokines and complement examined. Similarly, several studies have examined possible associations with altered lymphocyte phenotypes (Evans et al. 1988; Jennings et al. 1988; Jung et al. 1998; Halperin et al. 1998; Hoffman et al. 1986; Hosnijeh et al. 2012a, 2012b; Michalek et al. 1999b; Neubert et al. 1993, 1995; Ott et al. 1994; Tonn et al. 1996; Webb et al. 1989), B and T cell levels (Evans et al. 1988; Hosnijeh et al. 2012a, 2012b; Kim et al. 2003; Ott et al. 1994), and NK cell levels (Jennings et al. 1988; Ott et al. 1994), but the findings are not consistent across studies or populations; see Table 2-16 for individual study results.

A number of epidemiological studies have evaluated the potential impairment of immune function. Halperin et al. (1998) reported an impaired response to the mitogens concanavalin and pokeweed in lymphocyte proliferation tests and a normal response to phytohemagglutinin among workers at two 2,4,5-T production facilities. Other studies found no response to phytohemagglutinin (Evans et al. 1988; Hoffman et al. 1986; Jennings et al. 1988; Jung et al. 1998; Neubert et al. 1993, 1995; Tonn et al. 1996; Webb et al. 1989), pokeweed (Evans et al. 1988; Hoffman et al. 1986; Jung et al. 1998; Neubert et al. 1993, 1995; Tonn et al. 1996; Webb et al. 1989), concanavalin A (Evans et al. 1988; Hoffman et al. 1986; Neubert et al. 1993, 1995; Webb et al. 1989), or tetanus toxoid (Evans et al. 1988; Hoffman et al. 1986; Jung et al. 1998; Webb et al. 1989). Zober et al. (1994) reported an increased incidence of infectious and parasitic diseases among highly exposed workers, and Jung et al. (1998) reported no association between serum 2,3,7,8-TCDD levels and the frequency of infectious diseases in former workers. Delayed-type hypersensitivity was reported in one of the studies examining residents exposed to contaminated soil (Hoffman et al. 1986), but not in the other two studies (Evans et al. 1988; Webb et al. 1989). A general population study reported an inverse trend for atopic dermatitis and serum total CDD/CDF levels and no association for allergic rhinitis (Nakamoto et al. 2013).

2,3,7,8-TCDD—Animal Studies. A large number of studies have evaluated the immunotoxicity of 2,3,7,8-TCDD in laboratory animals. These studies reported alterations in immune tissue weights and histopathology and immunosuppressive outcomes.

Effects on primary and secondary immune organs. Decreased thymus weights have been observed in several animal species; the lowest LOAELs are 0.66 µg/kg in rats (Fletcher et al. 2001) and 1 µg/kg in mice (Silkworth et al. 1989b; Smialowicz et al. 1997) following acute-duration oral exposure and 0.014 µg/kg/day in rats (Van Birgelen et al. 1995) and 0.005 µg/kg/day in guinea pigs (Decaprio et al. 1986) following intermediate-duration oral exposure. Thymic atrophy is also commonly reported in laboratory animals; the lowest LOAELs are 70 µg/kg in monkeys (McConnell et al. 1978a), 25 µg/kg in rats (De Heer et al. 1994b), 280 µg/kg in mice (Hanberg et al. 1989), 48 µg/kg in hamsters (Hanberg et al. 1989), and 0.8 µg/kg in guinea pigs (Hanberg et al. 1989) following acute-duration oral exposure; 0.016 µg/kg in rats (NTP 2006) and 0.03 µg/kg in guinea pigs (DeCaprio et al. 1986) following intermediate-duration oral exposure; and 0.0071 µg/kg/day in rats (NTP 2006) following chronic-duration oral exposure. A species comparison of effective doses resulting in thymic atrophy, reported ED_{50} values of 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). Depletion of cortical lymphocytes in the thymus has been observed in rats exposed to a single dose of 30 µg/kg (Luebke et al. 1999) and in mice exposed to a single dose of ≥1 µg/kg (Ao et al. 2009; Inouye et al. 2005). Age-related differences in the sensitivity of the thymus to 2,3,7,8-TCDD-induced toxicity have been examined in two studies. In 3-week-old C57BL/6 mice, decreases in thymus weight and number of thymocytes were observed following a single-dose administration of ≥1 µg/kg; however, in 6-week-old C57BL/6 mice, exposure to 1 or 3 µg/kg resulted in decreased thymus weights, but did not alter the number of thymocytes (Inouye et al. 2005). Similarly, a single dose administration of 10 µg/kg to 12-week-old B6C3F1 mice resulted in decreased thymus weight and number of thymocytes; however, no significant alterations were observed in similarly exposed 76-week-old mice (Luebke et al. 1999). Huang and Koller (1998, 1999) compared the toxicity of 2,3,7,8-TCDD following a single-dose exposure to that of equivalent multiple doses. Exposure of female Long-Evans rats to a single dose of 25 µg/kg resulted in a pronounced thinning of the thymic cortex with most of the thymus consisting of medulla (Huang and Koller 1998, 1999). In contrast, administration of 5 µg/kg/day for 5 days resulted in a less dramatic thinning of the thymic cortex, but no effect on cellular density (Huang and Koller 1999).

Like the effects observed in the thymus, acute-duration exposure to 2,3,7,8-TCDD resulted in decreased number of lymphocytes in the spleen and decreased spleen weight in mice administered ≥1 µg/kg (Ao et al. 2009; Ito et al. 2002; Luebke et al. 1999; Smialowicz et al. 1997) and F344 rats administered 30 µg/kg (Luebke et al. 1999). In contrast, Inouye et al. (2005) did not find alterations in the number of splenocytes in C57BL/6 mice administered ≤3 µg/kg. Age-related differences in 2,3,7,8-TCDD-induced effects in the spleen were also observed in B6C3F1 mice; decreased numbers of splenocytes and relative spleen weight were observed in 12-week-old mice administered ≥10 µg/kg, but not in 76-week-old mice (Luebke et al. 1999). Lymph node atrophy was observed in monkeys exposed to 0.011 µg/kg/day for 9 months (Allen et al. 1977).

A decrease in serum total hemolytic complement activity (CH50) was observed in B6C3F1 mice administered ≥0.01 µg/kg/day 2,3,7,8-TCDD for 14 days (White et al. 1986); complement component C3 levels were decreased at ≥0.5 µg/kg/day. When animals exposed to 1 µg/kg/day for 14 days were allowed to recover, serum CH50 levels were significantly lower than controls after 14 days of recovery but were not significantly different at 28 days post-exposure (White et al. 1986). In contrast, C3 levels returned to control levels by post-exposure day 14. Serum C3 levels were also significantly decreased in mice following a single dose exposure to 20 µg/kg 2,3,7,8-TCDD (Lin and White 1993).

Effects on adaptive immune function. A summary of animal studies examining immunosuppression is presented in Table 2-17. Several studies have found decreased cytokine production by spleen cells in response to an antigen in 2,3,7,8-TCDD exposed mice. When mice were immunized with ovalbumin immediately before or after 2,3,7,8-TCDD exposure, significantly decreased production of IL-5 levels in the spleen was observed at ≥0.3 µg/kg in C57BL/6 mice (Ao et al. 2009; Inouye et al. 2005; Ito et al. 2002) and at 20 µg/kg in BALB/c mice (Chen et al. 2013). A time-course study by Ito et al. (2002) examined the response of several cytokines in the spleen after 20 µg/kg 2,37,8-TCDD exposure of C57BL/6N mice immunized with ovalbumin immediately after exposure. Unlike control mice, which had biphasic increases in IL-2, IL-4, IL-5, and IL-6 levels in response to antigen exposure, 2,3,7,8-TCDD-exposed mice had significant decreases in these cytokine levels in the spleen. The significant decrease in IL-4 and IL-6 levels were observed as early as 1-day postexposure and IL-2 and IL-5 were first observed 4-days postexposure (Ito et al. 2002). IL-5 appeared to be the most sensitive to 2,3,7,8-TCDD exposure with no ovalbumin-induced increases in IL-5 levels from days 4 through 14 post-immunization. Exposure to 2,3,7,8-TCDD also significantly decreased IL-5 and IL-6 production by Th2 cells. In Long-Evans rats administered 25 µg/kg 2,3,7,8-TCDD and 2 days later exposed to *Staphylococcal*

Table 2-17. Results of Studies Evaluating Immunosuppression in Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose (µg/kg/day)	Delayed-type hypersensitivity	Host resistance	Immune response to antigen	Comments	Reference
C57BL/6 mouse, once	1			↓ (OVA)	Decreased IL-5 production	Ao et al. 2009
C57BL/6 mouse, once	20			↓ (OVA)	Decreased IL-5 production	Ao et al. 2009
B6C3F1 mouse, once	0.01		↓ (influenza A)		Increased mortality	Burleson et al. 1996
BALB/c mouse, once	20			↓ (OVA)	Decreased interferon-γ, IL-2, IL-4, IL-5, and IL-10 levels Decreased OVA-specific IgG1 and IgM levels	Chen et al. 2013
Sprague-Dawley rat, once	10	↑				Fan et al. 1996
B6C3F1/N mouse, once	0.1			↓ (sRBC)	Decreased antibody plaque forming cell response	Frawley et al. 2014
B6C3F1 mouse, once	1			↓ (sRBC)	Decreased IgM anti-sRBC antibody forming cells	Holsapple et al. 1986
B6C3F1 mouse, 14 days	1			↓ (sRBC)	Decreased IgM antibody-forming cells	Holsapple et al. 1986
Long-Evans rat, once	25		↓ (*Staphylococcal enterotoxin* B)		Increased IL-2 levels and no change in IL-1 or IL-6 levels	Huang and Koller 1998
C57BL/6 mouse, once	20			↓ (OVA)	Decreased IgM and IgG levels; suppressed increase in B cells; formation of germinal center and high affinity antibody forming cell generation in spleen	Inouye et al. 2003
C57BL/6N mouse, once	0.3			↓ (OVA)	Decreased IL-5 levels	Inouye et al. 2005

Table 2-17. Results of Studies Evaluating Immunosuppression in Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose (µg/kg/day)	Delayed-type hypersensitivity	Host resistance	Immune response to antigen	Comments	Reference
C57BL/6 mouse, once	20			↓ (OVA)	Decreased IL-2, IL-4, IL-5, and IL-6 levels Decreased $CD3^+$ and $CD4^+$ T cells and CD45R/B220 B cells	Ito et al. 2002
C57BL/6N mouse, once	1			↓ (OVA)	Decreased IgG1 and IL-5 levels	Ito et al. 2002
C57BL/6 mouse, once	5		↓ (influenza A)		Decreased plasma IgM and IgG levels and increased IgA levels Re-infection with influenza resulted in IgM and IgG2 levels lower than controls	Lawrence and Vorderstrasse 2004
B6C3F1 mouse (12 weeks of age), once	1			↓ (*Trichinella spiralis* antigen)	Decreased lymphoproliferative response in spleen Decreased response to LPS antigen at 10 µg/kg	Luebke et al. 1999
B6C3F1 mouse (76 weeks of age), once	1			↓ (*T. spiralis* antigen)	Decreased lymphoproliferative response in spleen No alteration in response to LPS antigen at 10 µg/kg	Luebke et al. 1999
B6C3F1 mouse, once	4.2			↓ (sRBC)	Decreased IgM antibody-forming cells	Matulka et al. 1997
B6C3F1 mouse, 14 days	1			↓ (sRBC)	Decreased IgM antibody-forming cells	Matulka et al. 1997
C57BL/6 mouse, once	10		↓ (influenza A)		Decreased $CD8^+$ T cell response	Mitchell and Lawrence 2003
B6C3F1 mouse, once	0.5		↔ (influenza A)		No change in mortality	Nohara et al. 2002

Table 2-17. Results of Studies Evaluating Immunosuppression in Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose (µg/kg/day)	Delayed-type hypersensitivity	Host resistance	Immune response to antigen	Comments	Reference
B6C3F1 mouse, once	1			↓ (sRBC)	Decreased antibody plaque forming cell response	Smialowicz et al. 1997
C57BL/6 mouse, once	1		↓ (influenza A)		Decreased IgG2 levels and increased IgA levels at 1 µg/kg Decreased survival and decreased IgG1 levels at 2.5 µg/kg Decreased $CD4^+$ cells at 5 µg/kg	Vorderstrasse et al. 2003
C57BL/6 mouse, once	10		↓ (influenza A)		Decreased $CD4^+$ and $CD8^+$ T cells Decreased IL-2 and interferon-γ levels Decreased plasma IgM, IgG1, and IgG2 levels and increased plasma IgA levels	Warren et al. 2000
Fischer 344 rat, 14 days	0.72		↓ (influenza A)		Suppression in virus-augmented NK cell activity	Yang et al. 1994
Siberian Hamster, once	2			↔ (allogeneic antigen)		Yellon et al. 2000
B6C3F1 mouse, 13 weeks	0.0011			↓ (sRBC)	Decreased antibody plaque forming cell response	Smialowicz et al. 2008
C57Bl/6Jfh mouse, 4 weeks	0.14		↓ (*Salmonella*)		Increased deaths	Thigpen et al. 1975
C57BL/6 mouse, 5–8 weeks	0.07			↓ (sRBC)	Decreased antibody plaque forming cell response	Vecchi et al. 1983
CD rat, 6 weeks	0.71	↔ (tuberculin)				Vos et al. 1973

Table 2-17. Results of Studies Evaluating Immunosuppression in Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose (µg/kg/day)	Delayed-type hypersensitivity	Host resistance	Immune response to antigen	Comments	Reference
B6D2F1 mouse, 4 weeks	0.71		↓ (graph versus host)			Vos et al. 1973
Hartley Guinea pig, 8 weeks	0.006	↓ (tuberculin)			Decreased skin diameter and thickness	Vos et al. 1973
Rhesus monkey, 3.5–4 years	0.00012			↓ (PHA)	Increased tumor necrosis factor-α levels	Rier et al. 2001a

↓ = impaired response; ↔ = no alteration in response; Ig = immunoglobulin; IL = interleukin; LPS = lipopolysaccharide; NK = natural killer; OVA = ovalbumin; PHA = phytohemagglutinin; sRBC = sheep red blood cell

enterotoxin B (SEB), there was a significant increase in IL-2 levels 2 hours after SEB injection, as compared to controls exposed to SEB; no changes in IL-6 or IL-1 levels were observed (as compared to SEB controls) (Huang and Koller 1998). Additionally, exposure to 2,3,7,8-TCDD without SEB exposure did not significantly alter cytokine levels, as compared to naïve controls (Huang and Koller 1999). Thirteen years after termination of 3.5–4 years of 2,3,7,8-TCDD exposure, significant increases in peripheral blood monocyte production of tumor necrosis factor-α in response to phytohemagglutinin were observed in Rhesus monkeys exposed to 0.00012 or 0.00064 μg/kg/day 2,3,7,8-TCDD in the diet (Rier et al. 2001a). Similarly, a significant increase in interferon-γ production in response to stimulation with phytohemagglutinin was observed in NC/Nga mice exposed to 5 μg/kg 2,3,7,8-TCDD; however, there was no effect at 20 μg/kg (Ito et al. 2008).

Suppression of the normal proliferation of CD45R/B220+ B cells in response to antigen exposure was observed in C57BL/6N mice administered a single dose of 20 μg/kg 2,3,7,8-TCDD and ovalbumin (Inouye et al. 2003; Ito et al. 2002). Examining the effect of 2,3,7,8-TCDD on germinal center formation, Inouye et al. (2003) found significant decreases in the number of germinal center B cells in C57BL/6N mice 7, 10, and 14 days after administration of 20 μg/kg 2,3,7,8-TCDD and immunization with ovalbumin. An apparent reduction in the size of the germinal center was observed in the spleen. To assess the effect of 2,3,7,8-TCDD exposure on high-affinity antigen-forming cells, mice were immunized with alum-precipitated (4-hydroxy-3-nitrophenyl) acetyl linked to chicken γ-globulin (NP-CG) immediately after dosing with 20 μg/kg 2,3,7,8-TCDD. Significant decreases in the total number of NP-specific antigen-forming cells were observed in the spleen and bone marrow of 2,3,7,8-TCDD exposed mice. A 96% reduction in the number of high-affinity, NP-specific antigen-forming cells in the spleen was observed 10 days postimmunization and a 64% reduction was observed on day 14; no significant alterations were observed in the bone marrow. Additionally, there were significant decreases in the production of total anti-NP and high-affinity anti-NP IgG1. Inouye et al. (2003) concluded that the inhibited generation of high-affinity antigen-forming cells and antibody production were likely caused by suppression of antigen-responding B cell proliferation induced by 2,3,7,8-TCDD during germinal center formation.

Numerous studies have examined the effects of 2,3,7,8-TCDD exposure on adaptive immune function by examining T cell subpopulations with or without exposure to an antigen. Suppression of the normal increase in $CD4^+$ T cells and/or $CD8^+$ T cells was observed in C57BL/6N or C57BL/10 mice administered a single dose of ≥5 μg/kg and exposed to ovalbumin (Ito et al. 2002) or influenza virus (Mitchell and Lawrence 2003; Vorderstrasse et al. 2003; Warren et al. 2000). Suppression of the normal increase in CD8+ T

cells is likely indicative of suppressed development of cytotoxic T lymphocyte response (Mitchell and Lawrence 2003). In the absence of an antigen, no alterations in splenic $CD4^+$ or $CD8^+$ T cell populations were observed in Long-Evans rats 2 days after administration of a single dose of 25 µg/kg 2,3,7,8-TCDD (Huang and Koller 1999). However, a decrease in the percentage of CD4+ cells in the spleen was observed when the rats were administered 5 µg/kg/day for 5 days (rats examined 2 days after the last dose); no change in $CD8^+$ T cells subpopulations were observed. Nohara et al. (2000) reported no alterations in the percentage of $CD4^+$ $CD8^+$, $CD4^-$ $CD8^-$, or $CD4^+$ T cell subpopulations in the thymus of Sprague-Dawley rats administered a single dose of 1 or 2 µg/kg 2,3,7,8-TCDD. However, there was a decrease in the ratio of $CD4^+$ T cells to $CD8^+$ T cells in the thymus and mesenteric lymph nodes at 1 µg/kg and an increase in the percentage of $CD8^+$ T cells in the thymus at 2 µg/kg. Similarly, Chen et al. (2013) and Oughton et al. (1995) found no alterations in splenic $CD3^+$, $CD4^+$, and/or $CD8^+$ cells in BALB/c mice administered 20 µg/kg or C57BL/6N mice administered 0.03 µg/kg/day for 14–15 months. However, in the chronic-duration study, alterations in splenic $CD4^+$ subsets (increases in naïve T helper cells and decreases in memory T cells) were observed (Oughton et al. 1995).

Acute-duration exposure to 2,3,7,8-TCDD also suppressed the production of antigen-specific IgM and IgG1 in C57BL/6N mice administered ≥2.5 µg/kg and immunized with ovalbumin (Inouye et al. 2003; Ito et al. 2002) or influenza A (Lawrence and Vorderstrasse 2004; Vorderstrasse et al. 2003; Warren et al. 2000) and in BALB/c mice administered 20 µg/kg 2,3,7,8-TCDD and immunized with ovalbumin (Chen et al. 2013). Increases in IgA levels have also been reported in C57BL/6 mice inoculated with influenza A and exposed to ≥1 µg/kg 2,3,7,8-TCDD (Lawrence and Vorderstrasse 2004; Vorderstrasse et al. 2003; Warren et al. 2000).

A number of studies have found that 2,3,7,8-TCDD exposure suppressed the primary antibody response to sheep red blood cells. Following an acute-duration exposure to 2,3,7,8-TCDD and sensitization with sheep red blood cells, suppression of the response (as measured by splenic plaque-forming cells or antibody-forming cells) was observed in B6C3F1 mice administered a single dose ≥0.1 µg/kg (Frawley et al. 2014; Holsapple et al. 1986; Matulka et al. 1997; Smialowicz et al. 1997), a 5-day exposure to 6 µg/kg/day (Kaplan et al. 2011), or a 14-day exposure to 1 µg/kg (Holsapple et al. 1986). Evaluating the effect of the time of administration of a single 14 µg/kg dose of 2,3,7,8-TCDD relative to sensitization with sheep red blood cells, Matulka et al. (1997) found immunosuppression (measured as total IgM antibody forming cells) when the 2,3,7,8-TCDD was administered 1, 2, or 3 days prior to sensitization, on the day of sensitization, and 1 or 2 days after sensitization; there was no significant effect when the 2,3,7,8-TCDD was administered 3 days after sensitization. Comparing total IgM antibody-forming cell

response in B6C3F1 and DBA/2 mice following a single exposure and repeated exposure, Matulka et al. (1997) found significant decreases at ≥14 µg/kg for a single exposure and ≥1 µg/kg/day for a 14-day exposure in the B6C3F1 mice, although the magnitude of the suppression was greater following repeated exposures than single exposure. In the DBA/2 mice, significant decreases were observed at 42 µg/kg and 14 µg/kg/day; at a given cumulative dose, the magnitude of the decrease was similar when the dose was administered once or over a 14-day period. Intermediate-duration exposure resulted in a lower adverse effect level; a significant decrease in the response to sheep red blood cells was observed in B6C3F1 mice administered 0.0011 µg/kg 5 days/week for 13 weeks (mice immunized with sheep red blood cells 3 days after the last exposure) (Smialowicz et al. 2008) and in C57BL/6 mice administered 0.5 µg/kg/day 1 day/week for 5–8 weeks (Vecchi et al. 1983).

Luebke et al. (1999) examined age-related differences in 2,3,7,8-TCDD-induced suppression of adaptive immune function in B6C3F1 mice administered 2,3,7,8-TCDD and infected with *Trichinella spiralis* larvae 7 days later. Exposure to ≥10 µg/kg resulted in a significant decrease in parasite elimination in 12-week-old mice, but not in 76-week-old mice. Similarly, there were no effects on the proliferative response to concanavalin A or lipopolysaccharide (LPS) in the spleen of aged mice, but a decreased response to LPS was observed in the spleen of young mice exposed to 10 µg/kg. However, a decrease in response to parasite antigens were observed in the spleen of young and aged mice exposed to ≥1 µg/kg. An increase in the splenic proliferative response to *Salmonella typhimurium* mitogen was also observed in aged rats administered 30 µg/kg and infected with *T. spiralis*; there were no alterations in the response to parasite antigens or concanavalin A (Luebke et al. 1999); the investigators noted that these results were in contrast to the enhanced response to concanavalin A and parasite antigens observed in young rats in other studies.

A significant decrease in lymphocyte proliferation, when measured during the light cycle, was observed in Siberian hamsters administered a single dose of 2 µg/kg 2,3,7,8-TCDD; however, no alterations were observed during the dark cycle (Yellon et al. 2000). Additionally, no alterations in lymphocyte proliferation in response to alloantigen were observed 2 or 20 weeks after 2,3,7,8-TCDD administration.

Burleson et al. (1996) reported a significant increase in mortality in B6C3F1 mice administered a single dose of ≥0.01 µg/kg 2,3,7,8-TCDD and infected with influenza A virus (A/Hong Kong/8/68 strain) 7 days later. Using the same protocol, Nohara et al. (2002) attempted to replicate these results. Groups of B6C3F1, BALB/c, C57BL/6N, and DBA/2 mice were administered a single dose of 2,3,7,8-TCDD and infected with influenza A virus (A/PR/34/8) 7 days later. No significant alterations in survival rate were

observed and the highest dose tested, 0.50 µg/kg, was considered a NOAEL in all four mouse strains. Vorderstrasse et al. (2003) reported increases in mortality in C57BL/6 mice administered ≥2.5 µg/kg and infected with influenza A virus (A/HKx31 strain); no deaths were observed in controls or mice administered 1 µg/kg. Although these results support the findings of Nohara et al. (2002), Vorderstrasse et al. (2003) cautioned that it is not appropriate to compare the results of their study with those of Burleson et al. (1996) and Nohara et al. (2002) because they utilized a virus strain that is not lethal to immunocompetent mice.

Increased mortality that was indicative of altered immunity was also observed in C57BL/6Jfh mice challenged with *Salmonella bern* following exposure to 0.14 µg/kg/day of 2,3,7,8-TCDD by gavage for 4 weeks (Thigpen et al. 1975); no significant effects were observed at 0.07 µg/kg/day. In the same study, using the same experimental design, doses of up to 2.8 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).

Delayed-type hypersensitivity response to tuberculin was observed in guinea pigs exposed to 0.006 µg/kg/day 2,3,7,8-TCDD for 8 weeks (Vos et al. 1973), but not in CD rats similarly exposed to 0.71 µg/kg/day (Vos et al. 1973). Another study reported a delayed-type hypersensitivity response in Sprague-Dawley rats sensitized with keyhole limpet hemocyanin following a single dose of 10 µg/kg 2,3,7,8-TCDD (Fan et al. 1996). Vos et al. (1973) also reported a suppressed response in the graft versus host test in B6D2F1 mice exposed to 0.71 µg/kg/day for 4 weeks.

In addition to the increased mortality, Vorderstrasse et al. (2003) reported a number of other alterations in immunological endpoints. At ≥1 µg/kg, there was a significant decrease in IgG2a levels and increase in IgA levels; at ≥2.5 µg/kg, there were decreases in IgG1 and IgG2b levels and decreases in the number of lymphocytes and macrophages in bronchoalveolar lavage fluid; and at 7.5 µg/kg, there was suppression of $CD8^+$ T cells in the mediastinal lymph node. The decreased IgG levels, increased IgA levels, and lack of alterations in IgM levels suggested that 2,3,7,8-TCDD affected antibody class switching. In addition to these alterations in adaptive immune function, there was also evidence of a dysregulation of the innate immune response to the influenza virus infection. A significant increase in the number of neutrophils (≥5 µg/kg) and a decrease in interferon-γ levels (10 µg/kg) were observed in bronchoalveolar lavage fluid. Similarly, Warren et al. (2000) reported decreases in $CD4^+$ and $CD8^+$ T cells, IL-2, and interferon-γ levels and cytotoxic T lymphocyte activity in the mediastinal lymph nodes; decreases in plasma IgM, IgG1, IgG2a, and IgG2b levels; increases in plasma IgA levels; and decreases in IL-2 and increases in interferon-γ levels in bronchoalveolar lavage fluid in mice administered 10 µg/kg 2,3,7,8-TCDD and

infected with influenza A virus (A/HKx31 strain). Increases in mortality were also observed; however, the data were not presented in a way that would facilitate identifying an adverse effect level.

Lawrence and Vorderstrasse (2004) examined the effect of 2,3,7,8-TCDD on immunological memory in C57BL/6 mice administered a single dose of 5 or 10 µg/kg and infected with influenza A virus (HKx31 strain). The primary infection resulted in significant suppression of IgM, IgG2a, and IgG2b levels in the plasma and bronchoalveolar lavage fluid 10 and 40 days after infection; a significant increase in plasma IgA levels was also observed at both examination periods. Upon re-infection, plasma levels of IgM, IgG2a, and IgG2b were still significantly lower than the vehicle controls for at least 7 days after reinfection. In contrast, there were no significant alterations in the number of IgG or IgA producing cells in the mediastinal lymph nodes after the primary infection or after re-infection. After the primary infection, a 70% decrease in $CD8^+$ cells was found in the mediastinal lymph nodes of mice exposed to 10 µg/kg. Examination of virus-specific memory $CD8^+$ T cells measured 60 days after the primary infection showed a 50% decrease in mice exposed to 2,3,7,8-TCDD. Upon re-infection, there was a delay in the expansion of virus-specific memory $CD8^+$ cells; 3 days after re-infection, there was a 70% difference between the number of virus-specific memory $CD8^+$ cells in the 2,3,7,8-TCDD group compared to the vehicle controls. However, 5-days after re-infection, the recall response was equivalent to that of the control group. To evaluate host resistance, survival and pulmonary virus titers were monitored for 7 or 21 days after the primary infection or re-infection, respectively, in two sets of animals. In the 10 µg/kg group, 37% of the mice died after the primary infection, compared to 3% mortality in the vehicle controls. In contrast, no deaths were observed in either group after re-infection. Additionally, no detectable virus was found in the lungs of exposed or control mice 3–14 days after the re-infection. The investigators noted that although exposure to 2,3,7,8-TCDD did not adversely affect the recall response to homotypic infection, it is likely that the decreased number of memory cytotoxic T lymphocytes would have a negative impact on host resistance to a heterosubtypic infection because the excess levels of IgA that are host-protective for homotypic infection would not be effective in a heterosubtypic infection.

Two studies demonstrated that oral exposure to ≥1 µg/kg 2,3,7,8-TCDD and oral immunization with ovalbumin resulted in impaired oral tolerance (Chmill et al. 2010; Kinoshita et al. 2006). Oral tolerance is defined as the antigen-specific inhibition of systemic IgG production by oral pre-administration of protein antigens. Both studies also found decreased fecal IgA levels that were indicative of impaired gut mucosal immunity.

Although not as well studied as other immunological endpoints, Yang et al. (1994) reported suppression of pulmonary NK cell activity in Fischer-344 rats infected with influenza A virus and exposed to 0.72 µg/kg/day 2,3,7,8-TCDD for 14 days. However, in the absence of infection, there was no alteration in pulmonary NK cell activity, and splenic NK cell activity was not altered by 2,3,7,8-TCDD exposure or by the virus.

Other CDD Congeners—Animal Studies. Other CDD congeners also appear to affect the immune system. Decreases in relative spleen and thymus weight were observed in C57BL/6 mice administered a single dose of ≥10 µg/kg 1,2,3,7,8-PeCDD (Ao et al. 2009) and decreases in the number of thymocytes were observed at ≥3 µg/kg. 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. 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 1 µg/kg/day for 13 weeks (NCI/NTP 1980).

Suppressed antibody response was reported in B6C3F1 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. 1986). Immunization with ovalbumin resulted in significant decreases in IL-5 levels in the spleen of mice exposed to ≥1 µg/kg 1,2,3,7,8-PeCDD (Ao et al. 2009). 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 B6C3F1 mice following 2 weeks of exposure to 1 µg/kg/day 1,2,3,6,7,8-HxCDD (White et al. 1986).

Immunological mechanisms. Many of the health effects of CDDs share a common initiating event in AhR binding. Section 2.21, Mechanisms of Toxicity, provides a detailed discussion of the evidence for this initiating event and its physiological sequelae. In this subsection, an overview of the mechanisms involved in immunotoxic effects is provided. Detailed mechanistic explanations are beyond the scope of this profile.

2,3,7,8-TCDD has been shown to induce a variety of effects on the immune system of experimental animals, including thymic involution, neutrophilia, and immune suppression manifested as decreased antibody production, reduced development of cytotoxic T-lymphocytes, and increased susceptibility to infections. Several detailed reviews of the mechanisms of immunotoxicity related to 2,3,7,8-TCDD have

been published (Corsini et al. 2011; Kerkvliet 2009, 2012; Marshall and Kerkvliet 2010; Prasad Singh et al. 2020) and provide the experimental evidence for the current understanding of the mechanisms. Key conclusions of these reviews are: (1) AhR is expressed in most immune system cells; (2) AhR is necessary for 2,3,7,8-TCDD immune suppression; (3) AhR responsive element (AhRE) sequences are found in many genes related to immune system function; (4) the primary pathway by which 2,3,7,8-TCDD suppresses immune function is via increasing the proportion of anti-inflammatory Treg cells; and (5) 2,3,7,8-TCDD effects on immune signaling depends on the physiological context (cell type and activation status, tissue, species, etc.).

Thymic involution is characteristic of exposure to 2,3,7,8-TCDD and structurally related chemicals in all species examined. The mechanism for 2,3,7,8-TCDD-induced thymic atrophy is not completely understood, but available data indicate that AhR activation is important. A recent study (Beamer et al. 2019) showed that AhR activation in dendritic cells is key to this effect because targeted deletion of the AhR in these cells prevented thymic atrophy in mice exposed to 2,3,7,8-TCDD. Thymic atrophy induced by 2,3,7,8-TCDD may, in part, result from apoptosis of thymocytes (Camacho et al. 2004), albeit not via Fas/Fas ligand signaling (Beamer et al. 2019; Nagai et al. 2006). Other studies have demonstrated that 2,3,7,8-TCDD can also decrease the proliferation of precursor thymocytes (Lai et al. 1998) and increase the migration of thymocytes out of the thymus (Poland et al. 1994; Temchura et al. 2005).

The innate immune response is largely mediated by myeloid cells including granulocytes, macrophages, dendritic cells, and NK cells. Microbial pathogens activate these cells via toll-like receptors (TLRs) that recognize structural components of common microbes. The TLRs initiate signaling to upregulate pro-inflammatory cytokines and complement activation. Many TLR and complement genes have been shown to contain AhRE sequences, suggesting potential susceptibility to modulation by 2,3,7,8-TCDD-liganded AhR (Kerkvliet 2009), although data to show the influence of 2,3,7,8-TCDD on these genes are lacking.

Exposure to 2,3,7,8-TCDD has been shown to induce dose-dependent increases in neutrophils (the most abundant type of granulocyte) in the blood, peritoneal cavity, spleen, and lungs of mice (Kerkvliet 2009). In addition, 2,3,7,8-TCDD alters the oxidative burst and cytolytic activity of neutrophils in a context-dependent fashion; under different circumstances, experiments have demonstrated suppression, enhancement, and absence of an effect of 2,3,7,8-TCDD on this function (Kerkvliet 2009). Similarly, the cytolytic activity of NK cells after 2,3,7,8-TCDD exposure varies from no response to either suppression or enhancement. The mechanisms by which 2,3,7,8-TCDD affects neutrophils and NK cells are not

known; however, several genes for neutrophil cytosolic factors and NK receptor subunits have AhRE sequences and may play a role (Kerkvliet 2009).

In mice exposed to 2,3,7,8-TCDD, a decrease in dendritic cell counts in the spleen was shown to occur 1 week after exposure, and *in vitro* studies showed that 2,3,7,8-TCDD enhanced both maturation and apoptosis of dendritic cells (Kerkvliet 2009). The mechanisms for these effects may include altered expression of apoptotic genes or upstream signaling pathways. For example, *in vitro* data show that 2,3,7,8-TCDD increased the expression of *Fadd*, a gene that mediates apoptosis, and also suppressed NFkB signaling (Kerkvliet 2009).

The adaptive immune response begins with activation of dendritic cells upon recognition of a pathogen. With prolonged interaction with activated dendritic cells, $CD4^+$ T cells are stimulated to differentiate, by one of several pathways, into T helper cells (TH1, TH2, TH17, Tregs). TH1, TH2, and TH17 cells facilitate the immune response to pathogens and are also involved in allergic and autoimmune responses. Tregs, in contrast, produce cytokines (e.g., IL-10, TGFβ) that suppress the immune response by modulating the activation and/or survival of T helper/effector cells and dendritic cells. A growing body of experimental data has shown that exposure to 2,3,7,8-TCDD enhances differentiation of T cells into Tregs and suppresses TH17 cells, tipping the balance toward suppression of all forms of adaptive immune responses, including not only pathogen responses but also allergic and autoimmune responses. There appear to be several pathways by which 2,3,7,8-TCDD influences T cell differentiation. For example, TCDD exposure has been shown to modulate expression of microRNAs, deoxyribonucleic acid (DNA) methylation, and histone modifications in the promoter region of the FoxP3 and IL-17 transcription factors, which play critical roles in the differentiation of Treg and TH17 cells (respectively). In addition, many genes involved in T helper cell differentiation have one or more AhRE sequences, as shown in Table 2-18.

Table 2-18. Numbers of Aryl Hydrocarbon Receptor Responsive Elements (AhREs) in Genes Regulating T Helper Cell Differentiation

Gene	Number of AhREs	Gene	Number of AhREs	Gene	Number of AhREs
Tgfb1	10	Il17b	3	Stat2	5
Tgfb2	15	Il17d	8	Stat3	5
Tgfb3	5	Il21	4	Stat4	4
Il2	3	Il32a	5	Stat5a	9
Il4	2	Gata3	10	Stat5b	7

Table 2-18. Numbers of Aryl Hydrocarbon Receptor Responsive Elements (AhREs) in Genes Regulating T Helper Cell Differentiation

Gene	Number of AhREs	Gene	Number of AhREs	Gene	Number of AhREs
Il6	6	Foxp3	5	Stat6	12
Il10	3	Jak1	5	Socs1	18
Il12a	3	Jak2	9	Socs2	8
Il12b	3	Jak3	20	Socs3	11
Il17	3	Stat1	9		

Source: Kerkvliet 2009

In summary, the mechanisms and pathways by which 2,3,7,8-TCDD modulates immune responses are complex and depend upon the physiological milieu in which the exposure occurs. Most of the data on immune mechanisms are from studies in mice, and there are well-known differences in the responses of various species to TCDD exposure, suggesting the need for studies in other species to better evaluate species differences in immune effects.

2.15 NEUROLOGICAL

Overview. A small number of epidemiological studies have evaluated the neurotoxicity of CDDs. The most studied neurological endpoint is peripheral neuropathy, which has been examined in workers, Vietnam War veterans, and the Seveso cohort. The results are inconsistent across studies and populations.

The potential neurotoxicity of 2,3,7,8-TCDD has not been well studied in laboratory animals. Two studies examined motor activity and found decreased activity. The scope of the remaining studies was limited to histopathological examination of nervous tissues in which no alterations were found.

Epidemiological Studies. The potential neurotoxicity of CDDs has been examined in a number of epidemiological studies with mixed results (Table 2-19). Apart from peripheral neuropathy, most neurological outcomes have only been investigated by a couple of studies. A number of studies have investigated the potential association between CDD exposure and peripheral neurotoxicity; studies have examined the incidence of peripheral neuropathy, clinical signs of neuropathy, and motor conduction velocity. The results have been inconsistent across studies. An increased occurrence of peripheral neuropathy has been reported in workers (Pazderova-Vejlupková et al. 1981) and Vietnam War veterans

Table 2-19. Neurological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Occupational			
Mannetje et al. 2018 Cross-sectional study in former employees (n=245) of a phenoxy herbicide production facility in New Zealand	Work history and 2007–2008 serum 2,3,7,8-TCDD levels ≥10 pg/g lipid	Frequent mood changes	↔
		Trouble sleeping	↔
		Abnormal reflexes	↑, serum level ≥25 pg/g lipid
Moses et al. 1984 Cross-sectional study of current and former workers at a 2,4,5-T production facility in West Virginia (n=226 workers; 117 with current or history of chloracne)	Chloracne used as a surrogate for heavy exposure to 2,3,7,8-TCDD	Pin prick sensation	↓, workers with chloracne
Singer et al. 1982 Cross-sectional study of current and former workers at a phenoxy herbicide production facility in Arkansas (n=45 workers and 25 controls)	Not evaluated	Nerve conduction velocity	
		Median motor nerve	↓
		Median sensory nerve	↔
		Sural sensory nerve	↓
Sweeney et al. 1993 Cross-sectional study of former workers at two 2,4,5-T, 2,4,5-trichlorophenate, and 2,4-D production facilities in New Jersey and Missouri (n=281 workers and 260 referents)	Mean serum 2,3,7,8-TCDD level: 220 pg/g lipid (workers) and 7 pg/g lipid (referents)	Peripheral neuropathy	↔
Pazderova-Vejlupková et al. 1981 Case series of 55 male workers at an herbicide production facility in the former Czechoslovakia (no comparison group was used)	Not reported	Polyneuropathy	↑, 31% of subjects

Table 2-19. Neurological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Thömke et al. 1999 Cross-sectional study of 121 workers at a pesticide production facility in Germany	Comparisons made between workers with chloracne (n=35) and without chloracne (n=86)	Nerve conduction velocity Sural nerve Peroneal nerve Ulnar nerve	 ↔ ↔ ↔
		Neurophysiological abnormalities	↑
		Diminished vibration sense	↑
Thömke et al. 2002 Cross-sectional study of 121 workers at a pesticide production facility in Germany	Comparisons made between workers with chloracne (n=35) and without chloracne (n=86) Median blood CDD/CDF TEQ: 871 pg/g lipid in chloracne group and 330 pg/g pg/g lipid in non-chloracne group	Visual evoked potential	↔
		Brainstem auditory evoked potential	↔
		Blink reflex	↔
Urban et al. 2007 Cross-sectional study of 15 workers exposed to CDDs at an herbicide production facility in the former Czechoslovakia (no comparison group was used); follow-up to the Pazderova-Vejlupková et al. (1981) study	Mean plasma 2,3,7,8-TCDD level: 128 pg/g lipid	Symptoms of polyneuropathy	↑, 60% of subjects
		Nerve conduction velocity Median, ulnar, tibial, and sural motor and sensory nerve fibers	↔
		Visual evoked potential abnormalities	↑, 33% of subjects
		Neuropsychological tests	↑, correlations with plasma 2,3,7,8-TCDD levels
		Color confusion index	↔
Seveso, Italy			
Ames et al. 2018 Retrospective cohort study of women (n=159 for physical function subgroup and 459 for working memory subgroup) participating in the Seveso Women's Health Study; physical function subgroup was evaluated in 1996 and working memory subgroup was evaluated in 2008	Median serum 2,3,7,8-TCDD levels (measured at time of accident): 45.2 ppt for physical function subgroup and 60.1 ppt for working memory subgroup	Walking speed	↔
		Manual dexterity	↔
		Lower body flexibility	↔
		Grip strength	↑, lower serum levels ↓, higher serum levels
		Verbal or spatial working memory	↔

Table 2-19. Neurological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Assennato et al. 1989 Retrospective cohort study of 133 subjects who developed chloracne after the accident and 191 referents	Not measured	Nerve conduction velocity Median motor nerve Peroneal motor nerve Sural sensory nerve	 ↔ ↔ ↔
Filippini et al. 1981 Retrospective cohort study of 197 subjects living in Seveso at the time of the accident; 305 referents were used to establish reference values	Not measured	Peripheral neuropathy (as assessed via motor nerve conduction velocity)	↑, among subjects with indicators of exposure (chloracne or increased serum enzymes [γ-glutamyl transferase, ALT, AST])
Vietnam War veterans and Operation Ranch Hand veterans			
Beard et al. 2016 Case-control study of U.S. veterans with amyotrophic lateral sclerosis (n=621 cases and 958 controls)	Military service during Vietnam War, self-reported exposure to Agent Orange in the field	Amyotrophic lateral sclerosis	↑
Beard et al. 2017 Cross-sectional study of 616 U.S. veterans with amyotrophic lateral sclerosis	Military service during Vietnam War, self-reported exposure to Agent Orange in the field	Amyotrophic lateral sclerosis survival	↓
Lee et al. 2022 Retrospective study of 348 Korean Vietnam veterans with exposure to defoliants and 670 veterans without defoliant exposure	Military service during Vietnam War and reported exposure to defoliants	Brain atrophy progression	↑

Table 2-19. Neurological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Levy 1988 Cross-sectional study of 6 U.S. Vietnam veterans with chloracne and 25 Vietnam veterans without chloracne	Chloracne used as a surrogate for Agent Orange exposure	Posttraumatic stress disorder	↑
Martinez et al. 2021 Cross-sectional study of U.S. Vietnam War veterans (n=316,351; 12.1% had presumed Agent Orange exposure)	Presumed Agent Orange exposure based on self-reported exposure and clinician indicated that a health care encounter was associated with Agent Orange	Dementia	↑
USAF 1991 Cross-sectional report of 866 Operation Ranch Hand personnel and a comparison group of 1,198	Not measured	Peripheral neuropathy	↔
		Coordination abnormalities	↑, high group
		CNS index (based on coordination, tremor, gait)	↑, high group
Wolfe et al. 1995 Retrospective study of the offspring of 454 male veterans involved in Operation Ranch Hand and 570 comparison fathers	Background serum dioxin level: <10 ppt Low exposure serum dioxin level: ≤110 ppt High exposure serum dioxin level: >110 ppt	Nerve conduction velocity	↔
		Scores on functional and performance psychological tests	↔
Yi et al. 2013 Cross-sectional study of a group of 114,562 Korean veterans of the Vietnam War exposed to Agent Orange	Exposure based on military record	Central nerve disorders	↔
		Peripheral neuropathy	↔
		Multiple nerve palsy	↑
		Multiple sclerosis	↑
		Amyotrophic lateral sclerosis	↔

Table 2-19. Neurological Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Yi et al. 2014 Cross-sectional study of a group of 111,726 Korean veterans of the Vietnam War exposed to Agent Orange	Self-reported exposure; veterans divided in low- and high-exposure groups	Spinal muscular atrophy	↔
		Parkinson's disease	↔
		Alzheimer's disease	↑
		Multiple sclerosis	↔
		Epilepsy	↑
		Polyneuropathies of peripheral nervous system	↑
		Paralytic syndromes	↔

↑ = association; ↓ = inverse association; ↔ = no association; 2,4-D = 2,4-dichlorophenoxyacetic acid; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; CNS = central nervous system; TCDD = tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalency

(Yi et al. 2014); other studies of workers (Sweeney et al. 1993) and Vietnam War veterans (USAF 1991; Yi et al. 2013) have not found increases. Pazderova-Vajlupková et al. (1981) reported a high incidence of fatigue and weakness in the lower extremities in workers. In studies of workers with chloracne (used as a biomarker of exposure to high levels of 2,3,7,8-TCDD), a decrease in pin prick sensation (Moses et al. 1984) and an increase in the incidence of simultaneous occurrence of sensory and deep tendon reflex abnormalities (Thömke et al. 1999) were observed. However, the Thömke et al. (1999) study did not find increases in the incidence of symptoms (such as paresthesia, numbness, or cramps) suggestive of peripheral neuropathy or differences in deep tendon reflexes or sensation to touch or pain in comparisons between workers with or without chloracne. In a follow-up of 15 surviving workers examined in the Pazderova-Vajlupková et al. (1981) study, no associations between symptoms of polyneuropathy and serum 2,3,7,8-TCDD levels were found (Urban et al. 2007); however, diminished sensation to touch and pain, diminished vibration sense, and bilateral or lost ankle and/or knee jerks were observed in 9 of the 15 workers. Another study reported an association between serum 2,3,7,8-TCDD levels and abnormal reflexes (Mannetje et al. 2018). Decreased nerve conduction velocities were observed in the median motor nerve and sural sensory nerve in workers (Singer et al. 1982) and among Seveso residents with chloracne or increased serum enzymes (γ-glutamyl transferase, ALT, AST) (Filippini et al. 1981). No alterations in nerve conduction velocity were observed in other studies of workers (Suskind and Hertzberg 1984; Sweeney et al. 1993; Urban et al. 2007), Seveso residents (Assennato et al. 1989), or Operation Ranch Hand veterans (Wolfe et al. 1985); Thömke et al. (1999) did not find alterations in sural, peroneal, or ulnar nerve conduction velocities in workers with chloracne, but did find an increase in the number of individuals with one or two, or with two and more, neurophysiologic abnormalities in the workers with chloracne.

Three studies evaluated possible associations between CDD exposure and amyotrophic lateral sclerosis (ALS) in Vietnam War veterans. An increased incidence of ALS (Beard et al. 2016) and decreased survival (Beard et al. 2017) was found among U.S. veterans with self-reported exposure to Agent Orange; a study of Korean veterans found no association between self-reported exposure to Agent Orange and ALS (Yi et al. 2013). A study in workers did not find evidence of altered cranial nerve function as evidenced by no difference in auditory brainstem evoked potential, visual evoked potential, or blink reflex in comparisons between workers with and without chloracne (Thömke et al. 2002). 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, 2–60, or >60 ppt was not dose-

related. Another study found visual evoked potential abnormalities in 33% of workers (Urban et al. 2007). Several neurological effects have been reported in single studies including altered grip strength (Ames et al. 2018), posttraumatic stress disorder (Levy 1988), dementia (Martinez et al. 2021), coordination abnormalities (USAF 1991), multiple sclerosis (Yi et al. 2013), multiple nerve palsy (Yi et al. 2013), Alzheimer's disease (Yi et al. 2014), epilepsy (Yi et al. 2014), and brain atrophy (Lee et al. 2022). Additional research is needed to assess the possible relationship between CDD exposure and these neurological effects.

2,3,7,8-TCDD—Animal Studies. Limited information was obtained regarding neurological effects in animals. Decreased motor activity was observed in Sprague-Dawley rats after a single dose of 15 µg/kg 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 pregnancy day 21 (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 and female B6C3F1 mice dosed with up to 0.3 µg/kg/day for 104 weeks did not result in significant histological alterations in the brain, spinal cord, or sciatic nerve (NTP 1982b); no histological alterations were observed in the brain of female Sprague-Dawley rats administered up to 0.071 µg/kg/day 2,3,7,8-TCDD for 2 years (NTP 2006).

2.16 REPRODUCTIVE

Overview. Epidemiological studies have evaluated a number of reproductive outcomes in men and women. Overall, epidemiological studies examining reproductive hormone levels have not found associations in several populations including male workers, males living in areas with contaminated soil, Seveso residents, and the general population. No associations between 2,3,7,8-TCDD levels and menstrual cycle or ovarian function were observed in Seveso women. Mixed results were found in studies examining the possible association between CDDs and risk of endometriosis. A study of Seveso men exposed as young boys found alterations in sperm parameters that were not found in men who were young adults at the time of the accident. Increased time-to-pregnancy was observed in two studies of Seveso women.

Laboratory animal studies of 2,3,7,8-TCDD provide strong evidence that the reproductive system is a sensitive target of toxicity. The observed effects include decreases in sperm production, count, viability, and motility; decreased ovulation; decreased female fertility; and altered nursing behavior. Alterations in sperm parameters have been observed at ≥0.1 and ≥0.001 µg/kg/day following acute or intermediate

durations, respectively. In females, reproductive effects have been observed at doses of 1 µg/kg/day (altered nursing behavior), 0.05 µg/kg/day (decreased implantation sites), and 0.00012 µg/kg/day (endometriosis) following acute-, intermediate-, or chronic-duration exposures, respectively. The potential for reproductive toxicity has not been evaluated for other CDD congeners.

Epidemiological Studies. Epidemiological studies have evaluated possible associations between CDD exposure and reproductive hormone levels in several populations. As presented in Table 2-20, most of these studies have not found associations in male workers (Egeland et al. 1994), male Operation Ranch Hand veterans (Henriksen et al. 1996; USAF 1991), male residents in Agent Orange contaminated areas in Vietnam (Sun et al. 2017, Van Luong et al. 2018), male Seveso residents (Mocarelli et al. 2008), female Seveso residents (Warner et al. 2007), or the post-menopausal general population (Lambertino et al. 2021). Some studies did find associations (Egeland et al. 1994; Gupta et al. 2006; Lambertino et al. 2021; Mocarelli et al. 2008; Van Luong et al. 2018) but the findings were not consistent across studies.

Several studies investigated the impact of TCDD exposure on women's menstrual cycles >20 years following the Seveso incident. The individual TCDD serum levels were not related to the age at menarche in a group of women who were premenarcheal at the time of initial exposure in 1976 (Warner et al. 2004). Eskenazi et al. (2002b) reported an increased menstrual cycle length in female adults who were premenarcheal at the time of the initial exposure; however, the confidence intervals included unity. The cycle length increased 0.93 days for each 10-fold increase in TCDD levels. There was also a dose-related association between the TCDD levels and an increased risk of early menopause in the Seveso women (Eskenazi et al. 2005). However, the relationship was not demonstrated at the highest exposures (>100 ppt). When indicators of ovarian function (ovarian cysts, ovarian follicles, ovulation rate) were evaluated in Seveso women, no clear evidence of TCDD-induced effects was observed (Warner et al. 2007).

The relationship between CDD exposure and endometriosis has been evaluated in several studies. In Seveso residents, no significant increase in the risk of endometriosis was found in a cohort of women from zones A and B (Eskenazi et al. 2002a). The risk of uterine leiomyoma (fibroids) associated with exposure to 2,3,7,8-TCDD for women who resided near Seveso in 1976 was investigated (Eskenazi et al. 2007). In total, about 26% of the women had confirmed fibroids. However, higher levels of serum 2,3,7,8-TCDD were found to be associated with lower risk for the fibroid development. Apart from the Seveso studies, there were several case-control and cross-sectional studies in the general population. Increased risks of deep endometriotic (adenomyotic) nodules and peritoneal endometriosis were associated with CDD/CDF TEQ serum levels in a study of Belgian women (Heilier et al. 2005). The

Table 2-20. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Occupational			
Egeland et al. 1994 Cross-sectional study of 248 male workers employed at two 2,4,5-T production facilities and 231 referents	Past serum dioxin levels were estimated from current levels. Workers divided into four quartiles (pg/g): • Q1: <20 • Q2: 20–75 • Q3: 76–240 • Q4: 241–3,400	LH	↑, for trend
		FSH	↔, for trend
		Testosterone	↔, for trend
Vietnam veterans and Operation Ranch Hand veterans			
Gupta et al. 2006 Prospective cohort study of 971 veterans involved in Operation Ranch Hand and 1,266 Air Force veterans not involved in spraying	Mean serum 2,3,7,8-TCDD level: 26.93 ppt in Ranch Hand veterans and 4.57 ppt in referent veterans Ranch Hand mean serum levels (ppt) • Q1: 4.14 • Q2: 8.95 • Q3: 18.40 • Q4: 76.16	Benign prostatic hyperplasia	↑, Ranch Hand veterans ↓, comparison veterans
		Serum testosterone	↓, Q2

Table 2-20. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Henriksen et al. 1996 Retrospective study of participants in the Air Force Health Study; 848–918 Operation Ranch Hand veterans and 1,011–1,154 non-exposed Air Force veterans, subjects were examined in 1982, 1985, 1987, and 1992	Serum "dioxin" levels at the end of veteran's tour of duty were estimated using current serum levels. Median serum "dioxin" levels in Ranch Hand veterans: ≤10 ppt (background group), >10 ppt (low-exposure group) and 130 ppt (high-exposure group) 98th percentile of serum dioxin levels in comparison group was 10 ppt	Serum testosterone <400 ng/mL in 1982 or <260 ng/mL in 1987 or 1992	↑, low-exposure group only in 1987 ↔, all other groups and time periods
		Serum FSH >25 IU in 1982 or >17.2 IU in 1987, or >15 IU in 1992	↔, all groups and time periods
		Serum LH >30 IU/mL in 1982, >25.1 IU/mL in 1987, or >9.8 IU/mL in 1992	↔, all groups and time periods
		Testicular abnormality (atrophic or missing)	↔, all groups and time periods
		Sperm counts ≤60 million/mL	↔, all groups and time periods
USAF 1991 Cross-sectional report of 866 Operation Ranch Hand personnel and a comparison group of 1,198 unexposed Air Force veterans	Not reported	Serum testosterone <260 ng/dL	↔ ↓ when body fat was not considered
Wolfe et al. 1985 Retrospective study of 1,278 Operation Ranch Hand personnel	Not measured	Sperm count	↔
Seveso, Italy			
Eskenazi et al. 2002a Retrospective cohort study of women participating in the Seveso Women's Health Study (n=637, 97 from zone A and 540 from zone B)	Median serum 2,3,7,8-TCDD levels: 257 ppt for zone A and 47.0 for zone B	Endometriosis	↔

Table 2-20. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Eskenazi et al. 2002b Retrospective cohort study of women participating in the Seveso Women's Health Study (n=301)	Median serum 2,3,7,8-TCDD level: 67.5 ppt	Menstrual cycle length	↔
		Days of menstrual flow	↔
		Risk of irregular cycle	↓
Eskenazi et al. 2003 Retrospective cohort study of women participating in the Seveso Women's Health Study (n=510, 888 total pregnancies)	Median 2,3,7,8-TCDD serum level at the time of the incident: 46.6 ppt	Spontaneous abortion	↔
Eskenazi et al. 2005 Retrospective cohort study of women participating in the Seveso Women's Health Study (n=616)	Median serum 2,3,7,8-TCDD level: 43.7 ppt	Early menopause	↔
Eskenazi et al. 2007 Retrospective cohort study of women participating in the Seveso Women's Health Study (n=956)	Serum 2,3,7,8-TCDD tertiles (ppt): • T1: ≤20 • T2: 20.1–75.0 • T3: >75	Uterine fibroids	↓, T2
Eskenazi et al. 2010 Retrospective cohort study of women participating in the Seveso Women's Health Study (n=278)	Median serum 2,3,7,8-TCDD level: 49.7 ppt at time of accident Extrapolated median serum 2,3,7,8-TCDD level at time of pregnancy: 13.4 ppt	Time to pregnancy	↑
		Infertility	↑
Eskenazi et al. 2021 Retrospective cohort study of women participating in the Seveso Women's Health Study (n=446)	Median serum 2,3,7,8-TCDD level at the time of accident: 61.4 ppt Estimated median serum 2,3,7,8-TCDD level at pregnancy: 12.8 ppt	Time to pregnancy	↑, initial level ↑, at pregnancy
		Infertility	↑, initial level ↔, at pregnancy

Table 2-20. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Mocarelli et al. 2000 Retrospective cohort study of 296 mothers and 239 fathers living in zones A, B, or R at the time of the accident	Median serum 2,3,7,8-TCDD levels: 96.5 ppt in fathers and 62.75 ppt in mothers	Sex ratio (male:female)	↓, fathers with 2,3,7,8-TCDD levels >15 ppt ↔, mothers with 2,3,7,8-TCDD levels >80 ppt
Mocarelli et al. 2008 Retrospective cohort study of 135 males (age at the time of the accident: 71 aged 1–9 years, 44 aged 10–17 years, and 20 aged 18–26 years) living in zones A, B, or R at the time of the accident and 372 referents	Median serum 2,3,7,8-TCDD level: 175 ppt for whole group, 201 ppt for ages 1–9 years, 164 ppt for ages 10–17 years, and 123 ppt for ages 18–26 years	Sperm concentration	↓, exposure at 1–9 years ↔, exposure at 10–17 years ↔, exposure at 18–26 years
		Progressive sperm motility	↓, exposure at 1–9 years ↔, exposure at 10–17 years ↔, exposure at 18–26 years
		Motile sperm	↓, exposure at 1–9 years ↓, exposure at 10–17 years ↔, exposure at 18–26 years
		Serum 17β-estradiol	↓, exposure 1–9 years ↓, exposure 10–17 years ↔, exposure at 18–26 years
		Serum FSH	↑, exposure 1–9 years ↑, exposure 10–17 years ↔, exposure at 18–26 years
		Serum LH	↔, all age groups
		Serum testosterone	↔, all age groups
		Serum inhibin B	↔, all age groups
Warner et al. 2004 Retrospective cohort study of women participating in the Seveso Women's Health Study (n=446)	Median serum 2,3,7,8-TCDD level in 1976: 140.3 ppt	Age at onset of menarche	↔

Table 2-20. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Warner et al. 2007 Retrospective cohort study of women participating in the Seveso Women's Health Study (n=282 premenarcheal at exposure)	Median serum 2,3,7,8-TCDD level: 77.3 ppt	Ovarian follicles >10 mm	↔
		Ovulation	↔
		Serum progesterone	↔
		Serum estradiol	↔
Populations living in contaminated areas of Vietnam			
Van Luong et al. 2018 Cross-sectional study of 42 men living in areas of Vietnam with Agent Orange contamination	Geometric mean serum levels (pg/g lipid) • 2,3,7,8-TCDD: 7.3 • 1,2,3,7,8-PeCDD: 10.0 • 1,2,3,4,7,8-HxCDD: 7.5 • 1,2,3,6,7,8-HxCDD: 14.5 • 1,2,3,7,8,9-HxCDD: 9.2 • 1,2,3,4,6,7,8-HpCDD: 28.1 • OCDD: 648.6	FSH	↔, all congeners
		LH	↔, all congeners
		Progesterone	↔, all congeners
		Prolactin • 2,3,7,8-TCDD • 1,2,3,7,8-PeCDD • 1,2,3,4,7,8-HxCDD • 1,2,3,6,7,8-HxCDD • 1,2,3,7,8,9-HxCDD • 1,2,3,4,6,7,8-HpCDD • OCDD	 ↑ ↑ ↔ ↑ ↔ ↑ ↔
		Estradiol • 2,3,7,8-TCDD • 1,2,3,7,8-PeCDD • 1,2,3,4,7,8-HxCDD • 1,2,3,6,7,8-HxCDD • 1,2,3,7,8,9-HxCDD • 1,2,3,4,6,7,8-HpCDD • OCDD	 ↔ ↔ ↔ ↔ ↔ ↓ ↔

Table 2-20. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Testosterone • 2,3,7,8-TCDD • 1,2,3,7,8-PeCDD • 1,2,3,4,7,8-HxCDD • 1,2,3,6,7,8-HxCDD • 1,2,3,7,8,9-HxCDD • 1,2,3,4,6,7,8-HpCDD • OCDD	 ↔ ↓ ↔ ↔ ↓ ↔ ↔
Sun et al. 2016 Cross-sectional study of men living in areas of Vietnam with Agent Orange contamination (n=50) or in non-sprayed areas (n=48)	Geometric mean serum levels in hotspot (pg TEQ/g lipid) • 2,3,7,8-TCDD: 2.63 • 1,2,3,7,8-PeCDD: 8.32 • 1,2,3,4,7,8-HxCDD: 0.50 • 1,2,3,6,7,8-HxCDD: 1.91 • 1,2,3,7,8,9-HxCDD: 0.65 • 1,2,3,4,6,7,8-HpCDD: 0.28 • OCDD: 0.10 Geometric mean serum levels in non-sprayed area (pg TEQ/g lipid) • 2,3,7,8-TCDD: 145 • 1,2,3,7,8-PeCDD: 2.40 • 1,2,3,4,7,8-HxCDD: 0.27 • 1,2,3,6,7,8-HxCDD: 0.45 • 1,2,3,7,8,9-HxCDD: 0.07 • 1,2,3,4,6,7,8-HpCDD: 0.28 • OCDD: 0.02	Prostate specific antigen	↔, all congeners
Sun et al. 2017 Cross-sectional study of men living in areas of Vietnam with Agent Orange contamination (n=50) or in non-sprayed areas (n=48)	Geometric mean serum 1,2,3,7,8-PeCDD levels in hotspot and non-sprayed area: 9.5 and 2.2 pg/g lipid	Reproductive hormones • Testosterone • DHT • DHEA • Estradiol • 3β-HSD	↔

Table 2-20. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Geometric mean serum 1,2,3,6,7,8-HxCDD levels in hotspot and non-sprayed area: 21.6 and 3.8 pg/g lipid	Reproductive hormones • Testosterone • DHT • DHEA • Estradiol • 3β-HSD	↔
	Geometric mean serum 1,2,3,4,6,7,8-HpCDD levels in hotspot and non-sprayed area: 33.2 and 6.2 pg/g lipid	Reproductive Hormones • Testosterone • DHT • DHEA • Estradiol • 3β-HSD	↔
General population			
Cai et al. 2011 Cross-sectional study of infertile women in Japan with (n=10) or without (n=7) endometriosis	Mean CDD/CDF levels in peritoneal fluid in endometriosis and control groups: 12.2 and 10.8 pg TEQ/g lipid	Endometriosis	↑
De Felip et al. 2004 Case-control study in 22 Italian and 18 Belgian women with and without endometriosis	Total TEQs in women without endometriosis: 18 pg/g lipid (Italian) and 45 pg/g lipid (Belgium)	Endometriosis	↔
Fierens et al. 2003 Volunteer-case study in Belgium; environmental exposure to CDDs, CDFs, PCBs+12 marker PCB (not TEQs)	Total TEQs (geometric mean): Cases (n=10) 34.6 pg TEQ/g lipid Controls (n=132) 34.5 pg TEQ/g lipid	Endometriosis	↔

Table 2-20. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Heilier et al. 2005 Case control study of 25 women with peritoneal endometriosis, 25 women with deep endometriotic nodules, and 21 controls in Belgium	Geometric mean CDD/CDF serum levels (pg TEQ/g lipid): 20.9 in women with peritoneal endometriosis, 26.0 in women with deep endometriotic nodules, and 15.5 in controls	Peritoneal endometriosis	↑
		Deep endometriotic nodules	↑
Lambertino et al. 2021 Cross-sectional study using the NHANES (1999–2000 and 2001–2002) database of 89 post-menopausal women	Mean serum CDD/CDF/PCB levels: 0.11 pg TEQ/g	LH	↓
		FSH	↔
Martínez -Zamora et al. 2015 Case-control study of 32 women with deep infiltrating endometriosis and 34 controls (Spain)	Median adipose levels (pg/g lipid) in cases • 2,3,7,8-TCDD: 0.70 • 1,2,3,7,8-PeCDD: 2.41 • 1,2,3,4,7,8-HxCDD: 1.45 • 1,2,3,6,7,8-HxCDD: 9.20 • 1,2,3,7,8,9-HxCDD: 1.21 • 1,2,34,6,7,8-HpCDD: 7.80 • OCDD: 68.10 Median adipose levels (pg/g lipid) in controls • 2,3,7,8-TCDD: 0.40 • 1,2,3,7,8-PeCDD: 1.67 • 1,2,3,4,7,8-HxCDD: 1.23 • 1,2,3,6,7,8-HxCDD: 8.40 • 1,2,3,7,8,9-HxCDD: 1.10 • 1,2,34,6,7,8-HpCDD: 10.22 • OCDD: 61.20	Deep infiltrating endometriosis • 2,3,7,8-TCDD • 1,2,3,7,8-PeCDD • 1,2,3,4,7,8-HxCDD • 1,2,3,6,7,8-HxCDD • 1,2,3,7,8,9-HxCDD • 1,2,34,6,7,8-HpCDD • OCDD	 ↑ ↑ ↔ ↔ ↔ ↔ ↔

Table 2-20. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Pauwels et al. 2001 Cases of infertile women with endometriosis and 27 infertile controls in the Netherlands	Median serum CDD/CDF/PCB levels (pg TEQ/g lipid): 29 in cases and 27 in controls	Endometriosis	↔

↑ = association; ↓ = inverse association; ↔ = no association; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; 3β-HSD= 3β-hydroxysteroid dehydrogenase; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; DHEA = dehydroepiandrosterone; DHT = dihydrotestosterone; FSH = follicle-stimulating hormone; HpCDD = heptachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; LH = luteinizing hormone; NHANES = National Health and Nutrition Examination Survey; OCDD = octachlorodibenzo-*p*-dioxin; PCB = polychlorinated biphenyl; PeCDD = pentachlorodibenzo-*p*-dioxin; Q = quartile; T = tertile; TCDD = tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalency

cases presented themselves at a gynecology ward of a university hospital. In contrast to previous studies in the literature, the control group was not recruited from the infertility clinic. A second case-control study found associations between the risk of deep infiltrating endometriosis and adipose 2,3,7,8-TCDD levels and 1,2,3,7,8-PeCDD levels but not with 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, or OCDD levels (Martínez-Zamora et al. 2015). An increased risk of endometriosis was also associated with CDD/CDF peritoneal fluid levels in a cross-sectional study of Japanese women (Cai et al. 2011). In contrast, other population-based, case-control studies reported no association between total TEQs of dioxins and endometriosis (De Felip et al. 2004; Fierens et al. 2003; Pauwels et al. 2001).

A small number of studies have examined reproductive endpoints in males. An increased risk of benign prostatic hyperplasia was observed among Operation Ranch Hand veterans (Gupta et al. 2006). No associations between prostate specific antigen levels and serum levels of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, or OCDD were observed in men living in areas of Vietnam contaminated with Agent Orange (Sun et al. 2016). Among Seveso residents, inverse associations between serum 2,3,7,8-TCDD levels and sperm concentration, progressive sperm motility, and total number of motile sperm were observed among males who were 1–9 years of age at the time of the accident (Mocarelli et al. 2008). An inverse association was also observed for total number of motile sperm in men aged 10–17 years at the time of the accident. No associations between serum 2,3,7,8-TCDD levels and sperm parameters were observed in men aged 18–26 years at the time of the accident. No alterations in sperm count were observed in two studies of Operation Ranch Hand veterans (Henriksen et al. 1996; Wolfe et al. 1985).

Fertility has been evaluated in a small number of epidemiological studies. Two studies of female Seveso residents reported associations between serum 2,3,7,8-TCDD levels and increased time to pregnancy and infertility (Eskenazi et al. 2010, 2021). In another study of Seveso residents, a decrease in male:female sex ratio was observed among fathers with 2,3,7,8-TCDD levels >15 ppt; no association was found among females with serum 2,3,7,8-TCDD levels >80 ppt (Mocarelli et al. 2000).

2,3,7,8-TCDD—Animal Studies. A number of animal studies have evaluated the effect of oral exposure to 2,3,7,8-TCDD on reproductive hormone levels; the results of these studies are summarized in Table 2-21. In male rats and mice, 2,3,7,8-TCDD exposure resulted in decreased levels of serum testosterone (Dhanabalan et al. 2010, 2011; Ma et al. 2010; Moore et al. 1985; Yin et al. 2012) and

Table 2-21. Effects on Reproductive Hormone Levels in Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, exposure duration	Dose (µg/kg/day)	Testosterone	Estradiol	Progesterone	Luteinizing hormone	Follicle-stimulating hormone	Reference
Sprague-Dawley rat, once	10				↑ (F)	↑ (F)	Li et al. 1995a
Sprague-Dawley rat, once	12.5	↓ (M)					Moore et al. 1985
Sprague-Dawley rat, once	10		↔ (F)	↔ (F)			Petroff et al. 2000
Sprague-Dawley rat, once	32				↑ (F)	↑ (F)	Petroff et al. 2002
Line C rat, once	30	↓ (M)					Simanainen et al. 2004a
Sprague-Dawley rat, once	20		↔ (F)	↔ (F)	↔ (F)		Son et al. 1999
Sprague-Dawley rat, once	32		↑ (F)	↔ (F)			Ushinohama et al. 2001
Sprague-Dawley rat, 29 weeks	0.02		↓ (F)				Chen et al. 2009
Wistar/NIN rats, 15 day	0.1	↓ (M)					Dhanabalan et al. 2010
Wistar/NIN rat, 15 days	0.1	↓ (M)					Dhanabalan et al. 2011
Sprague-Dawley rat, 29 weeks	0.05	↓ (M)					Ma et al. 2010
Sprague-Dawley rat, 29 weeks	0.125				↓ (M testicular)	↓ (M testicular)	Ma et al. 2010
NIH mouse, GDs 1–3, 1–8, or 4–8	0.002			↓ (F)			Li et al. 2006

Table 2-21. Effects on Reproductive Hormone Levels in Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, exposure duration	Dose (µg/kg/day)	Testosterone	Estradiol	Progesterone	Luteinizing hormone	Follicle-stimulating hormone	Reference
NIH mouse, GDs 1–3, 1–8, or 4–8	0.01		↔ (F)				Li et al. 2006
BALB/c mouse, 28 days	0.09	↑ (F)	↔ (F)				Maranghi et al. 2013
BALB/c mouse, 28 days	0.0009	↔ (F)	↔ (F)				Rasinger et al. 2018
Mouse (strain NS), 7 weeks	0.1	↓ (M)			↓ (M testicular)	↓ (M testicular)	Yin et al. 2012
Cynomolgus monkey, once	4		↔ (F)	↓ (F)			Morán et al. 2001

↑ = association; ↓ = inverse association; ↔ = no association; F = females; GD = gestation day; M = males

decreased testicular levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Ma et al. 2010; Yin et al. 2012). In female rats, exposure to 2,3,7,8-TCDD resulted in increased serum testosterone levels (Maranghi et al. 2013). Although there is some inconsistency, most studies of rats and mice have not found alterations in serum estradiol levels in females (Li et al. 2006; Maranghi et al. 2013; Morán et al. 2001; Petroff et al. 2000; Rasinger et al. 2018; Son et al. 1999). Decreased progesterone levels have been observed in female monkeys (Morán et al. 2001) and mice (Li et al. 2006) acutely exposed but have not been found in rats following acute-duration exposure (Son et al. 1999; Petroff et al. 2000; Ushinohama et al. 2001). Increased serum LH and FSH levels have been observed in rats acutely exposed (Li et al. 1995a; Petroff et al. 2002), although a third study did not find an alteration in LH levels at a similar dose level (Son et al. 1999). In a study of pregnant mice, decreased progesterone and estradiol levels were observed on GD 17; no alterations in prolactin levels were observed (Vorderstrasse et al. 2004).

A variety of reproductive effects have been observed in male and female animals orally exposed to 2,3,7,8-TCDD; the results of these studies are summarized in Table 2-22. Several studies in rats have reported decreased epididymal sperm counts, daily sperm production, sperm viability, and sperm motility (Dhanabalan et al. 2010, 2011; El-Tawil and Elsaieed 2005; Latchoumycandane et al. 2002; Ma et al. 2010; Simanainen et al. 2004a). The lowest LOAEL was 0.001 µg/kg/day observed in Wistar rats administered 2,3,7,8-TCDD for 45 days (Latchoumycandane et al. 2002). A dose-related decrease in epididymal sperm counts was observed; the magnitudes of the changes were approximately 9, 23, and 36% at 0.001, 0.01, and 0.1 µg/kg/day, respectively. Decreased sperm motility, decreased sperm viability, and increased sperm head and tail abnormalities were observed at ≥0.05 µg/kg/day (Dhanabalan et al. 2010, 2011; El-Tawil and Elsaieed 2005). In the only study examining sperm parameters in mice, decreased testicular spermatozoa and necrosis of spermatocytes and spermatogonia were observed in mice (strain not specified) administered 0.1 µg/kg/day for 7 weeks (Yin et al. 2012).

Table 2-22. Reproductive Effects in Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)

Species, duration of exposure	Dose (µg/kg/day)	Effect	Reference
Males			
Line C rat, once	10	↓ daily sperm production and caudal sperm reserve	Simanainen et al. 2004a
Wistar/NIN rat, 15 days	0.1	↓ epididymal count, ↓ sperm viability, ↓ sperm motility	Dhanabalan et al. 2010

Table 2-22. Reproductive Effects in Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)

Species, duration of exposure	Dose (µg/kg/day)	Effect	Reference
Wistar/NIN rat, 15 days	0.1	↓ epididymal count, ↓ sperm viability, ↓ sperm motility ↓ testicular daily sperm production	Dhanabalan et al. 2011
Sprague-Dawley rat, 60 days	0.05	↓ sperm counts, motility ↑ sperm mortality and abnormalities	El-Tawil and Elsaieed 2005
ICR mouse, 5 weeks	0.1	↓ male/female ratio	Ishihara et al. 2007
ICR mouse, 5 weeks	0.1	↓ male/female ratio	Ishihara et al. 2010
Wistar rat, 45 days	0.001	↓ epididymal sperm count	Latchoumycandane et al. 2002
Sprague-Dawley rat, 29 weeks	0.05	↓ sperm counts	Ma et al. 2010
Mouse (strain NS), 7 weeks	0.1	↓ testicular spermatozoa; necrosis of spermatocytes and spermatogonia	Yin et al. 2012
Females			
CRCD rat, 2 weeks	2	↓ corpora lutea, ↑ pre-implantation loss	Giavini et al. 1983
Sprague-Dawley rat, once	32	Inhibition of ovulation	Jung et al. 2010
Sprague-Dawley rat, once	10	↓ ovulation (number of animals ovulating and number of ova recovered)	Li et al. 1995a
Sprague-Dawley rat, once	10	↓ ovulation; irregular estrous cycle	Li et al. 1995b
Sprague-Dawley rat, once	10	↓ ovarian weight, ↓ ovulation	Petroff et al. 2000
Sprague-Dawley rat, once	32	inhibition of ovulation	Petroff et al. 2002
Cynomolgus monkey, once	4	↑ uterine antral follicle size, anovulation, lack of menstrual cycle	Morán et al. 2001
Cynomolgus monkey, once	1	Squamous metaplasia in endocervix	Scott et al. 2001
Sprague-Dawley rat, once	20	↓ ovulation	Son et al. 1999
Wistar rat, once (GD 15)	1	↓ maternal nursing behavior and milk ejection volume	Takeda et al. 2020
Sprague-Dawley rat, once	32	Delayed ovulation	Ushinohama et al. 2001
Siberian hamster, once	2	↑ time to pregnancy	Yellon et al. 2000
C57BL/6J mouse, GD 0, 7, 14	1	Suppression of mammary gland differentiation	Vorderstrasse et al. 2004
Wistar rat, 15 weeks	0.046	↔ mating, fertility, or fecundity indices	Bell et al. 2007b

Table 2-22. Reproductive Effects in Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)

Species, duration of exposure	Dose (µg/kg/day)	Effect	Reference
Sprague-Dawley rat, 2–6 doses in 3–15 weeks	10	↑ endometriotic growth	Cummings et al. 1996
B6C3F1 mouse, 2–6 doses in 3–15 weeks	3	↑ endometriotic growth	Cummings et al. 1996
Wistar rat, 16 weeks	0.14	Decreased number of ovarian follicles at the post-primordial phase and corpus luteum	Gül et al. 2018
Holtzman rat, 9 weeks	0.02	↔ sex ratio	Ikeda et al. 2005b
B6C3F1 mouse, 5 doses in 12 weeks	0.6	↑ endometriotic growth	Johnson et al. 1997
NIH mouse, GDs 1–3, 1–8, or 4–8	0.05	↓ implantation sites	Li et al. 2006
Sprague-Dawley rat, 90 days prior to mating and throughout gestation	0.1	↓ fertility	Murray et al. 1979
Sprague-Dawley rat, 15 or 31 weeks	0.071	↔ histopathology	NTP 2006
Sprague-Dawley rat, 105 weeks	0.016	Dilation of clitoral gland ducts	NTP 2006
Rhesus monkey, 3.5–4 years	0.00012	↑ endometriosis	Rier et al. 1993
Rhesus monkey, 3.5–4 years	0.00064	↓ reproductive success	Bowman et al. 1989a, 1989b; Hong et al. 1989; Schantz and Bowman 1989; Schantz et al. 1986, 1992

↑ = increase; ↓ = decrease; ↔ = no change; GD = gestation day; NS = not specified

Reproductive effects have also been observed in female animals, including effects on ovarian function, menstrual cycle, endometriosis, and fertility (summarized in Table 2-22). Effects on ovarian function include decreased number of ovarian follicles in the post-primordial phase and corpus luteum in rats administered 0.14 µg/kg/day for 16 weeks (Gül et al. 2018) and decreased ovulation, inhibition of ovulation, or delayed ovulation in rats receiving a single dose of ≥10 µg/kg/day (Jung et al. 2010; Li et al. 1995a, 1995b; Petroff et al. 2000, 2002; Son et al. 1999; Ushinohama et al. 2001). Anovulation and an increase in antral follicle size were observed in monkeys administered a single dose of 4 µg/kg and examined 443–625 days post-exposure (Morán et al. 2001). Alterations in menstrual cycle have also been

observed. Morán et al. (2001) reported a lack of menstrual cycle in monkeys administered a single dose of 4 µg/kg 2,3,7,8-TCDD; prolonged periods of diestrus with a loss of proestrus and estrus phases was observed in rats following administration of a single dose of 10 µg/kg.

Rier et al. (1993) reported a dose-related increase in the incidence and severity of endometriosis in monkeys chronically exposed to 0.00012 or 0.00064 µg/kg/day for 3.5–4 years of 2,3,7,8-TCDD in the diet and maintained for 10 years. In a follow-up study of these monkeys, blood levels of 2,3,7,8-TCDD and other related compounds were measured in blood samples taken 13 years post-exposure termination (Rier et al. 2001b). An increased level of the PCB congener, 3,3′,4,4′-tetrachlorobiphenyl, was observed in both groups of 2,3,7,8-TCDD exposed animals in a 2,3,7,8-TCDD dose-related manner. 2,3,7,8-TCDD levels did not significantly differ in animals with or without endometriosis. However, serum 3,3′,4,4′-tetrachlorobiphenyl levels were associated with endometriosis; elevated 3,3′,4,4′-tetrachlorobiphenyl levels were only observed in animals with endometriosis and exposed to 2,3,7,8-TCDD and the severity of the endometriosis was correlated with 3,3′,4,4′-tetrachlorobiphenyl levels. These data suggest that 3,3′,4 4′-tetrachlorobiphenyl may have been the causative agent rather than 2,3,7,8-TCDD. 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 in rats (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 (route of exposure not reported) 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) noted that pre-exposure to 2,3,7,8-TCDD results in endometriosis development due to immune suppression rather than an estrogen responsive disease. A study in Rhesus monkeys found that exposure to 0.0035 µg/kg/day 2,3,7,8-TCDD exposure for 12 months resulted in increased survival of an endometrial implant (Yang et al. 2000).

In a 3-generation study in rats, decreased fertility indices were observed in the F0 rats exposed to 0.1 µg/kg/day for 90 days prior to mating and during gestation (Murray et al. 1979). Following a 12-month exposure, a decreased fertility index was observed when the exposed females were mated with

unexposed younger males; no effect was observed when males were mated with unexposed younger females (Murray et al. 1979). A decrease in reproductive success (evaluated using an ordinal scale of offspring survival time) was observed in monkeys exposed to 0.0064 µg/kg/day for 7 or 27 months (Bowman et al. 1989a, 1989b; Hong et al. 1989; Schantz and Bowman 1989; Schantz et al. 1986, 1992). No effects on fertility or fecundity were observed in rats exposed to 0.046 µg/kg/day for 15 weeks (Bell et al. 2007b).

A study in Wistar rats examined maternal behaviors following exposure to 1 µg/kg 2,3,7,8-TCDD on GD 15 (Takeda et al. 2020). Maternal licking behavior was significantly reduced on postnatal days (PNDs) 2, 4, 7, and 10; there were no alterations in time spent crouching, nesting, or retrieving. A decrease in milk ejection volume was also observed. The study also found decreased levels of circulating prolactin in the dams on PNDs 2, 4, 7, and 10, which corresponded to the decreased maternal licking behavior. A group of 2,3,7,8-TCDD-exposed dams were administered prolactin intracerebroventricularly, which resulted in a significant increase in maternal licking behavior; licking time was no longer significantly different from controls.

Two studies reported histological alterations in female reproductive tissue. Squamous metaplasia was observed in the endocervix of monkeys administered a single dose of 1 µg/kg 2,3,7,8-TCDD and examined 1.2–2.7 years post exposure (Scott et al. 2001). Dilation of clitoral gland ducts were observed in rats exposed to 0.016 µg/kg/day for 2 years (NTP 2006); histological alterations were observed from exposure to 0.071 µg/kg/day for 15 or 31 weeks (NTP 2006). Other reproductive effects observed in females orally exposed to 2,3,7,8-TCDD include decreased pre-implantation sites in NIH mice exposed to 0.05 µg/kg/day 2,3,7,8-TCDD on GDs 1–3, 4–8, or 1–8 (Li et al. 2006), increased pre-implantation loss in CRCD rats exposed to 2 µg/kg/day (Giavini et al. 1983), decreased sex ratio in the offspring of female Holtzman rats exposed to 0.02 µg/kg/day for 9 weeks prior to mating (Ikeda et al. 2005b) and of the offspring of male ICR mice exposed to 0.1 µg/kg/day for 5 weeks (Ishihara et al. 2007, 2010), increased time to pregnancy in Siberian hamsters exposed to a single dose of 2 µg/kg (Yellon et al. 2000), and suppression of mammary gland differentiation in mice exposed to 1 µg/kg/day on GDs 0, 7, and 14 (Vorderstrasse et al. 2004). No effects on mating, fertility, or fecundity indices were observed in female Wistar rats exposed to 0.046 µg/kg/day for 15 weeks (Bell et al. 2007b).

2.17 DEVELOPMENTAL

Overview. The potential developmental toxicity of CDDs in humans has been extensively investigated in highly exposed populations and the general population examining birth outcome, birth defects, endocrine and other systemic effects, immunological development, neurological development, and reproductive development.

- In general, studies involving paternal exposure to high levels of CDDs did not find associations between CDD levels and birth outcomes or birth defects; inconsistent results have been reported in highly exposed populations (male and female exposures) and birth outcome.
- One study of the Seveso cohort found an association between maternal blood 2,3,7,8-TCDD levels and neonatal TSH levels and the risk of elevated TSH levels; no associations were found in adult children. General population studies have not found consistent associations between maternal CDD levels and thyroid hormone levels in children.
- General population studies have not found consistent results for associations between maternal CDD levels and infections. The small number of general population studies evaluating associations between maternal CDD exposure and the child's risk of other immune responses such as asthma, wheezing, allergies, sensitization, or vaccine antibodies have not found consistent effects.
- Associations between exposure to CDDs and neurodevelopment have been evaluated in several prospective cohort studies of highly exposed populations and the general population. Results of these studies are mixed, with some studies finding associations between increasing exposure concentrations and decreasing performance on tests of cognition and behavior, but most studies found no associations.
- Epidemiological studies evaluating development of the reproductive system have found associations between CDD exposure and impaired development (decreases in sperm concentrations and delayed puberty) in boys in highly exposed populations.

The developmental toxicity of 2,3,7,8-TCDD has been extensively investigated, particularly following acute-duration oral exposure in rats and mice. The observed effects include increased offspring mortality, structural malformations and anomalies, impaired growth, impaired development of respiratory, cardiovascular, skeletal, and gastrointestinal systems, impaired thyroid function, and impaired development and function of the immune, nervous, and reproductive systems.

- Increased fetal/newborn mortality have been observed at ≥0.7 μg/kg/day in acute-duration studies, often at doses associated with no or minimal maternal toxicity. Intermediate-duration exposure to ≥0.01 μg/kg/day resulted in decreased neonatal survival.
- The most reported structural anomalies are cleft palate at doses ≥1 μg/kg/day and hydronephrosis at doses ≥0.5 μg/kg/day.

- Decreases in birth weight and growth were observed at doses ≥0.7 μg/kg/day.
- Impaired development of the lungs, heart abnormalities and decreased heart rate, decreased bone mineral density, impaired mandible and tooth development, and gastrointestinal hemorrhages have been reported.
- Decreased thymus weight and thymic atrophy and functional alterations in the response to bacteria, viruses, or mitogens at doses ≥0.011 μg/kg/day.
- Neurodevelopmental effects including morphological alterations, delays in neurodevelopmental milestones, hyperactivity, alterations in motor activity, alterations in social behaviors, and impaired learning have been reported in rats, mice, and/or monkeys. The lowest LOAELs for neurodevelopmental effects are ≥0.1, ≥0.046, and ≥0.00012 μg/kg/day following acute-, intermediate-, and chronic-duration exposure, respectively.

Developmental effects have also been observed in animals exposed to other CDD congeners.

- Cardiac myofibril edema in rat offspring exposed to 2,7-DCDD.
- Decreased thymus weight in rat offspring exposed to 1,2,3,7,8-PeCDD.
- Decreased fetal growth and skeletal and soft tissue anomalies in rat offspring exposed to mixed HxCDD congeners.
- No developmental effects were observed following oral exposure to 2-MCDD, 2,3-DCDD, 1,2,3,4-TCDD, or OCDD.

Epidemiological Studies. 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 and Vietnamese residents living in contaminated areas. In most of the human studies, exposure was poorly characterized; however, most studies used serum and/or human milk levels of CDDs as a biomarker of exposure. A summary of the epidemiological studies is presented in Table 2-23.

Birth outcomes. Among potentially highly exposed populations, no associations between dioxin or 2,3,7,8-TCDD exposure and an increased risk of spontaneous abortions were found (Aschengrau and Monson 1990; Schnorr et al. 2001; Townsend et al. 1982; Wesselink et al. 2014; Wolfe et al. 1995). Apart from the Wesselink et al. (2014), which used maternal biomarkers to examine the possible association, the other studies evaluating spontaneous abortions involved paternal exposure to CDDs. It

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Occupational			
Dimich-Ward et al. 1996 Nested case control study of 9,512 male sawmill production and maintenance workers at 10 sawmills in Canada using chlorophenate	Cumulative hours of exposure estimated based on work history. Workers divided into three cumulative exposure groups and a group of workers with the maximum exposure	Prematurity	↔
		Small for gestational age	↔
		Low birth weight	↔
		Stillborn	↔
		Neonatal deaths	↔
		Spina bifida or anencephaly	↑, maximum exposure
		Cataracts	↑, two highest cumulative exposure groups and maximum exposure group
		Undescended testicles	↑, highest cumulative exposure group
Lawson et al. 2004 Cross-sectional study of 176 male workers at two sodium trichlorophenol (or one of its derivatives) or 2,4,5-T production facilities in the United States and 217 neighborhood referents	Serum 2,3,7,8-TCDD levels estimated at the time of conception using current TCDD levels; median level of 254 pg/g lipid in workers and 6 pg/g lipid in referents.	Birth weight	↑, pregnancy occurred during employment
		Preterm birth	↔
Mannetje et al. 2017 Cross-sectional study of 355 children of 127 male workers and 21 female workers at a phenoxy herbicide production facility in New Zealand	Mean serum 2,3,7,8-TCDD level: 9 pg/g lipid High exposure: ≥4 pg/g lipid	Probability of male children of male workers	↓
		Probability of male children of female workers	↔

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Schnorr et al. 2001 Cross-sectional study of 259 male workers (300 pregnancies before exposure and 332 pregnancies after exposure) at two sodium trichlorophenol or one of its derivatives production facilities in the United States and 243 neighborhood referents (707 pregnancies)	Median paternal 2,3,7,8-TCDD serum level at pregnancy: 254 ppt in workers with pregnancies during exposure and 6 ppt in workers with pregnancies before exposure and in referents 4th quartile serum 2,3,7,8-TCDD level: ≥1,120 ppt	Spontaneous abortion	↔, 4th quartile
		Sex ratio	↔, 4th quartile
Smith et al. 1982 Cross-sectional study of 548 male workers spraying 2,4,5-T in New Zealand and 441 referents working as agricultural contractors	Not measured	Congenital defect	↔
		Miscarriage	↔
Townsend et al. 1982 Cross-sectional study of children of 370 male workers involved in chlorophenol processing and 345 male workers at the same facility but not exposed to dioxins	Exposure to 2,3,7,8-TCDD only Exposure to any dioxin Exposure levels were not reported	Spontaneous abortion	
		2,3,7,8-TCDD exposure	↔
		Dioxin exposure	↔
		Stillbirths	
		2,3,7,8-TCDD exposure	↔
		Dioxin exposure	↔
		Infant deaths	
		2,3,7,8-TCDD exposure	↔
		Dioxin exposure	↔
		Health defects	
		2,3,7,8-TCDD exposure	↔
		Dioxin exposure	↔
		Congenital malformations	
		2,3,7,8-TCDD exposure	↔
		Dioxin exposure	↔

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Vietnam veterans and Operation Ranch Hand veterans			
Aschengrau and Monson 1989 Case-control study of 201 women having spontaneous abortions through gestation week 27 and 1,119 controls (United States)	CDD levels were not measured; paternal military service in Vietnam was used as a surrogate for exposure to CDDs	Spontaneous abortion	↔
Aschengrau and Monson 1990 Case control study of 1,314 women delivering infants with one or more congenital anomalies, 121 women delivering stillborn infants without anomalies, 76 women with infants without anomalies dying shortly after birth, and 1,490 controls delivering infants without anomalies (United States)	CDD levels were not measured; paternal military service in Vietnam was used as a surrogate for exposure to CDDs	Congenital anomalies	↔
		Stillbirth	↔
		Newborn death	↔
Erickson et al. 1984 Case control study of 1,659 cases of major congenital abnormalities and 1,047 control infants living in Atlanta, Georgia	An Exposure Opportunity Index (EOI) was calculated to estimate the likelihood of exposure to Agent Orange among paternal Vietnam veterans	Multiple defects	↔
		Spina bifida	↑, highest EOI score
		Cleft palate	↔
		Cleft lip without cleft palate	↑, highest EOI score
		Other neoplasms	↑, highest EOI score
Grufferman et al. 2014 Case-control study of children with rhabdomyosarcoma (n=319 cases and 319 controls) in United States	Not measured	Rhabdomyosarcoma association with parental military service, particularly Vietnam War veterans	↔
Michalek et al. 1998 Retrospective study of 2,082 children (859 children of fathers involved in Operation Ranch Hand and 1,223 children in the comparison group)	Background group dioxin level: <10 ppt Low-exposure group dioxin level: ≤79 ppt High-exposure group dioxin level: >79 ppt	Preterm births	↔
		Intrauterine growth retardation	↔
		Infant deaths	↑, background, high

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Ngo et al. 2010 Meta-analysis of 7 studies examining 330 cases of spina bifida and 134,884 non-cases associated with paternal Agent Orange exposure	Not reported	Spina bifida	↑
Wolfe et al. 1995 Retrospective study of the offspring of 454 male veterans involved in Operation Ranch Hand and 570 comparison fathers	Background dioxin level: <10 ppt Low-exposure dioxin level: ≤110 ppt High-exposure dioxin level: >110 ppt	Spontaneous abortion	↑, low exposure ↔, high exposure
		Stillbirth	↔, low and high exposure
		Congenital defects	↑, low exposure ↔, high exposure
Populations living in contaminated areas in Vietnam			
Anh et al. 2017 Prospective study of 52 mother-infant pairs living in Bien Hoa, Vietnam; the control group of 52 mother-infant pairs lived in a noncontaminated area in northern Vietnam	Geometric mean CDDs/CDFs human milk levels: 9.19 and 3.48 pg TEQ/g lipid in Bien Hoa residents and controls, respectively	Salivary DHEA in 1-year-old children	↑, as compared to controls
		Salivary cortisol in 1-year-old children	↔, as compared to controls
Boda et al. 2018 Prospective study of 162 mother-newborn pairs living near the Bien Hoa airbase	Geometric mean levels in human milk: 2,3,7,8-TCDD: 2.2 pg/g lipid 1,2,3,6,7,8-HxCDD: 4.5 pg/g lipid	Estradiol cord blood levels	
		2,3,7,8-TCDD	↔, boys and girls
		1,2,3,6,7,8-HxCDD	↔, boys and girls
		Testosterone cord blood levels	
		2,3,7,8-TCDD	↔, boys and girls
		1,2,3,6,7,8-HxCDD	↔, boys and ↓ girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Dao et al. 2016 Cross-sectional study of 58 mother-infant pairs living near the Phu Cat airbase and 62 mother infant pairs living in a similar rural area in North Vietnam that was not sprayed with Agent Orange	Mean human milk levels of total CDDs 7.432 and 2.064 pg TEQs/g lipid in exposed and control populations Estimated dietary intake of CDDs and CDFs in infants: 54.2 and 18.0 pg TEQ/kg/day in exposed and control 8–9-week-old infants and 42.7 and 12.3 pg TEQ/kg/day in 12–14-week-old infants	Height	↔, compared to controls
		Weight	↔, compared to controls
		Head circumference	↔, compared to controls
		Chest circumference	↔, compared to controls
Nguyen et al. 2018 Prospective study of 185 mother-child (3 years of age) pairs living in Da Nang, Vietnam	Median human milk levels in mothers of boys and girls, respectively: 2,3,7,8-TCDD: 1.5 and 1.7 pg/g lipid 1,2,3,7,8-PeCDD: 4.2 and 4.5 pg/g lipid 1,2,3,4,7,8-HxCDD: 2.2 and 2.4 pg/g lipid 1,2,3,7,8,9-HxCDD: 8.3 and 8.4 pg/g lipid 1,2,3,4,6,7,8-HpCDD:2.5 and 2.7 pg/g lipid OCDD: 63.9 and 68.1 pg/g lipid	Food approach score (food responsiveness, enjoyment of food, desire to drink, and emotional overeating)	↔ for all CDD congeners
		Food avoidance score (satiety responsiveness, slowness in eating, fussiness, and emotional undereating)	↔ for all CDD congeners
Nishijo et al. 2012 Prospective study of 210 mother-infant pairs living in Da Nang, Vietnam	4th quartile CDD/CDF human milk level: 25.09 pg TEQ/g lipid	Weight	↔, boys at birth, 1 month, and 4 months ↓, boys 0–4 months ↔, girls at birth, 1 month, and 4 months ↓, girls 0–4 months
		Length	↔, boys at birth, 1 month, and 4 months ↔, boys 0–4 months ↔, girls at birth, 1 month, and 4 months ↔, girls 0–4 months

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Head circumference	↔, boys at birth, 1 month, and 4 months ↔, boys 0–4 months ↑, girls at birth, 1 month, and 4 months ↑, girls at 0–4 months
		Abdominal circumference	↔, boys at birth, 1 month, and 4 months ↔, girls at birth, 1 month, and 4 months
		BMI	↔, boys at birth, 1 month, and 4 months ↓, boys at 0–4 months ↔, girls at birth, 1 month, and 4 months ↓, girls at 0–4 months
Nishijo et al. 2014 Prospective study of 153 3-year-old children living in Da Nang, Vietnam follow-up to the Tai et al. (2013) study	Human milk 2,3,7,8-TCDD levels of <3.5 pg/g fat or ≥3.5 pg/g lipid Human milk CDDs/CDFs levels of <17.6 pg TEQ/g fat or ≥17.6 pg TEQ/g lipid	Bayley neurodevelopmental test	TCDD
		Cognitive total score	↔, boys and girls
		Language total score	↔, boys and girls
		Motor total score	↔, boys and girls
		Adaptive behavior total score	↔, boys and girls
		Autism Spectrum Rating Scale	TCDD
		Total score	↑, boys and girls
		DSM-IV-TR Scale	↑, boys and girls
		Social communication	↑, boys; ↔, girls
		Unusual behavior	↔, boys and girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Bayley neurodevelopmental test	CDDs/CDFs TEQ
		Cognitive total score	↓, boys; ↔, girls
		Language total score	↓, boys; ↔, girls
		Motor total score	↓, boys; ↔, girls
		Adaptive behavior total score	↓, boys; ↔, girls
		Autism Spectrum Rating Scale	CDDs/CDFs TEQ
		Total score	↔, boys and girls
		DSM-IV-TR Scale	↔, boys and girls
		Social communication	↔, boys and girls
		Unusual behavior	↔, boys and girls
Nishijo et al. 2021 Prospective study of 181 8-year-old children (follow-on study to Nishijo et al. 2014)	Mean human milk 2,3,7,8-TCDD levels of 1.1–1.7 pg/g lipid Mean human milk CDDs/CDFs levels of 11.6–13.7 pg TEQ/g fat	C-SHARP Aggression scores	CDDs/CDFs TEQ
		Verbal	↔
		Bullying	↔
		Covert	↔
		Hostility	↔
		Physical	↔
		C-SHARP Aggression scores	TCDD
		Verbal	↔
		Bullying	↔
		Covert	↑
		Hostility	↔
		Physical	↔
Pham et al. 2015 Prospective study of 214 mother-infant (1 year of age) pairs living in Da Nang, Vietnam; follow-up to the Tai et al. (2013) study	4th quartile human milk 2,3,7,8-TCDD levels of >3.5 pg/g lipid 4th quartile human milk CDDs/CDFs levels of ≥17.6 pg TEQ/g fat 4th quartile estimated dietary dioxin intake (DDI) of infants ≥118.2 pg TEQ/kg/day	Neurodevelopmental scores	↔, CDDs/CDFs TEQ
		Cognitive	↔, TCDD
			↔, DDI
		Neurodevelopmental scores	↔, CDDs/CDFs TEQ
		Motor	↔, TCDD
			↔, DDI
		Social emotional score	↓, CDDs/CDFs TEQ
			↓, TCDD
			↔, DDI
		Adaptive behavioral score	↔, CDDs/CDFs TEQ
			↔, TCDD
			↔, DDI

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Pham et al. 2019 Prospective study of 226 mother-child pairs living in Bien Hoa, Vietnam and 75 mother-child pairs living in a non-exposed area of Vietnam; children were tested at 2 years of age	Human milk level 2,3,7,8-TCDD 2nd tertile: 1.8–5.5 pg/g lipid 3rd tertile: ≥5.5 pg/g lipid 1,2,3,7,8-PeCDD 2nd tertile: 3.1–4.9 pg/g lipid 3rd tertile: ≥4.9 pg/g lipid 1,2,3,4,7,8-HxCDD 2nd tertile: 1.4–2.7 pg/g lipid 3rd tertile: ≥2.7 pg/g lipid 1,2,3,6,7,8-HxCDD 2nd tertile:2.9–9.2 pg/g lipid 3rd tertile: ≥9.2 pg/g lipid 1,2,3,7,8,9-HxCDD 2nd tertile: 1.7–3.6 pg/g lipid 3rd tertile: ≥3.6 pg/g lipid 1,2,3,4,6,7,8-HpCDD 2nd tertile: ≥4.5–22.0 pg/g lipid 3rd tertile: ≥22.0 pg/g lipid OCDD 2nd tertile: 54.0–162 pg/g lipid 3rd tertile: ≥162 pg/g lipid CDDs TEQ 2nd tertile: 5.3–11.9 pg TEQ/g lipid 3rd tertile: ≥11.9 pg TEQ/g lipid	Bayley scale test scores Cognitive Composite language Composite motor	TCDD ↔, boys, girls ↔, boys, girls ↓, boys 2nd tertile, ↔, girls
		Bayley scale composite score	1,2,3,7,8-PeCDD ↓, boys 2nd tertile ↔, girls
		Bayley scale composite score	1,2,3,4,7,8-HxCDD ↓, boys 2nd tertile ↔, girls
		Bayley scale composite score	1,2,3,6,7,8-HxCDD ↓, boys 2nd tertile ↔, girls
		Bayley scale composite score	1,2,3,7,8,9-HxCDD ↓, boys 2nd tertile ↔, girls
		Bayley scale composite score	1,2,3,4,6,7,8-HpCDD ↔, boys ↓, girls 2nd tertile
		Bayley scale composite score	OCDD ↓, boys 2nd tertile ↔, girls
		Bayley scale composite score	CDDs TEQ ↓, boys 2nd tertile ↓, girls 3rd tertile

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Pham et al. 2020b Prospective study of 815 mother-child (8 years of age) pairs living in Da Nang, Vietnam; follow-up to the Tai et al. (2013, 2016), Pham et al. (2015), Nishijo et al. (2012, 2014), and Tran et al. (2016) studies	Human milk CDD levels 2,3,7,8-TCDD 2nd tertile: 1.8–3.5 pg/g lipid 3rd tertile: ≥3.5 pg/g lipid CDD/CDF TEQ 2nd tertile: 11.5–17.6 pg TEQ/g lipid 3rd tertile: ≥17.6 pg TEQ/g lipid	Feminine index of gaze behavior in response to biological stimuli (human line drawing)	2,3,7,8-TCDD ↔, boys ↑, girls, 3rd tertile
		Feminine index of gaze behavior in response to biological stimuli (human line drawing)	CDD/CDF TEQ ↑, boys, 3rd tertile ↔, girls
		Feminine index of gaze behavior in response to non-biological stimuli (toy photos)	2,3,7,8-TCDD ↔, boys ↑, girls
		Feminine index of gaze behavior in response to non-biological stimuli (toy photos)	CDD/CDF TEQ ↔, boys ↔, girls
Pham et al. 2021 Prospective cohort study of 51 mother-newborn pairs living in Bien Hoa, Vietnam	Human milk CDD levels, geometric mean 2,3,7,8-TCDD: 2.2 pg/g lipid CDD/CDF TEQ: 7.9 pg TEQ/g lipid	Alterations in EEG power values in the quiet sleep stage	↑, 2,3,7,8-TCDD
Phuong et al. 1989 Retrospective cohort study of 1,249 families living in area that was heavily sprayed with Agent Orange and 1,224 families living in a non-sprayed area	Not measured	Hydatidiform mole	↑

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Sun et al. 2020	Human milk levels median	Salivary DHEA	
	2,3,7,8-TCDD	1-year-old children	
Prospective cohort study; follow-on study to Anh et al. (2017); examined 26 exposed and 26 unexposed children examined at 1, 3, and 5 years of age	Exposed: 1.8 pg/g lipid	2,3,7,8-TCDD	↔
	Unexposed: 0.5 pg/g lipid	1,2,3,7,8-PeCDD	↔
	1,2,3,7,8-PeCDD	1,2,3,4,7,8-HxCDD	↔
	Exposed: 2.6 pg/g lipid	1,2,3,6,7,8-HxCDD	↔
	Unexposed: 1.0 pg/g lipid	1,2,3,7,8,9-HxCDD	↔
	1,2,3,4,7,8-HxCDD	1,2,3,4,6,7,8-HpCDD	↑
	Exposed: 1.4 pg/g lipid	OCDD	↑
	Unexposed: 0.7 pg/g lipid	3-year-old children	
	1,2,3,6,7,8-HxCDD	2,3,7,8-TCDD	↔
	Exposed: 4.5 pg/g lipid	1,2,3,7,8-PeCDD	↔
	Unexposed: 1.3 pg/g lipid	1,2,3,4,7,8-HxCDD	↔
	1,2,3,7,8,9-HxCDD	1,2,3,6,7,8-HxCDD	↔
	Exposed: 1.5 pg/g lipid	1,2,3,7,8,9-HxCDD	↔
	Unexposed: 0.5 pg/g lipid	1,2,3,4,6,7,8-HpCDD	↔
	1,2,3,4,6,7,8-HpCDD	OCDD	↔
	Exposed: 8.0 pg/g lipid	5-year-old children	
	Unexposed: 2.6 pg/g lipid	2,3,7,8-TCDD	↓
	OCDD	1,2,3,7,8-PeCDD	↓
	Exposed: 56.3 pg/g lipid	1,2,3,4,7,8-HxCDD	↔
	Unexposed: 13.5 pg/g lipid	1,2,3,6,7,8-HxCDD	↓
		1,2,3,7,8,9-HxCDD	↓
		1,2,3,4,6,7,8-HpCDD	↓
		OCDD	↓

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Salivary testosterone	
		3-year-old children	
		2,3,7,8-TCDD	↓
		1,2,3,7,8-PeCDD	↔
		1,2,3,4,7,8-HxCDD	↔
		1,2,3,6,7,8-HxCDD	↓
		1,2,3,7,8,9-HxCDD	↔
		1,2,3,4,6,7,8-HpCDD	↔
		OCDD	↔
		5-year-old children	
		2,3,7,8-TCDD	↔
		1,2,3,7,8-PeCDD	↓
		1,2,3,4,7,8-HxCDD	↔
		1,2,3,6,7,8-HxCDD	↔
		1,2,3,7,8,9-HxCDD	↔
		1,2,3,4,6,7,8-HpCDD	↓
		OCDD	↓
Tai et al. 2013 Prospective study of 216 mother-infant (4 months of age) pairs living in Da Nang, Vietnam	Human milk levels 2,3,7,8-TCDD Moderate group: 1.8–3.5 pg/g lipid CDDs High group: ≥12.3 pg TEQ/g lipid	Bayley Scales of Infant and Toddler Development Cognitive score Language composite score Motor composite score	 ↓, moderate TCDD ↔, high CDDs ↓, moderate TCDD ↔, high CDDs ↓, moderate TCDD ↔, high CDDs
Tai et al. 2016 Prospective study of 217 mother-child (3 years of age) pairs living in Da Nang, Vietnam; follow-up to the Tai et al. (2013) and Pham et al. (2015) studies	Mean human milk levels of 2,3,7,8-TCDD 1.4 pg/g lipid and CDD/CDF 12.5 pg TEQ/g lipid	Bayley Scales of Infant and Toddler Development Cognitive score Language composite score Motor composite score	 ↔, TCDD ↔, CDD/CDF ↔, TCDD ↔, CDD/CDF ↓, TCDD (boys only) ↔, CDD/CDF

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Weight	TCDD ↓, boys ↔, girls CDDs ↓, boys ↔, girls
		Height	TCDD ↓, boys ↔, girls CDDs ↔, boys ↔, girls
		Head circumference	TCDD ↓, boys ↔, girls CDDs ↓, boys ↔, girls
		Abdominal circumference	TCDD ↓, boys ↔, girls CDDs ↓, boys ↑, girls
		BMI	TCDD ↔, boys ↔, girls CDDs ↓, boys ↔, girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Tai et al. 2020 Prospective study of 185 mother-child (8 years of age) pairs living in Da Nang, Vietnam; follow-up to the Tai et al. (2013, 2016), Tran et al. (2016), and Pham et al. (2015) studies	Geometric mean human milk levels Boys 2,3,7,8-TCDD: 1.34 pg/g fat 1,2,3,7,8-PeCDD: 4.21 pg/g fat 1,2,3,4,7,8-HxCDD: 2.21 pg/g fat 1,2,3,6,7,8-HxCDD: 8.11 pg/g fat 1,2,3,7,8,9-HxCDD: 2.59 pg/g fat 1,2,3,4,6,7,8-HpCDD: 11.94 pg/g fat OCDD: 68.23 pg/g fat Girls 2,3,7,8-TCDD: 1.46 pg/g fat 1,2,3,7,8-PeCDD: 4.17 pg/g fat 1,2,3,4,7,8-HxCDD: 2.39 pg/g fat 1,2,3,6,7,8-HxCDD: 8.24 pg/g fat 1,2,3,7,8,9-HxCDD: 2.68 pg/g fat 1,2,3,4,6,7,8-HpCDD: 12.22 pg/g fat OCDD: 68.71 pg/g fat	Colorado Learning Difficulties Questionnaire-math score CDD TEQs 2,3,7,8-TCDD 1,2,3,7,8-PeCDD 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-HpCDD OCDD	 ↔, boys, girls ↔, boys, girls ↔, boys, girls ↔, boys, girls ↔, boys, girls ↔, boys, girls ↔, boys, girls ↔, boys, girls
		Colorado Learning Difficulties Questionnaire-reading score CDD TEQs 2,3,7,8-TCDD 1,2,3,7,8-PeCDD 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-HpCDD OCDD	 ↔, boys, girls ↔, boys, ↓, girls ↔, boys, ↔, girls ↔, boys, ↔, girls ↔, boys, ↔, girls ↔, boys, ↔, girls ↔, boys, ↔, girls ↔, boys, ↔, girls
		Math achievement tests CDD TEQs 2,3,7,8-TCDD 1,2,3,7,8-PeCDD 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-HpCDD OCDD	 ↔, boys, girls ↓, boys, ↔, girls ↔, boys, ↔, girls ↓, boys, ↔, girls ↔, boys, ↔, girls ↓, boys, ↔, girls ↓, boys, ↔, girls ↔, boys, ↔, girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Language achievement tests	
		CDD TEQs	↔, boys, girls
		2,3,7,8-TCDD	↔, boys, girls
		1,2,3,7,8-PeCDD	↔, boys, girls
		1,2,3,4,7,8-HxCDD	↔, boys, girls
		1,2,3,6,7,8-HxCDD	↔, boys, girls
		1,2,3,7,8,9-HxCDD	↔, boys, girls
		1,2,3,4,6,7,8-HpCDD	↔, boys, girls
		OCDD	↔, boys, girls
		Oral reading tests, reading speed	
		CDD TEQs	↔, boys, girls
		2,3,7,8-TCDD	↔, boys, girls
		1,2,3,7,8-PeCDD	↔, boys, girls
		1,2,3,4,7,8-HxCDD	↔, boys, girls
		1,2,3,6,7,8-HxCDD	↔, boys, girls
		1,2,3,7,8,9-HxCDD	↔, boys, girls
		1,2,3,4,6,7,8-HpCDD	↔, boys, girls
		OCDD	↔, boys, girls
		Oral reading tests, reading errors	
		CDD TEQs	↑, boys, ↔, girls
		2,3,7,8-TCDD	↔, boys, ↔, girls
		1,2,3,7,8-PeCDD	↑, boys, ↔, girls
		1,2,3,4,7,8-HxCDD	↑, boys, ↔, girls
		1,2,3,6,7,8-HxCDD	↑, boys, ↔, girls
		1,2,3,7,8,9-HxCDD	↑, boys, ↔, girls
		1,2,3,4,6,7,8-HpCDD	↑, boys, ↔, girls
		OCDD	↔, boys, ↔, girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Tran et al. 2016 Prospective study of 181 mother-infant pairs living in Da Nang, Vietnam; children were evaluated at 5 years of age; follow-up to the Tai et al. (2013, 2016) and Pham et al. (2015) studies	Mean human milk levels in the low-, middle-, and high-exposure groups for CDDs/CDFs: 8.3, 13.9, 21.1 pg TEQ/g lipid for boys and 7.2, 14.4, and 22.6 pg TEQ/g lipid for girls Mean human milk levels in the low-, middle-, and high-exposure groups for 2,3,7,8-TCDD: 0.86, 1.6, 2.3 pg/g lipid for boys and 0.62, 1.6, 3.3 pg/g lipid for girls	Movement tests (manual dexterity, aiming and catching, and balance)	CDDs/CDFs TEQ ↓, high-exposure boys ↔, high-exposure girls
		Cognitive function tests (nonverbal index, short term memory, visual processing)	TCDD ↓, high-exposure boys ↔, high-exposure girls
Seveso, Italy			
Ames et al. 2019 Prospective study of 161 (82 males and 79 females) 7–17 years old, born after the Seveso accident	Maternal serum TCDD levels in 1976: 74.6 ppt Estimated serum TCDD levels during pregnancy: 4.5 ppt	Performance on neuropsychological tests per 10-fold increase in maternal serum TCDD	↔, 1976 serum levels ↔, pregnancy levels
Baccarelli et al. 2008 Retrospective cohort study on 1,014 children born to the 1,772 women of reproductive age in the most contaminated zones; 1,772 age-matched women controls	Geometric mean plasma 2,3,7,8-TCDD: 6.1, 17.6, and 60.5 ppt for control, zone B and zone A, respectively.	Neonatal blood TSH	↑, zones B and A
		Risk of blood TSH >5 μU/mL	↑, zone A
Eskenazi et al. 2003 Retrospective cohort study of women participating in the Seveso Women's Health Study (n=510, 888 total pregnancies)	Median maternal TCDD serum level at the time of the incident: 46.6 ppt	Low birth weight	↔
		Small for gestational age	↔
		Preterm delivery	↔
Mastroiacovo et al. 1988 Retrospective cohort study of 15,291 infants born to mothers living in zone A (n=26), zone B (n=435), zone R (n=2,439), and non-exposed areas (n=12,391)	Not reported	Total birth defects	↔

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Mocarelli et al. 2011 Retrospective cohort study of 39 sons (mean age 22.5 years) of mothers living in the highest TCDD exposure area and 58 controls whose mothers did not live in the TCDD exposed area	Estimated serum TCDD levels at the time of conception using 1976 blood levels: 19.0 ppt in breastfed sons and 27.9 ppt in formula-fed sons	Semen volume (comparison between exposed and controls)	↔, all ↔, breastfed ↔, formula fed
		Sperm concentration (comparison between exposed and controls)	↓, all ↓, breastfed ↔, formula fed
		Sperm count (comparison between exposed and controls)	↓, all ↓, breastfed ↔, formula fed
		Sperm progressive motility (comparison between exposed and controls)	↔, all ↓, breastfed ↔, formula fed
		Progressive motile sperm count (comparison between exposed and controls)	↓, all ↓, breastfed ↔, formula fed
		FSH (comparison between exposed and controls)	↔, all ↑, breastfed ↔, formula fed
		Inhibin B (comparison between exposed and controls)	↔, all ↓, breastfed ↔, formula fed
Warner et al. 2020a Retrospective cohort study of 426 children (≥18 years of age) born to 383 mothers exposed to TCDD	Maternal initial 1976 serum 2,3,7,8-TCDD levels: Q2: 28.0–60.9 ppt Q3: 61.0–149.0 ppt Q4: 150.0–914.0 ppt	Total T4	↔
		Free T4	↓, Q2
		Free T3	↓, Q2
		TSH	↔
Warner et al. 2020b Retrospective cohort study of 570 adult children born to 303 mothers exposed to TCDD	Maternal initial 1976 serum 2,3,7,8-TCDD levels and 2,3,7,8-TCDD levels estimated at pregnancy were not reported	Insulin Initial 1976 serum levels Estimated pregnancy levels	 ↔, men; ↔, women ↔, men; ↓, women
		Blood glucose Initial 1976 serum levels Estimated pregnancy levels	 ↔, men; ↔, women ↔, men; ↔, women

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		HOMA2-IR	
		Initial 1976 serum levels	↔, men; ↔, women
		Estimated pregnancy levels	↔, men; ↔, women
		HOMA2-β	
		Initial 1976 serum levels	↔, men; ↔, women
		Estimated pregnancy levels	↔, men; ↓, women
Wesselink et al. 2014 Retrospective study of 617 women participating in the Seveso Women's Health Study	Median 1976 serum 2,3,7,8-TCDD level: 55.0 ppt Median estimated 2,3,7,8-TCDD level at pregnancy: 9.9 ppt	Spontaneous abortion	↔
		Birth weight	↔
		Small for gestational age	↔
		Gestational age	↔
Ye et al. 2018 Retrospective study of 676 children (2–38 years of age) of 438 mothers participating in the Seveso Second Generation Health Study	Maternal 1976 serum 2,3,7,8-TCDD level: 64.7 ppt	Eczema (doctor diagnosed)	↓
		Asthma	↔
		Hay fever	↔
Communities with contaminated soil			
Burns et al. 2016 Prospective study of 315 boys aged 17–18 years living in Chapaevsk, Russia	Median serum CDD/CDF/PCB TEQ: 21.1 pg TEQ/g lipid • 2nd Q: 14.6–21.0 pg TEQ/g lipid • 3rd Q: 21.1–33.2 pg TEQ/g lipid • 4th Q: 33.3–174.7 pg TEQ/g lipid	Pubertal onset	
		• Testicular volume >3 mL	↓, 3rd Q
		• Genitalia stage ≥2	↓ 2nd Q
		• Pubarche stage ≥2	↔, 4th Q
		Sexual maturity	
		• Testicular volume >3 mL	↓, 2nd Q
		• Genitalia stage ≥2	↓, 3rd Q
		• Pubarche stage ≥2	↔, 4th Q
Hanify et al. 1981 Cross-sectional study of a community in Northland, New Zealand with contaminated soil from 2,4,5-T spraying	Not reported	Birth malformations	↑
		Anencephaly	↔
		Spina bifida	↔
		Cleft lip	↑
		Isolated cleft palate	↔
		Heart malformations	↑

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Hypospadias, epispadias	↑
		Talipes	↑
Korrick et al. 2011 Prospective study of 473 boys aged 8–9 years living in Chapaevsk, Russia	4th quartile blood CDD, CDF, and dioxin-like PCBs: 30–175 pg TEQ/g lipid 4th quartile 2,3,7,8-TCDD: 4.0–45 pg TEQ/g lipid 3rd quartile CDDs: 8–12.9 pg TEQsg lipid	Puberty onset, as assessed by a testicular volume of >3 mL	↔, total TEQs ↓, 2,3,7,8-TCDD ↓, CDD TEQs
		Puberty onset, as assessed by genitalia at ≥stage 2	↔, total TEQs ↔, 2,3,7,8-TCDD ↔, CDDs
Nelson et al. 1979 Retrospective study of a residents living in counties in Arkansas in which 2,4,5-T was sprayed on rice crops (1,201 cases of cleft lip and/or cleft palate)	Cases divided into high-, medium-, or low-exposure groups based on rice acreage in the county	Cleft lip and/or cleft palate	↑, high and low groups
Stockbauer et al. 1988 Retrospective study of a community in eastern Missouri; 402 births to exposed mothers and 804 births to unexposed mothers in 1972–1982 and 235 and 470 births to exposed and unexposed mothers, respectively, in 1978–1982	Not reported	Fetal deaths, infant deaths, perinatal deaths	↔, 1972–1982
		Very low birth weight	↔, 1972–1982
		Intrauterine growth retardation	↔, 1972–1982
		Low birth weight	↔, 1972–1982 ↔, 1978–1982
		Birth defects	↔, 1972–1982
Communities in China near electronic waste recycling facilities			
Wang et al. 2019 Longitudinal study of 27 mother infant pairs living in Taizhou, China (an electronic waste recycling area) and Jiaxing (an area with almost no residents involved in electronic waste recycling)	Mean human milk 2,3,7,8-TCDD level: 2.7 and 0.5 pg/g lipid in exposed and control groups Mean human milk CDDs: 6.3 and 2.2 pg TEQ/g lipid	Height 6 months TCDD CDDs 3 years TCDD CDDs	 ↔, boys, girls ↔,boys, girls ↔, boys, ↑, girls ↓, boys, ↑, girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Weight	
		6 months	
		TCDD	↔, boys, girls
		CDDs	↔, boys, girls
		3 years	
		TCDD	↔, boys, girls
		CDDs	↔, boys, ↑, girls
		BMI	
		6 months	
		TCDD	↔, boys, girls
		CDDs	↔, boys, girls
		3 years	
		TCDD	↔, boys, girls
		CDDs	↔, boys, girls
		Head circumference	
		6 months	
		TCDD	↔, boys, girls
		CDDs	↔, boys, girls
		3 years	
		TCDD	↔, boys, girls
		CDDs	↔, boys, girls
		Chest circumference	
		6 months	
		TCDD	↔, boys, girls
		CDDs	↔, boys, girls
		3 years	
		TCDD	↔, boys, girls
		CDDs	↔, boys, girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Communities living near municipal incinerators			
Lin et al. 2006 Cross-sectional study of 3,025 infants of mothers living near a municipal incinerator in Taiwan; comparison group was 3,421 infants born prior to the operation of the incinerator	Mean emission concentration of CDD/CDF levels in exhaust air: 6.47 ng TEQ/m^3	Birth weight	↔
		Gestation length	↔
		Preterm birth	↔
General population			
Alaluusua et al. 1996 Prospective cohort study of 6–7-year-old children (n=102) in Finland	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	Hypomineralization of teeth in 6–7-year-old children	↑, frequency and severity
Cao et al. 2008 Cross-sectional study of 104 mother-infant pairs in Duisburg, Germany	Maternal blood fat CDDs/CDFs: 15.3 pg TEQ/g Milk fat CDDs/CDFs in milk fat: 13.1 pg TEQ/g	Cord serum testosterone	↓, females
		Cord serum estradiol	↓, males
Caspersen et al. 2016a Longitudinal prospective study of 1,024 children (mean age 3.5 years) participating in the Norwegian Mother and Child Cohort Study	Median maternal dietary exposure to 17 2,3,7,8-substiuted CDDs/CDFs and 13 dioxin-like PCBs: 0.6 pg TEQ/kg/day	Performance on tests for ADHD	↔
Caspersen et al. 2016b Longitudinal prospective study of 44,092 3-year-old children participating in the Norwegian Mother and Child Cohort Study	Median maternal dietary exposure to 17 2,3,7,8-substiuted CDDs/CDFs and 13 dioxin-like PCBs: 0.6 pg TEQ/kg/day Low exposure:≤14 ng TEQ/kg/day High exposure:>14 ng TEQ/kg/day	Incomplete grammar	↑
		Moderate language delay	↔
		Severe language delay	↑
		Speech problem	↔
		Low score for communication skills	↔, boys and girls ↔, boys ↑, girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Darnerud et al. 2010 Prospective study of 180 mother-infant pairs living in Sweden	Median CDD/CDF human milk level: 9 pg TEQ/g lipid	Infant TSH	↑, 3 weeks ↔, 3 months
		Infant total T3	↔, 3 weeks ↔, 3 months
		Infant free T4	↔, 3 weeks ↔, 3 months
Hui et al. 2016, 2019 Prospective study of 161 11-year-old children born in Hong Kong	4th quartile mean human milk: 22.5 pg CALUX-TEQ/g lipid[a]	Wechsler Intelligence Scale Children IV (Hong Kong)	↔
		Hong Kong List Learning test	↔
		Test for Everyday Attention in Children	↔
		Grooved Peg Board Test	↔
Huisman et al. 1995a Prospective study of 418 mother-infant (newborns) pairs living in the Netherlands	Human milk levels (median concentration): 2,3,7,8-TCDD: 3.61 pg/g lipid 1,2,3,7,8-PeCDD:10.25 pg/g lipid, 1,2,3,4,7,8-HxCDD: 8.71 pg/g lipid, 1,2,3,6,7,8-HxCDD: 45.98 pg/g lipid, 1,2,3,7,8,9-HxCDD: 6.72 pg/g lipid, 1,2,3,4,6,7,8-HpCDD: 57.38 pg/g lipid OCDD: 660.64 pg/g fat	Neurological optimality score 2,3,7,8-TCDD 1,2,3,7,8-PeCDD 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,6,7,8-HpCDD OCDD	 ↔ ↑ ↑ ↑ ↑ ↑ ↔
Huisman et al. 1995b Prospective study of 418 mother-child (age 18 months) pairs living in the Netherlands	Same children as Huisman et al. (1995a)	Neurological optimality score for motor function	↔, dioxins
Ikeno et al. 2018 Prospective study of 141 mother-child (age 42 months) pairs living in Japan	Maternal blood levels (median concentration) 1,2,3,7,8-PeCDD:: 4.1 pg/g lipid 1,2,3,6,7,8-HxCDD: 13.9 pg/g lipid 1,2,3,7,8,9-HxCDD: 2.2 pg/g lipid 1,2,3,4,6,7,8-HpCDD 24.3 pg/g lipid OCDD: 437.7 pg/g lipid Total CDD: 488.5 pg/g lipid	Cognitive development—achievement scale	↑, 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD in females ↔, other congeners and total CDDs
		Cognitive development—mental processing scale	↔, all congeners and total CDDs

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Ilsen et al. 1996 Prospective study of 38 mother-infant pairs living in the Netherlands	Mean human milk CDD/CDF levels: 18.5 pg TEQ/g lipid for the low-exposure group and 37.3 pg TEQ/g lipid for the high-exposure group	Bayley Scales of Infant Development at age 2 years	↔
		Neurological suboptimality score at age 2.7 years	↔
		Reflex score-suboptimality at age 2.7 years	↓, high-exposure group compared to low-exposure group
Iszatt et al. 2016 Prospective study using data from three European birth cohort studies (Belgium, Norway, Slovenia)	Mean prenatal exposure (estimated using human milk or cord blood samples): 31.2, 7.9, and 15.5 pg DR-CALUX/g lipid[a] in the Flemish, Norwegian, and Slovak cohorts, respectively	Infant growth	↔
		BMI at age 7 years	↔, boys and girls ↔, boys ↑, girls
		Risk of overweight BMI	↔, boys and girls ↔, boys ↑, girls
Kono et al. 2015 Prospective study of 175 mother-infant pairs in Japan	Median human milk levels of CDDs, CDFs, and dioxin-like PCBs from a human milk survey: 8.3 and 8.6 in boys and girls, respectively Median estimated dioxin exposure based on human milk survey levels and breastfeeding ratio during first year: 14.0 and 18.8 ng TEQ (CDDs, CDFs, PCBs)/kg/day boys and girls, respectively	Psychosocial behavioral development in 6–10- or 11–13-year-old children	↔, human milk levels ↔, estimated dioxin exposure levels
Koppe et al. 1991 Cross-sectional study of 14 mothers in the Netherlands	Human milk level of 2,3,7,8-TCDD: 5.35–17.0 pg/g milk fat (mean of 9.79)	Abnormal bleeding	↑
Koopman-Esseboom et al. 1994	12.44–76.43 (mean of 32.06 pg TEQ/g milk fat) High-exposure group: >30.75 pg TEQ/g milk fat	Total T3	↔, high exposure versus low exposure
		Total T4	↓, high exposure versus low exposure

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Prospective study of 78 mother-infant (2 weeks of age) pairs living in the Netherlands	Low-exposure group: ≤30.75 pg/TEQ/g milk fat	Free T4	↔, high exposure versus low exposure
		TSH	↑, high exposure versus low exposure
Miyashita et al. 2018a Prospective study of 183 mother-infant pairs in Japan	Median maternal blood levels of CDDs: 7.05 pg TEQ/g lipid for all subjects and 7.24 and 6.95 for boys and girls, respectively	Cord blood estradiol	↔
		Cord blood testosterone	↔
		Cord blood testosterone/estradiol ratio	↔, all ↔, boys ↔, girls
		Cord blood androstenedione	↔
		Cord blood DHEA	↔, all ↑, boys ↔, girls
		Cord blood cortisol	↔, all ↔, boys ↔, girls
		Cord blood cortisone	↔, all ↔, boys ↔, girls
		Cord blood adrenal androgen/ glucocorticoid ratio	↔, all ↔, boys ↔, girls
		Cord blood sex hormone binding globulin	↔, all ↔, boys ↔, girls
		Cord blood prolactin	↔
		Cord blood LH	↔, boys
		Cord blood FSH	↔, boys
		Cord blood inhibin B	↓, boys
		Cord blood insulin-like factor	↔, boys

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Miyashita et al. 2018b Prospective study of newborns and children in Japan. Three groups of children examined at birth (n=239), age 3.5 years (n=327), and 7 years of age (n=264)	Maternal median CDDs, CDFs, and dioxin-like PCBs: 14.0, 14.2, and 15.0 pg TEQ/g lipid in the birth, 3.5 years, and 7 years groups, respectively	Cord blood IgE	↔, all ↓, boys ↔, girls
		Allergy	Age 3.5 years ↔, all ↔, boys ↔, girls Age 7 years ↔, all ↔, boys ↔, girls
		Food allergy	Age 3.5 years ↔, all ↔, boys ↔, girls Age 7 years ↔, all ↔, boys ↔, girls
		Eczema	Age 3.5 years ↔, all ↔, boys ↔, girls Age 7 years ↔, all ↔, boys ↔, girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Wheezing	Age 3.5 years ↔, all ↑, boys ↔, girls Age 7 years ↔, all ↔, boys ↔, girls
		Infections	Age 3.5 years ↔, all ↔, boys ↔, girls Age 7 years ↔, all ↔, boys ↔, girls
		Otitis media infections	Age 3.5 years ↔, all ↔, boys ↔, girls Age 7 years ↔, all ↔, boys ↔, girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Respiratory infections	Age 3.5 years ↔, all ↔, boys ↔, girls Age 7 years ↔, all ↔, boys ↔, girls
Neugebauer et al. 2015 Prospective study of 117 school age children participating in the Duisburg Birth Cohort Study	Median maternal blood CDD/CDF level: 12.99 pg TEQ/g lipid Median human milk CDD/CDF level: 59.81 TEQ ng	Attentional performance test of distractibility	↔, maternal blood ↑, human milk
		Attentional performance test of divided attention	↑, maternal blood ↔, human milk
		ADHD-associated behavior assessed via parent questionnaire	↔, maternal blood ↔, human milk
Nowack et al. 2015 Prospective study of 116 9–10-year-old children participating in the Duisburg Birth Cohort Study	Median maternal blood CDDs/CDFs: 12.91 pg TEQ/g lipid for boys and girls, 12.79 pg TEQ/g lipid for boys, and 13.74 pg TEQ/g lipid for girls	Social responsiveness total score which measures autistic traits	↓ boys and girls ↔, boys ↓, girls
		Empathy-Systemizing Quotient, which measures sex-specific behaviors	↔, boys and girls, boys only, and girls only
Papadopoulou et al. 2013 Prospective study of 50,651 mother-infant pairs participating in the Norwegian Mother Child Cohort Study	Estimated maternal dietary intake of CDDs/CDFs/dioxin-like PCBs: 0.55 pg TEQ/kg body weight/day 2nd quartile estimated intake: 0.39–0.55 pg TEQ/kg body weight/day	Birth weight	↓, 2nd quartile
		Birth length	↓, 2nd quartile
		Birth head circumference	↓, 2nd quartile
Papadopoulou et al. 2014 Multicountry (Greece, Spain, Norway, Denmark, United Kingdom) cross-sectional study of 537 mother-infant pairs	Maternal dioxin-diet score	Birth weight	↓, 3rd tertile
		Gestational age	↔

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Pluim et al. 1993b Prospective study of 38 mother-infant pairs living in the Netherlands	Human milk CDD/CDF mean levels: 18.6 and 37.5 pg TEQ/g fat in the low- and high-exposure groups, respectively	Total T3	High exposure versus low exposure ↔, cord blood ↔, 11 weeks
		Total T4	High exposure versus low exposure ↔, cord blood ↑, 1 week ↑, 11 weeks
		Free T4	High exposure versus low exposure ↔, cord blood
		TSH	High exposure versus low exposure ↔, cord blood ↔, 1 week ↑, 11 weeks
		TBG	High exposure versus low exposure ↔, cord blood ↔, 1 week ↔, 11 weeks
Pluim et al. 1994a Prospective study of 35 mother-infant pairs living in the Netherlands	Human milk CDD/CDF levels: 8.7–62.7 pg TEQ/g fat (mean of 28.1 pg TEQ/g fat) Cumulative intake at 11 weeks: 5.7–123.7 ng TEQ (mean of 44.7 ng TEQ)	GGT	↔, cord blood ↔, 1 week ↔, 11 weeks ↔, cumulative intake
		AST	↔, cord blood ↔, 1 week ↔, 11 weeks ↑, cumulative intake
		ALT	↔, cord blood ↔, 1 week ↔, 11 weeks ↑, cumulative intake

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Plasma cholesterol	↔, cord blood ↔, 1 week ↔, 11 weeks ↔, cumulative intake
		Total and conjugated bilirubin	↔, cord blood ↔, 1 week ↔, 11 weeks ↔, cumulative intake
		Leukocytes	↔, cord blood ↔, 1 week ↔, 11 weeks ↔, cumulative intake
		Platelets	↔, cord blood ↔, 1 week ↔, 11 weeks ↓, cumulative intake
Pluim et al. 1994b Prospective study of 32 mother-infant pairs living in the Netherlands	Human milk CDD/CDF levels: 13.7–62.6 pg TEQ/g fat (mean of 29.4 pg TEQ/g fat)	Vitamin K	↔,cord blood ↔, 11 weeks
		PIVKA-II	↔,cord blood ↔, 11 weeks
Pluim et al. 1996 Prospective study of 32 mother-infant pairs living in the Netherlands	Mean human milk CDD/CDF levels: 18.1 and 37.4 pg TEQ/g fat in the low- and high-exposure groups, respectively	Gestation age	High exposure versus low exposure ↔
		Birth weight	High exposure versus low exposure ↔
		Body weight	High exposure versus low exposure ↔, 10 weeks of age ↔, 20 weeks of age

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Head circumference	High exposure versus low exposure ↔, 10 weeks of age ↔, 20 weeks of age
		Neurological optimality score	High exposure versus low exposure ↔
Rennert et al. 2012 Prospective study of 111 6–7- and 8–9-year-old children participating in the Duisburg Birth Cohort Study	Geometric mean CDD/CDF levels in maternal blood: 13.50 pg TEQ/g fat Geometric mean CDD/CDF levels in human milk: 10.94 pg TEQ/g fat	DHEA-S levels	↔, maternal blood ↑, human milk
Stølevik et al. 2011 Prospective study of 195 mother-infant (1 year old) participating in a subcohort study of the Norwegian Mother and Child Cohort Study	Median estimated maternal dietary intake of CDD/CDF/dioxin-like PCB 0.56 pg TEQ/kg body weight/day	Eczema	↓
		Wheeze	↑
		Otitis media	↔
		Gastric flu	↔
		Chicken pox	↔
		Exanthema subitem	↑
		Upper respiratory infections	↑
Stølevik et al. 2013 Prospective study of 162 mother-infant (1–3 years old) participating in a subcohort study of the Norwegian Mother and Child Cohort Study	Median estimated maternal dietary intake of CDD/CDF/dioxin-like PCB 0.59 pg TEQ/kg body weight/day	Eczema 0–3 years of age 2–3 years of age	 ↔ ↔
		Atopic eczema 0–3 years of age 2–3 years of age	 ↑ ↔
		Allergy 0–3 years of age 2–3 years of age	 ↔ ↔
		Asthma 0–3 years of age	 ↔

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Asthma medication	
		0–3 years of age	↔
		2–3 years of age	↔
		Wheeze	
		0–3 years of age	↔
		2–3 years of age	↔
		Otitis media	
		0–3 years of age	↔
		2–3 years of age	↔
		Chicken pox	
		0–3 years of age	↔
		Exanthema subitem	
		0–3 years of age	↔
		Gastroenteritis	
		0–3 years of age	↑
		2–3 years of age	↔
		Upper respiratory tract infection	
		0–3 years of age	↑
		2–3 years of age	↑
		Sensitization	↔
		Measles vaccine antibodies	↓
		Rubella vaccine antibodies	↔
		Tetanus vaccine antibodies	↔
		Hib vaccine antibodies	↔
Su et al. 2010 Prospective study of 92 mother-child pairs living in Taiwan; children examined at 2 and 5 years of age; follow-up to the Wang et al. (2005) study	Placental CDD/CDF levels Low exposure: <15 pg TEQ/g lipid High exposure: ≥15 pg TEQ/g lipid	Serum T3 at 2 years	↓
		Serum TSH at 2 years	↑
		Serum free T4 x TSH at 2 years	↑
		Serum TTR at 2 years	↔

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Su et al. 2012 Follow-up study to the Su et al. (2010) study of 56 children aged 8 years	Low CDD/CDF/PCB group: <14.83 pg TEQ/g lipid High CDD/CDF/PCB group: ≥14.83 pg TEQ/g lipid	Estradiol	↓
		FSH	↔
		LH	↔
		Testosterone	↔
		Sex characteristics	↔, boys ↔, girls
Su et al. 2015 Follow-up study to the Su et al. (2010) study of 56 children aged 8 years	Low CDD/CDF/PCB group: <14.83 pg TEQ/g lipid High CDD/CDF/PCB group: ≥14.83 pg TEQ/g lipid	Growth hormone	↔
		Total T3	↔
		Total T4	↔
		Free T4	↔
		TSH	↔
		TBG	↑, boys ↔, girls
		Aldosterone	↔, boys ↓, girls
ten Tusscher et al. 2014 Prospective cohort study of children living in Netherlands examined at ages 7–12 years (n=41) and 14–18 years (n=33); same group of children examined in the Ilsen et al. (1996) study	Estimated dioxin intake (calculated using human milk levels) for the pre-adolescents and Serum CDD/CDF levels in adolescents 2.2 pg TEQ/g lipid	Social problems	↑, pre-adolescents
		Aggressive behavior	↑, pre-adolescents
		Thought problems	↑, pre-adolescents
		Anxious/depressed feelings	↑, pre-adolescents
		External behavioral problems	↑, adolescents
		Wechsler Intelligence Scale	↔, pre-adolescents
Vafeiadi et al. 2013 Prospective study of 237 newborns and 462 young children (16 months of age) living in Greece or Spain	Mean maternal blood DR CALUX levels for newborns: 52.3 pg TEQ/g lipid Mean maternal blood DR CALUX levels for children: 49.7 pg TEQ/g lipid	Anogenital distance in newborns	↓, boys ↔, girls
		Anogenital distance in children	↔, boys ↔, girls

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Vafeiadi et al. 2014 Cross-sectional study of 967 mother-infant pairs living in Denmark, Greece, Norway, Spain, or England	3rd tertile maternal serum DR CALUX: 47.9–129.1 pg TEQ/g lipid 3rd tertile cord blood DR CALUX: 43.3–156 pg TEQ/g lipid	Birth weight Maternal serum Cord blood	 ↔ ↔
		Head circumference Maternal serum Cord blood	 ↔ ↔
		Gestational age Maternal serum Cord blood	 ↔ ↓
Vartiainen et al. 1998 Prospective study of 84 mother-infant pairs living in Finland	Human milk CDD/CDF levels: 10.8–96.3 pg TEQ/g fat	Birth weight	↔
Virtanen et al. 2012 Case-control study of 280 infants (95 cases and 185 controls) in Denmark and Finland	Median placental CDD/CDF levels: 8.47 and 9.78 pg TEQ/g lipid for Finnish controls and cases 10.88 and 11.75 for Danish controls and cases	Cryptorchidism	↔
		FSH	↔
		LH	↔
		Sex hormone binding globulin	↔
Wang et al. 2005 Prospective study in the general population of female (n=62) and male (n=57) newborns in the Taiwanese cohort	Placental levels of CDD/CDF/PCBs Low-exposure group: <15.1 1 pg TEQ/g lipid Higher-exposure group: >15.1 pg TEQ/g lipid dioxin/PCB	Cord TSH levels	↑, CDDs/CDFs
		Cord T4 levels	↑, CDDs/CDFs

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Weisglas-Kuperus et al. 1995 Prospective study of 207 mother-infant pairs in the Netherlands	Not reported; human milk CDD/CDF/PCB TEQ levels	Rhinitis, bronchitis, tonsillitis, otitis	↔
		Antibodies to mumps, measles, and rubella at 18 months of age	↔
		White blood cell counts Monocytes Granulocytes Lymphocytes	 ↓, 3 months ↓, 3 months ↔
		T cell markers	↔
		B cell markers	↓, 3 months
		NK cell markers	↔
Wilhelm et al. 2008 Prospective study of the Duisburg, Germany birth cohort of 189 mother-infant pairs	Blood levels (n=182) of CDDs/CDFs/PCBs ranged 3.8–58.4 pg TEQ/g lipid Human milk levels (n=149) of CDDs/CDFs/PCBs ranged 2.6–52.4 pg TEQ/g lipid	Thyroid hormones (cord blood) TSH T3 Free T3 T4 Free T4	Blood and human milk ↔ ↔ ↔ ↔ ↔
		Neurological optimality score Age 2 weeks Age 18 months	Blood and human milk ↔ ↔
		Bayley Scales of Infant Development Age 12 months Age 24 months	Blood and human milk ↔ ↔

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Winneke et al. 2014 Prospective study of 121 mother-child (mean age 6.6 years) pairs participating in the Duisburg birth cohort study in Germany	Mean CDD/CDF in maternal serum: 14.5 pg TEQ/g lipid Mean CDD/CDF in human milk: 11.6 pg TEQ/g lipid	Preschool Activities Inventory to assess sexually dimorphic behavior Maternal blood Masculine score Feminine score	 ↔, boys, girls ↔, boys, girls
		Preschool Activities Inventory Human milk Masculine score Feminine score	 ↔, boys; ↓, girls ↑, boys, ↔,girls
Wohlfahrt-Veje et al. 2014 Longitudinal study of 417 mother-child pairs participating in the Copenhagen Mother Child Cohort of Growth and Reproduction; children examined at 0, 3, 18, and 36 months of age	Median CDD/CDF/PCB human milk level: 20.2 pg TEQ/g lipid	Body weight 0 months 3 months 18 months 36 months	 ↔ ↔ ↔ ↔
		Body weight change 0–3 months 0–18 months 0–36 months	 ↔ ↑ ↔
		Skinfold fat 0 months 3 months 18 months 36 months	 ↓ ↔ ↔ ↔
		Length/height 0 months 3 months 18 months 36 months	 ↔ ↔ ↑ ↔

Table 2-23. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Change in height	
		0–3 months	↑
		0–18 months	↑
		0–36 months	↑
		IGF1 at 3 months	↑

[a]Chemical activated luciferase (CALUX) is a cell-based assay used to measure dioxin levels.

↑ = association; ↓ = inverse association; ↔ = no association; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; ADHD = attention deficit hyperactivity disorder; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; CALUX = chemical-activated luciferase gene expression; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorodibenzofuran; C-SHARP = Children's Scale of Hostility and Aggression; DHEA = dehydroepiandrosterone; DHEA-S = dehydroepiandrosterone sulfate; DR CALUX = dioxin-responsive chemical-activated luciferase gene expression; DSM-IV-TR = Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision; EEG = electroencephalogram; FSH = follicle-stimulating hormone; GGT = gamma-glutamyl transferase; HiB = *Haemophilus influenzae* type b; HOMA2-β = homeostatic model assessment of pancreatic beta-cell function; HOMA2-IR = homeostatic model assessment of insulin resistance; HpCDD = heptachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; Ig = immoglobulin; IGF1 = insulin-like growth factor 1; LH = luteinizing hormone; NK = natural killer; OCDD = octachlorodibenzo-*p*-dioxin; PCB = polychlorinated biphenyl; PeCDD = pentachlorodibenzo-*p*-dioxin; PIVKA-II = protein-induced by vitamin K absence-II; Q = quartile; T3 = triiodothyronine; T4 = thyroxine; TBG = thyroxine-binding globulin; TCDD = tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalency; TSH = thyroid-stimulating hormone; TTR = transthyretin

should be noted that Wolfe et al. (1995) found an association between low dioxin levels and spontaneous abortion but did not find an association among highly exposed Operation Ranch Hand veterans.

Similarly, no alterations in miscarriages were observed in a study of male pesticide workers (Smith et al. 1982). No associations between paternal exposure to dioxins and increased risk of stillbirths were found in male workers (Dimich-Ward et al. 1996; Townsend et al. 1982) or Vietnam veterans (Aschengrau and Monson 1990; Wolfe et al. 1995).

No associations between neonatal or infant deaths and CDD exposure were found in the offspring of male workers at a chlorophenol manufacturing facility exposed to any dioxin or to 2,3,7,8-TCDD only (Dimich-Ward et al. 1996; Townsend et al. 1982), offspring of male Operation Ranch Hand veterans (Aschengrau and Monson 1990; Wolfe et al. 1985), or offspring of the Missouri cohort (Stockbauer et al. 1988). Michalek et al. (1998) reported an increased risk of infant deaths in the infants of fathers involved in Operation Ranch Hand, as compared to the referent group of veterans in Southeast Asia not exposed to Agent Orange. Disorders related to short gestation and low birth weight were the most common causes of infant deaths. 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.

Several studies have evaluated prematurity or premature births and have not found associations with paternal (Dimich-Ward et al. 1996; Lawson et al. 2004; Michalek et al. 1998) or maternal (Eskenazi et al. 2003; Lin et al. 2006) exposure to CDDs. Gestational age was not associated with CDD levels in a study of women in Seveso (Wesselink et al. 2014) or in general population studies (Papadopoulou et al. 2014; Pluim et al. 1996). A general population study (Vafeiadi et al. 2014) did find an inverse association between cord blood dioxin levels and gestational age, but no association when maternal serum dioxin levels were used as a biomarker of exposure. No associations were found between paternal (Dimich-Ward et al. 1996) or maternal (Eskenazi et al. 2003; Wesselink et al. 2014) exposure to CDDs and the risk of small for gestational age.

Inconsistent results have been reported in studies examining birth weight and/or infant body weight. Several studies examining the children of residents living in contaminated areas of Vietnam have reported inverse associations between 2,3,7,8-TCDD or CDD biomarker levels and birth weight/infant weight (Dao et al. 2016; Nishijo et al. 2012; Tai et al. 2016). Two other high exposure studies (Wang et al. 2019;

Wesselink et al. 2014) did not find associations. Two general population studies conducted by Papadopoulou et al. (2013, 2014) found an inverse association between estimated maternal dietary intake of dioxins (CDD/CDF/dioxin-like PCBs) and birth weight. Other general population studies did not find associations with birth weight or body weight (Pluim et al. 1996; Vafeiadi et al. 2014; Vartiainen et al. 1998; Wohlfahrt-Veje et al. 2014). Similarly, no associations between CDDs and intrauterine growth (Michalek et al. 1998; Stockbauer et al. 1988) or low birth weight/very low birth weight (Dimich-Ward et al. 1996; Eskenazi et al. 2003; Stockbauer et al. 1988) were found among the children of highly exposed parents. Length/height in boys was inversely associated with 2,3,7,8-TCDD levels in studies of contaminated areas of Vietnam (Tai et al. 2016). A study of children living in a contaminated area of China (Wang et al. 2019) found no associations at 6 months of age and found an association between CDDs and 2,3,7,8-TCDD human milk levels and height in girls and an inverse association between CDDs levels in human milk and height in boys. Other studies in Vietnam (Dao et al. 2016; Nishijo et al. 2012) and in the general population (Papadopoulou et al. 2013; Pluim et al. 1996) did not find associations with length/height. Wohlfahrt-Veje et al. (2014) reported an association between human milk CDD/CDF/PCB levels and length/height, but only in children at 18 months of age; no associations were observed at birth, 3 months, or 36 months of age. Mixed results have also been reported in studies of head/chest circumference. In studies of children living in contaminated areas of Vietnam, an association between CDD/CDF human milk levels and head circumference was found in girls, but not in boys (Nishijo et al. 2012), whereas another study found an inverse association between human milk 2,3,7,8-TCDD and CDD levels and head circumference in boys, but not in girls. Other studies have not found associations in studies of Vietnamese children (Dao et al. 2016) or in children living in a contaminated area of China (Wang et al. 2019). In general population studies, one study reported in inverse association between maternal dietary intake of CDDs/CDFs/PCBs and birth head circumference (Papadopoulou et al. 2013) and two studies found no associations between maternal human milk (Pluim et al. 1996) or maternal serum levels of dioxins (Vafeiadi et al. 2014) and head circumference.

Birth defects. The potential for CDDs to induce birth defects or other congenital anomalies has been investigated in several populations including male workers, Vietnam veterans, Seveso residents, and communities living in contaminated areas. In the offspring of male workers at a chlorophenol manufacturing facility (Townsend et al. 1982) or males spraying 2,4,5-T (Smith et al. 1982), no significant increases in the incidence of congenital malformations were observed. An increased risk of spina bifida or anencephaly was observed in the offspring of male sawmill workers with the highest maximum exposure to chlorophenate (Dimich-Ward et al. 1996); an increased risk of cataracts was also observed in the offspring.

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 overall risk of having a child with birth defects was not increased in the Vietnam veterans in the Erickson et al. (1984) study. 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 et al. 1984). In the Aschengrau and Monson (1990) study, no increase in the risk of fathering a child with birth defects was observed for the Vietnam veterans. Among the children with birth defects, an increased risk of having one or more major systemic malformations 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 congenital malformations was observed in veterans in the low-exposure group, but not in the high-exposure group. The study also found an increase in nervous system defects with increasing paternal serum lipid 2,3,7,8-TCDD levels (statistical analysis was not performed due to the small number of defects: 3/981 in comparison group, 0/283 in Ranch Hand veterans in the background group, 2/241 in veterans in the low-exposure group, and 3/268 in veterans in the high-exposure group). However, the study authors cautioned 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. A meta-analysis of seven studies evaluating birth defects in the offspring of male Vietnam veterans (including Erickson et al. 1984 and Wolfe et al. 1995) found an increased risk of spina bifida (Ngo et al. 2010).

In residents of Seveso, a 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 study authors suggested 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 study authors noted 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 that 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, major defects, or minor defects (Mastroiacovo et al. 1988). The small number of observed birth defects limits the statistical power of this study to detect increases in a specific defect.

In a study of residents of Northland, New Zealand exposed to 2,4,5-T during aerial spraying, an increase in the total number of birth defects was 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). Alterations in specific defects have also been observed; increases in cleft lip, heart malformations, talipes (club foot), and hypospadias or epispadias were found. There were no alterations in the occurrence of anencephaly, spina bifida, or isolated cleft palate. Stockbauer et al. (1988) studied the Missouri cohort and found no excess risk of birth defects among infants from exposed mothers compared to an unexposed referent group. 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 study authors attributed this to better case-ascertainment rather than 2,4,5-T exposure. In Vietnamese families potentially exposed to 2,3,7,8-TCDD-contaminated herbicides during the Vietnam War, an increase in the incidence of unspecified congenital anomalies was observed as compared with a nonexposed population (Phuong et al. 1989). 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.

Endocrine and other systemic effects. Several epidemiological studies have evaluated possible associations between exposure to CDDs and offspring thyroid hormone levels. An association between maternal blood 2,3,7,8-TCDD levels and neonatal TSH levels was found in the Seveso cohort (Baccarelli et al. 2008). The study also found an increased risk of serum TSH levels >5 μU/mL, which has been established by the WHO as an indicator of potential thyroid problems in neonates. No association was found between maternal 2,3,7,8-TCDD blood levels at the time of the Seveso accident and TSH levels in the adult children (Warner et al. 2020c). Mixed results have been observed in general population studies. Wang et al. (2005) reported an association between CDD/CDF/PCB TEQ levels and cord blood TSH levels; other studies have not found this association (Pluim et al. 1993b; Wilhelm et al. 2008). Associations were also found in children aged 3 weeks, 11 weeks, and 2 years (Darnerud et al. 2010;

Pluim et al. 1993b; Su et al. 2010), but not in children aged 1 week, 3 months, or 8 years (Darnerud et al. 2010; Pluim et al. 1993b; Su et al. 2015).

Warner et al. (2020a) found an inverse association between maternal 2,3,7,8-TCDD levels and free T3 levels in adult children. General population studies have not found associations between CDD/CDF/PCB levels and free or total T3 levels (Darnerud et al. 2010; Koopman-Esseboom et al. 1994; Pluim et al. 1993b; Su et al. 2010; Wilhelm et al. 2008). Similarly, mixed results have been observed in general population studies examining associations with free or total T4 levels. One study reported an association between maternal CDD/CDF levels and free T4 levels in 2-year-old children (Su et al. 2010). Other studies have not found an association (Darnerud et al. 2010; Koopman-Esseboom et al. 1994; Pluim et al. 1993b; Wilhelm et al. 2008). Two general population studies found associations between maternal dioxin levels and total T4 levels (Pluim et al. 1993b; Wang et al. 2005); one study found an inverse association (Koopman-Esseboom et al. 1994) and one study found no association (Wilhelm et al. 2008). The Warner et al. (2020a) study of adult children of mothers exposed to 2,3,7,8-TCDD in Seveso found an inverse association for free T4 levels and no association with total T4 levels.

Several studies have evaluated other systemic effects; however, only one study examined each endpoint and no conclusions can be drawn. Warner et al. (2020b) evaluated the possible relationship between maternal 2,3,7,8-TCDD exposure from the Seveso accident and glucose metabolism in adult children. Inverse associations between estimated 2,3,7,8-TCDD levels at pregnancy and insulin and pancreatic beta cell function (assessed using a homeostatic model assessment of beta-cell function, HOMA-β) were found in the female adult children; no associations were found for blood glucose levels or insulin resistance in the females or for any measure in males. An increase in the frequency and severity of hypomineralization of teeth was observed in a general population study of 6–7-year-old children (Alaluusua et al. 1996). An association between human milk 2,3,7,8-TCDD levels and abnormal bleeding was observed in infants (Koppe et al. 1991). Pluim et al. (1994b) did not find associations between human milk CDD/CDF levels and vitamin K or protein-induced vitamin K absence-II (PIVK-II) levels in cord blood or blood from 11-week-old infants. Another general population study by this group found no associations between human milk CDD/CDF levels and GGT, AST, ALT, plasma cholesterol, bilirubin, leukocyte, or platelet levels in cord blood or blood from 1- or 11-week-old infants (Pluim et al. 1994a). When cumulative intake at 11 weeks was used as the biomarker of exposure, associations with AST and ALT levels and an inverse association with platelet levels were found.

Immunological development. Several general population studies have examined associations between CDDs and infections in children; most studies did not find associations. No associations were found for infections (Miyashita et al. 2018b), respiratory tract infections (Miyashita et al. 2018b; Weisglas-Kuperus et al. 1995), otitis media infections (Miyashita et al. 2018b; Stølevik et al. 2011, 2013), gastrointestinal infections (Stølevik et al. 2011, 2013), or chicken pox (Stølevik et al. 2011, 2013). Some studies did find associations between maternal CDDs and infections; associations were found between maternal dietary CDD/CDF/PCB TEQs and upper respiratory infections (Stølevik et al. 2011, 2013), gastroenteritis in 0–3-years old children, but not in 2–3-year-old children (Stølevik et al. 2013), and exanthema subitem in 1-year-old children (Stølevik et al. 2011) but not in 0–3-year-old children (Stølevik et al. 2013). General population studies also evaluated other immune endpoints; as with infections, most studies did not find associations. In the three studies examining eczema incidence, two found inverse associations (Ye et al. 2018; Stølevik et al. 2011), one found no association (Stølevik et al. 2013) but did find an association with atopic eczema in 0–3-year-old children, but not in 2–3-year-old children. Two studies reported associations between wheezing in children and maternal CDD/CDF/PCB TEQs in the diet (Stølevik et al. 2013) or in blood (Miyashita et al. 2018b). The Miyashita et al. (2018b) study only found the associations in 3.5-year-old children; no associations were found in 7-year-old children. Another study of young children did not find an association between maternal dietary CDD/CDF/PCB TEQs and wheezing (Stølevik et al. 2013). No associations were found for asthma (Ye et al. 2018; Stølevik et al. 2013), hay fever (Ye et al. 2018), allergy (Miyashita et al. 2018b), food allergy (Miyashita et al. 2018b), or sensitization (Stølevik et al. 2013). Stølevik et al. (2013) measured vaccine antibodies in children, an inverse association between maternal dietary CDD/CDF/PCB TEQs and antibodies for the measles vaccine; no associations were found for the Rubella, tetanus, or *Haemophilus influenza* type B vaccines.

Neurological development. A number of studies have evaluated potential neurodevelopmental effects in children living in areas of Vietnam with contamination from Agent Orange, living in Seveso, or in the general population. Interpretation of the results of these studies is difficult due to differences in the biomarkers of exposure, tests used, and ages of the children.

A series of studies have followed the neurodevelopment of a group of children living in an area of Vietnam contaminated with Agent Orange. In infants, impaired performances on the Bayley Scales of Infant and Toddler development tests were observed in infants of mothers with human milk 2,3,7,8-TCDD levels in the second tertile, but not in the third tertile (Tai et al. 2013); when human milk CDD/CDF TEQ was used as the biomarker of exposure, no associations were found. At 1 year of age, no associations between human milk CDD/CDF TEQs or 2,3,7,8-TCDD levels and neurodevelopmental

scores for cognition, motor function, or adaptive behavior were observed (Pham et al. 2015); impaired performance on social emotional tests was observed. When the children were 3 years of age, no alterations in performance on Bayley Scales tests of cognition or language were found using 2,3,7,8-TCDD or CDD/CDF TEQ human milk levels as biomarkers of exposure; impaired performance on motor function was found in boys only when 2,3,7,8-TCDD human milk level was used as a biomarker (Tai et al. 2016). Another study of the 3-year-old children found impaired performance on cognitive, language, motor, and adaptive behavior scores in the high CDD/CDF TEQs group, as compared to the lower CDD/CDF group (Nishijo et al. 2014). At 5 years of age, impaired performance on tests of coordinated movement was observed in boys of mothers with high CDD/CDF human milk levels; no effect was observed in girls (Tran et al. 2016). Cognitive function was also impaired in boys of mothers with high levels of 2,3,7,8-TCDD in human milk. When the children were examined at 8 years of age, impaired reading (greater number of errors) in boys was associated with 1,2,3,7,8-Pe CDD, 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 levels, but not with 2,3,7,8-TCDD or OCDD levels (Tai et al. 2020). Impaired performance on math achievement tests were associated with 2,3,7,8-TCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, and 1,2,3,4,6,7,8-HpCDD levels. No alterations in performance on tests of math or reading learning difficulties or language achievement were associated with CDD TEQ levels or individual congener levels. However, comparisons between groups with low and high 2,3,7,8-TCDD human milk levels demonstrated an inverse effect on reading errors and language achievement and an association with reading learning difficulties in boys (Tai et al. 2020). Comparisons between high- and low-exposure groups also demonstrated impaired performance on reading tests for CDD TEQs, 1,2,3,4,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD, math and language scores for 1,2,3,4,7,8-HxCDD, and math scores and reading learning disabilities for 1,2,3,4,6,7,8-HpCDD. This cohort of children has also been evaluated for other neurodevelopmental effects. An association between human milk 2,3,7,8-TCDD levels and impaired performance on tests of autism traits were found in 3-year-old boys, but not in girls (Nishijo et al. 2014); no associations were found when CDD/CDF TEQ levels were used as the biomarker of exposure. At 8 years of age, an association between scores of tests of covert aggression and 2,3,7,8-TCDD levels was observed (Nishijo et al. 2012). No associations were found between CDD congener levels in human milk and food approach or food avoidance scores in 3-year-old children (Nguyen et al. 2018).

One study evaluated potential neurodevelopmental effects in 7–17-year-olds whose mothers were exposed to 2,3,7,8-TCDD resulting from the Seveso accident (Ames et al. 2019). In general, performances on tests of executive functioning and reversal learning, non-verbal intelligence, attention

and hyperactivity, and memory were not affected by maternal serum 2,3,7,8-TCDD levels at the time of the accident or estimated 2,3,7,8-TCDD levels at the time of pregnancy.

A number of general population studies have evaluated neurodevelopmental endpoints in children of various ages. A study in newborns found an association between human milk CDD/CDF TEQs and neurological optimality score of motor function (Huisman et al. 1995a) but did not find associations for individual congeners. Another study found alterations in neurological optimality score in comparisons between newborns of mothers with high levels of CDD/CDF TEQs in human milk, as compared to those with low levels (Pluim et al. 1996). No associations were found when the children were 18 months of age (Huisman et al. 1995b) or in another study of 2.7-year-old children (Wilhelm et al. 2008). Other tests found enhanced neuromuscular maturation and higher reflexes in 2.7-year-old children (Ilsen et al. 1996). No alterations in tests of developmental delays (Bayley Scales of Infant and Toddler Development) were observed in infants 12 or 24 months of age (Ilsen et al. 1996; Wilhelm et al. 2008). Increased risks of severe language delays, low communication skills (girls only), and having incomplete grammar were found in 3-year-old children (Caspersen et al. 2016b). A study of 42-month-old children found an improvement in cognitive in cognitive development achievement score associated with 1,2,3,6,7,8-HxCDD (girls and boys and girls only) and 1,2,3,7,8-PeCDD (girls only) but not with other CDD congeners or total CDD congeners (Ikeno et al. 2018). In general, studies evaluating associations between CDD exposure and intelligence or learning have not found associations in general population studies. No alterations in performance on the Wechsler Intelligence Scale tests were found in 7–12-year-old children (ten Tusscher et al. 2014) or in 11-year-old children (Hui et al. 2016, 2019). No alterations were found in the Hong Kong List Learning test in 11-year-old children (Hui et al. 2016, 2019).

Several studies have examined behavior. No association between CDD/CDF/PCB human milk levels and psychosocial behavioral development was observed in 6–10- or 11–13-year-old children (Kono et al. 2015). In contrast, a study of 7–12-year-old children found associations between estimated dioxin intake and social problems, aggressive behavior, and external behavioral problems (ten Tusscher et al. 2014). Studies examining sex-specific behaviors have not found associations with maternal blood CDD/CDF TEQ levels in 6-year-old children (Winneke et al. 2014) or 9-year-old children (Nowack et al. 2015). Two studies evaluated possible associations between CDDs and attention deficit hyperactivity disorder (ADHD) in children; no associations were found between maternal dietary intake of CDDs/CDFs in 3.5-year-old children (Caspersen et al. 2016a).

A study of 10-year-old girls found an inverse association between maternal blood CDD/CDF TEQ levels and the score on a test measuring autistic traits; no association was found in males (Nowack et al. 2015). A second study examining autistic traits in 3-year-old children living in an area of Vietnam with Agent Orange contamination found associations between human milk 2,3,7,8-TCDD levels and performance on an autism spectrum rating scale (Nishijo et al. 2014); no associations were found between human milk CDD/CDF TEQs and performance on the autism tests or between maternal blood or human milk CDD/CDF TEQs in school-age children (Neugebauer et al. 2015). The Neugebauer et al. (2015) study did find associations between the attentional performance test of distractibility and human milk CDD/CDF TEQs and between attentional performance of divided attention and maternal blood CDD/CDF TEQs.

Reproductive development. A small number of epidemiological studies evaluated impaired development of the reproductive system. In a general population study, an inverse association between anogenital distance and maternal blood dioxin levels (as measured by dioxin-responsive chemical-activated luciferase gene expression [DR CALUX] bioassay) was observed in newborn boys, but not in young children (16 months of age) (Vafeiadi et al. 2013). Another general population study did not find an association between placental CDD/CDF levels and the occurrence of cryptorchidism (Virtanen et al. 2012). Decreased sperm concentration, count, and progressive motility were observed in the breastfed sons of women in the Seveso cohort (Mocarelli et al. 2011); however, no significant alterations were observed in formula-fed children. Higher blood 2,3,7,8-TCDD levels or CDDs TEQs levels were associated with later puberty onset in boys aged 8–9 years living in an area of Russia with contaminated soil (Korrick et al. 2011). When the boys were 17–18 years of age, CDD/CDF/PCB TEQs levels were also associated with delayed puberty and delayed sexual maturity (Burns et al. 2016). Su et al. (2012) found no associations between placental CDD/CDF/PCB TEQs and sex characteristics in boys and girls at 8 years of age.

A number of studies have evaluated the effects of developmental exposure on reproductive hormone levels. Interpretation of the results is complicated by the small number of studies examining a particular hormone and the different ages of the children. In studies of children living in areas of Vietnam contaminated by Agent Orange, an association between human milk CDD/CDF TEQs and salivary dehydroepiandrosterone (DHEA) was observed in 1-year-old children (Anh et al. 2017). When individual congeners were examined, associations were found for 1,2,3,4,6,7,8-HpCDD and OCDD, but not for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, or HxCDD congeners (Sun et al. 2020). Sun et al. (2020) also examined salivary DHEA levels when the children were 3 and 5 years of age; no associations were

observed at 3 years of age. At 5 years of age, inverse associations were found for 2,3,7,8-TCDD, 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. The study also evaluated salivary testosterone levels in 3- and 5-year-old children (Sun et al. 2020); the results were inconsistent at the two ages. At 3 years of age, inverse associations were found for 2,3,7,8-TCDD and 1,2,3,6,7,8-HxCDD; at 5 years of age, inverse associations were found for 1,2,3,7,8-PeCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD. In newborns, no associations were found for 2,3,7,8-TCDD and cord blood estradiol or testosterone levels; 1,2,3,6,7,8-HxCDD was inversely associated with cord blood testosterone levels in girls, but not in boys, and was not associated with estradiol levels (Boda et al. 2018). In the adult sons of mothers exposed during the Seveso accident, maternal serum 2,3,7,8-TCDD levels were associated with FSH levels and inversely associated with inhibin B levels among breastfed sons, but not in formula-fed sons (Mocarelli et al. 2011). Most general population studies have not found associations between CDD and testosterone (Miyashita et al. 2018a; Su et al. 2012), estradiol (Miyashita et al. 2018a), androstenedione (Miyashita et al. 2018a), sex hormone binding globulin (Miyashita et al. 2018a; Virtanen et al. 2012), FSH (Miyashita et al. 2018a; Su et al. 2012; Virtanen et al. 2012), LH (Miyashita et al. 2018a; Su et al. 2012; Virtanen et al. 2012), or inhibin B (Miyashita et al. 2018a). A couple of studies did find associations: an inverse association between maternal blood fat CDD/CDF TEQs and cord testosterone levels in females (Cao et al. 2008), inverse associations between estradiol levels and maternal blood fat CDD/CDF TEQs in infants (Cao et al. 2008) and CDD/CDF/PCB levels in 8-year-old children (Su et al. 2012), and an association between maternal CDD TEQs and cord DHEA levels in males (Miyashita et al. 2018a).

2,3,7,8-TCDD—Animal studies. The literature on developmental effects of 2,3,7,8-TCDD is extensive; over 150 studies have been published. The summary below includes representative examples with emphasis on low-dose studies that could help construct dose-response relationships and determine PODs for the various specific effects. The types of effects observed in the offspring of animals exposed to 2,3,7,8-TCDD include, but are not limited to, fetal/newborn mortality, altered growth, structural malformations, impaired development of the cardiovascular, respiratory, skeletal, and gastrointestinal systems, and impaired functional alterations of the immune, neurological, and reproductive systems.

Fetal/pup mortality. Several studies have reported increased mortality in the offspring of rodents and monkeys exposed to 2,3,7,8-TCDD during gestation. Fetal/newborn deaths have occurred at doses that were either nontoxic or minimally toxic to the mothers. Increased newborn mortality was observed in Han/Wistar rats exposed to 1 μg/kg on GD 15 (Bell et al. 2007a), Holtzman rats dosed with ≥0.7 μg/kg on GD 15 (Bjerke and Peterson 1994; Bjerke et al. 1994a; Ishimura et al. 2002) or GD 10 (Kransler et al.

2009), and Line C rats (defined as having no TCDD-resistant alleles) dosed with 1 µg/kg on GD 15 (Miettinen et al. 2006). Decreased litter sizes were observed in Dark-Agouti rats exposed to 0.7 µg/kg on GD 18 (Tomasini et al. 2012), Sprague-Dawley rats exposed to 0.8 µg/kg on GD 15 (Ikeda et al. 2002), and Wistar rats exposed to 1 µg/kg on GD 15 (Takeda et al. 2020). An increase in abortions was observed in monkeys after a single exposure to 1 µg/kg on GD 25, 30, 35, or 40 (McNulty 1984) and early fetal losses were observed after exposure on GD 12 (Guo et al. 2000). Exposure of pregnant C57BL/6 mice to 10 µg/kg 2,3,7,8-TCDD on GD 12 resulted in 90% lethality of the pups by PND 28 by a wasting-like syndrome (Mustafa et al. 2008); no deaths occurred at 0.2 µg/kg. Increased fetal mortality was also reported in Hartley guinea pigs following dosing of the dams with 1.5 µg/kg 2,3,7,8-TCDD on GD 14 (Kransler et al. 2007; Olson and McGarrigle 1992); no significant lethality was reported at 0.15 µg/kg.

Dietary exposure of female Han/Wistar rats to 0.046 µg/kg/day 2,3,7,8-TCDD for 12 weeks before mating with untreated males and during mating and gestation resulted in 8/27 females with total litter loss compared with 3/27 in controls; the difference between the two groups was not statistically significant (Bell et al. 2007b). However, the number of pups alive on day 1, expressed as a ratio to the number of pups born, was significantly decreased, and the number of pups surviving between days 1 and 4 (as a ratio of number of pups alive on day 1) was also statistically significantly reduced in the exposed group. Decreased neonatal survival was found in the F1 generation of Sprague-Dawley rats exposed via the feed to 0.001 µg/kg/day of 2,3,7,8-TCDD in a 3-generation study (Murray et al. 1979); decreased survival was also observed in the F2 generation at 0.01 µg/kg/day but was not observed in the F3 generation. Significantly reduced neonatal survival was reported in pups from C57BL/6J mice following exposure to a maternal dose of 0.5 µg/kg/day 2,3,7,8-TCDD administered on GDs 0, 7, and 14, and PND 2 (Vorderstrasse et al. 2006). A study in minks in which females were exposed to 2,3,7,8-TCDD in the diet for 35 days before mating with untreated males reported that maternal doses of 0.00003, 0.003, and 0.007 µg/kg/day resulted in 3-week survival rates of 83, 47, and 11%, respectively (Hochstein et al. 2001).

Structural malformations and anomalies. Skeletal malformations have been reported in a number of studies of laboratory animals. The most commonly reported skeletal malformation is cleft palate, which has been reported in rats and mice following acute-duration oral perinatal exposure to 2,3,7,8-TCDD at maternal doses ≥1 µg/kg (see Table 2-24 for citations). The other commonly reported anomaly occurs in the kidney (primarily hydronephrosis) of rats, mice, and hamsters at maternal doses ≥0.5 µg/kg (see Table 2-24 for citations).

Table 2-24. Structural Anomalies in Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, exposure	NOAEL (µg/kg/day)	LOAEL (µg/kg/day)	Effect	Reference
C57BL/6J mouse GD 10		12	Hydronephrosis	Abbott et al. 1987a
C57BL/6N mouse GD 10		12	Impaired function of the Bowman's capsule	Abbott et al. 1987b
C57Bl/6N mouse, GD 10 or 21		24	Cleft palate	Abbott and Birnbaum 1990
C57BL/6N mouse, GD 14		6	Hydronephrosis	Aragon et al. 2008a
Wild-type mouse, GD 12		24	Cleft palate, hydronephrosis	Bryant et al. 2001
CD-1 mouse, GDs 7–16		25	Hydronephrosis	Courtney 1976
CD-1 mouse, GDs 7–16		50	Cleft palate	Courtney 1976
C57BL/6N mouse PND 1 or 4		6	Hydronephrosis	Couture-Haws et al. 1991b
C57Bl/6J mouse, GD 9		15	Cleft palate	Dasenbrock et al. 1992
DBA2 mouse, GD 9		150	Cleft palate	Dasenbrock et al. 1992
ICR mouse, GD 12.5		40	Cleft palate	Fujiwara et al. 2008
CRCD rat, 2 weeks prior to mating	0.5	2	Cystic kidneys	Giavini et al. 1983
Syrian hamster, GD 11		2	Nephrosis	Gray et al. 1995
Long-Evans rats, GD 8	1	5	Cleft palate	Huuskonen et al. 1994
Hans/Wistar rats, GD 8 or 10	1	10	Hydronephrosis	Huuskonen et al. 1994
Golden Syrian hamster, GD 9		3	Hydronephrosis	Kransler et al. 2007
Holtzman rat, GD 10	6	18	Cleft palate	Kransler et al. 2007
C57BL/6J mouse, GD 10		24	Cleft palate	Li et al. 2010
EGFR mouse, GD 10	1.5	4.4	Hydronephrosis	Miettinen et al. 2004
C57BL/6J mouse, GD 12.5		40	Cleft palate, hydronephrosis	Mimura et al. 1997
C57BL/6 mouse, GD 10		1	Hydronephrosis	Moore et al. 1973
C57BL/6 mouse, GDs 10–13		1	Hydronephrosis	Moore et al. 1973

Table 2-24. Structural Anomalies in Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, exposure	NOAEL (µg/kg/day)	LOAEL (µg/kg/day)	Effect	Reference
C57BL/6 mouse, GDs 10–13		3	Cleft palate	Moore et al. 1973
C57BL/6 mouse, once at parturition		1	Hydronephrosis	Moore et al. 1973
NMRI mouse, GDs 6–15	0.3	3	Cleft palate	Neubert and Dillmann 1972
Holtzman rat, GD 15		1	Hydronephrosis	Nishimura et al. 2006
Golden Syrian hamster, GD 7 or 9		1.5	Hydronephrosis	Olson and McGarrigle 1992
C57Bl/6J mouse, GDs 6–15		0.5	Hydronephrosis	Silkworth et al. 1989b
DBA/2J mouse, GDs 6–15		0.5	Hydronephrosis	Silkworth et al. 1989b
C57Bl/6J mouse, GDs 6–15	2	4	Cleft palate	Silkworth et al. 1989b
DBA/2J mouse, GDs 6–15	4	8	Cleft palate	Silkworth et al. 1989b
CF-1 mouse, GDs 6–15	0.1	1	Cleft palate	Smith et al. 1976
C57BL/6N mouse, GD 10		12	Cleft palate	Weber et al. 1985
C57BL/6J mouse, GD 12.5		10	Cleft palate	Yamada et al. 2006
C57BL/6J mouse		28	Cleft palate	Yuan et al. 2017

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

A study on the role of the timing of exposure found that the highest incidence of cleft palate in mice was observed when 2,3,7,8-TCDD was administered on GDs 11.5–12.5, which is just before palatogenesis, as compared to other exposure days (GDs 8.5–14.5 tested) (Yamada et al. 2006). A timing study for hydronephrosis found that the incidence and severity of hydronephrosis was greater in pups exposed *in utero* and/or during lactation, as compared to pups only exposed *in utero* (Nishimura et al. 2006). Mimura et al. (1997) examined the role of the AhR in the development of 2,3,7,8-TCDD-induced cleft palate and hydronephrosis and found that almost all of wild-type (*AhR*+/+) fetuses exhibited cleft palate and hydronephrosis following dosing of dams with 40 µg/kg 2,3,7,8-TCDD on GD 12.5; neither defect was observed in similarly exposed AhR-null mice. In contrast, most of the offspring from heterozygous AhR mutant genotype (*AhR*+/−) exhibited hydronephrosis, but only 24–28% exhibited cleft palate indicating the haplo-insufficiency of the *AhR* gene in the incidence of cleft palate.

Effects on growth. Decreases in offspring body weights were observed in Holtzman rats administered 0.7 or 1 µg/kg on GD 15 (Bjerke and Peterson 1994; Bjerke et al. 1994a; Hattori et al. 2014; Nishimura et al. 2006); however, no effects on body weight were observed in offspring exposed to ≤0.8 µg/kg on GD 15 (Ikeda et al. 2005a; Nishimura et al. 2003). Neonatal weight was significantly reduced in pups from Sprague-Dawley rats administered 1 µg/kg 2,3,7,8-TCDD on GD 15; no significant effects were reported at 0.5 µg/kg (Nayyar et al. 2002). In Wistar rats, fetal weight was significantly reduced on GD 19 following maternal administration of 0.1 µg/kg 2,3,7,8-TCDD on GDs 9– 19 (Nishijo et al. 2007). Significant decreases in body weight on PNDs 7, 21, and 30 were observed in the offspring of C57BL/6 mice administered 1 µg/kg/day on 4 lactation days (Jin et al. 2010); a decrease in body length was also observed on PNDs 30 and 60. Doses of up to 106 µg/kg 2,3,7,8-TCDD given to a strain of mice heterozygous for the epidermal growth factor receptor ($EGFR^{+/-}$) on GD 10 did not significantly affect fetal weight on GD 18 (Miettinen et al. 2004).

Impaired development of respiratory, cardiovascular, skeletal, and gastrointestinal systems. 2,3,7,8-TCDD has been shown to alter lung development in perinatally exposed rats (see Table 2-25). Treatment of Holtzman rats with ≥1.5 µg/kg 2,3,7,8-TCDD on GD 10 resulted in morphological changes in the lungs of GD 20 fetuses and PND 7 pups indicative of immaturity and hypoplasia (Kransler et al. 2009). These changes were associated with alterations in mechanical properties of the lungs examined on PND 7. 2,3,7,8-TCDD-treated rats required more pressure to achieve comparable changes in lung volume than control rats. The study also showed the presence of responsive AhR and aryl hydrocarbon receptor nuclear translocator (ARNT) mRNA and protein in the developing alveolar and bronchiolar epithelium.

Table 2-25. Systemic Effects Observed in the Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species/exposure	LOAEL[a] (µg/kg/day)	Effect	Reference
Respiratory system			
Holtzman rat, GD 10	1.5	Altered lung morphology and mechanical properties	Kransler et al. 2009
Cardiovascular system			
C57BL/6N mouse, GD 14	6	↑ relative left ventricle plus septum weight	Aragon et al. 2008a
C57BL/6N mouse, GD 14.5	6	↑ susceptibility to hypertension in adulthood	Aragon et al. 2008b
C57BL/6J mouse, PND 1	20	Hypertrophy of left ventricle	Fujisawa et al. 2019

Table 2-25. Systemic Effects Observed in the Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species/exposure	LOAEL[a] (µg/kg/day)	Effect	Reference
C57BL/6J mouse GD 0.5, GD 7.5, and PND 10	1	↑ systolic blood pressure and arterial pressure in response to angiotensin stress	de Gannes et al. 2021
C57BL/6N mouse, GD 14.5	3[b]	↓ relative heart weight	Thackaberry et al. 2005a
Skeletal system			
Sprague-Dawley rat, GD 11	1	↓ bone strength	Finnilä et al, 2010
Line C rat, GD 15	0.03	↓ molar size	Kattainen et al. 2001
C57BL/6N mouse, GD 13	1[c]	Altered molar and mandible shape	Keller et al. 2007
C3H/HeJ mouse, GD 13	0.01	Altered mandible shape	Keller et al. 2008
Line C rat, GD 11	1	Arrested molar development	Miettinen et al. 2002
Line C rat, GD 15	1[d]	Morphological and mechanical alterations in bone	Miettinen et al. 2005
Linc C rat, GD 15	0.03	Enhanced dental caries susceptibility	Miettinen et al. 2006
Gastrointestinal system			
Han/Wistar rat, GD 12	10[e]	Gastrointestinal hemorrhage	Huuskonen et al. 1994
Wistar rat, GDs 6–15	0.25[f]	Gastrointestinal hemorrhage	Khera and Ruddick 1973
Holtzman rat, GD 10	1.5	Intestinal hemorrhage	Kransler et al. 2007
Holtzman rat, GDs 7–19	1	Intestinal hemorrhage	Shiverick and Muther 1983
Sprague-Dawley rat, GDs 6–15	0.125[g]	Intestinal hemorrhage	Sparschu et al. 1971
Endocrine system and metabolic effects			
Wistar rat, GD 1–LD 30	0.2	↓ T3, T4, growth hormone ↑ TSH	Ahmed 2001
Long-Evans rat, GD 15	1	↓ serum TSH levels at PND 25 and 60 ↓ T4 at PND 60	Fenton et al. 2002
Long-Evans rat, GD 15	1	↓ core body temperature	Gordon et al. 1995
Long-Evans rat, GD 15	1	Altered thermoregulation	Gordon and Miller 1998
Holtzman rat, GD 15	0.8[h]	↓ serum T4 and ↑ TSH at PND 21; thyroid hyperplasia	Nishimura et al. 2003
Holtzman rat, GD 15	1	↓ serum T4 and ↑ TSH at PND 21; thyroid hyperplasia	Nishimura et al. 2005b

Table 2-25. Systemic Effects Observed in the Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species/exposure	LOAEL[a] (µg/kg/day)	Effect	Reference
Sprague-Dawley rat, GDs 10–16	1[i]	↓ T4	Seo et al. 1995

[a]Unless noted, studies did not identify NOAELs.
[b]NOAEL of 1.5 µg/kg.
[c]NOAEL of 0.1 µg/kg/day.
[d]NOAEL of 0.3 µg/kg.
[e]NOAEL of 1 µg/kg.
[f]NOAEL of 0.125 µg/kg/day.
[g]NOAEL of 0.03 µg/kg/day.
[h]NOAEL of 0.2 µg/kg.
[i]NOAEL of 0.025 µg/kg/day.

↑ = increase; ↓ = decrease; GD = gestation day; LD = lactation day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PND = postnatal day; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone

Heart abnormalities have been reported in mice following perinatal exposure to 2,3,7,8-TCDD, as summarized in Table 2-25. In C57BL/6N mice dosed with ≥3 µg/kg 2,3,7,8-TCDD on GD 14.5, relative fetal heart weight on GD 17.5 was significantly decreased (Thackaberry et al. 2005a). Maternal doses ≥6 µg/kg significantly reduced cardiocyte proliferation; this was seen throughout the developing heart but was most evident in the interventricular septum. In offspring examined on PND 21, but not PND 7, maternal doses ≥6 µg/kg 2,3,7,8-TCDD significantly increased relative heart weight, which was found to be associated with increased expression of the cardiac hypertrophy marker, atrial natriuretic factor. An electrocardiogram (EKG) performed in 21-day-old anesthetized pups showed no evidence of cardiac arrhythmias, but gestational plus lactational exposure significantly reduced heart rate. However, responsiveness to isoproterenol stimulation of the heart rate was not changed. Microarray gene analysis of the fetal heart showed that 2,3,7,8-TCDD significantly altered the expression of a number of genes involved in drug metabolism, cardiac homeostasis, extracellular matrix production/remodeling, and cell cycle regulation (Thackaberry et al. 2005b). Left ventricle hypertrophy was observed in the offspring of C57BL/6J mice administered 20 µg/kg on PND 1 (Fujisawa et al. 2019). Other studies showed that the 2,3,7,8-TCDD-induced changes required the AhR since gene expression was not altered in *AhR* knockout fetuses (Aragon et al. 2008a). Furthermore, evaluation of 3-month-old offspring showed that cardiac abnormalities seen in fetuses persisted through adulthood and increased the susceptibility of offspring to hypertension (Aragon et al. 2008a, 2008b). Studies examining blood pressure in adult offspring have found increased systolic blood pressure in rats administered 0.2 µg/kg/day on GDs 14 and 21 and PNDs 7

and 22 (Hsu et al. 2018, 2020) and an increase in systolic blood pressure and arterial pressure in response to angiotensin pathological stress (de Gannes et al. 2021).

Perinatal exposure to 2,3,7,8-TCDD can also affect bone and teeth development, as presented in Table 2-25. A study in a strain of rat with no TCDD-resistant alleles (referred to as "Line C") reported that a single maternal dose of 1 μg/kg 2,3,7,8-TCDD, but not ≤0.3 μg/kg, on GD 15 resulted in the following effects in female pups (males were not monitored) on PND 35: decreased cortical bone mineral density in the tibia and femur, decreased cross-sectional area of the cortex of femur, decreased periosteal and endosteal circumference in the femur, and decreased polar cross-sectional moment of inertia of the femur; bone length was not significantly affected (Miettinen et al. 2005). To determine a critical time of exposure, male and female pups were examined on PND 40 after dosing the dams with 1 μg/kg 2,3,7,8-TCDD at various times from GD 11 to PND 4. Effects varied somewhat between males and females and, in general, earlier exposures caused more severe effects and decreases in bone mineral density were not observed in offspring only receiving postnatal exposure. In a separate experiment, the investigators showed that at 1 year of age, most of the effects induced by gestational and lactational exposure to 1 μg/kg on GD 15 were reversed (Miettinen et al. 2005). A maternal dose of 1 μg/kg 2,3,7,8-TCDD administered to Sprague-Dawley rats on GD 11 significantly decreased parameters of mineralization, geometry, and strength in the tibias from pups on PNDs 35 and 70 (Finnilä et al. 2010). Results of nanoindentation tests showed that exposure to 2,3,7,8-TCDD disturbs the age-dependent maturation process causing the tibias of pups to be more ductile, softer, and less able to store energy than control bone. The results suggested that the reduced bone strength is associated more with the mineralization level and altered bone geometry than with changes in bone material properties.

Dosing rats of a strain with no TCDD-resistant alleles with 1 μg/kg 2,3,7,8-TCDD on GD 15 completely prevented the development of the lower third molar in 50% of female pups and 60% of male pups sacrificed on PNDs 35 and 70, respectively (Kattainen et al. 2001). 2,3,7,8-TCDD also reduced the size of the lower third molar at ≥0.03 μg/kg in females and ≥0.3 μg/kg in males. Further studies by the same group of investigators showed that effects were limited to third molars and that maternal exposure on GD 11 resulted in more missing molars than exposure at later times (Miettinen et al. 2002). 2,3,7,8-TCDD also decreased eruption frequency of developed third molars and effects were more marked in pups exposed *in utero* plus lactation than only *in utero* or only during lactation. The results suggested that the critical window for the third molar is during early morphogenesis, from tooth initiation to the early bud stage, and that the dental epithelium is the likely target for 2,3,7,8-TCDD. In a more recent study, it was shown that *in utero* exposure to 2,3,7,8-TCDD rendered rat molars more susceptible

to caries and this could not be explained by changes in mineral composition (Miettinen et al. 2006). 2,3,7,8-TCDD has also been shown to affect mandible size and shape in mice. Exposure on GD 13 of five different strains of mice, all containing the sensitive b allele at the AhR locus, showed that 2,3,7,8-TCDD affected mandible size and shape in the offspring, but the sensitivity differed among the inbred strains (Keller et al. 2007, 2008). A significant association between mandible size and 2,3,7,8-TCDD exposure was observed in male C3H/HeJ mice. Mandible shape was also affected significantly in male C3H/HeJ mice at 0.01 µg/kg and in C57BL/6J and C57BL/10J mice at higher doses. The investigators hypothesized that beyond AhR-related effects, variation in response to 2,3,7,8-TCDD reflects differences in the genetic architecture controlling the trait being evaluated.

Gastrointestinal hemorrhage was observed in the offspring of Wistar, Han/Wistar, or Holtzman rats at doses ≥0.125 µg/kg/day during GDs 6–15 or GD 8, 12, or 20 (see Table 2-25 for citations and summaries).

Impaired thyroid function and metabolic effects. Several studies examined thyroid hormone levels in 2,3,7,8-TCDD exposed offspring and reported conflicting results; see Table 2-25 for a summary of results. Fenton et al. (2002) reported significant increases in serum TSH levels in 25-day-old female pups from Long-Evans rats exposed once to 1 µg/kg 2,3,7,8-TCDD on GD 20 or PND 1, 3, 5, or 10; no effects were observed when maternal exposure occurred on GD 15; serum T3 and T4 levels were not significantly altered. However, in 60-day-old offspring (exposed on GD 15), serum TSH was significantly elevated and T4 was significantly decreased. In contrast, Seo et al. (1995) reported decreased T4 levels in weanling offspring of rats exposed to 0.1 µg/kg/day 2,3,7,8-TCDD on GDs 10–16, but no alterations in T3 or TSH levels. Decreased serum T3 and T4 levels and increased TSH levels were observed in the offspring of Wistar rats administered 0.2 µg/kg/day 2,3,7,8-TCDD from GD 1 to lactation day (LD) 30 (Ahmed 2011); hormone levels were measured in fetuses on GDs 16 and 19 and in the pups on LDs 10, 20, and 30. Significantly increased serum TSH levels were reported in 21- and 49-day-old offspring from Holtzman rats dosed with 0.8 µg/kg 2,3,7,8-TCDD on GD 15 (Nishimura et al. 2003). The increases in TSH were more pronounced in male pups. At 0.2 µg/kg, significant decreases in T4 levels and increases in T3 levels were observed in 21-day-old offspring, but not in 49-day-old offspring. Microscopic examination of the thyroid on PND 49 showed that exposure to 0.8 µg/kg 2,3,7,8-TCDD induced diffuse hyperplasia of follicular cells in males. Immunocytochemistry showed a significant increase in the number of proliferating cell nuclear antigen-positive cells indicating the ability of 2,3,7,8-TCDD to induce proliferation. In a subsequent study, cross-fostering experiments revealed that serum total and free T4 levels were reduced significantly, mostly due to lactational exposure (dams were

dosed with 1 µg/kg on GD 15); serum total T3 levels were not significantly altered by exposure to 2,3,7,8-TCDD (Nishimura et al. 2005b). Additionally, serum TSH levels were significantly elevated due to lactational exposure to 2,3,7,8-TCDD. Microscopic examination of the thyroid on PND 49 revealed proliferative lesions of follicular cells including hyperplasia in pups exposed via the milk but not in those exposed only *in utero*. In another study in which AhR-null mouse pups were evaluated, the same group of investigators showed that the disruption of thyroid homeostasis is mediated entirely via AhR (Nishimura et al. 2005a).

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). A follow-up study showed that exposure to 2,3,7,8-TCDD affected the 24-hour pattern of core temperature by reducing nocturnal temperature, particularly at 7 and 11 months of age (Gordon and Miller 1998). Motor activity was reduced in a parallel manner. The hypothermic effects of 2,3,7,8-TCDD were more pronounced at cooler ambient temperatures. Behavioral thermoregulation was not affected by 2,3,7,8-TCDD. The investigators noted that the normal behavioral regulation of core temperature suggested that hypothalamic thermoregulatory centers are not permanently altered by gestational exposure to 2,3,7,8-TCDD.

Impaired development and functional alterations of the immune system. The immune system is a sensitive target following gestational and/or lactational exposure to 2,3,7,8-TCDD; a summary of studies examining immune endpoint is presented in Table 2-26. The observed effects include decreases in lymphoreticular organ weight (particularly the thymus and spleen), decreases in thymic cellularity, alterations in the T cell and mature B cell phenotypes, and functional impairment.

Table 2-26. Immunological Effects in the Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect			
Species, exposure	Thymus	Thymocyte	Function	Reference
C57BL/6 mouse GDs 6–14	1.5: atrophy	1.5: delayed maturation		Blaylock et al. 1992
C57BL/6 mouse, GD 15.5			10: ↓ decreased resistance to infection	Ding et al. 2018
F344 rat, LD 0, 7, and 14	5: ↓ weight (44–52% on PND 25)		5: ↓ response to PHA and ConA	Faith and Morre 1977
BALB/cGa mouse, GD 14	10: atrophy			Fine et al. 1989

Table 2-26. Immunological Effects in the Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect			
Species, exposure	Thymus	Thymocyte	Function	Reference
Fischer 344 rat, GD 14	1: ↔ 3: ↓ weight (38% on GD 19)	3: ↑ $CD4^-/CD8^+$ and ↓ $CD4^+/CD8^+$		Gehrs et al. 1997a
Fischer 344 rat, GD 14	3: ↓ weight (27%)	3: ↓ $CD4^-/CD8^-$	3: delayed-type hypersensitivity to BSA	Gehrs et al. 1997b
Fischer 344 rat, GD 14	1: ↔ weight	1: ↓ $CD4^-/CD8^-$	1: delayed-type hypersensitivity to BSA	Gehrs et al. 1997b
Sprague-Dawley rat, LD 1	10: Atrophy			Håkansson et al. 1987
C57BL/6 mouse, GD 0, 7, and 14 and LD 2			0.17: ↓ CD8+ response to viral infection	Hogaboam et al. 2008
B6C3F1 mouse, GDs 6–14	1.5: atrophy	1.5: ↓ $CD4^+CD8^+$ and ↑ $CD4^-/CD8^-$	1.5: ↓ cytotoxic T lymphocytes 1.5: ↔ response to PHA, ConA, or LPS	Holladay et al. 1991
Long-Evans rat, GD 8	1: ↔ 5: atrophy			Huuskonen et al. 1994
Han/Wistar rat, GD 8	1: ↔ 10: atrophy			Huuskonen et al. 1994
B6C3F1 mouse, GD 14, LDs 1, 7, and 14	1: ↔ 5: ↓ weight (41%)		1: ↔ 5: Impaired response to *Listeria monocytogenes* challenge and to PHA	Luster et al. 1980
C57BL/5 mouse GD 12	2.5: ↓ weight (14%)	5: ↓ $CD4^+CD8^+$		Mustafa et al. 2008
Holtzman rat, GD 15		0.8: ↔ CD4 CD8		Nohara et al. 2000
C57BL/6NCji mouse, LDs 0–17	0.011: ↔ weight	0.001: ↔ 0.011: ↑ $CD4^+$	0.001: ↔ 0.011: impaired response to *Listeria* challenge	Sugita-Konishi et al. 2003
Swiss-Webster mouse, 4 weeks prior to mating and during gestation and lactation	0.13: ↔ 0.325: atrophy		0.13: ↔ 0.325: ↓ response to sRBC	Thomas and Hinsdill 1979
C57BL/6J mouse, GD 0,7, and 14 and LD 2			0.04: ↔ 0.1: ↓ response to influenza virus	Vorderstrasse et al. 2006

↑ = increase; ↓ = decrease; ↔ = no change; BSA = bovine serum albumin; ConA = concanavalin A; GD = gestation day; LD = lactation day; LPS = lipopolysaccharide; PHA = phytohemagglutinin: sRBC = sheep red blood cell

Decreased thymus weights were observed in the offspring of rats and mice orally exposed to ≥1 µg/kg/day for acute durations (Faith and Moore 1977; Gehrs et al. 1997b; Luster et al. 1980; Mustafa et al. 2008) or 0.011 µg/kg/day for an intermediate duration (Sugita-Konishi et al. 2003). Thymic atrophy was found in pups of rats and mice exposed to acute doses ≥1.5 µg/kg/day (Blaylock et al. 1992; Fine et al. 1989; Håkansson et al. 1987; Holladay et al. 1991; Huuskonen et al. 1994) and at 0.325 µg/kg/day in the offspring of mice exposed for 4 weeks prior to mating and during gestation and lactation (Thomas and Hinsdill 1979). 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 µg/kg on GD 14 but not on GD 22 (Gehrs et al. 1997a). Similarly, transient thymus atrophy was observed in the neonates of BALB/cGa mice exposed to 10 µg/kg on GD 14 but was not observed on PND 18 (Fine et al. 1989).

Evaluation of 24-week-old offspring from C57BL/6 mice administered a single dose of 5 µg/kg 2,3,7,8-TCDD on GD 12 showed significant changes in thymic T cell differentiation, in T cell phenotypes in the spleen and lymph nodes, in the phenotype of mature B cells in the spleen, and in B lymphoid progenitors in bone marrow; the immune dysregulation often appeared to be gender-specific (Mustafa et al. 2008). This study also reported increased deposition of anti-IgG and anti-C3 immune complexes in the kidneys of both male and female offspring that, according to the investigators, were suggestive of early stages of autoimmune glomerulonephritis. Subsequent studies by the same group of investigators of a strain of mice that spontaneously develop an immune complex-mediated glomerulonephritis showed that gestational exposure to 2,3,7,8-TCDD exacerbated a type III hypersensitivity lupus-like autoimmune disease, which was more severe in males than in females (Mustafa et al. 2009).

Studies have also examined functional alterations in immune response in offspring from dams exposed perinatally to 2,3,7,8-TCDD. Suppressed responses to phytohemagglutinin-P and concanavalin A mitogens and a suppressed delayed hypersensitivity response to oxazolone were observed in the offspring of F344 rats exposed to 5 µg/kg on LDs 0, 7, and 14 or on GD 18 and LDs 0, 7, and 14; no alteration in antibody production in response to bovine gamma globulin was observed (Faith and Moore 1977). Gehrs et al. (1997b) also found a suppression of the delayed hypersensitivity response to bovine serum albumin in 5-month-old male offspring receiving *in utero* and lactational exposure.

An impaired response to sheep red blood cells was observed in the offspring of Swiss Webster mice exposed to 0.325 µg/kg/day for 4 weeks prior to mating and during gestation and lactation (Thomas and Hinsdill 1979). Exposure of C57BL/6NCji mice pups to 0.011 µg/kg/day 2,3,7,8-TCDD via lactation resulted in reduced clearance of *Listeria monocytogenes* from the spleen 2 days after infection (Sugita-

Konishi et al. 2003). Decreased survival in response to a *Streptococcus agalactiae* infection was observed in the pups of C57BL/6 mice administered 10 µg/kg 2,3,7,8-TCDD on GD 15.5 (Ding et al. 2018). Exposure of C57BL/6J mice on GDs 0, 1, and 14 and PND 2 to a time-weighted average (TWA) dose of 0.1 µg/kg/day suppressed the increase in total cellularity and significantly reduced the number of $CD8^+$ cytotoxic T lymphocytes recovered from the mediastinal lymph node from female pups in response to infection to influenza A virus; no significant alterations were observed in the male pups (Vorderstrasse et al. 2006). To rule out that the observed effects in functional immunity resulted from overt toxicity to the immune organs rather than altered responsiveness following infection, the investigators examined the percentage and number of specific immune cell populations in the bone marrow, thymus, and spleen in 2,3,7,8-TCDD-treated mice not exposed to virus. No changes were detected in total cellularity of these tissues or in the percentage or number of any cell subpopulation. In another study in C57BL/6 mice that used the same exposure protocol, exposure to a TWA dose of 0.17 µg/kg/day 2,3,7,8-TCDD (only dose tested) resulted in a 66% reduction in the number of virus-specific $CD8^+$ cells in the mediastinal lymph node 9 days after infection, relative to unexposed offspring (testing was conducted at the age of 6–12 weeks) (Hogaboam et al. 2008). Nine days after infection of dams, the number of $CD8^+$ cells in the mediastinal lymph node was equivalent to control dams. Antibodies in response to immunization with ovalbumin also were reduced in offspring exposed during development, but not in treated dams. These results suggested that AhR activation in adults does not cause long-lasting deregulation of the mature immune system; however, inappropriate activation of the AhR during ontogeny of the hematopoietic system results in long-lasting functional deregulation. Furthermore, results of cross-fostering experiments showed that $CD8^+$ production in response to viral infection was significantly reduced in all adult offspring groups except those exposed only during gestation. Increased neutrophils were found in pups of B6C3F1 mice exposed to 1 µg/kg/day 2,3,7,8-TCDD on GD 14 and LDs 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 or 3 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 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.

Impaired development and functional alterations of the nervous system. Numerous studies have reported neurological effects (morphological and neurobehavioral) in offspring following perinatal exposure to 2,3,7,8-TCDD. For example, Moran et al. (2004) reported morphological alterations in the neural tube of Cynomolgus monkeys following a maternal dose of 4 μg/kg 2,3,7,8-TCDD on GD 15. The investigators suggested that alterations in critical fatty acid mobilization during pregnancy, which were documented, may have played a role in the morphological effects observed. A lower dose of 0.7 μg/kg 2,3,7,8-TCDD (only dose tested) administered to pregnant rats on GD 18 resulted in delayed myelination in several areas in the pups' brain, some of which persisted until adulthood (Fernández et al. 2010). Treatment of Sprague-Dawley rats with 0.18 μg/kg 2,3,7,8-TCDD on GD 8 shifted hemispheric dominance from right to left in male pups examined on PND 90 (Hojo et al. 2006). The shift in hemispheric dominance was judged by changes in cell numbers and size distribution in the cerebral cortex. A much higher dose of 20 μg/kg 2,3,7,8-TCDD administered to pregnant C57BL/6N mice on GD 7 induced a significant reduction (15%) in the thickness of the somatosensory cortex (Mitsuhashi et al. 2010); the thickness of the deeper cortical layers was reduced by 24%, whereas no significant changes were seen in the superficial layers.

Delays in negative geotaxis and cliff avoidance reflexes were observed in the offspring of Sprague-Dawley rats administered 0.2 μg/kg/day on GDs 8–14 (Zhang et al. 2018b). Doses of up to 1 μg/kg 2,3,7,8-TCDD given on GD 15 to Wistar rats did not cause treatment related alterations in tests of learning ability or motor activity or in a functional observation battery conducted on postnatal weeks 12–13 (Bell et al. 2007a). The same group of investigators reported similar observations in the offspring of rats dosed with up to 0.008 μg/kg/day 2,3,7,8-TCDD via the diet for 12 weeks before mating and continued during mating and gestation (Bell et al. 2007b); at 0.046 μg/kg/day, a decrease in motor activity was observed. Dosing of Long-Evans rats with up to 0.8 μg/kg 2,3,7,8-TCDD on GD 15 did not affect spontaneous activity in male offspring on PNDs 100–110 (Kakeyama et al. 2003). Using benchmark methodology to estimate a POD, Markowski et al. (2001) calculated an ED_{10} of 0.007 μg/kg 2,3,7,8-TCDD with a 95% lower bound of 0.005 μg/kg for neurobehavioral alterations that suggested reduced responsiveness to environmental contingencies in offspring of Holtzman rats dosed once on GD 18. The same group reported that a maternal dose of 0.18 μg/kg 2,3,7,8-TCDD on GD 15 caused impaired performance on operant behavior tests in Holtzman rats (Markowski et al. 2002). The investigators noted that rather than a global learning deficit, this effect appeared to be more a function of an inability to inhibit or delay voluntary behavior. Hojo et al. (2002) calculated an ED_{10} of 0.003 μg/kg 2,3,7,8-TCDD with 95% lower bounds of 0.002 μg/kg 2,3,7,8-TCDD for alterations in two different schedule-controlled, food reinforced operant procedures in 80-day-old offspring from Sprague-Dawley

rats dosed on GD 8. Improved performance of 80-day-old offspring of Sprague-Dawley rats administered 0.1 µg/kg/day on GDs 10–16 was observed in a radial arm maze working memory task (Seo et al. 1999). The investigators suggested that the improvement in the spatial task was specific to the radial arm maze and might have been related to response patterning (Seo et al. 1999). This study also reported that 0.1 µg/kg 2,3,7,8-TCDD significantly impaired visual reversal learning in 80-day-old offspring. A follow-up study by this group (Seo et al. 2000) confirmed the finding of improvement performance on the radial arm maze test in rats exposed to 0.1 µg/kg/day on GDs 10–16; however, this improvement was not observed at 0.2 µg/kg/day. A subsequent study by this group found that alterations in spatial and visual reversal learning observed in Sprague-Dawley rats administered 0.1 µg/kg/day on GDs 10–16 was likely due to either attentional or associative processing effects (Widholm et al. 2003). Hojo et al. (2008) reported an increase in response rate on schedule-controlled operant behavior tests in female offspring of Long-Evans rats administered 0.2 µg/kg on GD 15; this effect was not observed at the next highest dose (0.8 µg/kg). The investigators suggested that the increased response rate was likely due to hyperactive behavior rather than enhanced learning performance. Sha et al. (2021) reported alterations in activity in an open field test suggestive of hyperactivity in the offspring of C57BL/6J mice administered 0.1 µg/kg/day 2,3,7,8-TCDD on GD 0.5, GD 12.5, and PND 7.5. Increased motor activity and decreased social activity were observed at 1 µg/kg in the offspring of Wistar rats administered 1 µg/kg on GD 15 (Nguyen et al. 2013a). Similarly, Kakeyama et al. (2007) reported anxiety-like behavior and inhibition of acquisition of paired-associative memory in male offspring of Long-Evans rats administered 0.2 µg/kg during pregnancy; however, these effects were not observed at 0.8 µg/kg. The results of a study in mice with different genotypes suggested that the effects of 2,3,7,8-TCDD on learning as well as on hippocampal morphology are mediated through the AhR since they were absent in *AhR*-knockout mice (Powers et al. 2005). Impaired acquisition and retention of fear memory were observed in the male offspring of C57BL/6J mice administered 3 µg/kg 2,3,7,8-TCDD on GD 12.5 and examined at 25–28 weeks of age (Haijima et al. 2010). In Wistar rats administered 1 µg/kg on GD 15, an impaired response in contextual fear conditioning tests was observed in male offspring, but not in female offspring (Mitsui et al. 2006). In the adult offspring of C57BL/6 mice administered 0.6 µg/kg 2,3,7,8-TCDD on GD 12.5, impaired attainment of rapid behavioral shifts in tests of behavioral flexibility, compulsive repetitive behavior, and low competitive dominance were observed (Endo et al. 2012); the latter two effects were not observed at 3 µg/kg. Delayed habituation and reduced exploration of novel objects were observed in the offspring of C57BL/6 mice administered 0.25 µg/kg/day on GD 7, GD 14, and PND 2 (Sobolewski et al. 2014). Delayed avoidance learning and reduced motor activity were reported in Wistar rat pups following maternal dosing with 0.1 µg/kg/day 2,3,7,8-TCDD on GDs 9–19 (Nishijo et al. 2007). A different type of study examined the effect of gestational exposure to 2,3,7,8-TCDD on sensory cortex

function in rats (Hood et al. 2006). In 45-day-old offspring of Long-Evans rats dosed with 0.7 µg/kg 2,3,7,8-TCDD on GD 15, the mean spontaneous electrical activity of cells assayed in the primary sensory cortex was reduced approximately 50% relative to controls, even after ≥60 days of postnatal recovery. Responses evoked by sensory stimulation were also reduced by 50% at every level of stimulus intensity compared with controls. The reduction in activity was associated with decrements in specific glutamate receptor subunits.

The neurodevelopmental toxicity of 2,3,7,8-TCDD was evaluated in a multi-breeding study in monkeys reported in several papers (Bowman et al. 1989a, 1989b; Schantz and Bowman 1989; Schantz et al. 1986, 1992). The study evaluated three cohorts of offspring of mothers exposed to 2,3,7,8-TCDD in the diet and mated to unexposed males. Cohort I consisted of offspring of mothers mated after 7 months of exposure with an average of 16.2 months of exposure prior to birth; cohort II consisted of offspring of mothers mated after 27 months of exposure with an average of 36 months of exposure prior to birth; and cohort III consisted of infants of mothers exposed for 3.5–4 years and mated 10 months post-exposure and born 18 months post-exposure. Alterations in peer-group behavior (Bowman et al. 1989b; Schantz et al. 1992) and cognitive deficits (Bowman et al. 1989a; Schantz and Bowman 1989) were observed in the cohort I offspring of monkeys exposed to 0.00012 µg/kg/day. 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; cognitive function was also altered in the cohort II monkeys (Schantz and Bowman 1989). In cohort III, there was increased and prolonged maternal care (increased time in mutual ventral contact and nipple contact) at 0.000012 and 0.00064 µg/kg/day (Schantz et al. 1986) and altered social behavior (rough tumble play) was observed at 0.00064 µg/kg/day (Schantz et al. 1992).

Ototoxicity was observed in the offspring of C57Bl/6 mice administered 0.5 µg/kg 2,3,7,8-TCDD on GD 12 (Safe and Luebke 2016). A 5–20 dB shift in auditory brainstem response was observed at frequencies of 11.3–30 kHz. There was no alteration in distortion-product otoacoustic emissions at the same frequencies suggesting a mild auditory neuropathy. No structural abnormalities in the cochlea. In contrast, no signs of ototoxicity were observed in similarly exposed offspring of CBA or Balb/C mice (Safe and Luebke 2016).

Impaired development and functional alterations of the reproductive system. A large 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 and/or lactation.

Studies examining outcomes indicative of impaired development of the reproductive system in the offspring of pregnant animals administered 2,3,7,8-TCDD are summarized in Table 2-27. Gestational (and lactational) exposure resulted in impaired development of the prostate and vagina in male and female offspring. Alterations in the formation of prostatic epithelial buds have been observed in mice at 5 µg/kg (Abbott et al. 2003; Allgeier et al. 2009; Ko et al. 2002). Other alterations in male reproductive tissue include degenerative changes in the testes, decreases in seminiferous tubular diameter and epithelial thickness, and an increase in the percentage of abnormal seminiferous tubules in rats at 0.5 µg/kg (Mai et al. 2020); altered development of the seminal vesicles was also observed at 1 µg/kg (Hamm et al. 2000).

Decreases in testis, prostate, seminal vesicle, and cauda epididymis weights have also been observed in male offspring at ≥0.064 µg/kg (Bjerke and Peterson 1994; Gray et al. 1995; Jin et al. 2010; Lin et al. 2002b; Mably et al. 1992a, 1992c; Ohsako et al. 2002). A cross-fostering study in mice showed that the decrease in relative ventral prostate weight was greater in offspring exposed *in utero* or *in utero* and during lactation than in offspring exposed during lactation only (Lin et al. 2002b). The study also found a greater decrease in ventral prostate weight in offspring exposed on GD 13 compared to those exposed on GD 16.

In female offspring, maternal exposure to 1 µg/kg 2,3,7,8-TCDD on GD 15 resulted in altered vaginal morphogenesis as early as GD 18 (Dienhart et al. 2000; Hurst et al. 2002); observed effects included an increase in the thickness of mesenchymal tissue between the caudal Mullerian ducts, which resulted in a failure of the Mullerian ducts to fuse (a process normally completed before birth) and impaired the regression of the Wolffian ducts by increasing the size of the interductal mesenchyme and by preventing fusion of the Mullerian ducts. Changes in the spatial and temporal expression of growth factors in response to 2,3,7,8-TCDD appeared to be implicated in the pathogenesis of the vaginal thread (Hurst et al. 2002). A vaginal thread has been observed at doses ≥0.2 µg/kg (Flaws et al. 1997; Gray and Ostby 1995; Gray et al. 1997a), but not at 0.05 µg/kg (Gray et al. 1997a). Partial clefting of the phallus was also observed in the female offspring at the same dose levels (Flaws et al. 1997; Gray and Ostby 1995; Gray et al. 1997a). Impaired development of mammary glands, specifically impairment of mammary gland differentiation, was observed in female offspring of dams exposed to 0.5 or 1 µg/kg/day (Brown et al. 1998; Fenton et al. 2002; Filgo et al. 2016; Lewis et al. 2001).

Table 2-27. Impaired Development of Reproductive System in Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect					
				Puberty		
Species, exposure	Morphological alterations	Organ weights	Anogenital distance (males)	Males	Females	Reference
C57BL/6J mouse, GD 12	5: impaired prostatic bud development					Abbott et al. 2003
C57BL/6J mouse, GD 13.5	5: impaired prostate budding					Allgeier et al. 2009
Han Wistar rat, GD 15				0.2: ↔ 1: ↓		Bell et al. 2007a
Holtzman rat, GD 15			0.7: ↔	0.7: ↓		Bjerke et al. 1994a
Holtzman rat, GD 15		1: ↓, prostate, seminal vesicle, testis, cauda epididymis		1: ↓		Bjerke and Peterson 1994
Sprague-Dawley rat, GD 15	1: impaired mammary gland differentiation					Brown et al. 1998
Holtzman rat, GD 15	1: altered vaginal morphogenesis					Dienhart et al. 2000
Long-Evans rat, GD 15	1: Delayed development of mammary gland					Fenton et al. 2002
Sprague-Dawley rat, GDs 15 and 18	0.5: Delayed development of mammary gland					Filgo et al. 2016
Holtzman rat, GD 11, 15, or 18	1: ↑ vaginal phallus clefting and vaginal thread					Flaws et al. 1997
Holtzman rat, GD 15	1: ↑ vaginal phallus clefting and vaginal thread				1: ↔	Gray and Ostby 1995
Long-Evans rat, GD 15	1: ↑ vaginal phallus clefting and vaginal thread				1: ↔	Gray and Ostby 1995
Long-Evans rat, GD 8	1: ↑ vaginal phallus clefting 1: ↔ vaginal thread				1: ↓	Gray and Ostby 1995

Table 2-27. Impaired Development of Reproductive System in Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect					
				Puberty		
Species, exposure	Morphological alterations	Organ weights	Anogenital distance (males)	Males	Females	Reference
Holtzman rat, GD 8 or 15		1: ↓, testis, cauda epididymis				Gray et al. 1995
Long-Evans rat, GD 8 or 15				1: ↓		Gray et al. 1995
Golden Syrian hamster, GD 11		1: ↓, testis, cauda epididymis		2: ↓		Gray et al. 1995
Long-Evans rat, GD 15	0.05: ↔ 0.2: ↑ vaginal phallus clefting and vaginal thread					Gray et al. 1997a
Long-Evans rat, GD 15				0.05: ↔ 0.2: ↓		Gray et al. 1997b
Long-Evans rat, GD 15	1: altered development of seminal vesicles					Hamm et al. 2000
Long-Evans rat, GD 15	1: altered vaginal morphogenesis					Hurst et al. 2002
C57Bl/6 mouse, PNDs 1–4		1: ↓, testis, cauda epididymis	1: ↓			Jin et al. 2010
Long-Evans rat, GD 15					0.2: ↔ 0.8: ↑	Kakeyama et al. 2008
C57BL/6J mouse, GD 13	5: inhibition of prostate lobe branching					Ko et al. 2002
Holtzman rat, GD 15	1: impaired mammary gland differentiation					Lewis et al. 2001
C57BL/6J mouse, GD 13	5: inhibition of prostate development					Lin et al. 2002a
C57BL/6J mouse, GD 13 or 16		5: ↓, prostate and seminal vesicle				Lin et al. 2002b

Table 2-27. Impaired Development of Reproductive System in Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect					
				Puberty		
Species, exposure	Morphological alterations	Organ weights	Anogenital distance (males)	Males	Females	Reference
Holtzman rat, GD 15		0.064: ↓, prostate 0.16: ↓, seminal vesicle	0.064: ↔ 0.16: ↓	0.064: ↔ 0.16: ↓		Mably et al. 1992a
Holtzman rat, GD 15		0.064: ↓, testis, cauda epididymis				Mably et al. 1992c
Wistar rat, GD 15	0.5: alterations in testes and seminiferous tubules					Mai et al. 2020
Holtzman rat, GD 15			0.0125: ↔ 0.05: ↓			Ohsako et al. 2001
Sprague-Dawley rat, GD 15		1: ↓, testis, prostate, epididymis	1: ↓			Ohsako et al. 2002
Sprague-Dawley rat, GD 18			1: ↓			Ohsako et al. 2002
Line C rat, GD 15			0.3: ↔ 1: ↓			Simanainen et al. 2004b
Long-Evans rat, GD 15			0.8: ↔	0.05: ↔ 0.2: ↓	0.2: ↔ 0.8: ↓	Yonemoto et al. 2005
Sprague-Dawley rat, GDs 8–14					1: ↑ (F3)	Yu et al. 2019
Wistar, 12 weeks premating and during gestation and lactation				0.0024: ↓		Bell et al. 2007b

↑ = increase; ↓ = decrease; ↔ = no change; GD = gestation day; PND = postnatal day

Other effects associated with impaired development of the reproductive system include alterations in anogenital distance and onset of puberty. Decreases in anogenital distance has been observed in male rat offspring at 1 µg/kg (Jin et al. 2010; Ohsako et al. 2002; Simanainen et al. 2004b); lower LOAEL values have been reported (Mably et al. 1992a; Ohsako et al. 2001) but these overlap with NOAEL values from other studies (Bjerke et al. 1994a; Simanainen et al. 2004b; Yonemoto et al. 2005). Delays in puberty, as measured by testis descent or date of preputial separation, were observed in rats at doses as low as 0.2 µg/kg (Bell et al. 2007a; Bjerke et al. 1994a; Gray et al. 1995, 1997a; Yonemoto et al. 2005) following acute-duration exposure and 0.0024 µg/kg/day following intermediate-duration exposure (Bell et al. 2007b) and in hamsters at 2 µg/kg (Gray et al. 1995). In one study, a delay in the onset of puberty, as measured by date of vaginal opening, was observed in the female offspring of rats exposed to 1 µg/kg on GD 8, but not in the offspring of rats exposed on GD 15 (Gray and Ostby 1995). Yonemoto et al. (2005) found delays at 0.8 µg/kg in the offspring of dams exposed on GD 15. In contrast, Kakeyama et al. (2008) reported shortened time to vaginal opening at 0.8 µg/kg; this effect was also observed in the F3 generation at 0.5 µg/kg (Yu et al. 2019).

Examination of the offspring after sexual maturity has revealed functional alterations in males and females; summarized in Tables 2-28 and 2-29, respectively. Decreased daily sperm production has been inconsistently observed in several studies. Decreases have been observed in offspring of rats administered ≥0.064 µg/kg 2,3,7,8-TCDD on GD 15 (Mably et al. 1992c; Simmanainen et al. 2004b; Sommer et al. 1996) but not in other studies testing doses as high as 1 µg/kg (Gray et al. 1995; Ohsako et al. 2001, 2002; Rebourcet et al. 2010; Yonemoto et al. 2005). Decreases in cauda epididymal sperm counts have been consistently found at doses ≥0.8 µg/kg (Bruner-Tran et al. 2014; Gray et al. 1995; Jin et al. 2010; Mai et al. 2020; Ohsako et al. 2002; Simanainen et al. 2004b). Studies examining sexual behavior in male offspring have reported demasculinization and feminization. Demasculinized sexual behavior, as measured by decreases number and/or increases in latency of mounts and intromission (in the Ikeda et al. [2005a] study, demasculinization was assessed by measuring brain aromatase levels), was observed at ≥0.2 µg/kg (Bjerke et al. 1994b; Ikeda et al. 2005a; Kakeyama et al. 2003; Mably et al. 1992b; Taura et al. 2014). Feminized sexual behavior, as measured by frequency and intensity of lordotic behavior, was also observed in male offspring castrated and primed with ovarian steroid at maternal doses ≥0.16 µg/kg (Bjerke and Peterson 1994; Bjerke et al. 1994b; Mably et al. 1992b). A small number of studies evaluated male fertility. In males mated with unexposed females an increase in preterm births and decrease in gestation length was observed at 10 µg/kg (Ding et al. 2011), an increase in pre- and post-implantation losses with no effect on mating or fertility indices was observed at ≥0.5 µg/kg (Mai et al.

Table 2-28. Functional Alterations in the Reproductive System of Male Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect					
Species, exposure	Daily sperm production	Cauda epididymal sperm count	Demasculinization	Feminization	Fertility	Reference
Holtzman rat, GD 15				1: ↑		Bjerke and Peterson 1994
Holtzman rats, GD 15			0.7: ↑	0.7: ↑		Bjerke et al. 1994b
C57BL/6 mouse, GD 15.5		10: ↓				Bruner-Tran et al. 2014
C57BL/6 mouse, GD 15.5					10: ↑, preterm births and ↓ gestation length	Ding et al. 2011
Holtzman rat, GD 8 or 15					1: ↓	Gray et al. 1995
Long-Evans rat, GD 15		1: ↓				Gray et al. 1995
Golden Syrian hamster, GD 11		2: ↓				Gray et al. 1995
Long-Evans rat, GD 15	0.05: ↓ ejaculated sperm count					Gray et al. 1997a
Long-Evans rat, GD 15	0.8: ↔	0.8: ↓				Gray et al. 1997b
Holtzman rat, GD 15			0.2: ↑			Ikeda et al. 2005a
C57BL/6 mouse, PNDs 1–4		1: ↓				Jin et al. 2010

Table 2-28. Functional Alterations in the Reproductive System of Male Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect					
Species, exposure	Daily sperm production	Cauda epididymal sperm count	Demasculinization	Feminization	Fertility	Reference
Long-Evans rat, GD 15			0.8: ↑			Kakeyama et al. 2003
Holtzman rats, GD 15			1: ↑	0.16: ↑		Mably et al. 1992b
Holtzman rat, GD 15	0. ↓					Mably et al. 1992c
Wistar rat, GD 15		0.5: ↔ 1: ↓			2: ↔ 0.5: ↑, pre- and post-implantation losses	Mai et al. 2020
Holtzman rat, GD 15	0.8: ↔					Ohsako et al. 2001
Sprague-Dawley rat, GD 15	1: ↔	1: ↓				Ohsako et al. 2002
Sprague-Dawley rat, GD 18		1: ↔				Ohsako et al. 2002
Sprague-Dawley rat, GD 15	0.2: ↔				0.2: ↔	Rebourcet et al. 2010
Line C rat, GD 15	0.3: ↔ 1: ↓	1: ↓				Simanainen et al. 2004b
Holtzman rat, GD 15	1: ↓	1: ↓				Sommer et al. 1996
Wistar rat, GD 15			1: ↑			Taura et al. 2014

Table 2-28. Functional Alterations in the Reproductive System of Male Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect					
Species, exposure	Daily sperm production	Cauda epididymal sperm count	Demasculinization	Feminization	Fertility	Reference
Long-Evans rat, GD 15	0.8: ↔	0.8: ↔				Yonemoto et al. 2005

↑ = increase; ↓ = decrease; ↔ = no change; GD = gestation day; PND = postnatal day

Table 2-29. Functional Alterations in the Reproductive System of Female Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect				
Species, exposure	Ovarian follicles	Estrous	Fertility	Other effects	Reference
C57BL/6 mouse, GD 15.5			10: ↓, F1, F2, and F3		Bruner-Tran and Osteen 2010
Sprague-Dawley rat, GD 8				1: ↔ endometriotic lesion diameter	Cummings et al. 1999
C57BL/6 mouse, GD 8				3: ↔ endometriotic lesion diameter	Cummings et al. 1999
C57BL/6 mouse, GD 8, PNDs 77, 98, 119, 140, and 161				3: ↑ endometriotic lesion diameter	Cummings et al. 1999
C57BL/6 mouse, GD15.5			10: ↓	10: ↑, preterm births and ↓ gestation length	Ding et al. 2011
Holtzman rat, GD 11, 15, or 18	1: ↔				Flaws et al. 1997
Sprague-Dawley rat, PND 29		10: ↑, premature onset of abnormal or absent cyclicity			Franczak et al. 2006

Table 2-29. Functional Alterations in the Reproductive System of Female Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	Dose (µg/kg/day): effect				
Species, exposure	Ovarian follicles	Estrous	Fertility	Other effects	Reference
Long-Evans rat, GD 8		1: ↑, constant estrus	1: ↓		Gray and Ostby 1995
Long-Evans rat, GD 15		1: ↔, constant estrus	1: ↓, litter 5		Gray and Ostby 1995
Long-Evans rat, GD 15			0.8: ↔ 0.8: ↓, time to pregnancy		Gray et al. 1997a
Holtzman rat, GD 15	1: ↓, antral and preantral follicles				Heimler et al. 1998
Long-Evans rat, GD 15		0.2: ↔, first day of estrus 0.8: ↑, first day of estrus 0.8: ↔, estrus cyclicity			Kakeyama et al. 2008
Sprague-Dawley rat, GD 15		1: ↓, days in estrous			Salisbury and Marcinkiewicz 2002
Syrian hamster, GD 11.5			2: ↓		Wolf et al. 1999
Long-Evans rat, GD 15		0.8: ↔, estrus cyclicity			Yonemoto et al. 2005
Sprague-Dawley rat, GDs 8–14	0.1: ↓, primordial follicles 0.1: ↑, primary and secondary follicles				Yu et al. 2020
Sprague-Dawley rat, GDs 8–14	0.1: ↓, primordial follicles 0.1: ↑, primary and secondary follicles				Zhang et al. 2018b
Lewis-Furth rat, GDs 14 and 21, PNDs 7 and 14, and PNDs 21–24		0.007: ↑, onset of acyclicity			Jablonska et al. 2010

↑ = increase; ↓ = decrease; ↔ = no change; GD = gestation day; PND = postnatal day

2020), a decrease in the number of implants was observed at 1 µg/kg (Gray et al. 1995), and no effect on pregnancy rate was observed in rats at 0.2 µg/kg (Rebourcet et al. 2010).

A smaller number of studies have examined functional alterations in female offspring (summarized in Table 2-29). A study by Yu et al. (2020) in the offspring of Sprague-Dawley rats administered 0.1 µg/kg on GDs 8–14 found decreased primordial follicles and increased primary and secondary follicles and Heimler et al. (1998) reported decreased antral and preantral follicles in the offspring of Holtzman rats administered 1 µg/kg on GD 15. In contrast, Flaws et al. (1997) found no alterations in primordial follicles in the offspring of Holtzman rats administered 1 µg/kg on GD 11, 15, or 18. Studies examining the estrus cycle have reported a delay in the first day of estrus at 0.8 µg/kg (Kakeyama et al. 2008), no effect on estrus cyclicity at 0.8 µg/kg (Kakeyama et al. 2008; Yonemoto et al. 2005), a decrease in days in estrous at 1 µg/kg (Salisbury and Marcinkiewicz 2002), and premature onset of abnormal or absent cyclicity at 0.007 or 10 µg/kg/day (Franczak et al. 2006; Jablonska et al. 2010). Exposure to 1 or 3 µg/kg on GD 8 followed by surgically induced endometriosis on PND 98, did not result in an increase in endometriotic lesions in Sprague-Dawley rats or C57BL/6 mice, respectively (Cummings et al. 1999). However, an increase in lesions were observed in mice prenatally exposed to 2,3,7,8-TCDD and receiving a 3 or 10 µg/kg dose of 2,3,7,8-TCDD on PNDs 77, 98, 119, 140, and 161. Several studies have reported decreases in transgenerational fertility in female rats, mice, and hamsters. Decreases in fertility were observed in the female offspring exposed to ≥1 µg/kg 2,3,7,8-TCDD *in utero* and mated to unexposed males (Bruner-Tran and Osteen 2011; Ding et al. 2011; Gray and Ostby 1995; Wolf et al. 1999); no alteration in fertility was observed in rats exposed to 0.8 µg/kg (Gray et al. 1997a). Bruner-Tran and Osteen (2011) also demonstrated decreased pregnancy rates in the F2 and F3 generations (only the P0 generation was administered 10 µg/kg 2,3,7,8-TCDD). Increased preterm births and decreased gestation length were observed in the offspring of mice exposed *in utero* to 10 µg/kg (Ding et al. 2011). Increased preterm births were also observed in another study of mice (Bruner-Tran and Osteen 2011); however, this was only observed in a colony contaminated with mouse parvovirus and in mice exposed to 2,3,7,8-TCDD and injected with lipopolysaccharide. The results suggest that TCDD-induced increased sensitivity to inflammation negatively impacted gestation length as the mouse parvovirus or lipopolysaccharide did not affect the rate of preterm births in controls.

Several studies have evaluated reproductive hormone levels in male offspring of rats orally administered 2,3,7,8-TCDD during pregnancy; the results of these studies are summarized in Table 2-30. Decreased plasma testosterone levels were observed at 1 µg/kg in the fetuses of Hans/Wistar and Long Evans rats (Haavisto et al. 2001) and 90-day-old rats (Sanabria et al. 2016), but were not observed in the male

Table 2-30. Alterations in Reproductive Hormones in the Male Offspring of Laboratory Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, exposure	Age	Plasma testosterone	Testicular testosterone	Plasma luteinizing hormone	Pituitary luteinizing hormone	Follicle stimulating hormone	Reference
Sprague-Dawley rat, GD 11	19-day fetuses		0.3: ↓				Adamsson et al. 2008
Han/Wistar rat, GD 13.5	19.5-day fetuses	0.1: ↔ 0.5: ↓	1: ↔		0.5: ↔ 1: ↓		Haavisto et al. 2001
Sprague-Dawley rat, GD 13	PND 14		1: ↔	1: ↔		1: ↔	Haavisto et al. 2006
Holtzman rat, GD 15	17-, 18-, 19-, 20-, or 21-day fetuses	1: ↓					Mably et al. 1992a
Holtzman rat, GD 15	PND 32					1: ↓	Mably et al. 1992c
Holtzman rat, GD 15	PND 42, 63, or 120					1: ↔	Mably et al. 1992c
Sprague-Dawley rat, GD 15	PND 28, 40, 67, or 145		0.2: ↔				Rebourcet et al. 2010
Wistar rat, GD 15	PND 90	1: ↓		1: ↔		1: ↔	Sanabria et al. 2016

↓ = decrease; ↔ = no change; GD = gestation day; PND = postnatal day

offspring of Holtzman rats exposed to 1 µg/kg/day (Mably et al. 1992a). Three of four studies examining testicular testosterone levels did not find significant alterations at ≤1 µg/kg (Haavisto et al. 2001, 2006; Rebourcet et al. 2010). Haavisto et al. (2006) and Sanabria et al. (2016) did not find alterations in plasma LH or FSH levels. Mably et al. (1992c) found decreases in FSH levels in rats on PND 32, but not on PND 42, 63, or 120. In the female offspring of Holtzman rats administered 1 µg/kg 2,3,7,8-TCDD on GD 15, a decrease in serum estrogen levels was observed (Chaffin et al. 1996) and no alterations in serum FSH, LH, or progesterone levels were observed (Chaffin et al. 1997).

Other CDD Congeners—Animal Studies. Other CDD congeners have also been found to induce developmental toxicity. Khera and Ruddick (1973) reported edematous separation of the cardiac myofibrils in rat offspring exposed *in utero* to 2,000 µg/kg/day 2,7-DCDD (Khera and Ruddick 1973); however, the study did not include statistical analysis. 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-PeCDD (Madsen and Larsen 1989). Subcutaneous edema was found in the offspring of Sprague-Dawley rats exposed to 1 µg/kg/day of mixed HxCDD isomers during GDs 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 GDs 6–15; however, the incidence was not significant when evaluated on a litter basis (Schwetz et al. 1973). No developmental effects were observed in mice exposed to 20 µg/kg/day of OCDD during GDs 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 GDs 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 GDs 6–15 (Khera and Ruddick 1973).

Developmental Mechanisms. Many of the health effects of CDDs share a common initiating event in AhR binding. Section 2.21, Mechanisms of Toxicity, provides a discussion of this initiating event and its physiological sequelae. In this subsection, an overview of the mechanisms involved in developmental effects is provided. Detailed mechanistic explanations are beyond the scope of this profile.

Hydronephrosis. Hydronephrosis has been induced by 2,3,7,8-TCDD in both rats and mice exposed in utero or during the neonatal period (Yoshioka and Tohyama 2019). While the AhR is necessary for both fetal and neonatal 2,3,7,8-TCDD-induced hydronephrosis, two distinct mechanisms have been elucidated, differing on the developmental stage of exposure (fetal or neonatal) (Yoshioka and Tohyama 2019). Fetal hydronephrosis is obstructive: a direct hyperplastic action of 2,3,7,8-TCDD on the uretic epithelium results in occlusion of the ureter (Abbott et al. 1987a) leading to accumulation of urine, expansion of the ureter and pyelocaliceal space of the kidney, and destruction of the renal parenchyma (Yoshioka and Tohyama 2019). In contrast, anatomical obstruction has not been observed in neonatal hydronephrosis, which has been shown to be associated with increased urine production (Yoshioka and Tohyama 2019).

In addition to AhR, neonatal hydronephrosis in 2,3,7,8-TCDD exposed animals also requires cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1), both of which are upregulated in the kidneys of exposed animals. Inhibition of COX-2 and genetic ablation of mPGES-1 can each block the development of neonatal hydronephrosis in 2,3,7,8-TCDD exposed animals (Yoshioka and Tohyama 2019). The increased expression of COX-2 and mPGES-1, which are key enzymes in the production of prostaglandin E2 (PGE2), leads to increased excretion of PGE2. Yoshioka and Tohyama (2019) suggested that higher levels of PGE2 in the renal tubules could interfere with water reabsorption, resulting in an increase in urine volume and backpressure on the renal pelvicalyceal space. In adult mice exposed to 2,3,7,8-TCDD, no increase in COX-2 or mPGES-1 expression was seen, urine volume was not affected, and hydronephrosis did not occur (Yoshioka and Tohyama 2019).

Both rats and mice are susceptible to hydronephrosis, and the AhR is necessary in both species. However, while AhR-null mice do not develop 2,3,7,8-TCDD-induced hydronephrosis, the AhR is required for normal development of the rat urinary tract, and AhR-null rats develop abnormalities in the absence of 2,3,7,8-TCDD (Yoshioka and Tohyama 2019).

Cleft palate. AhR binding is also necessary for 2,3,7,8-TCDD-induced cleft palate. Cleft palate was observed in nearly all wild-type ($AhR^{+/+}$) fetuses, in 24–28% of heterozygous AhR mutant genotype ($AhR^{+/-}$) fetuses, and not in any AhR-null fetuses after dosing of maternal mice with 40 µg/kg 2,3,7,8-TCDD on GD 12.5 (Mimura et al. 1997).

Jacobs et al. (2011) showed that, in addition to AhR, all-trans-retinoic acid (atRA) signaling was necessary for the development of cleft palate after 2,3,7,8-TCDD exposure in mice, and that the atRA signaling controlled AhR expression in the nasal mesenchyme. In mice bearing null mutations for

enzymes that synthesize atRA or for retinoic acid receptor G (RARG), gestational exposure to 2,3,7,8-TCDD (30 µg/kg on GD 10.5) did not result in cleft palates in the offspring. Further, in mice lacking the RALDH3 enzyme (the only retinoic acid synthesizing enzyme in the nasopalatal region during the critical developmental period, and transduced by RARG), Ahr mRNA levels were significantly decreased relative to wild-type mice (Jacobs et al. 2011).

Several mechanisms have been proposed to explain the development of cleft palate in animals exposed to 2,3,7,8-TCDD, including: (1) 2,3,7,8-TCDD may induce palatal split after fusion of the palatine processes; (2) 2,3,7,8-TCDD may inhibit the development of the palatine processes so that they do not make contact; or (3) 2,3,7,8-TCDD may inhibit palatal fusion by impairing the apoptosis of epithelial cells and mesenchymal tissues in the medial epithelium seam. Post-fusion split was demonstrated by Yamada et al. (2014), who examined the palatal forms of E14–E18 mouse fetuses after 2,3,7,8-TCDD exposure (40 µg/kg on E12.5). Yamada et al. (2014) observed palatal fusion in 3–18% of fetuses between days E14 and E16, but by E18, all of the palates were separated, suggesting that, in some instances, the split occurred after fusions.

Other studies have shown that 2,3,7,8-TCDD can alter the proliferation, migration, and apoptosis of epithelial and mesenchymal cells involved in palate development. Immunohistochemistry showed that 2,3,7,8-TCDD exposure decreased cell proliferation (bromodeoxyuridine [BrdU]-positive cells) in fetal palatal mesenchyme when pregnant mice were given 64 µg/kg by gavage on GD 10 and sacrificed on GD 13, 14, or 15 (Tao et al. 2020). 2,3,7,8-TCDD also altered apoptosis (terminal deoxynucleotidyl transferase dUTP nick end labeling [TUNEL]-positive cells) in the palatal mesenchyme, but the effect differed by GD. Decreased apoptosis was observed at sacrifice on GD 13, while on GD 15, apoptosis was increased by 2,3,7,8-TCDD exposure, and no difference from control was observed on GD 14 (Tao et al. 2020). In an *in vitro* study, Chen et al. (2020) compared the effects of 2,3,7,8-TCDD on primary epithelial and mesenchymal cells from GD 14 mouse embryo palatal tissue. At a lower exposure level (10 nmol/L), 2,3,7,8-TCDD increased cell proliferation and migration in mesenchymal cells, while decreasing epithelial cell proliferation with no effect on motility (Chen et al. 2020). At a higher exposure level (100 nmol/L), 2,3,7,8-TCDD exposure resulted in decreased proliferation of both cell types, decreased motility of mesenchymal cells, and increased apoptosis of mesenchymal cells (with no effect on epithelial cell motility or apoptosis). The study authors proposed that the mechanism for cleft palate formation by 2,3,7,8-TCDD may differ with dose, consistent with the observed dose-dependence seen *in vitro*.

Male reproductive tract development. Johnson et al. (2020) outlined a proposed adverse outcome pathway for effects of 2,3,7,8-TCDD on the developing male reproductive tract via influence on the pituitary. In this proposed scheme, absorption of 2,3,7,8-TCDD into the fetal pituitary and testis leads to binding and activation of the AhR, which triggers alterations in intracellular signaling pathways in the pituitary that result in reductions in the secretion of LH and FSH. Decreased LH secretion reduces the expression of steroidogenic genes and subsequently the production of androgens in Leydig cells. In the testes, AhR is proposed to downregulate the expression of cholesterologenic genes in Leydig cells, which also reduces the production of androgens. Coupled with a decrease in FSH secretion, the diminished production of androgens leads to impaired proliferation of Sertoli cells, which are necessary for spermatogenesis: the result is decreased sperm production (Johnson et al. 2020).

Transcriptomic studies in male rats exposed to 2,3,7,8-TCDD *in utero* have shown effects of exposure on the expression of pituitary hormone genes. Takeda et al. (2014) observed decreases in LH subunit β [*Lhb*] mRNA in rat offspring exposed *in utero* to 1 μg/kg 2,3,7,8-TCDD on GD 15 and removed on GD 20, GD 21, or PND 0. Johnson et al. (2020) reported decreased pituitary expression of Fshb, but not Lhb in GD 20 male fetuses after *in utero* exposure to 6 or 10 μg/kg on GDs 8–20 or 10 μg/kg on GD 15. Testicular expression of inhibin subunit alpha (*Inha*), a glycoprotein that suppresses FSH secretion, was also decreased at the same doses (Johnson et al. 2020).

2,3,7,8-TCDD exposure during gestation leads to feminization of sexual behavior in male offspring. Mably et al. (1992b) suggested that the demasculinization/feminization of sexual behavior might result from impaired sexual differentiation of the central nervous system, which is dependent on the presence of androgens during early development. However, Bjerke et al. (1994b) observed no effects of 2,3,7,8-TCDD exposure on the volume of the sexually dimorphic nucleus in the preoptic area of the hypothalamus or on the sexual differentiation of ER concentrations in brain nuclei, which exhibit sexual dimorphism, suggesting 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. More recently, Del Pino Sans et al. (2016) showed that exposure of male pups to 2,3,7,8-TCDD via lactation (maternal exposure on PND 1) resulted in a significant increase in the number of gamma-aminobutyric acid (GABA)/glutamate neurons in the anteroventral periventricular nucleus of the brain (compared to untreated male pups). During normal development of male pups, these estradiol-sensitive dual-phenotype neurons are lost, preventing them from responding to estradiol signals to induce the female LH surge release pattern. Exposure to 2,3,7,8-TCDD via lactation prevented the loss of these neurons in male pups and resulted in GABA/glutamate neuron content in the anteroventral periventricular nucleus (AVPV)

more similar to female pups. Del Pino Sans et al. (2016) also observed that 2,3,7,8-TCDD exposure downregulated the expression of *cugpbp2* (CUG triplet repeat, ribonucleic acid [RNA] binding protein 2). This gene encodes a protein that is proapoptotic, may be involved in signaling sexual differentiation of neural structures, and is usually upregulated in the AVPV of males.

Takeda et al. (2014) demonstrated that direct injection of equine chorionic gonadotropin (eCG, a hormone that mimics LH) into rat fetuses reversed the inhibition of masculine sexual behavior induced by 2,3,7,8-TCDD. When evaluated at sexual maturity, male rats exposed *in utero* to 2,3,7,8-TCDD exhibited reduced mount frequency and prolonged latency to mount, while those receiving eCG 2 days after maternal 2,3,7,8-TCDD exposure on GD 15 exhibited behavior similar to controls (not treated with 2,3,7,8-TCDD) (Takeda et al. 2014). This finding suggests that the effects of 2,3,7,8-TCDD on sexual behavior may stem from reductions in LH or gonadotropin-releasing hormone (GnRH), which stimulates the production of LH.

These studies also showed that 2,3,7,8-TCDD exposure alters the testicular expression of several genes important to steroidogenesis (including steroidogenic acute regulatory protein [*Star*], scavenger receptor class B member 1 [*Scarb1*], *Cyp17a1*, and *Cyp11a1*). Administration of 1 µg/kg 2,3,7,8-TCDD on GD 15 to pregnant Wistar rats resulted in decreased mRNA levels of *Star* and *CYP17* in the testes of offspring removed between GD 19 and PND 2 (Takeda et al. 2014). Similarly, repeated exposures of maternal rats to doses of 6 or 10 µg/kg on GDs 8–20 resulted in decreased mRNA levels of *Star*, *Cyp17a1*, *Cyp11a1*, and *Scarb1* mRNA in fetal testes collected on GD 20 (Johnson et al. 2020). A single 10 µg/kg dose on GD 15 resulted in decreases in *Star* and *Scarb1* expression, but not *Cyp17a1* or *Cyp11a1* (Johnson et al. 2020). In contrast to these results, steroidogenesis measured by testosterone production and expression of *Star* was not impacted by 2,3,7,8-TCDD exposure in cultures of primary mouse Leydig cells (Naville et al. 2011).

Abnormal prostate development (smaller dorsolateral and anterior prostate, fewer main ducts and ductal tips, and agenesis or smaller size of ventral prostate) has been demonstrated in mice exposed to 2,3,7,8-TCDD *in utero* or during lactation (Yoshioka and Tohyama 2019). Like other effects of 2,3,7,8-TCDD, AhR expression is necessary for effects on the developing prostate; AhR-null mice exhibit resistance to prostate abnormalities. The role of AhR in 2,3,7,8-TCDD-induced prostate effects is not simple, however, because a functional AhR is required for normal development of the prostate. There is some evidence that AhR-mediated changes in the Wnt/β-catenin signaling pathway may be involved in ventral prostate agenesis mediated by 2,3,7,8-TCDD. Specifically, organ culture experiments showed

that 2,3,7,8-TCDD-induced reductions in prostatic buds can be reversed by treatment with an antibody against Wnt5a (Yoshioka and Tohyama 2019).

Developmental neurotoxicity. *In utero* and lactational exposure to 2,3,7,8-TCDD has been associated with neurobehavioral effects in laboratory rodents. Efforts to investigate the mechanisms underlying these changes have been focused on neuromorphology, GABA/glutamate neurotransmission, and gene expression. Kimura et al. (2015) showed that 14-day-old offspring of pregnant mice given 0.6 or 3.0 μg/kg 2,3,7,8-TCDD on GD 12.5 exhibited altered dendritic branch lengths in pyramidal neurons of the hippocampal CA1 and BLA regions. Dendritic branch length differences were not seen in groups of offspring evaluated at 16 months of age; however, at this time point, dendritic spine density in the hippocampal CA1 was significantly decreased in treated compared with control offspring. Excitatory synapses expressing glutamate receptors occur in dendritic spines and are important in neuronal transmission; furthermore, decreased spine density is thought to be involved in memory impairments (Kimura et al. 2015).

Studies in neonatal rats have suggested that 2,3,7,8-TCDD may alter GABA and glutamate neurotransmission in the developing brain. In cerebral cortical neurons obtained from rat pups treated *in utero* on GD 18, both basal and potassium-evoked glutamate transmission were reduced, as was cellular uptake of ^{3}H glutamate (Tomasini et al. 2012). This effect was seen in cells obtained from 1-day-old rats and also in cerebral cortical slices from 14- and 60-day-old rats, indicating the persistence of the change. Nguyen et al. (2013b) treated rats with 2,3,7,8-TCDD during gestation and analyzed the numbers of parvalbumin (PV)- and calbindin (Calb)-immunoreactive neurons (GABAergic neurons) in the brains of the offspring at 14 weeks of age. The effects of 2,3,7,8-TCDD varied by sex of the pups and by region of the brain. Increases in the numbers and sizes of PV-immunoreactive neurons were noted in the medial prefrontal cortex of female pups, but not male pups. In exposed offspring of both sexes, decreases in the numbers of immunoreactive neurons were observed in the basolateral amygdala (PV-immunoreactive only) and hippocampus (both PV- and Calb-immunoreactive). In the superior colliculus, decreases in PV-immunoreactive neurons were seen in both sexes, while only females exhibited a decrease in Calb-immunoreactive neurons (Nguyen et al. 2013b). The study authors suggested that impaired functioning of GABAergic neurotransmission could be a factor in the neurobehavioral effects of 2,3,7,8-TCDD.

Kimura et al. (2016) identified two genes upregulated in the olfactory bulb of neonatal mice treated *in utero* under the same regimen. These genes, *Sema3b* and *Sema3g*, which encode proteins that control axonal projections, were upregulated in groups sacrificed on PNDs 3, 7, and 14. The study authors showed that these genes were selectively upregulated in the brain; expression of these genes in the kidney, liver, lung, and spleen was not affected by exposure (Kimura et al. 2016). The study authors noted that the olfactory bulb, along with the hippocampus and amygdala, has been shown to be involved in behavioral changes.

In microarray analysis subsequently confirmed with reverse transcriptase polymerase chain reaction (RT-PCR), Mitsui et al. (2011) observed upregulation of the chemokine genes *Cxcl4* and *Cxcl7* in GD 18.5 whole brains of both male and female mice. *In situ* hybridization was used to determine that the *Cxcl4* mRNA was located on the surface of the cerebral cortex (Mitsui et al. 2011). Mitsui et al. (2011) noted that chemokines in the brain have been suggested to play roles in neuronal regeneration and apoptosis, hippocampal structure formation, blood-brain barrier disruption, and disorders of the central nervous system.

Using an *in vitro* blood:brain barrier model, Miyazaki et al. (2016) showed that 2,3,7,8-TCDD exposure prior to adult developmental function of the barrier resulted in increased permeability as measured by transendothelial electrical resistance (TEER), as well as decreased expression of the tight junction proteins ZO-1 and claudin-5. These effects were shown to be mediated by suppressed expression of glial cell line-derived neurotrophic factor (GDNF), as the effects were mitigated when exogenous GDNF was added to the culture medium (Miyazaki et al. 2016).

2.18 OTHER NONCANCER

No data were located on other noncancer effects in humans or animals following inhalation, oral, or dermal exposure to CDDs.

2.19 CANCER

The carcinogenicity of CDDs has been evaluated in a number of cohorts of workers at chlorophenoxy herbicide or trichlorophenol manufacturing facilities and phenoxy herbicide applicators, Vietnam War veterans exposed to Agent Orange, Seveso residents, communities living near municipal incinerators, and the general population. Meta-analysis of the occupational exposure cohorts has found increased risks of all cancers associated with serum CDD TEQ levels. IARC concluded that there was consistent evidence of associations between CDDs and several specific cancer types: lung cancer, soft tissue sarcoma, and non-Hodgkin lymphoma.

Several animal studies have evaluated the carcinogenicity of 2,3,7,8-TCDD and found increases in several tumor types including hepatocellular carcinoma, thyroid follicular cell adenoma, squamous cell carcinoma in the lungs, squamous cell carcinoma in hard palate and tongue, and gingival squamous cell carcinoma in oral mucosa. Hepatocellular carcinomas were also observed in mice orally exposed to a mixture of HxCDD congeners or 2,7-DCDD.

Epidemiological Studies. 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 War veterans exposed to Agent Orange, residents of Seveso exposed to high levels of 2,3,7,8-TCDD resulting from an industrial accident, communities living near a municipal incinerator, and the general population. A major weakness in many of these studies is the lack of adequate exposure data. Most studies did not measure exposure levels or 2,3,7,8-TCDD body burdens; 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.

Increases in the overall cancer risk were observed in a number of large cohort mortality studies of chemical manufacturing workers and phenoxy herbicide applicators; these studies are briefly summarized in Table 2-31. Most of the subjects in these studies were males working in chlorophenoxy herbicide or trichlorophenol manufacturing facilities.

Table 2-31. Cancer Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Occupational			
Becher et al. 1996 Retrospective cohort mortality study of 2,479 workers at four phenoxy acid herbicides and chlorophenols production facilities in Germany (1,144 male workers in subcohort 1); one facility was also examined by Manz et al. (1991)	2,3,7,8-TCDD blood level: 3–2,252 pg/g lipid in subcohort 1 (samples from 120 workers)	All cancer deaths	↔, whole cohort ↑, subcohort 1
		Lung cancer deaths	↑, whole cohort ↑, subcohort 1
		Lymphatic and hematopoietic cancer deaths	↔, whole cohort ↑, subcohort 1
		Non-Hodgkin lymphoma deaths	↑, whole cohort ↑, subcohort 1
		Leukemia	↔, subcohort 1
Boers et al. 2010 Retrospective cohort mortality study of 1,021 male workers (in factory A) in the Netherlands; follow-up to the Bueno de Mesquita et al. (1993) and Hooiveld et al. (1998) studies	Not reported	All cancer deaths	↔
		Respiratory cancer deaths	↔
		Lymphatic and hematopoietic cancer deaths	↔
		Non-Hodgkin lymphoma	↔
		Leukemia	↔
Boers et al. 2012 Retrospective cohort study; follow-up to the Bueno de Mesquita et al. (1993), Hooiveld et al. (1998), and Boers et al. (2010) studies	Predicted serum 2,3,7,8-TCDD level: high-exposure group ≥20.1 pg/g	All cancer deaths	↔, high exposure
		Respiratory cancer deaths	↔, high exposure
		Lymphatic and hematopoietic cancer deaths	↔
		Non-Hodgkin lymphoma deaths	↑, high exposure
		Leukemia	↔
Bueno de Mesquita et al. 1993 Retrospective cohort mortality study of 549 male workers at 2,4,5-T production facility (factory A) in the Netherlands	Not reported	All cancer deaths	↔
		Respiratory tract cancer deaths	↔
		Non-Hodgkin lymphoma	↔
		Hodgkin lymphoma	↔

Table 2-31. Cancer Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Coggon et al. 2015 Retrospective cohort mortality study of 8,036 male workers at five phenoxy herbicide facilities or were contract sprayers in the United Kingdom (facilities part of the IARC cohort)	Workers ever potentially exposed to phenoxy acids	All cancer deaths	↔
		Soft tissue sarcoma	↔
		Hodgkin lymphoma	↔
		Non-Hodgkin lymphoma	↑
		Leukemia	↔
Collins et al. 2016 Retrospective cohort study of 1,615 trichlorophenol and 2,4,5-T production workers in the United States	Mean serum 2,3,7,8-TCDD levels: 16 pg/g; high-exposure group: 1,500–112,272 pg/g-months	All cancer deaths	↔, high exposure
		Lung cancer deaths	↔, high exposure
		Non-Hodgkin lymphoma deaths	↔, high exposure
Eriksson et al. 1981 Case-control study of 110 cases with soft tissue sarcoma and 219 controls in Sweden	Exposure to phenoxyacetic acid herbicides and/or chlorophenols	Soft tissue sarcoma	↔, high exposure
Eriksson et al. 1990 Case-control study of 237 cases with soft tissue sarcoma and 237 controls in Sweden	Exposure to phenoxyacetic acid herbicides and/or chlorophenols	Soft tissue sarcoma	↑
Fingerhut et al. 1991 Retrospective mortality study of 5,172 workers at 12 facilities in the United States involving exposure to chemicals contaminated with 2,3,7,8-TCDD (NIOSH cohort)	Mean 2,3,7,8-TCDD serum level: 233 pg/g lipid (range of 2–3,400 pg/g) (samples from 253 workers at two facilities)	All cancer deaths	↑
		Respiratory tract cancer deaths	↔
		Lymphatic and hematopoietic cancer deaths	↔
		Soft-tissue sarcoma deaths	↑
		Non-Hodgkin lymphoma deaths	↔
		Hodgkin lymphoma deaths	↔

Table 2-31. Cancer Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Flesch-Janys et al. 1998 Retrospective mortality study of 1,189 male workers at facility producing trichlorophenol, 2,4,5-T, and other herbicides contaminated with 2,3,7,8-TCDD in Germany (follow-up to Manz et al. 1991 study)	Mean serum 2,3,7,8-TCDD serum level: 108.6 pg/g lipid (samples from 275 workers)	All cancer deaths	↑
		Lung cancer deaths	↑
		Lymphatic and hematopoietic cancer deaths	↑
Hardell and Eriksson 1988 Case-control study of 54 males with soft tissue sarcoma (18 alive and 36 dead), 311 population-based referents (208 alive and 103 dead) and 179 cancer referents (73 alive and 106 dead) in Sweden	Employment in industries associated with phenoxyacetic acids and chlorophenols (forestry, agriculture, horticulture, carpentry, saw mills)	Soft tissue sarcoma	↑,exposure to phenoxyacetic acids ↔, exposure to chlorophenols
Hardell and Sandström 1979 Case-control study of 52 males with soft tissue sarcoma and 208 controls in Sweden	Exposure to phenoxyacetic acids or chlorophenols	Soft tissue sarcoma	↑,exposure to phenoxyacetic acids ↑, exposure to chlorophenols
Hardell et al. 1995 Meta-analysis of Eriksson et al. (1981, 1990), Hardell and Eriksson (1988), and Hardell and Sandström (1979) studies	Phenoxyacetic acid herbicide or chlorophenol exposure	Soft-tissue sarcoma	↑
Hooiveld et al. 1998 Retrospective cohort mortality study of 549 male workers at 2,4,5-T production facility in the Netherlands (follow-up to Bueno de Mesquita et al. 1993 study)	Mean 2,3,7,8-TCDD serum levels: 96.3 pg/g lipid in 14 workers exposed to high levels during an accident and 16.6 in 17 workers not exposed during accident	All cancer deaths	↑
		Lung cancer deaths	↔
		Non-Hodgkin lymphoma deaths	↔
		Hodgkin lymphoma	↔
		Leukemia	↔

Table 2-31. Cancer Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Kogevinas et al. 1995 Nested case-control study of 11 cases of soft-tissue sarcoma (55 controls) and 32 cases of non-Hodgkin lymphoma (158 controls)	Phenoxy herbicide, chlorophenols, CDD/CDF, or 2,3,7,8-TCDD exposure	Soft-tissue sarcoma	↑, any phenoxy herbicide ↔, chlorophenols ↑, CDD/CDF ↔, 2,3,7,8-TCDD
		Non-Hodgkin lymphoma	↔, any phenoxy herbicide ↔, chlorophenols ↔, CDD/CDF ↔, 2,3,7,8-TCDD
Kogevinas et al. 1997 Retrospective mortality study of the IARC cohort expanded to 21,863 phenoxy herbicide or chlorophenol workers (20,851 males and 1,012 females) in 36 facilities in 12 countries; 13,831 workers exposed to phenoxy herbicides contaminated with 2,3,7,8-TCDD or higher chlorinated dioxins	Mean serum 2,3,7,8-TCDD levels in 573 workers in 10 cohorts: 17–401.7 pg/g lipid	All cancer deaths	↑, males only ↔, females only
		Lung cancer deaths	↔, phenoxy herbicide workers
		Soft-tissue sarcoma deaths	↑, phenoxy herbicide workers
		Non-Hodgkin lymphoma deaths	↔, phenoxy herbicide workers
Mannetje et al. 2005 Cross-sectional study of a total of 813 producers and 699 sprayers classified as exposed to dioxin and phenoxy herbicides in a New Zealand study	Job codes were used for exposure evaluation	All cancer deaths	↔, producers, sprayers
		Respiratory cancer deaths	↔, producers, sprayers
		Non-Hodgkin lymphoma deaths	↔, producers, sprayers
Manuwald et al. 2012 Retrospective cohort mortality study of 1,589 workers at an herbicide and insecticide (including 2,4,5-T) production facility in Germany; follow-up to the Manz et al. (1991), Flesch-Janys et al. (1998), and Becher et al. (1996) studies	Median serum cumulative job exposure level: 77.4 pg/g lipid for men and 19.5 ppt for women	All cancer deaths	↑, men ↔, women
		Respiratory cancer deaths	↑, men
		Lymphatic and hematopoietic tissue cancer deaths	↔, men, women
		Non-Hodgkin lymphoma	↔

Table 2-31. Cancer Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Manz et al. 1991 Retrospective cohort mortality study of 1,583 workers (1,184 males and 399 females) at a facility producing trichlorophenol, 2,4,5-T, and other herbicides contaminated with 2,3,7,8-TCDD in Germany	Mean serum 2,3,7,8-TCDD level: 296 pg/g lipid in 37 workers in high- exposure group and 83 pg/g lipid in 11 workers in medium-/low-exposure groups	All cancer deaths, compared to gas worker reference cohort	↑, total cohort ↑, high exposure cohort
		Lung cancer deaths, compared to gas worker reference cohort	↑, total cohort
McBride et al. 2009 Retrospective cohort mortality study of 1,754 workers at a phenoxy herbicide production facility in New Zealand	Not measured	All cancer deaths	↔
		Respiratory tract cancer deaths	↔
		Lymphatic and hematopoietic cancer deaths	↔
		Non-Hodgkin lymphoma deaths	↔
		Hodgkin lymphoma deaths	↔
		Soft tissue sarcoma deaths	↔
McBride et al. 2018 Retrospective cohort mortality study of 1,134 workers at a facility producing 2,4,5-trichlorophenol in New Zealand; follow-up to the McBride et al. (2009) study	Mean serum 2,3,7,8-TCDD level in samples from 241 workers in 2005: 9.9 pg/g lipid	All cancer deaths	↔
		Lung cancer deaths	↔
		Lymphatic and hematopoietic cancer deaths	↔
		Non-Hodgkin lymphoma deaths	↔
		Hodgkin lymphoma deaths	↔
Ott and Zober 1996 Retrospective cohort study; follow-up to the Zober et al. (1990) study	Half-life extrapolated 2,3,7,8-TCDD body burdens of <0.1, 0.1–0.99, and ≥1 µg/kg body weight	All cancer deaths	↔, high body burden group ↑, high body burden and 20-year lag
		Respiratory cancer deaths	↔, high body burden group ↑, high body burden and 20-year lag
		Lymphatic or hemopoietic tissue cancer deaths	↔, high body burden group

Table 2-31. Cancer Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Saracci et al. 1991 Retrospective mortality study of the IARC cohort of 18,875 in 20 facilities in 10 countries	Exposed workers sprayed chlorophenoxy herbicides or worked in factories producing chlorophenoxy herbicides or chlorinated phenols; probably exposed workers worked at facilities producing PCP or 2,4-D, 2,4-(dichlorophenoxy)butanoic acid, (4-chloro-2-methyl-phenoxy)acetic acid, and (4-chloro-2-methyl)propanoic acid	All cancer deaths	↔, exposed workers
		Trachea, bronchus, and lung cancer deaths	↔, exposed workers ↑, probably exposed workers
		Hodgkin lymphoma deaths	↔, exposed workers
		Soft-tissue sarcoma deaths	↑, exposed workers
Steenland et al. 1999 Retrospective mortality study of U.S cohort of 5,132 workers; follow-up to the Fingerhut et al. (1991) study	Cumulative exposure score	All cancer deaths	↑, total cohort
Zober et al. 1990 Retrospective cohort mortality study of 247 male workers exposed to 2,3,7,8-TCDD during an accident in a German 2,4,5-TCP production facility	Median serum 2,3,7,8-TCDD level: 24.5 pg/g lipid in 11 highly exposed workers	All cancer deaths	↔, highly exposed ↔, workers with chloracne ↑, workers with chloracne and exposure lagged 20 years
		Trachea, bronchus, lung cancer deaths	↔, highly exposed ↔, workers with chloracne
Vietnam veterans and Operation Ranch Hand veterans			
USAF 1991 Cross-sectional report of 866 Operation Ranch Hand personnel and a comparison group of 1,198	Not reported	All malignant cancers	↔
		Hodgkin lymphoma	↔
		Non-Hodgkin lymphoma	↔
		Soft tissue sarcoma	↔
Wolfe et al. 1985 Retrospective study of 1,278 Operation Ranch Hand personnel	Not reported	All malignant cancers	↔

Table 2-31. Cancer Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Yi and Ohrr 2014 Retrospective cohort study of Vietnam War veterans participating in the Korean Veterans Health Study (n=180,251 men)	Exposure to Agent Orange evaluated using data on the proximity of the military unit to area sprayed with Agent Orange	All cancers	↑
		Non-Hodgkin lymphoma	↔
		Hodgkin lymphoma	↔
		Soft tissue sarcoma	↔
		Lymphoid leukemia	↔
Seveso, Italy			
Bertazzi et al. 1993 Retrospective cohort study of adults living in Seveso aged 20–74 years (n= 724 in zone A, n=4,824 in zone B, and n=31,647 in zone R; referent population of 181,579)	Not measured	All cancers	↔, zone A, B, or R
		Soft-tissue sarcoma	↑, zone R, males
Bertazzi et al. 1997 Retrospective cohort mortality study of Seveso residents (n=805 in zone A, n=5,943 in zone B, and 38,625 in zone R); follow-up to Bertazzi et al. (1993) study	Not measured	All cancer deaths	↔, zone A, B, or R
		Lymphohemopoietic cancer deaths	↑, zone B, males
		Leukemia deaths	↑, zone B, males
Bertazzi et al. 2001 Retrospective cohort mortality study of Seveso residents (n=804 in zone A and n=5,941 in zone B); follow-up to the Bertazzi et al. (1993, 1997) studies	Not reported	All cancer deaths	↔
		Lung cancer deaths	↔
		Lymphatic and hemopoietic cancer deaths	↔, zone A ↑, zone B
		Non-Hodgkin lymphoma deaths	↑, 15–20 since first exposure
Consonni et al. 2008 Retrospective cohort mortality study of Seveso residents (n=804 in zone A, n=5,941 in zone B, and n=36,623 in zone R)	Not reported	All cancer deaths	↔
		Lung cancer deaths	↔
		Lymphatic and hematopoietic tissue cancer deaths	↑, zone B
		Non-Hodgkin lymphoma deaths	↑, zone A

Table 2-31. Cancer Effects in Humans Exposed to TCDD/CDDs

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Pesatori et al. 2009 Retrospective cohort study of Seveso residents (n=723 in zone A, n=4,821 in zone B, and n=31,643 in zone R)	Serum 2,3,7,8-TCDD in samples collected from some participants in 1976: 447.0 pg/g (n=296), 94.0 (n=80), and 48.0 (n=48) in zones A, B, and R, respectively	All cancers	↔, zone A
		Lung cancer	↔, zone A
		Lymphatic and hematopoietic cancers	↔, zone A ↑, zone B
		Non-Hodgkin lymphoma	↔, zone A
		Lymphatic leukemia	↔, zone A
Warner et al. 2011 Retrospective cohort study of female Seveso residents (n=981) residing in zone A or B at the time of the accident	Median 2,3,7,8-TCDD serum level: 55.9 pg/g lipid	All cancers	↑, per 10-fold increase in 2,3,7,8-TCDD serum level
Communities living near a municipal incinerator			
Floret et al. 2003 Case-control study of 222 residents in France living near a solid waste incinerator with non-Hodgkin lymphoma and 2,220 controls	Modeled CDD/CDF ground level concentration; high-exposure group: 0.0004–0.0016 pg/m^3	Non-Hodgkin lymphoma	↑, high dioxin exposure group
Viel et al. 2011 Case-control study of 34 men and women with non-Hodgkin lymphoma and 34 controls living near a solid waste incinerator in France	Serum CDD/CDF levels (values not reported)	Non-Hodgkin lymphoma	↑, CDD TEQ

↑ = association; ↓ = inverse association; ↔ = no association; 2,4-D = 2,4-dichlorophenoxyacetic acid; 2,4,5-T = 2,4,5-trichlorophenoxyacetic acid; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; IARC = International Agency for Research on Cancer; NIOSH = National Institute for Occupational Safety and Health; PCP = pentachlorophenol; TCDD = tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalency

The occupational exposure database consists of several large cohort studies, which are presented in Table 2-32. Mixed results were found for associations between 2,3,7,8-TCDD exposure and deaths from all cancers. Associations were found in the some of the cohorts (Becher et al. 1996; Fingerhut et al. 1991; Flesch-Janys et al. 1998; Hooiveld et al. 1998; Kogevinas et al. 1997; Manuwald et al. 2012; Manz et al. 1991; Ott and Zober 1996; Steenland et al. 1999; Zober et al. 1990) and no associations were found in other cohorts (Boers et al. 2010, 2012; Bueno de Mesquita et al. 1993; Collins et al. 2016; McBride et al. 2009, 2018; Saracci et al. 1991). Steenland et al. (2001) reported a dose-response relationship between estimated cumulative serum 2,3,7,8-TCDD levels and cancer mortality for the NIOSH cohort. A meta-analysis of the NIOSH cohort (Fingerhut et al. 1991; Steenland et al. 1999), German accident cohort (Ott and Zober 1996; Zober et al. 1990), and German cohort (Becher et al. 1996; Flesch-Janys et al. 1998) was conducted by Crump et al. (2003). The analysis found an increased risk of all cancer deaths (SMR=117, 95% CI: 104–130); a linear model predicted an increased relative risk of $6.3x10^{-6}$ (95% CI: $8.8x10^{-7}$–$1.3x10^{-5}$) per 1 ppt-year of cumulative lipid concentration.

Table 2-32. Occupational Cohort Studies Examining the Carcinogenicity of Chlorinated Dibenzo-*p*-Dioxins (CDDs)

Cohort	References
National Institute for Occupational Safety and Health (NIOSH) cohort	Fingerhut et al. 1991; Steenland et al. 1999
U.S. cohort	Collins et al. 2016
German cohort	Becher et al. 1996; Flesch-Janys et al. 1998; Manuwald et al. 2012; Manz et al. 1991
German accident cohort	Ott and Zober 1996; Zober et al. 1990
Dutch cohort	Boers et al. 2010, 2012; Bueno de Mesquita et al. 1993; Hooiveld et al. 1998
New Zealand cohort	McBride et al. 2009, 2018
International Agency for Research on Cancer (IARC) multi-nation cohort	Kogevinas et al. 1997; Saracci et al. 1991

Studies of Seveso residents have not found increased risk of all cancers (Bertazzi et al. 1993, 1997, 2001; Consonni et al. 2008). However, the most recent study of this cohort (Warner et al. 2011) did find an increase in all cancers among women. 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. Limitations of this study design include 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 that examined cancer incidences in "Vietnam veterans" may not be adequate to assess the carcinogenicity of 2,3,7,8-TCDD. Two studies of Operation Ranch Hand personnel did not find increases in the risk of all malignant cancers (USAF 1991; Wolfe et al. 1985); a third study of veterans stationed near areas sprayed with Agent Orange found an increased risk of all cancers (Yi and Ohrr 2014).

A large number of studies have evaluated possible associations between CDD exposure and specific tumor types. The National Academy of Sciences (NAS) review of the health of effects in Vietnam veterans from exposure to herbicides concluded that there was sufficient evidence of an association between the chemicals of interest (2,4-D, 2,4,5-T, picloram, dimethylarsinic acid, and 2,3,7,8-TCDD) and soft tissue sarcomas, B-cell lymphomas (Hodgkin lymphoma, non-Hodgkin lymphoma, chronic lymphocytic leukemia, hairy cell leukemia), and monoclonal gammopathy of undetermined significance (NAS 2018). IARC (2012) concluded that the most consistent evidence was for lung cancer, soft tissue sarcoma, and non-Hodgkin lymphoma. Increases in lung (or respiratory tract) cancer have been observed in the German cohort and German accident cohort, but not in the Dutch, U.S., NIOSH, IARC, or the New Zealand cohorts; see Tables 2-31 and 2-32 for citations. Increases in lung cancer risk was not observed in the Seveso cohort (Bertazzi et al. 2001; Consonni et al. 2008; Pesatori et al. 2009).

The possible association between 2,3,7,8-TCDD exposure and soft tissue sarcoma was first suggested by the results of a series of case-control studies that found 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 Sandström 1979), workers involved in manufacturing and application of phenoxy herbicides (Kogevinas et al. 1995), and New Zealand farmers (Smith et al. 1984). Increased risk of soft tissue sarcomas has also been reported in the NIOSH cohort (Fingerhut et al. 1991), IARC cohort (Kogevinas et al. 1997; Saracci et al. 1991), and Seveso cohort (Bertazzi et al. 1993); it has not been found in Operation Ranch Hand veterans (USAF 1991) or Korean Vietnam veterans (Yi and Ohrr 2014).

Increases in the risk of several types of lymphohematopoietic cancers have been associated with 2,3,7,8-TCDD exposure. Increases in deaths or incidences of non-Hodgkin lymphoma have been reported in the German cohort (Becher et al. 1996), Dutch cohort (Boers et al. 2012), U.K. cohort (Coggon et al. 2015), and Seveso cohort (Bertazzi et al. 2001; Consonni et al. 2008); it was also found in two case-control studies of residents living near a solid waste incinerator (Floret et al. 2003; Viel et al. 2011). No increases in non-Hodgkin lymphoma risk were found in the U.S. cohort (Collins et al. 2016), NIOSH cohort (Fingerhut et al. 1991), IARC cohort (Kogevinas et al. 1995, 1997), New Zealand cohort

(Mannetje et al. 2005; McBride et al. 2009), German cohort (Manuwald et al. 2012), or Vietnam veterans (USAF 1991; Yi and Ohrr 2014). No increases in Hodgkin lymphoma were found in large occupational exposure studies (Bueno de Mesquita et al. 1993; Coggon et al. 2015; Fingerhut et al. 1991; Hooiveld et al. 1998; McBride et al. 2009, 2018) or in Operation Ranch Hand veterans (USAF 1991).

A meta-analysis of five studies of workers involved in chlorophenol pesticide production found a risk of prostate cancer deaths (standard mortality rate of 1.2, 95% CI of 1.02–1.42) (Kabir et al. 2018).

2,3,7,8-TCDD—Animal Studies. The carcinogenicity of 2,3,7,8-TCDD has been demonstrated in several experiments in animals; the cancer sites include the liver, lungs, oral cavity, and thyroid. A summary of these studies is presented in Table 2-33. Hepatocellular carcinomas and neoplastic nodules have been observed in rats and mice chronically exposed to ≥0.01 µg/kg/day (Della Porta et al. 1987; Kociba et al. 1978; NTP 1982b, 2006; Toth et al. 1979). In Sprague-Dawley rats, females were more affected than males (Kociba et al. 1978). Cholangiocarcinomas have also been observed in female Sprague-Dawley rats exposed to 0.071 µg/kg/day for 2 years (NTP 2006). In the thyroid, follicular cell adenomas were observed in rats and mice (NTP 1982b) and c-cell adenomas were reported in rats (NTP 2006). Lung lesions in rats include squamous cell carcinoma and cystic keratinizing epithelioma (Kociba et al. 1978; NTP 2006). Chronic-duration oral exposure also resulted in squamous cell carcinoma in the hard palate or nasal turbinates and oral mucosa gingiva (Kociba et al. 1978; NTP 2006). NTP (2006) also found a nonsignificant increase in the combined incidence of adenoma or carcinoma in the pancreas in rats exposed to 0.071 µg/kg/day; however, the incidence was higher than historical controls and there was a significant positive trend. The study also found an increased incidence of squamous cell carcinoma of the uterus at 0.032 µg/kg/day, but not at 0.0711 µg/kg/day.

Table 2-33. Carcinogenic Effects in Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose (µg/kg/day)	Tumor type	Reference
Liver			
B6C3 mouse, 1 year	0.36	Hepatocellular carcinomas (males and females) and adenomas (females)	Della Porta et al. 1987
Female Sprague-Dawley rat	0.1	Hepatocellular carcinoma	Kociba et al. 1978
Female Sprague-Dawley rat, 2 years	0.01	Hepatocellular hyperplastic nodules	Kociba et al. 1978

Table 2-33. Carcinogenic Effects in Animals Orally Exposed to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

Species, duration	Dose (µg/kg/day)	Tumor type	Reference
Female Osborne-Mendel rat, 2 years	0.071	Neoplastic nodules in liver and hepatocellular carcinoma	NTP 1982b
Male B6C3F1 mouse, 2 years	0.071	Hepatocellular carcinoma	NTP 1982b
Female Sprague-Dawley rat, 2 years	0.071	Hepatocellular adenomas, cholangiocarcinomas	NTP 2006
Swiss mouse, 1year	0.1	Hepatomas and hepatocellular carcinomas	Toth et al. 1979
Thyroid			
Male Osborne-Mendel rat, 2 years	0.0071	Thyroid follicular cell adenoma	NTP 1982b
Female B6C3F1 mouse, 2 years	0.3	Thyroid follicular cell adenoma and histiocytic lymphoma	NTP 1982b
Female Sprague-Dawley rat, 2 years	0.071	c-cell adenoma in thyroid gland	NTP 2006
Lung			
Female Sprague-Dawley rat, 2 years	0.1	Squamous cell carcinoma in lungs	Kociba et al. 1978
Female Sprague-Dawley rat, 2 years	0.071	Cystic keratinizing epithelioma in lungs	NTP 2006
Oral cavity			
Sprague-Dawley rat, 2 years	0.1	Squamous cell carcinoma in hard palate or nasal turbinates	Kociba et al. 1978
Female Sprague-Dawley rat, 2 years	0.071	Gingival squamous cell carcinoma in oral mucosa	NTP 2006

Dermal application of ≥0.036 µg/kg 2,3,7,8-TCDD 3 times/week for 26 weeks (equivalent to 0.015 µg/kg/day) to the shaved skin of groups of 20 female Tg.AC transgenic mice (genetically initiated, tumor promoter-sensitive epidermal tumorigenesis model) resulted in significant increases in the incidence of skin squamous cell papillomas (Wyde et al. 2004); an increase in squamous cell carcinomas was observed at ≥0.052 µg/kg/day. No significant alterations were observed at lower doses (0.0021 or 0.0073 µg/kg/day).

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 mice with 0.01 µg 2,3,7,8-TCDD inhibited the development of skin papillomas otherwise initiated by 7,12-dimethylbenzathracene (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/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). No ovarian tumors were observed in Sprague-Dawley rats administered 0.125 µg/kg 2,3,7,8-TCDD in corn oil via gavage biweekly for 14, 30, or 60 weeks (Davis et al. 2000); however, in rats administered a single dose of diethylnitrosamine (175 mg/kg, intraperitoneal) and followed by biweekly doses of 2,3,7,8-TCDD for 60 weeks (administered 2 weeks after initiation), there was a significant increase in ovarian tumors. Similarly, no observable tumors were observed in female Sprague-Dawley rats administered 1.25 µg/kg/day 2,3,7,8-TCDD in corn oil biweekly for up to 60 weeks (Walker et al. 2000). Initiation with diethylnitrosamine and biweekly exposure to 1.25 µg/kg/day 2,3,7,8-TCDD for 60 weeks resulted in a non-statistically significant increase in the incidence of liver tumors. In a chronic-duration 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/week for 2 years (NTP 1982a).

Initiation with 100 µg DMBA applied to the dorsal shaved skin of male ICR mice followed by application of 0.0025, 0.025, and 0.125 µg 2,3,7,8-TCDD in 100 µL acetone 2 times/week for up to 20 weeks did not result in skin papillomas (Wu et al. 2004).

Other Congeners—Animal Studies. Experiments with other congeners showed that chronic-duration 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 0.34 µg/kg/day in female Osborne-Mendel rats and at 0.71 µg/kg/day in male B6C3F1 mice (NCI/NTP 1980). Furthermore, chronic-duration exposure to $6.5x10^5$ 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 B6C3F1 mice (NCI/NTP 1979). In contrast, no cancer effects were observed following chronic-duration exposure of Osborne-Mendel rats to $5x10^5$ µg/kg/day of 2,7-DCDD (NCI/NTP 1979) in the feed.

Rozman et al. (2005) reported an increase in the prevalence of lung tumors (squamous cell carcinoma) in female Sprague-Dawley rats receiving a single dose of 2.8 mg/kg 1,2,3,4,6,7,8-HpCDD in corn oil; an increased prevalence of liver tumors (hepatocarcinoma and cholangiocarcinoma) was observed at 3.4 mg/kg. Similarly, increases in the prevalence of lung tumors and liver tumors were observed in rats repeatedly exposed to a TWA dose of 0.0065 or 0.012 mg/kg/day, respectively.

Cancer Classification. HHS has classified 2,3,7,8-TCDD as known to be a human carcinogen (NTP 2021). EPA (IRIS 2012) has not established a cancer classification for 2,3,7,8-TCDD. IARC (2012) has determined that 2,3,7,8-TCDD is carcinogenic to humans (Group 1) based on limited evidence in humans and sufficient evidence in animals.

EPA (1987) categorized the mixture of 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD as a probable human carcinogen (Group B2) based on sufficient evidence of carcinogenicity in animals. IARC (1997) concluded that other CDDs are not classifiable as to their carcinogenicity in humans (Group 3) based on limited evidence for a mixture of 1,2,36,7,8-HxCDD and 1,2,3,7,8,9-HxCDD in animals and inadequate evidence for 1,2,3,7,8-PeCDD in animals.

Cancer Mechanisms. Information pertaining to the mechanisms of carcinogenicity for CDDs is primarily from studies of 2,3,7,8-TCDD. As with many other effects of 2,3,7,8-TCDD, its carcinogenicity is expected to result from AhR activation. Section 2.21, Mechanisms of Toxicity, provides more information on the interaction between 2,3,7,8-TCDD and the AhR as well as the diversity of gene expression changes and cellular events that ensue from this interaction. This section provides an overview of the proposed mechanism(s) by which 2,3,7,8-TCDD induces carcinogenic effects based on published reviews (Chen et al. 2023; IARC 2012; Knerr and Schrenk 2006; Opitz et al. 2023; Patrizi and Siciliani de Cumis 2018; Schwarz and Appel 2005). Detailed mechanistic explanations are beyond the scope of this profile.

Studies to date have indicated that 2,3,7,8-TCDD does not act as a direct genotoxic carcinogen (see Section 2.20), but acts primarily by perturbing cellular growth, differentiation, and programmed death mechanisms (IARC 2012; NTP 2006). These changes are believed to result from persistent AhR activation due to the long half-life of 2,3,7,8-TCDD in the body (Chen et al. 2023; IARC 2012; Schwarz and Appel 2005). Sustained cell proliferation may increase the frequency of spontaneous mutations, induce the accumulation of epigenetic changes, and promote the growth of initiated cells.

Increases in cell proliferation have been observed in several tissues (including liver and skin), after both *in vivo* and *in vitro* exposure to 2,3,7,8-TCDD (Chen et al. 2023; IARC 2012). While activation of AhR is known to be an initial step leading to proliferation, it is likely that several key events follow from AhR activation. Downstream effects of 2,3,7,8-TCDD-liganded AhR activation include modulation of growth factors, cytokines, hormones, and metabolic pathways related to cell proliferation or differentiation,

including (for example): epidermal growth factor (EGF), vitamin A, tumor necrosis factor-α, interleukin-1-β, gonadotrophin-releasing hormone, testosterone, plasminogen activator inhibitor-2, and many others (Knerr and Schrenk 2006; NTP 2006; see also Section 2.21). 2,3,7,8-TCDD also inhibits cellular apoptosis and senescence (Knerr and Schrenk 2006; NTP 2006; Ray and Swanson 2009; Schwarz and Appel 2005), effects that may foster the clonal expansion of initiated cells.

In addition to its effects on cell growth and proliferation, 2,3,7,8-TCDD is believed to indirectly increase oxidative stress and oxidative DNA damage via prolonged upregulation of metabolic enzymes (IARC 2012). Induction of cytochrome P450 (CYP) can lead to uncoupling of the P40 catalytic cycle, with concomitant production of excess reactive oxygen species (ROS) and oxidative DNA damage (IARC 2012; Knerr and Schrenk 2006; Veith and Moorthy 2018). Increases in oxidative stress, along with DNA damage and mutations, have been observed in rats and mice exposed *in vivo* to 2,3,7,8-TCDD, as well as in *in vitro* studies (IARC 2012; Knerr and Schrenk 2006).

Upregulation of CYPs leads to increased levels of reactive intermediates from the metabolism of both exogenous and endogenous compounds (IARC 2012; Knerr and Schrenk 2006; Veith and Moorthy 2018). For example, estrogen has been shown to markedly increase oxidative DNA damage in the livers of female rats, an effect postulated to result from redox cycling of the CYP-generated estradiol metabolite, 4-hydroxyestradiol (IARC 2012; Knerr and Schrenk 2006). CYP-mediated metabolism of estrogen and the related production of ROS has been proposed as a mechanism for the greater sensitivity of female rats to the hepatocarcinogenic effects of 2,3,7,8-TCDD (IARC 2012; Knerr and Schrenk 2006).

Enhanced metabolism may also perturb retinoid homeostasis in the liver. Exposure to 2,3,7,8-TCDD depleted hepatic stores of retinyl acid in several species, an effect that was demonstrated to depend on intact AhR (Knerr and Schrenk 2006). NTP (2006) noted that disruption of hepatic retinoid homeostasis leads to aberrant differentiation of epithelial cells in the lung to a keratinized squamous phenotype, proposing that this change could progress to squamous metaplasia and cystic keratinizing epitheliomas, a lung tumor observed at increased incidences in rats in their 2-year study.

As reviewed by Patrizi and Siciliani de Cumis (2018) and Opitz et al. (2023), 2,3,7,8-TCDD induces a variety of epigenetic changes that may contribute to its carcinogenic action. Experiments both *in vivo* and *in vitro* have shown that 2,3,7,8-TCDD exposure alters the expression of large non-coding RNAs (lncRNAs) that act as regulators of chromatin remodeling (including DNA methylation and histone modifications). In mice, alterations in DNA methylation (both demethylation and hypermethylation) have

been observed after exposure to 2,3,7,8-TCDD. Finally, histone modifications have been observed in human breast and hepatic cancer cell lines exposed to 2,3,7,8-TCDD (Patrizi and Siciliani de Cumis 2018). These epigenetic changes may play a role in the regulation of the AhR or its target genes and thereby modify carcinogenic action (Opitz et al. 2023). For example, regulation of expression of some CYPs (and therefore metabolic changes that may relate to cancer) is dependent on DNA methylation (Opitz et al. 2023). In addition, AhR hypomethylation has been associated with reduced survival in some cancers (Opitz et al. 2023).

A stop-exposure component in the NTP (2006) cancer bioassay of 2,3,7,8-TCDD demonstrated that protracted exposure was a requirement for its liver carcinogenicity in this animal model. Female rats exposed to 100 ng/kg 2,3,7,8-TCDD for 30 weeks developed significantly fewer liver tumors (cholangiocarcinomas and hepatocellular adenomas) than rats exposed to the same dose for 2 years (NTP 2006).

In summary, the carcinogenic effects of 2,3,7,8-TCDD are strongly linked to sustained AhR activation and its pleiotropic sequelae, rather than from a direct genotoxic action. As discussed further in Section 2.21, Mechanisms of Toxicity, there are marked differences in 2,3,7,8-TCDD-mediated AhR activation and ensuing changes across species, strains, sexes, and tissues. The variability in AhR activation and cellular responses to 2,3,7,8-TCDD exposure likely contributes to the diversity of tumor types seen in animals exposed *in vivo*. Further, the activation of AhR is a plausible mechanism for the carcinogenicity of other CDDs, especially those with physiological half-lives similar to that of 2,3,7,8-TCDD, but data with which to evaluate this potential mechanism were not located.

2.20 GENOTOXICITY

Information on studies regarding genotoxic effects in humans is provided in Table 2-34. The studies do not provide conclusive data regarding dioxin genotoxicity.

Table 2-34. Genotoxic Effects in Humans Exposed to 2,3,7,8-TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Baccarelli et al. 2004	20 years after the Seveso, Italy (1976) incident, 62 randomly selected individuals from the exposed zone and 59 controls from the noncontaminated area	2,3,7,8-TCDD in plasma (lipid adjusted) ranged from 3.5 to 90 pg/g (ppt)	AhR mRNA levels in uncultured lymphocytes were negatively correlated with plasma TCDD
Baccarelli et al. 2006	Among 144 healthy individuals from the previously exposed population in Seveso, there were 50 of the t(14,18)-translocation-positive subjects (34.7%)	TCDD in plasma: <10 ppt 10–50 ppt 50–475 ppt	The frequency of non-Hodgkin lymphoma-related t(14,18)-translocations (but not the prevalence) was associated with increased plasma levels in previously exposed individuals; clinical impact is not clear; similarly, increased frequency was detected in smokers.
Rowland et al. 2007	24 New Zealand Defense Force Vietnam War veterans and 23 matched controls	Not applicable	Significant increase in SCE (mean 11.05 versus 8.18).
Valic et al. 2004	A case-control study; two occupationally exposed workers (suspected oral exposure); 30 employees from the same workplace were in the normal TCDD range (1.2–8.6 pg/g blood lipids, average 3.0 pg/g), with the exception of three other employees with moderately increased TCDD levels of 93, 149, and 856 pg/g blood lipids and no clinical signs	2,3,7,8-TCDD at a concentration of 144,000 pg/g blood lipids in patient 1 and 26,000 pg/g blood lipids in patient 2 at first examination after manifestation of chloracne; 2 months later, when TCDD levels were 85,600 and 17,700 pg/g blood lipids, respectively, second examination	First examination: normal values (2.4 and 2.5 MN/500 binucleated cells; 6.7±2.2 and 6.0±2.5 SCEs/metaphase); second examination: MN had increased to 16 and 21.8 MN/500 binucleated cells, SCE remained within normal range. Within a period of 13 months, MN had returned to a nearly normal range in both patients. The comet assay tail factor (DNA damage level) in peripheral lymphocytes showed a very high value of 33.5% (at the time of 2nd evaluation).
Yoshida et al. 2006	Occupational exposure, municipal waste incinerator workers; concentrations of serum dioxins and lymphocytic 8-OH-dG were measured in 57 male workers; from the cohort, urinary 8-OH-dG and urinary mutagenicity was tested in 29 males	Mean CDD, CDF, and coplanar-PCB levels were 12.9, 12.4, and 13.6 pg TEQ/g lipids	Oxidative DNA damage and urinary mutagenicity tested. The lymphocytic 8-OH-dG level showed a negative association with the serum dioxin level (total TEQs). Dioxin did not increase the urinary 8-OH-dG level by oxidative DNA damage.

8-OH-dG = 8-hydroxydeoxyguanosine; AhR = aryl hydrocarbon receptor; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; DNA = deoxyribonucleic acid; MN = micronuclei; mRNA = messenger ribonucleic acid; PCB = polychlorinated biphenyl; SCE = sister chromatid exchange; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalency

Data regarding genotoxic effects in humans exposed to CDDs are inconclusive. *In vivo* genotoxicity studies are summarized in Table 2-35. 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). 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 1980). None of these studies included 2,3,7,8-TCDD dosimetry and all were limited by using exposed groups that were relatively small (<20 individuals) to have the statistical power to reliably assess the cytogenetic damage. Fewer birth defects due to chromosomal abnormalities in children of Vietnam veterans were reported in another study (Erickson et al. 1984).

Table 2-35. Genotoxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) *In Vivo*

Species (test system)	Endpoint	Results	Reference
Drosophila melanogaster	Recessive lethals	–	Zimmering et al. 1985
Rat, bone marrow	Chromosomal aberrations	–	Loprieno et al. 1982
Mouse, bone marrow	Chromosomal aberrations	+	Loprieno et al. 1982
Rat, bone marrow	Chromosomal aberrations	+	Green et al. 1977
Mouse, bone marrow	Chromosomal aberrations, SCE, micronucleus test	–	Meyne et al. 1985
Monkey, peripheral lymphocytes	Chromosomal aberrations, SCE	–	Lim et al. 1987
Rat	Dominant lethals	–	Khera and Ruddick 1973
Rat, liver	DNA adducts	–	Randerath et al. 1989
Rat, liver	DNA-single strand breaks	+	Wahba et al. 1989
Rat, 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

Table 2-35. Genotoxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) *In Vivo*

Species (test system)	Endpoint	Results	Reference
Human, peripheral lymphocytes	Chromosomal aberrations, SCE	–	Zober et al. 1993

– = negative result; + = positive result; DNA = deoxyribonucleic acid; SCE = sister chromatid exchange

One study examined the incidence of chromosomal aberrations and of sister chromatid exchanges in human lymphocytes in 27 workers with current 2,3,7,8-TCDD concentrations in blood >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 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 >10 sister chromatid exchanges. However, these associations were no longer significant when smoking status was included as a 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 are inconsistent and inconclusive. Human studies cited above were limited by several factors. Generally, the levels of exposure to 2,3,7,8-TCDD were not known and co-exposure 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 (<20 individuals) to have the statistical power to detect any changes. The lack of exposure data, small sample sizes, and inconsistent results preclude drawing conclusions from these studies.

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, an 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 at 24 hours, post treatment. 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 or 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-PeCDD 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 seven 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 2-36. 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).

Table 2-36. Genotoxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium				
TA1530	Reverse mutations	NA	–	Hussain et al. 1972
TA1532	Reverse mutations	NA	+	Hussain et al. 1972
TA1535	Reverse mutations	NA	–	Seiler 1973
TA1531	Reverse mutations	NA	–	Seiler 1973
TA1532	Reverse mutations	NA	(+)	Seiler 1973
TA1537	Reverse mutations	NA	(+)	Seiler 1973
TA1535	Reverse mutations	–	NA	Geiger and Neal 1981
TA100	Reverse mutations	–	NA	Geiger and Neal 1981
TA1537	Reverse mutations	–	–	Geiger and Neal 1981
TA1538	Reverse mutations	–	NA	Geiger and Neal 1981
TA98	Reverse mutations	–	NA	Geiger and Neal 1981
TA100	Reverse mutations	–	NA	Mortelmans et al. 1984
TA1535	Reverse mutations	–	NA	Mortelmans et al. 1984
TA1537	Reverse mutations	–	NA	Mortelmans et al. 1984
TA98	Reverse mutations	–	NA	Mortelmans et al. 1984
TA1530	Reverse mutations	–	NA	Gilbert et al. 1980
TA1535	Reverse mutations	–	NA	Gilbert et al. 1980
TA100	Reverse mutations	–	NA	Gilbert et al. 1980
TA1537	Reverse mutations	–	NA	Gilbert et al. 1980
TA1538	Reverse mutations	–	NA	Gilbert et al. 1980
TA98	Reverse mutations	–	NA	Gilbert et al. 1980
TA1535	Reverse mutations	–	–	Toth et al. 1984
TA100	Reverse mutations	–	–	Toth et al. 1984
TA1537	Reverse mutations	–	–	Toth et al. 1984
TA1538	Reverse mutations	–	–	Toth et al. 1984
TA98	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

Table 2-36. Genotoxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD) *In Vitro*

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
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; DNA = deoxyribonucleic acid; NA = not applicable; RNA = ribonucleic acid; SCE = sister chromatid exchange; UDS = unscheduled DNA synthesis

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, TA1530, TA1535, TA100, TA1950, and TA1975, revealing base pair substitutions; and with strains TA1531, TA1532, TA1534, TA1538, TA98, and TA1978, 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.

Inconclusive results were obtained regarding genotoxicity of CDDs in human studies 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.

2.21 MECHANISMS OF TOXICITY

Overview. 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 has been shown to be initiated by the binding of individual congeners (or ligands) with the AhR. The role of AhR binding in the toxicity of 2,3,7,8-TCDD was first discovered in the 1970s. Since that time, the extraordinary binding affinity of 2,3,7,8-TCDD for the AhR has led to its extensive use in experiments aimed at determining the mechanisms through which AhR binding influences physiological systems. As a result, the scientific literature on this topic is voluminous. It is beyond the scope of this profile to discuss these studies in detail. Instead, this section provides a brief overview of the role of the AhR in inducing gene expression changes and epigenetic effects believed to be involved in many of the diverse effects seen in humans and animals exposed to 2,3,7,8-TCDD. For more detailed discussions, there are numerous reviews on this topic, including some recent reviews that were used for this section: Denison et al. (2011); Gasiewicz et al. (2008); Patrizi and Siciliani de Cumis (2018); Xu et al. (2022); Wright et al. (2017).

The AhR is a cytosolic protein in the basic helix-loop-helix-Per-ARNT-Sim family of transcription factors. The AhR exists as a multimeric complex with a 90 KDa heat-shock protein (hsp-90) chaperone protein and the co-chaperones, x-associated protein 2 (XAP2) and p23. When 2,3,7,8-TCDD diffuses into the cytoplasm, it binds to inactive (unliganded) AhR. Upon ligand activation, the AhR undergoes a transformational change to expose a nuclear localization sequence(s) resulting in translocation of the complex into the nucleus. Within the nucleus, the AhR:ligand, released from the complex, forms a heterodimer complex with ARNT (also known as hypoxia inducible factor 1β or HIF-1β). The

ligand:AhR:ARNT heterodimer complex binds to specific DNA recognition sites within target genes, referred to as AhR responsive elements or AhREs, also called dioxin-responsive elements [DREs] or xenobiotic responsive elements [XREs]). Target genes include genes coding for phase I and II biotransformation enzymes and genes involved in regulation of development, proliferation, and differentiation. This is the canonical pathway for AhR signal transduction and is exemplified by the induction of CYP1A1.

In addition to induction of CYP1A1, the canonical liganded AhR-ARNT pathway leads to changes in gene expression that trigger a myriad of cellular level changes. Table 2-37 below shows some of the genes known to have functional AhRE sequences, and Table 2-38 shows examples of cellular level changes associated with TCDD-mediated induction of some of these genes. These tables demonstrate the wide distribution of the AhRE across the genome and the diversity of cellular-level effects that are induced by the AhR-ARNT pathway.

Table 2-37. Genes with Functional Aryl Hydrocarbon Response (AhR) Responsive Elements (AhREs)

Aldehyde dehydrogenase 3A1	HES-1
Aryl hydrocarbon receptor repressor (AhRR)	Hsp27
Bax	Insulin-like growth factor binding protein
c-jun	IgM μ gene
c-myc	Interleukin-2
Cathepsin D	junD
Cyclooxygenase-2	NAD(P)H-quinone oxidoreductase-1
CYP1A1	NF-E2 p45 –related factor (NRF2)
CYP1A2	$p21^{CIP1}$
CYP1B1	$p27^{KIP1}$
CYP2A5	pS2
CYP2S1	Slug
Epiregulin	Suppressor of cytokine signaling 2 (Socs2)
Gluthathione-S-transferase Ya	U=Uridine 5'-diphospho-glucuronosyltransferase (UDP)-glucuronosyltransferase 1A1
Filaggrin	UDP-glucuronosyltransferase 1A6

Source: Gasiewicz et al. 2008

Table 2-38. Examples of AhR:ARNT Canonical Pathway Effects

Gene expression change	Cellular level effect
CYP1A1 induction	• Generation of ROS/oxidative stress • Oxidative DNA damage • Activation of intracellular kinase signaling pathways (c-Jun, NFκB, etc.) • Endothelial dysfunction
TiPARP induction	• Suppression of hepatic gluconeogenesis
Nedd1/Hef1/Cas-L induction	• Changes in cell adhesion and shape • Cytoskeletal reorganization • Increased cell migration
SOS1 induction	• Activated Ras-GTP • Activation of extracellular signal related kinase • Accelerated cell proliferation
CYP1A1/1B1 induction	• Catabolism of estrogen
$p27^{kip1}$ and $p21^{Waf1/Cip1}$ induction	• Inhibition of CDKs • Inactivation of Rb • Repression of cell cycle

Source: Denison et al. 2011

CDK = cyclin-dependent kinase; DNA = deoxyribonucleic acid; NFκB = nuclear factor κB; Rb = retinoblastoma protein; ROS = reactive oxygen species

More recent studies have indicated that AhR can also mediate effects on genes that lack an identifiable AhRE. These changes in gene expression are postulated to occur via AhR-ARNT interaction with transcription sites other than the AhRE (Wright et al. 2017). Table 2-39 provides some examples of non-canonical AhR signaling pathways as reviewed by Denison et al. (2011).

Table 2-39. Examples of Non-canonical AhR Signaling

Signal pathway	Effects
AhR and ER crosstalk	• Binding of liganded AhR:ARNT complex to inhibitory DREs blocking gene activation by ER • Competitive sequestration of coactivators or DNA binding partners (p300, cAMP, CREB-binding protein, SRC1/2, ARNT) leading to repression of ER signaling • Direct binding of liganded AhR to ER leading to repression of ER signaling and ubiquitination/degradation of ER
Liganded AhR binding to hyperphosphorylated Rb	• Repression of cell cycle • Decreased cell proliferation
Interaction between AhR and E2F	• Recruitment of positive regulatory factors • Increased cell proliferation

Table 2-39. Examples of Non-canonical AhR Signaling

Signal pathway	Effects
AhR and NFkB crosstalk	• Binding to RelA dimer forming transcriptionally inactive dimer • Competition for coactivators • Binding to RelB forming transcriptionally active dimer • Alterations in immune and inflammatory responses
Opening plasma membrane calcium channels and inducing release of intracellular calcium via action on ryanodine receptors	• Rapid and sustained calcium influx into cells • Stimulation of protein kinase C activity and cAMP production • Enhanced transcriptional activity of ligand-activated AhR
Interaction with KLF6	• Induction of PAI1 • Induction of sustained p21Cip1 expression

Source: Denison et al. 2011

AhR = aryl hydrocarbon receptor; ARNT = aryl hydrocarbon receptor nuclear translocator gene; cAMP = cyclic AMP; CREB = cAMP response element-binding; DNA = deoxyribonucleic acid; DRE = dioxin-responsive element; E2F = E2 promotor-binding factor; ER = estrogen receptor; KLF6 = Kruppel-like factor 6; PAI1 = plasminogen activator inhibitor 1; SRC1/2 = steroid receptor coactivators 1/2

Species Differences. The AhR is present in essentially all tissues and is well conserved across species, with only a few amino acid differences in the ligand binding domain (LBD) (Denison et al. 2011; Xu et al. 2022). However, even small alterations in amino acid residues result in differing binding affinities for 2,3,7,8-TCDD. For example, when the Ala 375 residue in the mouse AhR LBD is replaced with Val (by mutation, or as seen in different mouse strains), binding affinity for 2,3,7,8-TCDD is reduced, and its toxicity is substantially decreased (Xu et al. 2022). Other species and strain differences have been identified in the AhR transactivation domain (TAD) and in the structure, distribution, location, and number of dioxin-responsive elements (Xu et al. 2022). Specifically, there appear to be important species differences in the C terminal region of the TAD, while the N terminal is well conserved across species (Wright et al. 2017; Xu et al. 2022). Xu et al. (2022) noted that the C terminus of the AhR TAD of humans is only 58% similar to that of mice. The Q-rich subdomain of the AhR TAD is an important determinant of AhR activation by TCDD, as shown by the observation that 2,3,7,8-TCDD LD_{50} values show a clear correlation with the number of glutamine residues in Q rich subdomain of the transactivation domain (Xu et al. 2022).

Variations in DNA sequences adjacent to the AhRE also contribute to species differences, as induction of Ah-responsive genes also depends on the presence of binding sites for coactivators or other transcription factors near the AhRE (Xu et al. 2022). As a result of these and potentially other variations, gene expression and physiological responses to 2,3,7,8-TCDD vary widely across species and strains. For

example, the number and spectrum of genes whose expression was up- or down-regulated by 2,3,7,8-TCDD differed markedly between mouse hepatocytes expressing the mouse Ahr and those expressing a human AhR (Denison et al. 2011). Furthermore, the potencies and targets of 2,3,7,8-TCDD toxicity differ by species and even by strain. The oral LD_{50} for 2,3,7,8-TCDD in hamsters is 5,000 µg/kg, ~5,000 times higher than the LD_{50} in guinea pigs (0.6–2.1 µg/kg) (Denison et al. 2011; Xu et al. 2022). Marked strain differences in lethality have also been demonstrated; the oral LD_{50} for 2,3,7,8-TCDD in Han/Wistar rats is >10,000 µg/kg while the LD_{50} in Long-Evans rats is only 17.7 µg/kg. Target organs also differ across species. Among the effects of acute-duration exposure to 2,3,7,8-TCDD, which include thymus, liver, nervous system, skin, and developmental effects, only thymic atrophy is consistently observed across all mammals (Xu et al. 2022). As an example, 2,3,7,8-TCDD is teratogenic in hamsters and rats, but not in guinea pigs (Xu et al. 2022).

Evidence from epidemiology studies and *in vitro* experiments suggests that humans may be less sensitive to the toxic effects of 2,3,7,8-TCDD than other mammals, due in part to the lower binding affinity of the human AhR compared with other mammals (Denison et al. 2011). For example, rat hepatocytes are 30 times more sensitive than human hepatocytes to 2,3,7,8-TCDD induction of CYP1A2 and 5 times more sensitive to induction of CYP1A1 (Xu et al. 2022). However, there also appears to be wide variability in the binding affinity of individual human AhR for 2,3,7,8-TCDD, as shown by experiments using human placental samples showing differences of more than 10-fold (Denison et al. 2011). The variability in AhR binding may help to explain why serum 2,3,7,8-TCDD levels in humans exposed to 2,3,7,8-TCDD in Seveso, Italy did not correlate with development of chloracne (Denison et al. 2011).

While the AhR is widely distributed in the body of mammals, there are tissue differences in levels of expression. The organs with the highest AhR expression are the liver, thymus, lung, kidney, spleen, and placenta (Wright et al. 2017).

Structure-Activity Relationships. Studies using *AhR* and *ARNT* knockout mice have demonstrated that these molecules are necessary for most, but not all, of 2,3,7,8-TCDD's toxic effects. 2,3,7,8-TCDD's remarkable potency for inducing AhR-mediated effects is attributed both to its relative affinity for the AhR as well as its stability. AhR ligands that are readily metabolized (for example, polycyclic aromatic hydrocarbons [PAHs]) remain in the cell only transiently because the induction of metabolic enzymes leads to their degradation. In contrast, 2,3,7,8-TCDD binding to AhR induces its persistent activation and leads to a wide spectrum of toxic effects (Denison et al. 2011).

While most of what is known about the mechanisms of CDD toxicity is from studies of 2,3,7,8-TCDD, there is abundant evidence that toxic effects of other CDDs are also mediated through AhR binding. For this reason, relative AhR binding has been used as one method to estimate the potency of other CDDs relative to 2,3,7,8-TCDD. To date, AhR binding affinity has been shown to correlate well with *in vivo* effects of CDDs on mortality, body weight loss, thymic atrophy, dermal effects, immunosuppression, and teratogenicity. TEFs used to estimate risks from CDDs other than 2,3,7,8-TCDD make use of relative AhR binding affinity in addition to relative potency estimates from *in vivo* data for a variety of endpoints (Ring et al. 2023). Section 2.1 provides a summary of existing TEF values for CDDs.

Exposure to TCDD has been shown to induce dose-dependent increases in neutrophils (the most abundant type of granulocyte) in the blood, peritoneal cavity, spleen, and lungs of mice (Kerkvliet 2009). In addition, TCDD alters the oxidative burst and cytolytic activity of neutrophils in a context-dependent fashion; under different circumstances, experiments have demonstrated suppression, enhancement, and absence of an effect of TCDD on this function (Kerkvliet 2009). Similarly, the cytolytic activity of NK cells after TCDD exposure varies from no response to either suppression or enhancement. The mechanisms by which TCDD affects neutrophils and NK cells are not known; however, several genes for neutrophil cytosolic factors and NK receptor subunits have AhRE sequences and may play a role (Kerkvliet 2009).

In mice exposed to TCDD, a decrease in dendritic cell counts in the spleen was shown to occur a week after exposure and *in vitro* studies showed that TCDD enhanced both maturation and apoptosis of dendritic cells (Kerkvliet 2009). The mechanisms for these effects may include altered expression of apoptotic genes or upstream signaling pathways. For example, *in vitro* data show that TCDD increased the expression of *Fadd*, a gene that mediates apoptosis and also suppressed NFkB signaling (Kerkvliet 2009).

In summary, the mechanisms and pathways by which TCDD modulates immune responses are complex and depend upon the physiological milieu in which the exposure occurs. Most of the data on immune mechanisms are from studies in mice, and there are well-known differences in the responses of various species to TCDD exposure, suggesting the need for studies in other species to better evaluate species differences in immune effects.