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ADDENDUM TO THE TOXICOLOGICAL PROFILE FOR CHLORINATED DIBENZO-p-DIOXINS (CDDs)

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ADDENDUM for CDDs

Supplement to the 1998 Toxicological Profile for Chlorinated Dibenzo-*p*-Dioxins (CDDs)

Background Statement

This addendum to the Toxicological Profile for Chlorinated Dibenzo-p-Dioxins supplements the profile that was released in 1998.

Toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). CERCLA mandates that the Administrator of ATSDR prepare toxicological profiles on substances on the CERCLA Priority List of Hazardous Substances and that the profiles be revised "no less often than once every three years". CERCLA further states that the Administrator will "establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" [Title 42, Chapter 103, Subchapter I, § 9604 (i)(1)(B)].

The purpose of this addendum is to provide, to the public and other federal, state, and local agencies a non-peer reviewed supplement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 1998.

Chapter numbers in this addendum coincide with the Toxicological Profile for Chlorinated Dibenzo-p-Dioxins (1998). This document should be used in conjunction with the profile. It does not replace the profile.

2. HEALTH EFFECTS

2.1 HUMAN STUDIES

2.1.1 Death

Mortality in 1,262 Operation Ranch Hand veterans (1982–1999) was contrasted with that in 19,078 comparison veterans in a follow-up study to Michalek et al.1990 (Ketchum and Michalek 2005). Mortality for all causes was borderline significantly increased (relative risk [RR] =1.15; p=0.06) and mortality from circulatory system diseases was significantly increased among enlisted ground crew workers (RR=1.7) who, according to the job description, had a high degree of skin contact with herbicides. However, cancer mortality risk was not increased. Dioxin levels were measured on a lipid weight basis in serum collected from veterans who completed the 1987 physical examination. Additional measurements were made in 1992 and 1997. For veterans whose dioxin levels were not measured in 1987, the subsequent measures were extrapolated back to 1987 by using a first-order kinetics model with a constant half-life of 7.6 years. The following categories were used in the study: background ≤10 ppt 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), initial low exposures ≤117.7 ppt TCDD, and initial high exposures >117.6 ppt TCDD.

Similarly, several other studies reported increased mortality following dioxin exposure linked to specific health effects. More complete descriptions of reported findings in the mortality studies are presented in the respective effect sections of the following text.

2.1.2 Systemic Effects

Cardiovascular Effects

Occupational Cohorts. Cardiovascular effects were reported in cohorts of factory workers. Atherosclerosis and vascular endothelial dysfunction was reported in a small cohort of workers more than 35 years following occupational exposure to herbicides in the Czech Republic (Pelclova et al. 2002, 2007; see Agency for Toxic Substances and Disease Registry 1998 for the original studies). This cohort was reported to be one of the highest chronically exposed cohort of workers, with an estimated body burden at the time of exposure ranging from 3,300 to 74,000 pg TCDD/g lipids.

A 6-year follow-up study was conducted on the original National Institute for Occupational Safety and Health (NIOSH) cohort of workers (see ATSDR 1998 for details on Fingerhut et al. 1991 and Sweeney et al. 1993) exposed to TCDD in occupational settings during production of phenoxy herbicide and chlorophenol (Steenland et al. 1999). Standardized mortality ratios (SMRs) for ischemic heart disease trend fell short of statistical significance (p=0.14). The SMR for the highest category was 1.28. However, internal analyses using Cox regression found statistically significant exposure-response trends. Only a small subset of workers (n=253) in the original cohort had dioxin serum levels analyzed. The mean TCDD serum level was back estimated to be 2,000 ppt (lipid adjusted) at the time of exposure (1960s–1983).

A total of 813 producers and 699 sprayers were classified as exposed to dioxin and phenoxy herbicides in a New Zealand study ('t Mannetje et al. 2005). In the production workers, the RRs for mortality from ischemic heart disease and all cardiovascular disease were 1.04 and 0.96, respectively. Interestingly, in the sprayers, the RRs were 0.49 and 0.52, respectively.

Vietnam Veterans' Cohorts. In the Ranch Hand veterans, the trend p-value of 0.07 was reported for all cardiovascular diseases (Ketchum and Michalek 2005). See Section 2.1.1 for study details. The odds ratios (ORs) for heart disease and hypertension were 1.52 and 1.32, respectively, in a large cohort of Vietnam veterans (Kang et al. 2006; see Table 3 for study details).

These studies support the association between dioxin exposure and cardiovascular disease (ischemic heart disease, in particular) reported previously in occupational cohorts (Agency for Toxic Substances and Disease Registry 1998) in studies by Flesch-Janys et al. (1995) and Vena et al. (1998). However, the major limitation of most of these studies is the lack of adjustment for confounding factors for cardiovascular diseases such as smoking, lack of exercise, diet, and alcohol consumption.

Information on studies regarding cardiovascular effects in humans is provided in Table 2-1.

Pulmonary Effects. Increased mortality (RR=2.53) from chronic obstructive pulmonary disease (7 cases in 723 exposed) was reported in a cohort of exposed individuals from zone A in the Seveso, Italy industrial accident (Consonni et al. 2008).

Hepatic Effects. Increased levels of β-lipoproteins, cholesterol, and triglycerides were associated with increased body burden of TCCD in workers from the Czech Republic who were exposed >30 years

prior to the examination (Pelclova et al. 2001). In another occupationally exposed cohort, increased chronic liver disease history was associated with higher exposures of chemical workers (former chloracne cases) (Neuberger et al. 1999). Compared to the controls, the γ-glutamyltransferase (γ-GGT), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT) enzyme levels were also significantly increased.

Statistically higher ORs for fatty liver and γ-GGT activity were found in subjects with higher serum chlorinated dibenzo-*p*-dioxin (CDD)/chlorinated dibenzofuran (CDF) levels (in toxic equivalents [TEQs]) and high body mass index in a case-control study in an environmental setting (Lee et al. 2006). Although the exposed cohort had a greater mean age (by 11 years) compared to the control group, and therefore a higher risk for fatty liver, a multivariant logistic regression model still found differences between the cohorts.

Information on studies regarding hepatic effects in humans is provided in Table 2-2.

Muscular and Skeletal Effects

Occupational Cohorts. A follow-up study was conducted in 13 workers exposed to TCDD occupationally in an herbicide production plant 30 years prior to the examination (Pelclova et al. 2001; see Agency for Toxic Substances and Disease Registry 1998 regarding the former investigation). The mean plasma level was 256 pg TCDD/g lipid (range: 14–760 pg/g lipid) in the follow-up study. Abnormal electromyography was observed in 23% of workers (a decrease from 38% incidence in the 1970s).

Seveso, Italy Cohorts. Twenty-five years after the Seveso, Italy incident (see Agency for Toxic Substances and Disease Registry 1998 for more details), 48 individuals from three exposed zones (R-, B-, and A-zones) and 65 controls were examined (Alaluusua et al. 2004). TCDD body burdens within a year of exposure ranged from 23 to 26,000 ng/kg serum lipids. Serum TCDD levels and corresponding responses were listed in three exposure groups with mean body burdens of 130, 383, and 1,830 ppt, for R-, B-, and A-zones, respectively. The controls had 15 ppt body burdens. 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. Clearly, a window of susceptibility exists in early childhood for the effect to manifest itself later in life. From other dental effects, 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, confirming a different (infectious) origin of the diseases. The incidence of all dental effects for the exposed groups was 10% (R-zone), 45% (B-zone), and 60% (A-zone). The reference group had a 26% incidence. The 383 ppt TCDD body burden is considered a lowest-observed-adverse-effect level (LOAEL) for the dental effects.

Dermal Effects. The toxicological profile (Agency for Toxic Substances and Disease Registry 1998) concluded that chloracne is the most commonly reported effect of TCDD exposure in humans. However, some data gaps were identified; specifically, differences in individual susceptibility needed further clarification.

Some insights may be inferred from a genetic polymorphism study in CYP1A1 and GSTM1 in human populations exposed to polychlorinated biphenyl (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 Agency for Toxic Substances and Disease Registry 1994 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 exposures (≤51 ppb PCB), GSTM1-null genotype was linked to dermal allergies in both CYP1A1 genotypic groups.

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 1993, 1997; see Agency for Toxic Substances and Disease Registry 1998 for details). 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; see Agency for Toxic Substances and Disease Registry 1998 for details) 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.

In summary, the studies clearly show the importance of chloracne as an indicator of exposure for TCDD and dioxin-like chemicals. Chloracne may persist 20–30 years postexposure. Interindividual differences in susceptibility do exist and may be linked to genetic polymorphism.

Endocrine Effects. Previous studies indicated that TCDD and other dioxin-like chemicals are endocrine disruptors (see Agency for Toxic Substances and Disease Registry 1998 for more details). The major concerns included potential alterations in glucose metabolism and in thyroid function. The latest studies further investigated these end points.

The OR for diabetes was 1.50 among those Vietnam veterans who sprayed herbicides (Kang et al. 2006). The multivariate-adjusted OR for diabetes among Vietnam veterans in the highest quartile of exposure compared to the lowest one was 1.71 (Longnecker and Michalek 2000). Lower odds were found after adjustments for serum triglycerides. In nondiabetic Vietnam veterans, mean (log) insulin levels were significantly higher in the high dioxin body burden category (Michalek et al. 1999a).

In environmentally exposed groups, diabetes was associated with higher levels of dioxins and dioxin-like chemicals in a case-based study (Fierens et al. 2003). However, the cases included only nine individuals. In individuals living in the vicinity of a Superfund hazardous wastes site, plasma insulin concentrations post glucose challenge were correlated with higher blood TCDD levels (Cranmer et al. 2000). The results suggest the possibility of insulin resistance in those affected. An overall increase in diabetes was found in the Seveso, Italy population exposed during an industrial accident (Bertazzi et al. 2001). The increase was particularly notable in women (RR=2.4). In an occupational setting, the prevalence of diabetes was not significantly different between the control group and the group of workers exposed in the production of 2,4,5-trichlorophenol >15 years prior to the study (Calvert et al. 1999). However, the highest exposures were correlated with increased serum glucose in those remaining after exclusion of treated diabetics. In addition, the highest exposures were also associated with a higher free thyroxine (FT4) index.

Increased thyroid stimulating hormone (TSH) means were reported at the 1985 and 1987 examinations of Vietnam veterans in the group that had TCDD body burdens >94 ppt (Pavuk et al. 2003). The exposed Air Force veterans were part of Operation Ranch Hand. The effects were not seen with low body burdens of TCDD. Similarly, multiple regression analysis failed to demonstrate a relationship between maternal serum level of dioxin-like chemicals and serum thyroid hormone levels in the general population (Foster et al. 2005).

Information on studies regarding endocrine effects in humans is provided in Table 2-3.

2.1.3 Immunological Effects

Increased odds of having lower counts of activated T cells were associated with TCDD exposure in a group of phenoxy herbicide workers exposed several years prior to the examination in two chemical plants (Halperin et al. 1998). Workers at both plants had potential exposure to other chemicals. The plants were part of a larger cohort (referred to as "the NIOSH cohort" in the literature) from 12 factories used for a mortality study in the past (see Agency for Toxic Substances and Disease Registry 1998 for details on Fingerhut et al. 1991).

Decreased IgG levels were reported in the Seveso residents 20 years following the industrial accident (Baccarelli et al. 2004) and in a small group of veterans exposed to Agent Orange in Vietnam (Kim et al. 2003). In contrast, no evidence of a consistent correlation between dioxin exposure and immune system alteration was found in Air Force veterans exposed to herbicides in Vietnam (Michalek et al. 1999b).

Information on studies regarding immunological effects in humans is provided in Table 2-4.

2.1.4 Neurological Effects

In 1965–1968, about 350 workers were accidentally exposed to TCDD during the production of trichlorophenoxyacetic acid-based herbicides in the former Czechoslovakia (Jirasek et al. 1976). Polyneuropathy and encephalopathy were reported in some workers in a 10-year follow-up (Pazderova-Vejlupkova et al. 1981) (see Agency for Toxic Substances and Disease Registry 1998 for details on the original studies). The estimated mean plasma concentration of TCDD at the time of exposure was about 5,000 pg/g of plasma lipids. The mean TCDD plasma concentration was 256 pg/g in 1996 (i.e., about 30 years postexposure). Abnormal electromyography (EMG), electrocardiography (ECG), and visual evoked potentials were observed in 23, 54, and 31% of exposed workers, respectively (Pelclova et al. 2001). However, only a small group of 13 workers was available for the examination. Abnormal electroencephalography (EEG) strongly correlated with plasma levels >200 pg/g. A low-voltage record with elevated beta activity was the most frequent observation. Neuropsychological impairments (such as decreased cognitive performance measured by Wechsler-Memory Scale tests) correlated with increased triglycerides and cholesterol levels. Most of the 12 former workers who had long-lasting chloracne experienced fatigue, headache, and sleeping and memory problems (Pelclova et al. 2002). Another follow-up of 15 workers was done 35 years postexposure (Urban et al. 2007). In 2003–2004, the mean

TCDD plasma concentration was 128 pg/g. Clinical signs of polyneuropathy (diminished sensation to touch and pain, diminished vibration sense, diminished or lost ankle and/or knee jerks) were observed in nine individuals and confirmed by nerve conduction velocity studies in three. Neurasthenic syndrome (headache, fatigue, emotional lability, memory disturbance) was present at eight individuals. EEG was abnormal in three cases and visual evoked potential was abnormal in five. Although the cohorts are very small, the results are suggestive of persistent neurological health effects of high-level TCDD exposure.

Workers with chloracne (159 cases) exposed during the production of herbicides in 1969–1975 in Austria were followed in several studies (see Agency for Toxic Substances and Disease Registry 1998 for details of Saracci et al. 1991 and Kogevinas et al. 1997). In 1996, a group of 50 workers from the original cohort and their matched controls were examined (Neuberger et al. 1999). Neurological symptoms such as sleep disturbance, headache, and neuralgia were reported in 44, 32, and 30% of individuals, respectively. A German study examined a group of workers from a plant producing 2,4,5-trichlorophenol herbicides (Thömke et al. 1999, 2002). Several individuals with known risk factors for the development of peripheral nerve problems such as diabetes, alcohol intake, isoniazid treatment, and cancer were excluded prior to the examination. In the final group, 35 workers had chloracne and 85 did not. Workers with chloracne had significantly higher exposure as illustrated by back-calculated lipid-adjusted dioxin levels. Workers with chloracne had significantly more frequent clinical signs of a sensory neuropathy restricted to the legs, more frequent neurophysiologic abnormalities, and lower mean amplitudes of the motor compound muscle potential of the peroneal nerve (Thömke et al. 1999). Dioxin exposure was not associated with cranial nerve dysfunction in these workers (Thömke et al. 2002). Changes in the brainstem auditory evoked potentials were observed more often in the chloracne workers, but did not reach statistical significance from the nonchloracne group. The authors concluded that a severe exposure to CDDs/CDFs is not followed by clinical signs of cranial nerve dysfunction.

In conclusion, as indicated by several studies, peripheral neuropathy (often of the lower extremities) is associated with high exposure to dioxins. The effects may persist for a long time after exposure.

Information on studies regarding neurological effects in humans is provided in Table 2-5.

2.1.5 Reproductive Effects

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). However, effects on the length of the menstrual cycle were recorded in female adults who were premenarcheal at the time of the initial exposure (Eskenazi et al. 2002b). The average age of the premenarcheal girls in 1976 was 6.8 years. The authors developed a regression model correlating the length of menstrual cycles to plasma TCDD concentrations from the initial exposure. The model estimated that the cycle length was 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, serum hormones) were evaluated in Seveso women, no clear evidence of TCDD-induced effects was observed (Warner et al. 2007).

TCDD-induced endometriosis was a topic discussed in several papers. The effect was originally reported in chronically exposed monkeys (Rier et al. 1993; see Agency for Toxic Substances and Disease Registry 1998 for details). In Seveso, 19 cases of endometriosis were identified (confirmed surgically or by ultrasound) in a cohort of 601 women (Eskenazi et al. 2002a). The relative risk ratios were 1.2 and 2.1 for the low- and high-dose groups, respectively. The risk of uterine leiomyoma (fibroids) associated with exposure to TCDD for women who resided near Seveso in 1976 was investigated in a group of 956 eligible individuals (Eskenazi et al. 2007). In total, about 26% of the women had confirmed fibroids. However, higher levels of serum TCDD were found to be associated with lower risk for the fibroid development.

Apart from the Seveso studies, there were several case-control studies in the general population. A significantly increased risk of deep endometriotic (adenomyotic) nodules (OR=3.3) for an increment of 10 pg TEQ/g lipids was reported in Belgium women (Heilier et al. 2005). The 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. An increased risk was also found for peritoneal endometriosis (OR=1.9) for total TEQ levels and for dioxins alone (OR=3.2). In contrast, other population-based, case-control studies reported no association between total TEQs of dioxins and endometriosis (De Filip et al. 2004; Fierens et al. 2003; Pauwels et al. 2001).

There was no association between $\log_{10}$ TCDD with spontaneous abortions (OR=0.8), low birth weight, or births that were small for gestational age in a retrospective cohort study of women exposed in the Seveso accident (Eskenazi et al. 2003). No association between total TEQs and low birth weight were

reported in newborns of mothers exposed to ambient air in the vicinity of an incinerator in Taiwan (Lin et al. 2006) or in newborns of fathers exposed occupationally (Lawson et al. 2004).

Body burden-related increases in time to pregnancy and infertility were observed among women living in Seveso at the time of the accident (Eskenazi et al. 2010). For every 10-fold increase in serum TCDD levels, there was a 25% increase in time to pregnancy and a doubling in the odds of infertility.

Changes in male/female sex ratio were reported previously in several studies (see Agency for Toxic Substances and Disease Registry 1998). In addition, in more recent studies, TCDD exposure of men was linked to a lowered male/female sex ratio in the offspring of a Seveso cohort (Mocarelli et al. 2000). According to the authors, the effect may persist for years after exposure. However, no changes in the sex ratio were reported following paternal occupational exposures during the Agent Orange production (Schnorr et al. 2001) or after parental exposures to ambient air around an incinerator (Lin et al. 2006).

Reproductive health of men was investigated in two studies. Herbicide production workers who had chloracne 2–20 years prior to the current study (n=35), complained significantly more often of sexual impotence (28.6% compared to 5.8%) (Thömke et al. 1999). Reproductive effects were recorded in men exposed as children during the Seveso incident (Mocarelli et al. 2008). Reductions in sperm concentration, percent progressive motility, total motile sperm count, and estradiol, and an increase in follicle-stimulating hormone (FSH) was found in a group (n=71) of men who were 1–9 years old in 1976 during the incident. Increased total sperm count, total motile sperm count, and FSH, and reduced estradiol was reported in a group (n=44) of men who were 10–17 years old at the time of the incident. No effects were observed in a group (n=20) exposed as adults.

Information on studies regarding reproductive effects in humans is provided in Table 2-6.

2.1.6 Developmental Effects

Developmental studies were done on several major population groups. One was the Seveso, Italy group where women were exposed environmentally in the aftermath of an industrial accident (Baccarelli et al. 2008; Eskenazi et al. 2003). The other groups were from the general population of Germany (Cao et al. 2008; Wilhelm et al. 2008), Japan (Nakajima et al. 2006), and Taiwan (Wang et al. 2005).

No association was found between TCDD exposure and low birth weight and small for gestational age category in the Seveso cohort (Eskenazi et al. 2003). Increased levels of TSH in newborns exposed to

TCDD *in utero* in the Seveso cohort indicated possible problems with regulation of thyroid hormone metabolism (Baccarelli et al. 2008). The authors reported that the mean TCDD levels correlated with TSH levels above or below 5 μU per mL serum. The 5 μU/mL standard is significant as it was established by the World Health Organization (WHO) as an indicator of potential thyroid problems in neonates. The authors noted that higher TCDD exposures across all three zones showed increased TSH concentrations. The group mean of 39 ppt TCDD was associated with TSH levels above the standard. Similarly, increased TSH levels were found to be correlated with increased CDDs and dioxin-like PCBs in the general population in Taiwan (Wang et al. 2005). In contrast, no changes in thyroid hormone levels were reported in the general population in Germany (Wilhelm et al. 2008). Decreased testosterone and estradiol levels in cord serum of female and male newborns, respectively, were reported in association with increased dioxin concentrations (Cao et al. 2008). Decreased sperm concentration and motility were observed in breastfed sons (aged 18–26 years at the time of examination) of women in the Seveso cohort (Mocarelli et al. 2010); however, no significant alterations were observed in formula-fed children.

In addition, no changes were found in the neurological development assessed by the neurological optimality score (at age 2 weeks and 12 months) and in the mental and motor development assessed by the Bayley Scales of Infant Development (at ages 12 and 24 months) (Wilhelm et al. 2008). The authors postulated that results of this study (sampling 2000–2002) reflected decreasing exposure to dioxins and PCBs in Europe over the last decade and compared their results with the Dutch cohort (sampling 1990–1992) (Koopman-Esseboom et al. 1996; see Agency for Toxic Substances and Disease Registry 1998 for details). However, some findings were still positive in the German cohort. Effects of prenatal exposure to PCBs and dioxins on mental and motor development were investigated in Japanese infants (Nakajima et al. 2006). One hundred thirty-four mother-infant pairs were involved in the study. Maternal blood was collected to be tested for CDDs, CDFs, and PCBs either in the last trimester of pregnancy or during delivery. The mean level of TEQs was 18.8 (4.0–51.2) pg/g blood lipids. The infants were tested at 6 months of age using Bayley Scales of Infant Development (BSID-II) to assess the mental developmental index (MDI) and psychomotor developmental index (PDI). Total TEQs were not associated with MDI or PDI, but 1,2,3,4,6,7,8-heptachloridibenzo-*p*-dioxin, total CDDs, and CDDs/CDFs were found to be negatively associated with MDI. No correlation was found with PCBs (coplanar or noncoplanar) levels. Postnatal exposure via breast milk was not evaluated in this study. However, approximately 58% of the infants were breastfed >3 months, which would suggest that the rest of the infants would potentially have lower body burden because of decrease in postnatal intake from contaminated breastmilk.

Information on selected studies regarding developmental effects in humans is provided in Table 2-7.

Breast Milk. Dioxin and dioxin-like chemicals are lipophilic, resistant to metabolic degradation, and have a tendency to bioaccumulate. Because of the high content of fat in human milk, dioxins are distributed to breast milk in considerable amounts (Agency for Toxic Substances and Disease Registry 1998). Several studies in the late 1990s attempted to link the background exposure to dioxins and adverse health effects in breast-fed infants. As summarized previously (Agency for Toxic Substances and Disease Registry 1998), some studies suggested a possible correlation between low environmental exposures and subtle effects in infants. Because of current studies limitations, dioxin studies via oral exposure designed to assess childhood susceptibility has been identified as Priority Data Needs (Agency for Toxic Substances and Disease Registry 2010).

Breast milk samples were collected from 240 Japanese mothers 30 days after delivery and tested for CDDs, CDFs, and coplanar PCBs (Tajimi et al. 2005). Overall, there was a slight negative correlation between the dioxin levels in breast milk and infant birth weights. However, some of the correlation coefficients were very low.

Health questionnaires regarding infectious and allergic diseases (n=175), white blood cell counts, and analyses of immunologic markers in lymphocytes (n=85) were used to investigate immunologic effects of background perinatal exposure to PCBs and dioxins in Dutch children (n=207) at 42 months (Weisglas-Kuperus et al. 2000) and at school age (n=167) (Weisglas-Kuperus et al. 2004). The studies were follow-ups to the Weisglas-Kuperus et al. (1995) study discussed in Agency for Toxic Substances and Disease Registry (1998). PCBs and dioxins were measured in maternal and cord plasma and in maternal milk in the original study. The authors confirmed the original observations that perinatal exposures to increased levels of PCBs and dioxins are associated with immunological effects and that the effects (especially susceptibility to infectious diseases) persist into childhood years. In contrast, no relationship was demonstrated between the postnatal dioxins exposure via breast feeding and the ratio of immunological markers and the level of humoral antibodies in 12-month-old Japanese infants (n=281 breastfed, n=20 bottle fed) (Kaneko et al. 2006). The authors compared the results with the Dutch study (Weisglas-Kuperus et al. 1995) and noted that the content of dioxins in breast milk in the Dutch study was almost 2-fold higher (30.75 pg TEQ/g lipids) than in their study (14.89/6.1 pg TEQ/g lipids). However, one of the explanations may be in the timing of the milk sampling. The Japanese study samples were collected later (30 days post partum).

Follow-up studies of the Dutch infant cohorts (Pluim et al. 1992, 1994; discussed in Agency for Toxic Substances and Disease Registry 1998) investigated lung function (ten Tusscher et al. 2001), liver function, and thyroid hormone levels (ten Tusscher et al. 2008) in association with perinatal exposure to dioxins in 7–12-year-old children. Spirometry results showed that decreased lung function in children (n=29) correlated with both pre- and postnatal (breast milk) exposures. In contrast, no relation was found between alanine transaminase (ALT) and aspartate aminotransferase (AST) enzymes or TSH and FT4 hormones and perinatal exposure to dioxins in the follow-up cohort (n=37). These results indicated that the abnormal readings reported in the cohort previously were transient and that the liver function and thyroid hormone levels get normalized. Infants in the breast-feeding (n=337) and bottle-feeding (n=53) cohorts were compared in Japan (Matsuura et al. 2001). Cohorts were obtained from several geographic areas with different breast milk levels of CDDs, CDFs, and co-PCBs. No differences in serum thyroxine (T4), triiodothyronine (T3), FT4, or TSH levels among the areas and no significant correlation between the mean serum levels of TSH and TEQ in breast milk were reported. There was no indication of thyroid effects.

Other examples of mixed exposure studies are the neurological and neurobehavioral longitudinal follow-up studies in Dutch cohorts (first reported in Koopman-Esseboom et al. 1996 and in Patandin et al. 1999). Prenatal exposure to PCBs (118, 138, 153, and 180) was measured in maternal and cord plasma; 17 CDDs and CDFs, 6 dioxin-like PCBs, and 20 nondioxin-like PCBs were analyzed in breast milk in the original study. In the follow-up, the McCarthy Scales of Children's Abilities were used to assess verbal skills, perceptual-performance, quantitative, memory, and motor skills in the 6.5-year-old children (Vreugdenhil et al. 2002a). The home environment was assessed and the verbal IQ of the primary parent was measured. However, only about one-half of the cohort was originally breast-fed (n=194), the other half was formula-fed (n=178). Differences in susceptibility to exposure-induced effects in the cognitive and motor areas were across both groups and were linked to less-than-optimal home environments. When the cohort was examined for the differences in play behavior of 7.5 year olds (n=158), higher prenatal dioxin levels were associated with more feminized play in both sexes (Vreugdenhil et al. 2002b).

Risk assessments for dioxins and dioxin-like chemicals in breast milk alone (Pohl et al. 1996) and in a mixture with other pollutants such as heavy metals and pesticides (Pohl et al. 2004) have been developed. Breastfeeding is widely recognized as offering the developing infant the benefits of balanced nutrition and passive immunity against microbial infections. The authors concluded that the benefits of breastfeeding outweigh concerns that these chemicals may have detrimental effects on the health and/or development of breastfed children in the general population.

2.1.7 Genotoxic Effects

Information on studies regarding genotoxic effects in humans is provided in Table 2-8. The studies do not provide conclusive data regarding dioxin genotoxicity.

2.1.8 Cancer

Limitations of many epidemiological studies in the past included among others few individual exposure data, short latency period, and small population size for certain cancer types. Although several follow-up studies emerged during the last decade (thus providing a longer latency period), many potentially confounding factors still persist. These include, for example, potential confounding by smoking, healthy worker effect, uncertainties of exposure assessment, and potential exposure to other occupational carcinogens.

NIOSH Cohorts. Cancer mortality was studied in a large cohort of workers (n=5,172) exposed to chemicals contaminated with TCDD in 12 U.S. factories (see Agency for Toxic Substances and Disease Registry 1998 for details on Fingerhut et al. 1991). Exposure was documented by reviewing job descriptions. Actual blood TCDD levels were measured in a small subcohort of workers. A follow-up study was done to include 6 additional years after exposure (Steenland et al. 1999). After some restrictions, the exposure-level subcohort included 3,538 workers and a subcohort of those who had chloracne included 608 workers. The SMR for all cancers combined was 1.13. However, an increasing trend for all cancers and lung cancer mortality was observed with increased exposure. The SMR for all cancers combined for the highest exposure group was 1.60.

Dutch Cohorts. Cause-specific mortality of Dutch chlorophenoxy herbicide workers exposed during 1955–1985 (factory A) or 1965–1986 (factory B) was reported first by Bueno de Mesquita et al. (1993) with a follow-up by Hooiveld et al. (1998) (see Agency for Toxic Substances and Disease Registry 1998 for more details). The third study of the cohorts extended the follow-up for an additional 15-year period up to the year 2006 (Boers et al. 2009, 2010). Mortality was investigated in male workers in factory A (539 exposed versus 482 unexposed) manufacturing predominantly 2,4,5-trichlorophenoxyacetic acid and in factory B (411 exposed versus 626 unexposed) manufacturing 4-chloro-2 methylphenoxyacetic acid, 4-chloro-2 methylphenoxy propanoic acid, and 2,4-dichlorophenoxyacetic acid. The exposure assessment was based on a job description and a small number of samples taken in 1993; TCDD levels were back-calculated to the time of exposure using the half-life of 7.1 years (Heederik et al. 1998). The highest

mean TCDD levels were found in the main production workers (608.2 ppt) versus low mean levels for the controls (7.6 ppt). Previously reported increased risk for mortality from non-Hodgkin lymphoma and respiratory cancers was not confirmed in the latest follow-up (Boers et al. 2010). However, slightly increased risk for all cancers was reported in factory A (hazard ratio [HR]=1.31) and B (HR=1.54). Increased risks for genital (HR=2.93) and urinary (HR=4.2) cancers were found in the exposed workers of factory A. The urinary cancers (bladder and kidney) were reported in 17 exposed versus two unexposed workers and prostatic cancer was reported in six exposed versus two unexposed workers.

New Zealand Cohorts. In New Zealand, workers were exposed to TCDD during the manufacturing of 2,4,5-trichlorophenol and 2,4,5-trichlorophenoxy acetic acid. Some of the workers were included in the international study (Kogevinas et al. 1997) discussed in the toxicological profile (Agency for Toxic Substances and Disease Registry 1998). A separate analysis of the New Plymouth workers was conducted later with a follow-up until the end of year 2000 (‘t Mannetje et al. 2005). The actual exposure occurred between 1969 and 1988; however, no data on exposure levels were available in the study. The authors found that the workers had increased total cancer mortality (43 observed versus 34.6 expected) and more multiple myeloma deaths than expected (3 observed versus 0.5 expected). Another investigation of the cohort was published within 4 years (McBride et al. 2009a). This investigation expanded the original cohort of exposed and control workers (>300 exposed individuals added), the follow-up was prolonged by 4 more years, and estimates of exposure based on samples of exposed and unexposed workers were back-calculated. Current TCDD levels were measured in 346 of the 1,599 workers (22%); 70% (241/346) were exposed in the sample (71% workers were exposed in the whole study population). The authors reported a possibly increasing relative risk with exposure level for all cancers combined; however, the two-sided p-value for this trend, either linear or quadratic, never reached significance. Another study in the plant included any workers (n=1,754) employed between 1969 and 2003 (McBride et al. 2009b). The all causes and all cancers SMRs were 0.97 and 1.1, respectively. Therefore, the mortality did not differ from the results reported for the rest of New Zealand.

Vietnam Veteran Cohorts. A case-control study was done in veterans who were diagnosed with prostate cancer (n=47) at the Veterans Affairs Medical Center (Giri et al. 2004). After adjusting for age and race, men with prostate cancer were approximately 2 times more likely to report previous exposure to Agent Orange (OR=2.06). Because data on actual exposure levels were not available, a number of studies relied on the self-reported exposure to Agent Orange as a measure of exposure. Veterans who received care in the Northern California Veterans Affairs Health System were categorized as exposed (n=6,214) or unexposed (n=6,930) to Agent Orange (Chamie et al. 2008). In the study, twice as many exposed men

were diagnosed with prostate cancer (OR=2.19), they developed the disease at a younger age, and they had a more aggressive variant of prostate cancer. In another study, an association between exposure to herbicides contaminated with TCDD and the development of prostate cancer was investigated in a cohort of U.S. Air Force veterans of Operation Ranch Hand (n=1,019) and controls who served in Southeast Asia (n=1,497) (Pavuk et al. 2006). The exposed veterans were categorized as "higher" and "lower" exposed based on their median 20-year cumulative TCDD level (body burden). Increased risk in the higher exposure group (RR=2.27) was reported; however, the numbers were small (n=15). In addition, a longer service in Southeast Asia was associated with increased risk of prostate cancer in comparison to veterans, indicating possible exposure to carcinogens other than TCDD.

Seveso, Italy Cohorts. Several follow-up studies of the general population exposed during the 1976 industrial accident in Seveso, Italy have been published during the last decade. These included studies on cancer mortality (Bertazzi et al. 2001; Consonni et al. 2008) and cancer incidence in this population (Pesatori et al. 2009). Combined "all cancer" mortality in the cohorts was not elevated in any of the studies or zones several years after exposure. RRs were as follows: 0.9 (0.6–1.3) in zone A and 1.1 (0.9–1.2) in zone B (Bertazzi et al. 2001), and 1.03 (0.76–1.34), again in zone A (Consonni et al. 2008). The small increase in lung cancer was not statistically significant in either zone, but mortality from rectal cancer was increased in zone B (RR=1.9) in the 1976–1996 follow-up (Bertazzi et al. 2001). Hodgkin's disease was increased in zone B (RR=3.5) and non-Hodgkin's lymphoma was increased in zone A (RR=3.3) (Bertazzi et al. 2001). However, the actual number of cases was too small, four and two cases in each respective category, to allow definitive conclusions. The findings by latency for zones A and B combined failed to reveal clear, definite, time-related mortality patterns. A mortality follow-up extension (1997–2001) reported increased RRs of 2.23 and 1.59 for zones A and B, respectively, in the category of lymphatic and hematopoietic tissue cancer (i.e., all related cancers pooled together) (Consonni et al. 2008). Concurrently with the mortality study, a cancer incidence study was conducted (Pesatori et al. 2009). This study confirmed an increased incidence of lymphatic and hematopoietic tissue neoplasms in zones A (RR=1.39) and B (RR=1.56).

A significantly increased HR (2.1) was reported for breast cancer in a cohort of 981 women who ranged in age from infants to 40 years old in 1976 at the time of the Seveso industrial accident and resided in the exposed area (Warner et al. 2002). No increase in "all cancer" mortality was found in women in the Seveso cohort.

Municipal Incinerator Cohorts. Whether exposure to low doses of environmental dioxins can lead to the development of cancer was investigated in a population living around a solid waste municipal incinerator in France. The incinerator was in service since 1971 and had two combustion chambers. In 1976, a third combustion chamber was opened. A geographic information system (GIS) investigation was performed to evaluate exposure to emissions of the surrounding population (Floret et al. 2003, 2006). Individuals living in the highest exposed zone were more likely to develop the non-Hodgkin lymphoma (OR=2.3). During the 16-year cancer study period (1980–1995), 80% of non-Hodgkin lymphoma developed within the last 6 years of the study (Floret 2003). A case-control study in women investigated the development of breast cancer in these cohorts (Viel et al. 2008). Among women <60 years old, no increased or decreased risk of breast cancer was found for any dioxin exposure category.

Information on studies regarding cancer effects in humans is provided in Table 2-9.

For the updated information regarding dioxin cancer risk assessment, see Chapter 7. With regard to the mechanism of carcinogenesis, the current view postulates that TCDD acts as both an initiator and a promoter (Portier et al. 1996).

2.2 ANIMAL STUDIES

2.2.2 Oral Exposure

Details regarding studies cited in this section are provided in Summary Tables (Table 2-10 to Table 2-14). For example, while the text in this section specifies the lowest-adverse-effect-levels (LOAELs) in a referenced study, the reader can learn more about higher doses tested in this study from the Summary Tables.

2.2.2.2 Systemic Effects

A large number of studies have been published since 1998 that examined the systemic toxicity of 2,3,7,8-TCDD following oral administration. Many of the studies involved acute exposure, confirmed the results of earlier studies, and examined the mechanism of action. Studies that provide new information or tested lower doses are discussed below and summarized in Table 2-10. In addition to acute-duration studies, several intermediate- and chronic-duration studies examining the toxicity of 2,3,7,8-TCDD were identified; these studies are also discussed below and summarized in Table 2-10. The discussion of systemic effects also includes summaries of the limited number of studies that examined the systemic toxicity of other CDD congeners.

Respiratory Effects. Increases in the incidence of alveolar epithelial metaplasia was observed 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.

Bronchiolar metaplasia of the alveolar epithelium was observed in the lungs of female Harlan Sprague-Dawley rats administered gavage doses of ≥0.003 µg/kg 2,3,7,8-TCDD 5 days/week for 2 years; histiocytic infiltration was observed at ≥0.022 µg/kg (NTP 2006). Bronchiolar metaplasia was also observed in a 0.1 µg/kg 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 0.1 µg/kg continuous exposure group (NTP 2006). No significant increases in respiratory tract lesions were observed in rats exposed to ≤0.1 µg/kg for 14 or 31 weeks (NTP 2006).

Cardiovascular Effects. Minimal to mild cardiomyopathy was observed in female Harlan Sprague-Dawley rats administered ≥0.01 µg/kg 2,3,7,8-TCDD in corn oil via gavage 5 days/week 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 0.1 µg/kg 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 0.1 µg/kg 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 0.1 µg/kg (Jokinen et al. 2003).

Significant increases in daytime and nighttime mean arterial blood pressure were observed in male C57BL/6 mice administered 0.18 µg/kg 2,3,7,8-TCDD via capsule, 5 days/week for 35 days (Kopf et al. 2010). The increases in blood pressure began on day 15 and plateaued at 25 days. 2,3,7,8-TCDD also increased acetylcholine-dependent vasorelaxation of the aortic rings, but did not affect the renin-angiotensin system, as evidenced by the lack of changes in angiotensin II levels, plasma angiotensin converting enzyme activity, or plasma renin activity. No cardiovascular effects were observed in CYP1A1 knockout mice, suggesting that hypertension and endothelial dysfunction are mediated by CYP1A1 induction.

Gastrointestinal Effects. Minimal to mild squamous hyperplasia of the forestomach was observed in female Harlan Sprague-Dawley rats receiving gavage doses of 0.1 µg/kg 2,3,7,8-TCDD 5 days/week for 2 years (NTP 2006). 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 (≥0.003 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.

Severe spaces in the villi in the proximal segment of the intestine were observed in male C57BL/6J mice administered a single dose of 100 µg/kg 2,3,7,8-TCDD and examined 10 days postexposure (Ishida et al. 2005). An increase in serum glucose was observed 10, 15, and 30 minutes after an oral glucose challenge; increases messenger ribonucleic acid (mRNA) levels for sodium-glucose co-transporter 1 and glucose transporter type 2 were also observed. These results suggest increased glucose absorption in response to 2,3,7,8-TCDD exposure. No alterations were observed in similarly exposed DBA/2J mice, suggesting that the mechanism for increased glucose absorption involves the Ah receptor (Ishida et al. 2005).

Hematological Effects. A single dose exposure to 0.4 µg/kg 2,3,7,8-TCDD resulted in a significant increase in hemoglobin levels 6 hours after dosing male Sprague-Dawley rats; however, at 1 or 7 days postexposure, an increase in hemoglobin was only observed at 40 µg/kg (Fletcher et al. 2005).

A dose-related decrease in plasma vitamin K-dependent coagulation factor VII was observed in female WAG/Rij rats administered a single dose of 0.096 µg/kg 2,3,7,8-TCDD and maintained on a vitamin K_3 deficient diet for 6–21 days; no effects were observed at 0.0096 µg/kg (Bouwman et al. 1999). Males were less sensitive than females, and no significant alterations in plasma factor VII levels were found.

Musculoskeletal Effects. 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). Cumulative exposure to 17 or 170 µg/kg over 20 weeks (0.12 or 1.2 µg/kg/day) resulted in discoloration, an opening of the pulp chamber to the lingual dental surface, and pulpal perforation to the lingual dental surface. 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.

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 pentachlorodibenzo-*p*-dioxin (PeCDD), hexachlorodibenzo-*p*-dioxin (HxCDD), or heptachlorodibenzo-*p*-dioxin (HpCDD), respectively. In Long-Evans rats, the ED_{50} values were 24, 130, or 630 µg/kg.

Hepatic Effects. Data published since 1998 support earlier studies that identify the liver as a sensitive target of 2,3,7,8-TCDD toxicity. Observed effects include hyperplasia, vacuolization, necrosis, alterations in fat metabolism, and alterations in vitamin A metabolism. Administration of single dose of ≥1 µg/kg to female ovariectomized C57BL/6 mice resulted in increases in the incidence of minimal to moderate cytoplasmic vacuolization in the periportal and midzonal regions of the liver within 24 hours of dosing (Boverhof et al. 2005). In a similarly designed study, hepatocellular necrosis was observed 24 hours after dosing with 300 µg/kg (Kopec et al. 2010). Another study by Kopec et al. (2008) reported cytoplasmic vacuolization and hepatocellular necrosis at 30 and 100 µg/kg 72 hours after exposure. In time course studies also conducted by Boverhof et al. (2005) and Kopec et al. (2008), gavage administration of 30 µg/kg once to female ovariectomized C57BL/6 mice resulted in cytoplasmic vacuolization in the periportal and midzonal regions within 18 hours of dosing; the severity and extent of the vacuolization increased at later time points (mice were examined until 168 hours after dosing). The vacuolization was due to lipid accumulation, particularly increased levels of triglycerides, free fatty acids, and cholesterol esters. Twenty-four hours after dosing, hepatocellular necrosis, increases in relative liver weight, and increases in serum alanine aminotransferase, free fatty acids, and triglyceride levels were observed; decreases in serum cholesterol levels were observed 72 hours after dosing. Gene expression analysis of liver tissues provided evidence of disruption of hepatic lipid uptake and metabolism leading to mobilization of peripheral fat and increased uptake of fatty acids by the liver.

In a similarly designed study, Boverhof et al. (2006) reported minimal to mild hepatocellular hypertrophy 24 hours after ovariectomized female Sprague-Dawley rats were administered 30 µg/kg 2,3,7,8-TCDD; no effects were observed at 10 µg/kg. In the time-course study, hepatocellular hypertrophy was observed in the centriacinar region of the liver beginning 24 hours after exposure to 10 µg/kg; the severity of the lesions increased with time after dosing. Significant increases in serum free fatty acids, triglycerides, and cholesterol were only observed 24 hours postexposure. Differences in the gene expression arrays were found between the rats and mice. Whereas the mouse-specific responses involved triglyceride and free fatty acid accumulation, the rat-specific responses involved cellular growth and lipid metabolism.

A biphasic alteration in serum cholesterol levels were observed in male Sprague-Dawley rats administered a single dose of 40 µg/kg 2,3,7,8-TCDD (Fletcher et al. 2005). Significant decreases in serum cholesterol levels were observed 6 hours after exposure; however, 1 and 7 days after exposure, there were significant increases in serum cholesterol levels. Increases in serum triglyceride levels were also observed 1 and 7 days postexposure and centrilobular hypertrophy was observed 7 days postexposure. Fletcher et al. (2005) also found broad changes in the expression of genes coding for cholesterol metabolism.

A 6-day exposure of male Sprague-Dawley rats to 10 µg/kg/day 2,3,7,8-TCDD resulted in increases in relative liver weight, increases in serum cholesterol and total bilirubin, decreases in serum alkaline phosphatase activity and serum triglyceride elvels, mild hepatocellular swelling and vacuolization, and inflammatory cell infiltration (Lu et al. 2010).

A series of studies conducted by Blackwell et al. (1998) found no alterations in serum cholesterol 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 2,3,7,8-TCDD. Similarly, repeated exposure to 0.0015 or 0.15 µg/kg/day 2,3,7,8-TCDD for 4, 8, or 12 weeks did not alter serum cholesterol levels. However, decreases in serum cholesterol levels (no statistical analyses of these data) were observed in mice on the diabetic diet administered ≥0.010 µg/kg/day for 16 weeks; no effects were observed in mice on the normal diet.

Hepatocyte hypertrophy was observed in female Harlan Sprague-Dawley rats administered 0.022 µg/kg 2,3,7,8-TCDD in corn oil 5 days/week for 14 or 31 weeks (NTP 2006); the incidence and severity of the lesion was dose-related. At 0.01 µg/kg for 31 weeks, there was a significant increase in the incidence of pigmentation. Diffuse fatty changes were observed at 0.1 µg/kg for 31 weeks.

Following chronic exposure (2 years), significant increases in the incidence of hepatocyte hypertrophy, inflammation, and oval cell hyperplasia were observed in female Harlan Sprague-Dawley rats administered ≥0.003 µg/kg 2,3,7,8-TCDD 5 days/week (NTP 2006). At the next highest dose (0.01 µg/kg), significant increases in the incidence of pigmentation, diffuse fatty changes, and necrosis were observed. Other hepatic effects included bile duct hyperplasia at ≥0.022 µg/kg, portal fibrosis at ≥0.046 µg/kg, and bile duct cysts at 0.1 µg/kg. To compare the severity of liver lesions across groups, NTP grouped all of the nonneoplastic liver changes together (referred to as toxic hepatopathy); the incidence of toxic hepatopathy was significantly increases at ≥0.01 µg/kg and the severity score increased

from 1.3 (minimal) at 0.01 µg/kg to 3.5 (moderate to marked) at 0.1 µg/kg. Significant increases in several types of neoplastic lesions were also present in animals exposed to 0.1 µg/kg. NTP (2006) also exposed groups of 50 female rats to 0.1 µg/kg for 30 weeks and then to vehicle for the remainder of the study. At the end of the 2 years, significant increases in hepatocyte hypertrophy, inflammation, pigmentation, diffuse fatty changes, and necrosis were observed, as compared to vehicle controls. The incidences of hypertrophy and diffuse fatty changes were significantly lower than in rats exposed to 0.1 µg/kg for 2 years.

Fletcher et al. (2001) compared 2,3,7,8-TCDD-induced reductions in hepatic vitamin A levels and vitamin A gain in four animal species 28 days after administration of a single gavage dose of 2,3,7,8-TCDD. In the study, the lowest doses associated with significant decreases in these parameters were observed at 0.12 µg/kg in Sprague-Dawley rats, 0.18 µg/kg in Hartley guinea pigs, and 1.6 µg/kg in C57BL/6 mice and Golden Syrian hamsters. Significant increases in relative liver weights were also observed at 0.12, 0.047, 8, and 1.6 µg/kg in the rats, guinea pigs, mice, and hamsters, respectively. Using model-based compartmental analysis, Kelley et al. (1998, 2000) concluded that exposure to 2,3,7,8-TCDD increases vitamin A mobilization from storage sites, which results in increases in vitamin A degradation, and that 2,3,7,8-TCDD did not affect the fractional uptake of plasma retinol. Hoegberg et al. (2003) and Schmidt et al. (2003) found significant decreases in retinyl esters levels and increases in all *trans*-retinoic acid levels in male Sprague-Dawley rats administered a single dose of 10 µg/kg and examined 1, 3, or 28 days after dosing. Increases in all-*trans*-retinoic acid were also observed in the liver (Schmidt et al. 2003). No alterations in serum or hepatic levels of all-*trans*-retinoic acid at 1 µg/kg 3 days after dosing were observed (Schmidt et al. 2003). Similarly, Yang et al. (2005) reported significant decreases in all-*trans*-retinol levels and increases in all-*trans*-retinoic acid levels in the liver of male C57BL/6 mice administered 0.1 µg/kg/day for 42 days. The investigators suggested that these alterations may be due to 2,3,7,8-TCDD-induced increases in hepatic retinal oxidase activity, which was also observed in the study.

An increase in the total number of nonmalignant liver lesions was observed in female Sprague-Dawley rats administered a single dose of 2.8 mg/kg 1,2,3,4,6,7,8-HpCDD (Rozman et al. 2005). The types of lesions included hepatitis, hyperplasia, dysplasia, bile duct proliferation, and necrosis; it is not known if all types of lesions were observed at all doses.

Renal Effects. Gavage administration of 10 µg/kg/day 2,3,7,8-TCDD for 12 days to male Sprague-Dawley rats resulted in damage to renal tubular cells, which was characterized as moderate swelling and

flattening of proximal tubular epithelial cells, tubular deformation and diminution, and tubulointerstitial congestion (Lu et al. 2009). Significant increases in serum creatinine and blood urea nitrogen (BUN) levels were also observed, indicating renal functional impairment.

NTP (2006) reported significant increases in the incidence of transitional epithelial hyperplasia in the kidneys of female Harlan Sprague-Dawley rats administered, via gavage, 0.046 or 0.1 µg/kg 2,3,7,8-TCDD 5 days/week for 2 years. Mild nephropathy was also observed in rats exposed to 0.1 µg/kg 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. No renal effects were observed in rats exposed to ≤0.1 µg/kg 5 days/week for 14 or 31 weeks (NTP 2006).

In male Sprague-Dawley rats administered a single gavage dose of 10 µg/kg and examined 1, 3, or 28 days after exposure, significant increases in renal all *trans*-retinoic acid levels were found; there was also an increase in retinyl esters levels, which was only observed 28 days postexposure (Hoegberg et al. 2003). An increase in lecithin retinol acyltransferase mRNA levels was also found in the kidney. An earlier study by this group (Fletcher et al. 2001) also found an increase in renal vitamin A levels in male Sprague-Dawley rats administered a single dose of 19 µg/kg (rats examined 28 days postexposure), but not in rats administered 100 µg/kg.

A variety of renal lesions that included glomerulonephritis, nephritis, nephropathy, hydronephrosis, and proteinuria were observed in female Sprague-Dawley rats administered a single dose of 1,2,3,4,6,7,8-HpCDD; the total incidence of lesions were increased at ≥3.4 mg/kg (Rozman et al. 2005).

Endocrine Effects. Significant decreases in serum total T4 levels were observed following a single dose administration of ≥10 µg/kg to male Long-Evans rats (Raasmaja et al. 1996) and ≥5 µg/kg 2,3,7,8-TCDD to male Sprague-Dawley rats (Viluksela et al. 2004). No significant alterations in serum T3 levels were observed in either strain. Time-course studies in which Sprague-Dawley rats were administered a single dose of 60 µg/kg showed that the serum thyronine levels plateaued at 5 minutes (Viluksela et al. 2004). Both studies examined the effect of 2,3,7,8-TCDD exposure on 5'-diodinase activity and found a decrease in type I iodothyronine diodinase activity in the liver and an increase in type II idothyronine diodinase activity in brown adipose tissue.

Using data from female Long-Evans rats administered 0.0001–10 µg/kg/day 2,3,7,8-TCDD in corn oil for 4 days, Crofton et al. (2005) estimated a 30% reduction in serum T4 levels (ED_{30}) at 0.15 µg/kg/day (95% confidence interval of 0.08–0.22 µg/kg/day).

Alterations in thyroid gland function have been reported in female Harlan Sprague-Dawley rats administered 2,3,7,8-TCDD in corn oil via gavage 5 days/week for 14, 31, or 53 weeks (NTP 2006). After 14 or 31 weeks of exposure, the effects included significant decreases in total and free thryoxine at 0.022 µg/kg and increases in total T3 and TSH (14 weeks only) at 0.046 µg/kg. Significant decreases in total T4 and increases in total T3 were also observed at 0.046 and 0.01 µg/kg, respectively, after 53 weeks of exposure. Follicular cell hypertrophy was also observed at ≥0.022 µg/kg after 14 weeks of exposure, ≥0.046 µg/kg after 31 weeks, and ≥0.022 µg/kg after 2 years.

NTP (2006; Tani et al. 2004) also found significant increases in the incidence of hyperplasia of the adrenal cortex in female rats administered ≥0.022 µg/kg 2,3,7,8-TCDD 5 days/week for 2 years; atrophy was observed at the highest dose tested (0.1 µg/kg). 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 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 adrenocortico-tropin-stimulated corticosterone secretion from the adrenal gland.

An ED_{30} for serum T4 levels (30% reduction in serum levels, as compared to controls) of 1.51 µg/kg/day (95% confidence interval of 1.10–1.92 µg/kg/day) was estimated in female Long-Evans rats administered 0.003–10 µg/kg/day PeCDD in corn oil for 4 days (Crofton et al. 2005).

Simanainen et al. (2002) estimated the ED_{50}-values for decreases in serum thyronine 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 PeCDD, HxCDD, and HpCDD, respectively. In Long-Evans rats, the ED_{50} values were 3.6, 21, and 47 µg/kg, respectively.

Dermal Effects. In hairless mice administered low doses of 2,3,7,8-TCDD (0.00003 µg/kg/day) for 54 days, a significant increase in scratching behavior was observed at the site of distilled water or acetone/olive oil application on the rostral part of the back (Ono et al. 2010). 2,3,7,8-TCDD alone (without application of water or acetone/olive oil) did not alter scratching behavior.

Metabolic Effects. 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 were 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. 2005); 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 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 in females 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, 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.

Other Systemic Effects. Intermediate-duration exposure to 0.1 µg/kg 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; Nyska et al. 2004); the lesions were observed after 31 weeks of exposure, but not after 14 weeks. A 2-year exposure to ≥0.046 µg/kg resulted in significant increases in the incidence of acinar cytoplasmic vacuolization (NTP 2006; Nyska et al. 2004). At 0.1 µg/kg, there were also significant increases in the incidence of chronic active inflammation and acinar atrophy.

2.2.2.3 Immunological Effects

The immune system is a sensitive target of 2,3,7,8-TCDD toxicity (Agency for Toxic Substances and Disease Registry 1998). Studies published since the toxicological profile for CDDs (Agency for Toxic Substances and Disease Registry 1998) confirm the results of older studies that 2,3,7,8-TCDD affects the primary and secondary immune organs and adaptive immune function and provides new mechanistic information. Selected studies are discussed in this section and summarized in Table 2-11.

Effects on Primary and Secondary Immune Organs. The effect of 2,3,7,8-TCDD exposure on the thymus has been well studied. Depletion of cortical lymphocytes in the thymus is an aryl hydrocarbon receptor (AhR)-dependent process and studies published since the toxicological profile (Agency for Toxic Substances and Disease Registry 1998) have identified adverse effects levels of ≥1.0 µg/kg in C57BL/6 mice following a single gavage exposure (Ao et al. 2009; Inouye et al. 2005) and 30 µg/kg in F344 rats

exposed to a single dose of 2,3,7,8-TCDD (Luebke et al. 1999). Related to this effect, significant decreases in thymus weight were observed in C57BL/6 mice administered ≥1.0 µg/kg (Ao et al. 2009; Inouye et al. 2005), B6C3F1 mice administered 1.0 µg/kg (Smialowicz et al. 1997), and Sprague-Dawley rats administered 1 µg/kg (Nohara et al. 2000). 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 ≥1.0 µg/kg; however, in 6-week-old C57BL/6 mice, exposure to 1.0 or 3.0 µg/kg resulted in decreased thymus weights, but did not alter the number of thymocytes. Similarly, a single-dose administration of 10 µg/kg to 12-week-old B6C3F1 mice resulted in decreases in 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, but no effect on cellular density (Huang and Koller 1999).

Similar to the effects observed in the thymus, acute exposure to 2,3,7,8-TCDD resulted in decreases in the number of lymphocytes in the spleen and decreases in spleen weight in C57BL/6 mice administered ≥1.0 µg/kg (Ito et al. 2002), B6C3F1 mice administered 1.0 µg/kg (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 ≤3.0 µg/kg. Age-related differences in 2,3,7,8-TCDD-induced effects in the spleen were also observed in B6C3F1 mice; decreases in the number 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).

Effects on Adaptive Immune Function. Several studies have found decreases in cytokine production by spleen cells in response to an antigen in 2,3,7,8-TCDD exposed mice. When C57BL/6 mice were immunized with ovalbumin immediately after 2,3,7,8-TCDD exposure, significant decreases in IL-5 levels in the spleen were observed at ≥0.3 µg/kg (Ao et al. 2009; Inouye et al. 2005; Ito et al. 2002). 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 in C57BL/6 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 decreases in IL-4 and IL-6 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, postimmunization. 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 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. 2001). 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/6 mice administered a single dose of 20 µg/kg 2,3,7,8-TCDD and ovalbumin (Inouye et al. 2003; Ito et al. 2002) and NC/Nga mice (animal model for atopic dermatitis) administered 20 µg/kg and dermally sensitized with picryl chloride prior to exposure (Ito et al. 2008). 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/6 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/6 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.

Acute exposure to 2,3,7,8-TCDD also suppressed the production of antigen-specific IgM and IgG1 in C57BL/6N mice administered 20 µg/kg and immunized with ovalbumin (Inouye et al. 2003). Unlike the control group, immunization with ovalbumin did not result in increases in ovalbumin-specific IgM and IgG1 production in the 2,3,7,8-TCDD exposed mice. Ito et al. (2002) also reported significantly lower antigen-specific IgG1 production in C57BL/6 mice administered ≥5 µg/kg and immunized with ovalbumin.

As reported previously, 2,3,7,8-TCDD exposure suppressed the primary antibody response to sheep red blood cells (Agency for Toxic Substances and Disease Registry 1998). Following an acute 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) were observed in B6C3F1 mice administered a single dose ≥1.0 µg/kg (Matulka et al. 1997; Smialowicz et al. 1997) or a 5-day exposure to 6 µg/kg/day (Kaplan et al. 2011).

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 ≥1.0 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. Similarly, intermediate-duration exposure resulted in a much lower adverse effect level; a significant decrease in the response to sheep red blood cells was observed in B6C3F1 mice administered 0.0015 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).

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 Con A or 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 Con A (Luebke et al. 1999); the investigators noted that these results were in contrast to the enhanced response to Con 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.

As discussed in the toxicological profile (Agency for Toxic Substances and Disease Registry 1998), 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 0, 0.005, 0.02, 0.10,

or 0.50 µg/kg 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 1.0 µg/kg. Although these results support the findings of Nohara et al. (2002), Vorderstrasse et al. (2003) cautions 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.

In addition to the increased mortality, Vorderstrasse et al. (2003) reported a number of other alterations in immunological end points. At ≥1.0 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 (≥5.0 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 re-infection. 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 23,7,8-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.

There are limited new data on immunological effects of other CDD congeners. 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. Stimulation with ovalbumin resulted in significant decreases in IL-5 levels in the spleen of mice exposed to ≥1.0 µg/kg 1,2,3,7,8-PeCDD (Ao et al. 2009).

2.2.2.5 Reproductive Effects

Details of selected reproductive studies are presented in Table 2-12. Recent studies have shown that acute- and intermediate-duration exposure results in significant decreases in sperm count and hormone levels in male rats. Daily sperm production and cauda epididymal sperm reserve were significantly reduced in wild-type rats lacking AhR-resistant alleles administered ≥10 µg/kg 2,3,7,8-TCDD (Simanainen et al. 2004a). Serum testosterone was decreased 60% in rats dosed with 30 µg/kg. Since rats with 2,3,7,8-TCDD-resistant alleles exhibited a similar decrease in serum testosterone, but the susceptible strain had a considerably more pronounced decrease in daily sperm production, the investigators suggested that the effect of TCDD on sperm numbers was not fully related to decreased serum testosterone. Epididymal sperm count was significantly reduced (approximately 11%) in Wistar rats dosed with 0.001 µg/kg/day 2,3,7,8-TCDD (the lowest dose tested) by gavage for 45 days; doses of 0.1 µg/kg/day induced a reduction of approximately 35% (Latchoumycandane et al. 2002a). A companion study found significant decreases in absolute and relative testes, epididymides, seminal

vesicle, and ventral prostate weights, but the reductions were <10% of control levels. Measurement of enzyme activities involved in oxidative stress in sperm showed significant reductions in superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase, and increased hydrogen peroxide and lipid peroxidation (Latchoumycandane et al. 2002b). Reduced activities of catalase and superoxide dismutase in epididymis were also reported in Wistar rats following administration of 0.1 μg/kg/day 2,3,7,8-TCDD (only dose tested) for 15 days (Dhanabalan et al. 2010). In that study, sperm count, viability, and motility were reduced by approximately 33, 12, and 10%, respectively, and there was a significant decrease in serum testosterone levels. Reduced sperm counts (21%) and decreases in serum testosterone levels were also reported in Sprague-Dawley rats dosed with ≥0.35 μg/kg 2,3,7,8-TCDD once per week (≥0.05 μg/kg/day) for 29 weeks (Ma et al. 2010). Serum levels of testosterone, FSH, and luteinizing hormone were all significantly reduced in rats dosed with 0.125 μg/kg/day (the highest dose tested). Analysis of protein expression profile in the testes showed several changes, some of which the investigators speculated might have been related to the decrease in sperm counts; others may have been associated with oxidative stress. Intermediate-duration gavage exposure (60 days) of Sprague-Dawley rats to 0.05 μg/kg/day resulted in significant decreases in relative testis, epididymis, seminal vesicles, and prostate weights and in sperm count and motility as well as an increase in sperm mortality and the occurrence of abnormalities (El Tawil and Elsaieed 2005).

Studies in females have found alterations in ovarian function, estradiol levels, and morphological changes in reproductive organs. Exposure of Sprague-Dawley rats to ≥0.14 μg/kg 2,3,7,8-TCDD once per week for 29 weeks (≥0.02 μg/kg/day) significantly decreased absolute and relative ovarian weight and serum estradiol levels (Chen et al. 2009). Analysis of proteins in ovarian tissue showed changes in protein levels that were consistent with acceleration of senescence and increased oxidative stress. Acute-duration exposure to 2,3,7,8-TCDD has been shown to inhibit ovulation. For example, cynomolgus monkeys examined over 1–2 years after receiving a single dose of 4 μg/kg of 2,3,7,8-TCDD during pregnancy showed histological evidence of anovulation, which was accompanied by significantly reduced levels of serum progesterone, but no significant alterations in serum estradiol levels, and the lack of menstrual cycles. No significant alterations in the ovaries were reported at 2 μg/kg (Morán et al. 2001). Decreased ovarian weight, ovulation rate, and number of ova released were reported in female Sprague-Dawley rats receiving a single gavage dose of ≥10 μg/kg (Jung et al. 2010; Petroff et al. 2000, 2002; Son et al. 1999; Ushinohama et al. 2001). Jung et al. (2010) suggested that 2,3,7,8-TCDD attenuated the progression of cell cycle by decreasing the levels of cell cycle regulators that are essential for the stimulatory action of FSH on cell cycle progression. Results from measurements of serum levels of hormones such as progesterone, estradiol, luteinizing hormone, FSH, and androstenedione in the studies mentioned above

were not always consistent between studies as they seemed to depend on the time they were measured after stimulation of follicular development.

No histological alterations were observed in the ovaries, uterus, or vagina of Sprague-Dawley rats administered up to 0.1 µg/kg/day 2,3,7,8-TCDD by gavage 5 days/week for 14, 31, or 53 weeks (NTP 2006). However, a 2-year exposure to 0.022 or 0.1 µg/kg 5 days/week resulted in a significant increase in the incidence of cystic dilatation of the clitoral gland ducts (NTP 2006). The incidence of atrophy of the ovaries was significantly reduced at 0.1 µg/kg/day, relative to controls. There were no significant histological alterations in the vagina at termination, but the incidence of squamous metaplasia in the uterus was significantly reduced; the investigators considered this finding of unclear significance. In rats administered 0.10 µg/kg 2,3,7,7-TCDD for 30 weeks followed by a 75-week recovery period, the incidence of uterine squamous metaplasia was significantly higher than rats administered 0.10 µg/kg for 2 years. The incidence of cystic dilatation in the 0.1 µg/kg stop exposure group was significantly lower than the 0.1 µg/kg continuous exposure group and was similar to the vehicle control group (NTP 2006). In cynomolgus monkeys examined 1.2–2.7 years after a single gavage dose of 1 µg/kg 2,3,7,8-TCDD, there was an increases in the incidence of squamous metaplasia in the endocervix (Scott et al. 2001). A single dose exposure to 1 or 3 µg/kg did not result in an increase in endometriotic lesion size in Sprague-Dawley rats or C57BL/6 mice, respectively, undergoing surgery to induce endometrosis (Cummings et al. 1999). However, prenatal exposure to 3 µg/kg (dams dosed on gestation day [GD] 8) followed by exposure to 3 or 10 µg/kg on postnatal day (PND) 77 resulted in an increase in endrometriotic lesions in mice (Cummings et al. 1999).

Suppression of mammary gland differentiation during pregnancy was reported in C57BL/6J mice administered a dose of 5 µg/kg 2,3,7,8-TCDD on GDs 0, 7, and 14 (approximately 1 µg/kg/day) and sacrificed on GD 9, 12, 17, or 19 (Vorderstrasse et al. 2004). Gland differentiation was significantly stunted in 2,3,7,8-TCDD-treated mice independent of the stage of pregnancy. Exposure to 2,3,7,8-TCDD significantly decreased serum progesterone on GD 17, appeared to decrease prolactin on GDs 17 and 19 (not significant), and marginally decreased estradiol ($p=0.09$) on GDs 17 and 19. Since suppressed gland differentiation was evident as early as on GD 9, the apparent decrease in blood hormones did not seem to be causally related to the gland effects. In contrast to the findings in pregnant mice, no alterations in mammary gland weight or morphology were observed in virgin mice administered two weekly doses of 5 µg/kg 2,3,7,8-TCDD (Vorderstrasse et al. 2004). A recent study that used mutant mice that expresses an AhR protein lacking the deoxyribonucleic acid (DNA) binding domain showed that defects in pregnancy-associated differentiation require AhR-DNA interactions (Lew et al. 2011). The study also

showed that activation of the AhR during pregnancy directly affects mammary tissue development both directly via mammary epithelial cells, and through changes in cells of the fat pad, and suggested that genes in mammary epithelial cells and stromal tissues are putative AhR targets.

Decreases in litter size, pre-implantation losses, abortions, and alterations in male:female ratio have also been reported in acute- and intermediate-duration studies. Increased time to first litter was reported in Siberian hamsters administered a single dose of 2 μg/kg 2,3,7,8-TCDD, but not 0.1 μg/kg (Yellon et al. 2000). Decreased litter size was observed in Holtzman rats following a single dose of 0.8 μg/kg on GD 15 with a no-observed-adverse-effect level (NOAEL) of 0.2 μg/kg (Nishimura et al. 2003). Increased preimplantation loss was reported in NIH mice dosed with ≥0.05 μg/kg/day 2,3,7,8-TCDD on GDs 1–3 and examined on GD 9 (Li et al. 2006). Similar results were obtained when the mice were dosed on GDs 1–8. The authors suggested that reduced levels of progesterone may have played a role in the pregnancy loss associated with early gestational exposure (Li et al. 2006). Furthermore, increased abortions (10 of 12) were observed in monkeys after a single gavage dose of 1 μg/kg (Guo et al. 1999, 2000). Maternal toxicity was noticeable in the study by Guo et al (2000) as evidenced by significant weight loss, poor appetite, hepatotoxicity, and facial and ocular changes. An intermediate-duration study in mink showed this species to be extremely sensitive to the effects of 2,3,7,8-TCDD (Hochstein et al. 2001). Female mink were exposed to 2,3,7,8-TCDD via the diet (approximately 0.00003, 0.0008, 0.003, 0.007, and 0.07 μg/kg/day) and were mated with untreated males 35 days after treatment started. No females in the 0.0008 and 0.07 μg/kg/day groups whelped.

Information is also available on the effects of 2,3,7,8-TCDD on the placenta. Administration of 0.8 or 1.6 μg/kg 2,3,7,8-TCDD to Holtzman rats on GD 15 increased fetal deaths on GD 20 but not on GD 16 (Ishimura et al. 2002b). Microscopic examination of the placenta as well as assays for glycogen showed that the glycogen content of the placentas from the 2,3,7,8-TCDD-exposed rats was higher than in controls. Reverse-transcription-polymerase chain reaction (RT-PCR) analysis showed that on GD 20, the level of expression of GLUT3 (a glucose transporter) mRNA was increased 2-fold in the treated rats compared to controls. GLUT3 expression was found in the labyrinth zone of the placenta where zone-specific expression of AhR and CYP1A1 mRNA induced by 2,3,7,8-TCDD were seen. The results suggested that 2,3,7,8-TCDD altered glucose kinetics in the placenta. In a companion paper, the investigators showed that the 2,3,7,8-TCDD-induced morphological alterations in the placenta correlated with an increase in a marker for hypoxia and suggested the possibility that the increased incidence in fetal death was due to placental hypoxia (Ishimura et al. 2002a). In a more recent study, Ishimura et al. (2006) reported that exposure to 2,3,7,8-TCDD suppresses the normal vascular remodeling that occurs in the

placenta during late gestation (GDs 16–20) by decreasing the number of apoptotic trophoblasts. A further study showed that Holtzman rats were more susceptible to the 2,3,7,8-TCDD-induced placental effects than Sprague-Dawley rats despite showing an identical sequence of placental AhR and very similar transcriptional activities of the AhR in terms of CYP1A1 induction (Kawakami et al. 2006). The latter results suggested that placental dysfunction and fetal death may be modulated by other factors in the genetic background of the two rat strains. Recently, Ding et al. (2010) showed that in C57BL/6 mice, developmental exposure of either parent (male or female F1 born to treated F0 females) was associated with preterm birth of F2 due to altered progesterone expression and placental inflammation.

Mixed results have been reported in studies examining sex ratio in the progeny of 2,3,7,8-TCDD-exposed animals. Ikeda et al. (2005a) exposed female Holtzman rats to an estimated mean dose of 0.02 μg/kg/day for several weeks before mating with untreated males; exposure continued during gestation and lactation. Sex ratio of fetuses examined on GD 20 or of newborns examined on PND 2 was not significantly different from controls. Rowlands et al. (2006) re-examined data from the 3-generation dietary study in Sprague-Dawley rats conducted by Murray et al. (1979), which had available (but did not publish) data on sex ratio. The diet provided doses of up to 0.1 μg/kg/day 2,3,7,8-TCDD to males and females. The re-analysis found no statistically significant treatment-related changes on PND 1 sex ratio in any generation of treated rats. Administration of up to 0. 2 μg/kg 2,3,7,8-TCDD to Sprague-Dawley rats on GD 15 did not affect sex ratio in F1 pups; the day the sex ratio was determined was not specified (Rebourcet et al. 2010). Similarly, exposure of Holtzman rats to up to 0.8 μg/kg 2,3,7,8-TCDD on GD 15 did not alter sex ratio in the offspring, but the day this was examined was not specified (Nishimura et al. 2003). Exposure of Wistar (Han) rats to up to 1 μg/kg 2,3,7,8-TCDD on GD 15 by gavage or up to 0.046 μg/kg/day in the diet for weeks before mating and during gestation did not significantly alter the sex ratio determined on PND 1 (Bell et al. 2007a, 2007b). A study of ICR male mice exposed to approximately mean concentrations of 0.0001 or 0.1 μg/kg/day 2,3,7,8-TCDD by gavage for several weeks before mating with untreated females found a significant reduction in male:female ratio of newborns on PND 0 (Ishihara et al. 2007). In a more recent study, Ishihara et al. (2010) examined the Y-bearing/X-bearing sperm ratio as well as the sex ratio of two-cell embryos of exposed male ICR mice to investigate when the sex ratio of the offspring decreases. The results showed that the Y-bearing/X-bearing sperm ratio was not significantly decreased in the 2,3,7,8-TCDD group. However, the sex ratio of the two-cell embryos of the 2,3,7,8-TCDD group was significantly lower than in the control group. The investigators noted that this may have been the result of decrease in fertility of Y-bearing sperm and suggested that the sex ratio of the offspring was decreased at fertilization and not during the spermatozoa stage. Rowlands et al. (2006) suggested that the inconsistencies between studies are likely due to random variation associated with

relatively small sample size, but differences between animal species and strains, dose regime, and day of evaluation of sex ratio may play a role.

No new studies examining the reproductive effects following oral exposure to other CDD congeners were identified.

2.2.2.6 Developmental Effects

The literature on developmental effects of 2,3,7,8-TCDD published after the toxicological profile for CDDs (Agency for Toxic Substances and Disease Registry 1998) is extensive; thus, mention of every published study is impossible. Therefore, the summary below includes representative examples with emphasis on low-dose studies that could help construct dose-response relationships and determine points of departure 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. Details of selected studies are presented in Table 2-13.

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 CRL:WI (Han) rats exposed to 1 μg/kg on GD 15 (Bell et al. 2007a), in Holtzman rats dosed with 1.6 μg/kg on GD 15 (Ishimura et al. 2002b) or 1.5 μg/kg on GD 10 (Kransler et al. 2009), and also in a rat strain with no TCDD-resistant alleles dosed with 1 μg/kg on GD 15 (Miettinen et al. 2006). 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); no significant lethality was reported at 0.15 μg/kg.

Decreased survival in offspring has also been reported in longer-term studies. Dietary exposure of female CRL:WI(Han) 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. Significantly reduced neonatal survival was reported in pups from C57BL/6J mice following a cumulative maternal dose of 10.5 μg/kg 2,3,7,8-TCDD over a 23-day period (doses were administered on GDs 0, 7, and 14, and PND 2) (Vorderstrasse et al. 2006). A study in mink 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 Abnomalies. Studies that have been published since the toxicological profile for CDDs support the findings of older studies that acute oral perinatal exposure to 2,3,7,8-TCDD results in increases in the incidence of cleft palate at maternal doses of ≥10 μg/kg (Bryant et al. 2001; Kransler et al. 2007; Mimura et al. 1997; Yamada et al. 2006) and kidney anomalies (primarily hydronephrosis) at ≥1 μg/kg (Aragon et al. 2008a; Bryant et al. 2001; Kransler et al. 2007; Miettinen et al. 2004; Mimura et al. 1997; Nishimura et al. 2006). Renal hydronephrosis was also observed in 90% of male offspring exposed to 6 μg/kg 2,3,7,8-TCDD on GD 14.5 and examined at 3.5 months of age (Aragon et al. 2008a). 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 Ah receptor 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 1 μg/kg on GD 15 (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). Administration of ≥3 μg/kg 2,3,7,8-TCDD to Holtzman rats on GD 10 resulted in significantly reduced fetal weight on GD 20; no significant effect was reported at 1.5 μg/kg (Kransler et al. 2007). 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, 30, and 60 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). Doses of up to 1.5 μg/kg 2,3,7,8-TCDD given to pregnant Hartley guinea pigs on GD 14 did not affect fetal weight on GD 56 (Kransler et al. 2007).

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. 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 Arnt mRNA and protein in the developing alveolar and bronchiolar epithelium.

Heart abnormalities have been reported in mice following perinatal exposure to 2,3,7,8-TCDD. 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 of ≥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 of ≥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 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). More recent 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).

Perinatal exposure to 2,3,7,8-TCDD can also affect bone and teeth development. A study in a strain of rat with no TCDD-resistant alleles 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 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. 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.

Consist with the older literature, Kransler et al. (2007) reported intestinal hemorrhage in fetuses from Holtzman rats examined on GD 20 following administration of a single dose of ≥1.5 μg/kg 2,3,7,8-TCDD to the dams on GD 10.

Impaired Thyroid Function and Metabolic Effects. 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 PNDs 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. 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 follow-up study to Gordon et al. (1995) 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. 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.

Older studies have reported thymic atrophy in the offspring of 2,3,7,8-TCDD exposed rats exposed to ≥5 μg/kg (Agency for Toxic Substances and Disease Registry 1998). A significant decrease in absolute thymus weight was reported in 24-week-old offspring from C57BL/6 mice administered a single dose of ≥2.5 μg/kg 2,3,7,8-TCDD on GD 12 (Mustafa et al. 2008). Female offspring also showed a significant decrease in thymic cellularity at 2.5 μg/kg. Neither absolute thymus nor spleen weight or thymic cellularity were significantly altered in male offspring from Holtzman rats dosed on GD 15 with up to 0.8 μg/kg 2,3,7,8-TCDD and examined on PND 21, 49, or 120 (Nohara et al. 2000). 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 phenotypes, 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 in 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 responses to infective agents in offspring from dams exposed perinatally to 2,3,7,8-TCDD. For example, exposure of C57BL/6Nji 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). Exposure of C57BL/6J mice on GDs 0, 1, and 14 and PND 2 to a time-weighted average 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 time-weighted average dose of 0.2 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 old) (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.

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 (only dose tested). 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 (only dose tested) 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.

Doses of up to 1 μg/kg 2,3,7,8-TCDD given on GD 15 to CRL:WI(Han) rats did not cause treatment-related alterations in tests of learning ability, 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 0.046 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). 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 point of departure, 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 pups (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. Hojo et al. (2004) also found a dose-specific response rate in an early study. Exposure to 2,3,7,8-TCDD on GD 15 resulted in a significant decreases in response rate at

0.05 µg/kg and an increase in response rate at 0.2 µg/kg; no significant difference in response rate was observed at 0.8 µg/kg. Similarly, Kakeyama et al. (2007) reported anxiety-like behavior in male offspring of Long-Evans rats administered 0.2 µg/kg during pregnancy (no additional information provided); however, this effect was not observed at 0.8 µg/kg. The results of a more recent 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 3.0 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). 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 (only dose tested) 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 receptors subunits.

Impaired Development and Functional Alterations of the Reproductive System. A number of studies have found impaired development of the reproductive system in male and female animals exposed to 2,3,7,8-TCDD during gestation. Effects have been observed in male and female offspring. Consistent with older studies (Agency for Toxic Substances and Disease Registry 1998), 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). Exposure to 2,3,7,8-TCDD resulted in 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. In addition, 2,3,7,8-TCDD 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). Examination of the offspring after sexual maturity revealed acceleration of corpora lutea and ovulation at 0.2 µg/kg (Kakeyama et al. 2008), delayed vaginal opening and decreased time to first estrous at 0.8 µg/kg (Kakeyama et al. 2008), a decrease in the amount of time spent in estrous at 1 µg/kg (Salisbury

and Marcinkiewicz 2002), and decreased ovulation rate and serum levels of estradiol at 2.5 µg/kg (Salisbury and Marcinkiewicz 2002). Exposure to 1 or 3 µg/kg on GD 8 followed by surgically induced endometrosis 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 PND 77.

Studies have shown that 2,3,7,8-TCDD can have transgenerational effects as illustrated by the following examples. Female Syrian hamsters born to dams treated with a single dose of 2 µg/kg 2,3,7,8-TCDD on GD 11.5 and mated with untreated males on PND 152–166 had a pregnancy rate of 79% compared to 100% in controls (Wolf et al. 1999). In addition, 38% of pregnant F1 females died before term vs. 5% in controls and those that delivered had smaller litter size. Survival of F2 to weaning was drastically reduced from 78% in controls to 15% in treated groups. Necropsy of exposed F1 females showed reduced weight of the liver, reproductive tract (uterus, cervix, and complete vagina), and ovaries and malformations of the external genitalia including a mild form of hypospadias, cleft phallus, and a decrease in the urethral-vaginal distance. In a study in C57BL/6 mice, dams were treated on GD 15.5 with 10 µg/kg 2,3,7,8-TCDD and produced the F1 generation, which was exposed *in utero* and through lactation (Bruner-Tan and Osteen 2010). Mating of F1 females with untreated males at the age of 10–12 weeks old resulted in a 44% pregnancy rate compared with 100% in unexposed F1 females. Delivery usually occurred early resulting in a high rate of offspring mortality. Mating of F2 females and F3 females with untreated males also resulted in a significant decrease in pregnancy rates. Complete infertility was observed in a subgroup of F1 females also exposed to 10 µg/kg gavage doses of 2,3,7,8-TCDD at 4 (prepubertal exposure) and 9 (pubertal exposure) weeks of age (Bruner-Tan and Osteen 2010).

2,3,7,8-TCDD has been shown to affect the rat pre-implantation embryo. Hutt et al. (2008) administered pregnant Sprague-Dawley rats 0.05 µg/kg 2,3,7,8-TCDD on GDs 7 and 14 and PNDs 7 and 14 then exposed the F1 female offspring directly to the same dose until 3 months of age. At this time, F1 females were mated to untreated males. Examination of post-coitum day 4.5 F2 embryos showed that neither ovulation, fertilization efficiency, or embryonic survival were affected by 2,3,7,8-TCDD. However, 2,3,7,8-TCDD significantly reduced the percentage of normal embryos (79.5% in controls vs. 36.6% in treated). 2,3,7,8-TCDD altered compaction-stage embryonic development, but did not prevent survival to the blastocyst stage. Specific alterations observed included formation of monopolar spindle, errors in chromosome segregation, and fragmentation resulting from aberrant cytokinesis. Similar observations

were made on day 4.5 or 5.5 post-mating embryos of Sprague-Dawley rats given a single dose of 0.05 μg/kg 2,3,7,8-TCDD on the evening of proestrus (Hutt et al. 2008). In a more recent study, it was reported that misregulation of centrosome number and localization may play a role to errors in chromosome segregation and cytokinesis following maternal exposure to 2,3,7,8-TCDD (Hutt et al. 2010).

Results from recent intermediate-duration exposure studies suggest that perinatal exposure to 2,3,7,8-TCDD followed by direct exposure of the female offspring until the age of 8–11 months affect ovarian function in offspring leading to premature reproductive senescence. Disruption of estrous cyclity was reported in Sprague-Dawley rats treated with ≥0.05 μg/kg 2,3,7,8-TCDD once per week (≥0.007 μg/kg/day) for ≥6 months, but not for shorter exposure durations (Franczak et al. 2006). No significant alterations in cyclicity occurred at 0.2 μg/kg once per week (0.029 μg/kg/day). Accelerated onset of reproductive senescence was reported in the same study in rats that received a single dose of 10 μg/kg 2,3,7,8-TCDD (only dose tested) on PND 29 (Franczak et al. 2006); this effect appeared to result from a combination of delayed puberty and a premature onset of abnormal cyclicity. Exposure to 2,3,7,8-TCDD did not significantly alter the number and size distribution of ovarian follicles or the number of corpora lutea, but significantly decreased serum concentration of estradiol (dose-related); the low dose significantly increased serum FSH (Franczak et al. 2006). In other studies in Sprague-Dawley rats that used the same exposure protocol, direct exposure to ≥0.05 μg/kg/week (0.007 μg/kg/day) after perinatal exposure delayed the age at vaginal opening and accelerated the loss of normal reproductive cyclicity with age without depletion of follicular reserves (Shi et al. 2007; Valdez et al. 2009). Doses of ≥0.005 μg/kg/week (0.0007 μg/kg/day) significantly reduced proestrus serum estradiol levels, but there were no significant effects on serum progesterone, FSH, or luteinizing hormone levels. Since exposure to 2,3,7,8-TCDD did not affect the responsiveness of the pituitary gland to GnRH, the authors suggested that 2,3,7,8-TCDD exerted direct effects on ovarian function. The studies also showed that exposure to 2,3,7,8-TCDD significantly increased the expression of 19 genes and decreased the expression of 31 genes in the ovary. Gene expression of 17α-hydroxylase decreased after treatment with 2,3,7,8-TCDD, suggesting that the decrease in estradiol biosynthesis may have been a consequence of decrease substrate. In a subsequent study, the investigators intended to identify the portion of the reproductive axis (ovary vs. hypothalamus and pituitary gland) by using an ovary transplantation model (Jablonska et al. 2010). The results showed a preferential acceleration of acyclicity with age following gestational and lactational exposure of the ovary to 2,3,7,8-TCDD.

Strain-specific differences on the effect of *in utero* 2,3,7,8-TCDD exposure on hormone levels have been reported in male offspring. Significant increases in plasma testosterone levels were observed in male fetuses of Han/Wistar rats exposed to 0.5 µg/kg 2,3,7,8-TCDD on GD 13.5 and examined on GD 19.5 (Haavisto et al. 2001); at 1.0 µg/kg, increases in pituitary luteinizing hormone levels were also increased. However, no changes in testicular or plasma testosterone levels or pituitary luteinizing hormone levels were observed in male fetuses of Long-Evans rats administered 1.0 µg/kg on GD 13.5 (Haavisto et al. 2001); serum levels of testosterone, FSH, or luteinizing hormone levels of male pups (examined on PND 14) of Sprague-Dawley rats administered 1 µg/kg on GD 13 (Haavisto et al. 2006); or testicular testosterone or 4-androstenedione levels of male pups of Sprague-Dawley rats administered 0.2 µg/kg on GD 15 (Rebourcet et al. 2010). Additional effects on androgenic status (androgen concentrations and androgen-dependent structures and functions) have been observed, including anogenital distance (Jin et al. 2010; Ohsako et al. 2001, 2002; Simanainen et al. 2004b), decreased prostate weight (Ko et al. 2002; Lin et al. 2002b; Ohsako et al. 2002; Simanainen et al. 2004b), decreased seminal vesicle weight (Hamm et al. 2000), decreased testicular testosterone and plasma luteinizing hormone levels (Adamsson et al. 2008), and prostatic bud formation (Abbott et al. 2003; Allgeier et al. 2009; Ko et al. 2002). A decrease in anogenital distance was observed in male pups of Sprague-Dawley rats (Ohsako et al. 2002) or Line C rats (a rat strain with no TCDD-resistant alleles) (Simanainen et al. 2004b) administered 1 µg/kg on GD 15. Cross-foster studies in C57BL/6J mice aimed at determining a critical window of vulnerability for effects of 2,3,7,8-TCDD on prostate and seminal vesicle development showed that the reduction in ventral prostate weight, measured on PND 35, was due primarily to *in utero* exposure and that exposure during GDs 13–16 was the most sensitive time (Lin et al. 2002b). Similar findings regarding a critical exposure period for development of the ventral prostate were reported by Ohsako et al. (2002). In the Lin et al. (2002b) study, relative seminal vesicle weight was practically unaffected by gestational or lactational exposures alone, but combined exposures significantly reduced the weight regardless of the day of exposure (GD 13 or 16). Another study of seminal vesicle development showed that reduction in weight was not significant until PND 32 following exposure on GD 15 (Hamm et al. 2000). Using AhR knockout mice, it was also shown that the AhR is necessary for normal dorsolateral prostate, anterior prostate, and seminal vesicle development, but apparently not for ventral prostate development (Lin et al. 2002a). The results of a study in three strains of rats with different sensitivity to 2,3,7,8-TCDD suggested that until PND 49, the reproductive system of the three rat strains was similarly affected by gestational and lactational 2,3,7,8-TCDD exposure (Simanainen et al. 2004b). While the sensitivity of developing accessory sex organs was not modified by the resistance AhR alleles, AhR type appeared to play a role in the sensitivity to 2,3,7,8-TCDD-induced decreases in sperm parameters. A study of the influence of null expression of epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) in the fetal

mouse on prostatic bud formation and on the response to 2,3,7,8-TCDD suggested that both are needed for the formation of prostatic buds and that their role may differ by region (Abbott et al. 2003). A recent study showed that estrogen signaling is not needed for 2,3,7,8-TCDD to inhibit prostatic epithelial budding (Allgeier et al. 2009).

Significant decreases in daily sperm production and decreased cauda epididymal sperm reserves were observed in pups of Line C rats administered 1 µg/kg 2,3,7,8-TCDD on GD 15 and examined on PND 70 (Simanainen et al. 2004b). Rebourcet et al. (2010) did not find significant alterations in sperm count in the caput, corpus, or cauda epididymis or daily sperm production in 67- or 145-day-old offspring of Sprague-Dawley rats administered doses up to 0.2 µg/kg 2,3,7,8-TCDD on GD 15. Similar results were reported by Ohsako et al. (2001) regarding no effect on daily sperm production in 49- and 120-day-old offspring from Holtzman rats administered up to 0.8 µg/kg 2,3,7,8-TCDD on GD 15. Offspring of CRL:WI (Han) rats exposed to up to 1 µg/kg 2,3,7,8-TCDD on GD 15 did not show alterations in total epididymal sperm count on PND 70, but mean path velocity and straight line velocity were significantly reduced (Bell et al. 2007a). A significant decrease in sperm count on PND 60 was also observed in C57BL/6 mice exposed to 1 µg/kg/day for 4 days during lactation (Jin et al. 2010). Mating of male rats exposed in *in utero* to 0.2 µg/kg (Ohsako et al. 2001; Rebourcet et al. 2010) or 1 µg/kg (Bell et al. 2007a) with untreated females did not significantly affect mating, fertility, litter size, or sex ratio. In contrast, a significant decrease in male/female ratio was observed in the F2 offspring of male Holtzman rats exposed during gestation and lactation (dams were also exposed prior to mating and during mating) to a time-weighted average dose of 0.02 µg/kg/day (Ikeda et al. 2005b).

Two studies have reported alterations indicative of demasculinzation of male offspring and masculization of female offspring. In a study by Ikeda et al. (2002a), an increased preference for saccharin consumption and decreased aromatase activity in the brain were found in male pups of Holtzman rats administered 0.2 µg/kg 2,3,7,8-TCDD on GD 15. Masculinization of female offspring was reported in a study that also used the saccharin preference test (Amin et al. 2000). In this study, exposure of Sprague-Dawley rats to 0.025 or 0.1 µg/kg/day 2,3,7,8-TCDD on GDs 10–16 significantly decreased saccharin consumption and preference in female offspring. 2,3,7,8-TCDD did not change saccharin consumption or saccharin preference in males. The results indicated that saccharin consumption is masculinized in female rats. The investigators suggested that the reduction in saccharin consumption and preference might be due to the antiestrogenic actions of 2,3,7,8-TCDD. Additionally, Kakeyama et al. (2003) reported altered sexual behavior (significant reductions in the number of mounts and number of intromissions in both castrated and noncastrated rats) in male offspring of Long-Evans rats administered 0.8 µg/kg on GD 15.

Consistent with older studies (Agency for Toxic Substances and Disease Registry 1998), perinatal exposure to 2,3,7,8-TCDD affected mammary gland development. Significant impairment of mammary gland differentiation was reported in 9-week-old female pups from Holtzman rats dosed with 1 μg/kg 2,3,7,8-TCDD on GD 15 (Lewis et al. 2001). In this study, offspring were ovariectomized and some received estradiol implants while others received a placebo implant. In 2,3,7,8-TCDD-exposed mammary glands, treatment with the high dose of estradiol induced differentiation of terminal ductal structures as evidenced by increased percentage of Lobules I and II. In unexposed mammary glands, exogenous estrogen did not significantly increase the percentage of Lobules I and II. Exogenous estrogen induced expression of progesterone receptor in both 2,3,7,8-TCDD-treated and untreated mammary glands. Exposure to 2,3,7,8-TCDD did not alter mammary epithelial cell proliferation in response to estrogen treatment. In addition, exposure to 2,3,7,8-TCDD did not alter the rate of mammary epithelial cells apoptosis in either estrogen treated or untreated glands. The results showed that gestational and lactational exposures to 2,3,7,8-TCDD impairs mammary gland differentiation, but exposed glands retain the ability to differentiate in response to estrogen. Examination of the mammary gland from Long-Evans rat pups at various times following administration of a single maternal dose of 1 μg/kg 2,3,7,8-TCDD on GD 15 showed that 2,3,7,8-TCDD-exposed glands on PND 4 already had reduced primary branches from the collecting duct, delayed migration from the epithelium through the fat pad, and fewer terminal branches and alveolar buds compared to controls (Fenton et al. 2002). The 2,3,7,8-TCDD-induced delay and stunting of mammary gland development was still evident on PNDs 68 and 110. Separate experiments aimed at determining a critical exposure period showed that GD 15 consistently triggered underdeveloped epithelium; exposure on GD 20 had no significant effects and neither did postnatal exposure, suggesting that gestational exposure was critical. Studies of the interaction between dietary fat and 2,3,7,8-TCDD in DBA/2J mice showed that gestational exposure to 2,3,7,8-TCDD (GD 12.5) primarily influenced mammary gland growth only in offspring fed a high-fat diet, but not in mice fed a low-fat diet (LaMerrill et al. 2009). 2,3,7,8-TCDD plus a high-fat diet significantly increased fat pad length and significantly decreased branch elongation, the number of terminal end buds, and the size of terminal end buds relative to vehicle-treated progeny fed a high-fat diet. A subsequent study from the same groups of investigators reported that gestational exposure to 2,3,7,8-TCDD doubled mammary tumor incidence induced by dimethylbenz(a)anthracene (DMBA) only in rats fed a high-fat diet (La Merrill et al. 2010). The findings were attributed to alterations in metabolism capability and the rate of breast development.

No studies examining developmental effects following exposure to other CDD congeners were identified in the update literature search.

2.2.2.8 Cancer

Several studies have examined the carcinogenicity of CDDs following oral exposure; the results of these studies are consistent with earlier studies summarized in Agency for Toxic Substances and Disease Registry (1998) and show that oral exposure to CDDs results in significant increases in tumor incidence at multiple sites. Details of these studies are summarized in Table 2-14. Gavage administration of 0.10 µg/kg 2,3,7,8-TCDD in corn oil to female Sprague-Dawley rats for 2 years resulted in significant increases in the incidence of a number of tumors including hepatocellular adenomas, cholangio-carcinomas, cystic keratinizing epithelioma in the lungs, gingival squamous cell carcinoma in oral mucosa, and c-cell adenoma in the thyroid gland (NTP 2006; Yoshizawa et al. 2005). Although the combined incidence of adenoma or carcinoma in the pancreas in rats exposed to 0.10 µg/kg was not significantly higher than concurrent controls, the incidence was higher than historical controls and there was a significant positive trend. In addition, an increased incidence of squamous cell carcinoma of the uterus was observed at 0.046 µg/kg, but not at 0.1 µg/kg.

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 nonstatistically significant increase in the incidence of liver tumors.

Exposure of a group of 32 neonatal (18 days of age) Sprague-Dawley rats to 2.5 µg/kg/day 2,3,7,8-TCDD in corn oil (via gavage) followed by an intraperitoneal injection of methylnitrososurea (30 mg/kg) on PND 21 resulted in a significant increase in the number of benign and malignant mammary tumors; 75% of the rats had tumors compared to 3.3% in the control group, which were detected within the first year after exposure (Desaulniers et al. 2001).

Gavage exposure to 1.250 µg/kg 2,3,7,8-TCDD in corn oil administered 5 days/week for 26 weeks (0.893 µg/kg/day) to 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 and carcinomas (Wyde et al. 2004).

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 time-weighted average dose of 0.0065 mg/kg/day or 0.012 mg/kg/day (see Table 2-14 for more detailed information on dosing schedule).

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).

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).

2.2.3 Dermal Exposure

2.2.3.1 Death

Increased mortality (60%) was observed in male ICR mice exposed twice weekly to 0.125 µg 2,3,7,8-TCDD dissolved in acetone brushed onto the shaved back skin for 20 weeks (Chang et al. 2005); survival was not affected at 0.025 µg.

2.2.3.2 Systemic Effects

Liver Effects. Increases in the incidence of edema with degranulation of centrilobular hepatocytes, centrilobular sinusoidal dilatation, and fatty changes (hepatocyte cytoplasmic vacuolization) were observed in the livers of male ICR mice exposed twice weekly to 0.0025, 0.025, or 0.125 µg

2,3,7,8-TCDD dissolved in acetone brushed onto the shaved back skin for 20 weeks (Chang et al. 2005). Centrilobular inflammatory infiltration was observed at ≥0.025 μg and increased liver weight and focal hepatic necrosis was observed at 0.125 μg.

2.3 TOXICOKINETICS

A large number of toxicokinetic studies have been published since the toxicological profile for CDDs (Agency for Toxic Substances and Disease Registry 1998). New studies reporting information that was reviewed in the toxicological profile and was well-supported with several references were not selected for inclusion. Rather, this section of the addendum focuses on studies that provide new information or information that was included in the toxicological profile but supported by limited evidence.

2.3.1 Absorption

2.3.1.2 Oral Exposure

Absorption of several CDDs (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, 1,2,3,4,6,7,9-HpCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD) from food was examined in seven volunteers using a mass balance protocol, collecting food (normal diet; not controlled) and feces over a 3-day period (Schlummer et al. 1998). Volunteers did not have any history of occupational or accidental exposure to CDDs. The highest net absorption observed for an individual volunteer was 62% for 2,3,7,8-TCDD in a 28-year-old male. However, estimates of absorption were highly variable and, in some individuals, net excretion rather than net absorption was observed. The study authors suggested that variability was related, in part, to the variability of food content of CDDs. Using a similar study design, the absorption of several CDDs (same as those evaluated by Schlummer et al. 1998) was estimated in five volunteers for both low- and high-CDD intake diets (Moser and McLachlan 2001). For high-intake diets, the net absorption of 2,3,7,8-TCDD, PeCDD, and HxCDD was >80%, with lower net absorption for HpCDDs (approximately 70%) and OCDD (approximately 50%). For low-intake diets, the net absorption of most CDDs could not be detected; findings are consistent with net excretion of CDDs from body stores under low-intake conditions.

The oral bioavailability of CDDs in soil relative to bioavailability in a reference material (relative bioavailability or RBA), such as corn oil, has been evaluated using several animal models, including rats (Budinksy et al. 2008; Finley et al. 2009; Lucier et al. 1986; Shu et al. 1988), guinea pigs (McConnell et al. 1984; Umbreit et al. 1986; Wendling et al. 1989), rabbits (Bonaccorsi et al. 1984), and swine

(Budinksy et al. 2008; Wittsiepe et al. 2007). Results of all studies show that the RBA of CDDs in soil is <100%, indicating that bioavailability of CDDs in soil is reduced compared to bioavailability of CDDs in the reference material. Relative bioavailability values for CDDs in soil were highly variable, ranging from <1 to 66% (Budinsky et al. 2008; Umbreit et al. 1986). Variability in RBA values may be related to several factors, including differences in soil characteristics, CDD conger composition of soil, experimental protocol, and/or species differences.

2.3.1.3 Dermal Exposure

Dermal absorption of 2,3,7,8-TCDD (70 mg total dose in acetone or in a low organic soil) was evaluated following application to shaved skin of female Sprague-Dawley under occluded conditions (Roy et al. 2008). After 96 hours, dermal absorption of 2,3,7,8-TCDD was 77.6 and 16.3% for acetone and soil applications, respectively.

2.3.2 Distribution

Distribution of CDDs in fetal and neonatal tissues is discussed in Section 3.5.4.5 (Elimination and Excretion, Transfer of CDDs Through the Placenta and Breast Milk).

2.3.2.2 Oral Exposure

Studies in animals have shown that 2,3,7,8-TCDD distributes preferentially to the liver and adipose tissue, and that distribution from blood to other tissues is rapid. Following single gavage administration of 50 or 100 ng/kg of 2,3,7,8-TCDD in corn oil to female Harlan Sprague-Dawley rats, TCDD tissue concentrations in blood, lung, liver, and adipose were measured at several time intervals up to 150 days (NTP 2006). The highest peak tissue concentration (per gram tissue) was observed in liver, followed by adipose>lung≈blood. Peak blood levels of 2,3,7,8-TCDD were observed within 24 hours of dosing, decreasing to nondetectable levels after 15 days; results are consistent with rapid distribution to tissues. Peak liver concentration was observed within 24 hours of dosing, whereas peak adipose concentration was observed in 20–40 days.

The dose- and time-dependent tissue distribution of 2,3,7,8-TCDD in mice has been examined in several single-dose oral exposure studies (Diliberto et al. 1998, 1999, 2000; Hakk et al. 2009; van Birgelen et al. 1996). Results show that distribution to liver and adipose is dose-dependent; at lower doses, distribution (as a percentage of the administered dose) to adipose is greater than to liver, whereas at higher doses,

distribution to liver is greater than to adipose. A typical example of the patterns for dose- and time-dependent distribution of 2,3,7,8-TCDD is provided in a study by Dilberbeto et al. (1995).

Dose-dependence of distribution of 2,3,7,8-TCDD to liver and adipose appears to be related to induction CYP1A2, a protein that is under aryl hydrocarbon receptor (AhR) transcriptional regulation and binds to 2,3,7,8-TCDD (Diliberto et al. 1998, 1999, 2000; Hakk et al. 2009).

To evaluate distribution of CDDs under steady-state or near steady-state conditions, studies have evaluated tissue distribution following intermediate-duration oral exposure (Birnbaum and Couture 1988; Birnbaum et al. 1989; DeVito et al. 1998; Diliberto et al. 2001; Fries and Marrow 1975; Laurent et al. 2005; Norback et al. 1975). In female B6C3F1 mice administered [^{3}H]-2,3,7,8-TCDD (1.5 or 150 ng/kg/day) in corn oil by gavage 5 days/week for 13 weeks, radioactivity was detected in all tissues examined (blood, adipose, liver, kidneys, lungs, skin, muscle, spleen, and thymus), with highest tissue concentrations in liver and adipose (Diliberto et al. 2001). As demonstrated in single-dose studies (discussed above), at the lower dose, distribution (as a percentage of the administered dose) to adipose was greater than to liver, whereas at the higher dose, distribution to liver was greater than to adipose. In female B6C3F1 mice administered 2,3,7,8-TCDD (1.5–150 ng/kg/day) or 1,2,3,7,8-PeCDD (90–9,000 ng/kg/day) in corn oil by gavage 5 days/week for 13 weeks, dose-dependent increases in tissues concentrations were observed for liver, adipose, skin, and blood (DeVito et al. 1998). After 13 weeks of treatment, tissue concentrations of CDDs were highest in liver, followed by adipose, skin, and blood. The study authors suggest that high liver concentrations of CDDs are consistent with an inducible hepatic binding protein for dioxin-like compounds. Male Sprague-Dawley rats fed diets containing a mixture of CDDs (TCDD, PeCDD, HxCDD, HPCDD, and OCDD) from contaminated milk for 120 days show that liver and adipose are the primary sites of distribution (Laurent et al. 2005). Liver and adipose levels of CDDs remained constant after approximately 1.5 months.

2.3.3 Metabolism

Little data were located regarding metabolic pathways of CDDs in humans. Two main metabolites, 2,3,7-trichloro-8-hydroxydibenzo-*p*-dioxin and 1,3,7,8-tetrachloro-2-hydroxydibenzo-*p*-dioxin, were identified in feces, urine, and blood serum of an individual poisoned with TCDD (Sorg et al. 2009). The patient's blood serum level of TCDD was 108,000 pg/g lipid three months after the poisoning. Results of an *in vitro* study using recombinant yeast microsomes containing human cytochrome P450 (CYP) isozymes from human liver show that 2,3,7-TriCDD undergoes sequential metabolized by cytochrome

P450 and UDP-glucuronsyltransferase (Kasai et al. 2004). Using the same *in vitro* model, several mono-, di-, and tri-CDDs have been shown to be metabolized by multiple forms of cytochrome P450 (Inouye et al. 2002). Metabolites included products of multiple reactions, including several types of hydrozylation reactions. Enzymes CYP1A1 and CYP1A2 exhibited the highest activity for mono-, di-, and tri-CDDs, although other P450 isozymes (PYP2C8, CYP2C9, and CYP3A4) did not show any significant activity for CDDs. None of the P450 CYP isozymes showed any activity toward 2,3,7,8-TCDD.

The role of CYP1A2 in the overall metabolism of CDDs has been studied in CYP1A2 knockout mice (lacking the CYP1A2 gene) following single-dose oral exposure (Hakk et al. 2002, 2003, 2009). Results show that mice with the CYP1A2 gene (CYP1A2+) only metabolize slightly more 2,3,7,8-TCDD or 1,2,3,7,8-PeCDD than CYP1A2 knockout mice, indicating that sequestration of CDDs by binding to CYP1A2 does not have an important effect on metabolism by other CYP isozymes or other enzymes (Hakk et al. 2002, 2003). Overall metabolism did not exhibit dose-dependence in either CYP1A2+ or CYP1A2 knockout mice (Hakk et al. 2009).

Metabolism of 1,3,6,8-TCDD was studied in hepatic microsomes obtained from male C57BL/6 mice administered a single oral dose of 2,3,7,8-TCDD in corn oil (Aozasa et al. 1996). Metabolites of 1,3,6,8-TCDD included several hydroyxylation products, which appear to be further metabolized to other compounds, including quinones, sulfate conjugates, and other smaller compounds (not identified). Metabolites isolated from urine, bile, and feces of Sprague-Dawley rats administered a single dose of [^{14}C]-1,2,7,8-TCDD (8 mg/kg) in corn oil by gavage include hydroxylation products, glucuronide conjugates, and sulfide conjugates (Hakk et al. 2001). Similar metabolic profiles were reported for 1,3,7,8- and 1,4,7,8-TCDD (Huwe et al. 1997, 1998; Pertoske et al. 1997).

2.3.4 Elimination and Excretion

In a 15-year follow-up of 97 Operation Range Hand veterans, Michalek et al. (1999) estimated a half-life of 7.6 years (95% confidence interval: 7.0–8.2 years). Michalek et al. (2002) conducted a combined analysis of Seveso adults and Operation Ranch Hand veterans. In the Seveso cohort, a period of fast elimination (half-life: 0.34 years) during the first 0.27 years after exposure, followed by a period of slower elimination (half-life: 6.9 years) from 3 to 16.35 years. In the Ranch Hand cohort, the half-life from 9 to 33 years (7.5 years) was similar to that of the Seveso population. The study authors noted that results in the Seveso cohort are consistent with a two-compartment model, with a distribution phase with rapid elimination, followed by a slower elimination phase.

Results of a study in two female patients with severe TCDD intoxication show that 2,3,7,8-TCDD undergoes cutaneous elimination (Geusau et al. 2001a). The TCDD exposure source for these patients is unknown (Geusau et al. 2001b). Cutaneous elimination was approximately 1–2% of the total daily TCDD elimination, when adjusted for skin surface area.

2.3.4.2 Oral Exposure

Elimination across the gastrointestinal tract is an important elimination pathway for absorbed chlorinated dibenzo-*p*-dioxins (CDDs) in humans. Results of a mass balance study in six men with high body burdens of CDDs show that fecal elimination of 2,3,7,8-TCDD and OCDD was 37 and 90%, respectively, of total elimination (Rohde et al. 1999). Fecal elimination of CDDs exceeded dietary intake, indicating gastrointestinal excretion of CDDs from diet or body stores. Similar results were reported in a mass balance study in 14 volunteers (7 males and 7 females), with fecal excretion exceeding dietary intake by approximately 2-fold (Schrey et al. 1998). Feces were the main route of eleimination also in a patient poisoned with high level of TCDD (Song et al. 2009). During the three year period of follow-up testing, the patient eliminated in feces and urine the total amout of the two major metabolites equivalent to about 95μg of TCDD (i.e., 38% of total TCDD eleiminated by all means). The half-life of TCDD in this patient was 15.4 months. The elimination pattern fits into a model predicting that expected half-life of TCDD ranges from less than 5 years in people exposed to high levels (more than 10,000 pg/g serum lipids of internal dose) to more than 10 years in those exposed to lower levels (less than 50 pg/g serum lipids of internal dose) (Aylward et al. 2005).

In C57BL/6N and CYP1A2 knockout mice administered single oral doses of [^{3}H]-2,3,7,8-TCDD (0.1 or 10 μg/kg), 24–31 and <5% of the administered dose were eliminated in feces and urine, respectively, within 4 days; no dose-related differences in excretion were observed (Hakk et al. 2009). Following oral administration of [^{14}C]-2,3,7,8-TCDD (1.25 mg/kg) to male Sprague-Dawley rats, only 0.27% of the administered dose of [^{14}C] was eliminated in urine within 3 days (Hakk et al. 1998). Of the [^{14}C] excreted in urine, approximately 8.8% of the urinary [^{14}C] was bound to albumin and approximately 64.2% was unbound. In bile-duct cannulated rats, 9.6% of [^{14}C] in bile was bound to an unidentified 89kDa protein and 76.6% was unbound.

In male Sprague-Dawley rats administered a single dose of [^{14}C]-1,2,7,8-TCDD (8 mg/kg) in peanut oil by gavage, 94.2% of the administered radioactivity was recovered in feces (79.8%) and urine (14.3%).

Biliary excretion was estimated as 32.4%, using biliary-cannulated rats (Hakk et al. 2001). Fecal excretion is also the predominant elimination pathway for 1,4,7,8-TCDD (Huwe et al. 1998). In male Sprague-Dawley rats administered a single dose of [^{14}C]-1,4,7,8-TCDD (2 mg total dose), 88.8 and 3.3% of the administered [^{14}C] were eliminated in feces and urine, respectively, within 3 days; biliary excretion was estimated as >30% of the administered [^{14}C]. Following oral administration of [^{14}C]-1,2,3,7,8-PeCDD (2.9 mg/kg) to male Sprague-Dawley rats, only 0.22% of the administered dose of [^{14}C] was eliminated in urine within 3 days (Hakk et al. 1999). Of the [^{14}C] excreted in urine, approximately 17.9% of the urinary [^{14}C] was bound to albumin and approximately 78.1% was unbound. In bile-duct cannulated rats, 7.2% of [^{14}C] in bile was bound to an unidentified 89kDa protein and 91.1% was unbound.

2.3.4.4 Transfer of CDDs through the Placenta and Breast Milk

Analysis of data obtained through the National Health and Nutrition Examination Survey (NHANES) provides support that breast milk levels of CDDs generally reflect blood lipid levels of CDDs, although these ratios may vary considerably between individuals and over time (Aylward et al. 2003). In a study of 21 Japanese women, statistically significant correlations have been reported between CDD concentrations (expressed as TEQs) in maternal blood and milk ($r=0.695$; $p=0.0007$), maternal adipose tissue ($r=0.913$; $p<0.0001$), and cord blood ($r=0.759$; $p<0.0001$) (Nakano et al. 2005).

Analysis of tissue CDDs obtained from 20 maternal-fetal pairs showed the highest levels of CDDs in perinatal venous serum, followed by placenta, cord serum, and breast milk (Wang et al. 2004). Transfer of CDDs from mothers to fetuses and infants was assessed by measuring CDDs in maternal blood, cord blood, placenta, maternal adipose tissue, and milk in 22 Japanese women (Suzuki et al. 2005). Results show that CDD congeners with high TEQs accumulate in the placenta relative to maternal blood and that CDD levels in milk are influenced, in part, by maternal adipose levels.

Maternal-fetal transfer has been evaluated in numerous studies in several animal models; results show that placental-fetal transfer is much lower than fetal transfer through lactation. Following administration of single oral doses of several CDDs to Long-Evans rats on GD 15, only 0.5–3% of the administered dose was transferred to the fetus, compared to postnatal transfer of 7–28% through milk (Chen et al. 2001). Following oral administration of a single dose of [^{3}H]-2,3,7,8-TCDD (1.15µg/kg in corn oil) to pregnant Long-Evans rats on GD 8, the amount of TCDD transferred to the fetus increased from 0.12 to 0.21% of the administered dose from GD 9 to 16 (Hurst et al. 1998a). Similar fetal tissue TCDD levels were observed on GD 21 following exposure of pregnant to Long-Evans rats to [^{3}H]-2,3,7,8-TCDD on GD 8 or

15 (Hurst et al. 1998b). Following administration of single oral doses [^{3}H]-2,3,7,8-TCDD (1, 10, or 30 mg/kg in corn oil) to pregnant Long-Evan rats (1.0 μg/kg in corn oil) on GD 15, fetal tissues concentration of TCDD were significantly and highly correlated with TCDD concentrations in maternal blood ($r=0.932$; $p<0.0001$) (Hurst et al. 2000a). Fetal tissue concentrations of pups born to female Long-Evans rats administered [^{3}H]-2,3,7,8-TCDD (1, 10, or 30 mg/kg in corn oil) 5 days/week for 13 weeks prior to mating and throughout gestation were similar to those observed following administration of single doses of [^{3}H]-2,3,7,8-TCDD (1, 10, or 30 mg/kg in corn oil) on GD 8 or 15 (Hurst et al. 2000b). Levels of CDDs in placenta exhibit a dose-dependent decrease, possibly due to increased maternal sequestration in the liver due to induction of CYP1A2 (Chen et al. 2000). In fetal liver, a dose-dependent increase in liver:fat TCDD levels, consistent with a similar hepatic sequestration CYP1A2 mechanism, may exist (Yonemoto et al. 2005).

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

Since 1998, several investigators have developed new PBPK models for CDDs and refined existing models. Aylward et al. (2005) modified the concentration- and age-dependent model of elimination developed by Carrier et al. (1995a, 1995b) to include the amount of 2,3,7,8-TCDD eliminated through partitioning from circulating lipids across the lumen of the large intestine into the fecal content. The modified model was fit to serial serum 2,3,7,8-TCDD sampling data from two Austrian subjects with a mean follow-up of 2.7–3 years and 36 subjects (19 males and 17 females) from Seveso with a mean follow-up of 16 years. The modified model allows for a better prediction of peak historical exposures using current serum 2,3,7,8-TCDD levels. Aylward et al. (2005) compared the estimated peak serum 2,3,7,8-TCDD levels for the NIOSH cohort back-extrapolated assuming first-order kinetics with a fixed half-life of 7–9 years to peak levels predicted by the modified model and found that assuming first-order kinetics resulted in an underestimation of maximum concentrations by several fold to an order of magnitude.

Kohn et al. (2001) updated the Kohn rat PBPK model (described in Agency for Toxic Substances and Disease Registry 1998) to include multiple TCDD-liganded Ah receptor binding sites for CYP1A1 and CYP1B1 genes, a lag of 0.2 days for production of mRNA and induced proteins, and stabilization of mRNA by a poly(A) tail. The model was validated using observed liver TCDD levels from the literature. In general, there was good agreement between the predicted and measured tissue TCDD concentrations and the dose-response curves for CYP1A1, CYP1A2, and CYP1B1

Emond et al. (2004), simplifying the Wang et al. (1997) model, developed a model with four compartments (liver, fat, placenta, and rest of the body) for the dam and one compartment for the fetus. The maternal compartments were described as diffusion limited and the model assumes simple diffusion of 2,3,7,8-TCDD between the placental and fetal compartments (no blood flow to the fetal compartment is assumed). Additionally, the model includes 2,3,7,8-TCDD induction of CYP1A2 and binding in the liver. All parameters for the nonpregnant animal (only fat, liver, and rest of the body compartments were activated) were adapted from Wang et al. (1997) and parameters for growth of the placental, blood, and fetal compartments and blood flow rates were based on data from O'Flaherty (1994) and Buelke-Sam et al. (1982a, 1982b). The model was validated by comparisons of simulations to the Wang et al. (1997) model and using experimental data for four scenarios: acute 2,3,7,8-TCDD exposure in nonpregnant rats, subchronic 2,3,7,8-TCDD exposure in nonpregnant rats, single exposure to 2,3,7,8-TCDD on gestation day 15, and subchronic 2,3,7,8-TCDD exposure prior to mating and continuing throughout gestation. Reasonable agreements were found in these comparisons (typically within 20–30% of the experimental data). The investigators noted that limited data are available to develop and validate the developmental model and that extrapolation to humans should be done with caution. The model was subsequently modified to include inducible hepatic elimination, which describes the elimination rate as a function of CYP1A2 induction (Emond et al. 2006). The Emond et al. (2004) model has also been extrapolated to humans (Emond et al. 2005). This model was optimized using serum 2,3,7,8-TCDD levels from 20 Ranch Hand veterans and data from a subject ingesting a single dose of 2,3,7,8-TCDD and followed for 40 days, and the model was validated using an additional 10 Ranch Hand veterans and data from 2 Austrian women. A good correlation between predicted blood concentrations and measured blood concentrations was found for both groups of subjects.

The Maruyama et al. (2002) PBPK model, which is a modification of the Lawrence and Gobase (1997) fugacity model, consists of six compartments: blood, liver, kidney, fat, muscle, and richly perfused tissue (brain, lung, and spleen). Exposure was assumed to be through diet only. Tissue:blood partition coefficients were determined using autopsy data from eight Japanese men and women exposed to background levels of CDDs and CDFs. For most congeners, the measured values were within the simulated ranges in liver, kidney, and blood; the model underestimated 1,2,3,6,7,8-HxCDD concentrations in the kidney, fat, blood, and muscle. For model validation, the estimated mean concentrations of CDD and CDF congeners were compared to measured values from autopsy data for 30 different Japanese persons. Overall, there was good agreement between the estimated and measured values, although the model tended to underestimate CDD levels and overestimate CDF levels. Maruyama et al. (2001) also developed a PBPK model that would allow for the estimation of dioxin concentrations

in Japanese breastfed infants. This model was composed of six compartments (liver, kidney, fat, blood, muscle, and richly perfused tissues) and the source of exposure was presumed to be breast milk exclusively.

2.4 MECHANISMS OF ACTION

2.4.2 Mechanisms of Toxicity

The mechanism of toxicity of CDDs is not completely understood but has been extensively studied, particularly for 2,3,7,8-TCDD. The toxicological profile for CDDs (Agency for Toxic Substances and Disease Registry 1998) provides an overview of the research on the mechanisms of toxicity published from the 1970s through the late 1990s. An extensive number of papers have been published since 1998 that provide additional insight into the mechanisms of 2,3,7,8-TCDD toxicity. Since essentially all of the toxic end points are dependent on the AhR, considerable research has been published on the physiological function of the AhR, structure of the AhR, and regulation of the AhR. A detailed discussion of these studies is beyond the scope of this addendum; an overview of these data relying on authoritative reviews is presented in this section of the addendum. In addition, end-point-specific data regarding the mechanisms of toxicity are discussed in Section 2.2.2.

The AhR is a cytosolic protein in the bHLH-PAS family of transcription factors, which 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 a 23 kDa protein referred to as p23 (Bock and Köhle 2006; Denison and Nagy 2003; Gasiewicz et al. 2008). Following the binding of CDD to the AhR, 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 the AhR nuclear translocator protein (Arnt; also known as hypoxia inducible factor 1β or HIF-1β). The ligand:AhR:Arnt heterodimer complex binds to a specific DNA recognition site within target genes referred to as AhR responsive elements (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 (Bock and Köhle 2006; Gasiewicz et al. 2008). The core recognition sequence for the AhREs is 5'-TNGCGTG-3' located upstream from the mammalian CYP1A1 gene and other AhR responsive genes (Gasiewicz et al. 2008; Okey 2007); a list of other AhR responsive genes is presented in Table 2-15. Studies published within the last 10 years or so provide data to suggest that ligand binding to the AhR can also initiate changes in other signal transduction pathways that are not dependent on

interactions of the AhR with DNA. There is evidence that AhR can directly interact with the transcription factor NF-κB and with retinoblastoma protein (Rb); the interaction of AhR with these proteins can affect the cell cycle (Bock and Köhle 2006; Carlson and Perdew 2002; Gasiewicz et al. 2008). However, data from mice carrying a mutation in the nuclear localization sequence of the AhR suggest that the toxicity of CDDs primarily results from nuclear uptake and interaction with AhREs.

The AhR is well conserved across species and is present in essentially all tissues, prompting researchers to hypothesize a physiological role for the AhR. AhR-null mice are viable and fertile; however, a number of abnormalities are detected as the animal matures. Abnormalities include impaired liver histopathology and development (small livers, portal fibrosis, bile duct inflammation, and transient fatty changes), impared immune function (decreased lymphocytes in spleen and lymph nodes), cardiomyopathy, impared vascular development, and the development of reproductive tissues (rate of follicle formation, dorsolateral and anterior prostate, seminal vesicle) (Gasiewicz et al. 2008; Okey 2007). In support of the normal physiological role of the AhR, a number of endogenous AhR ligands have been identified including flavonoids and carotinoids (Denison and Nagy 2003).

As reviewed by Gasiewicz et al. (2008), numerous studies have examined the regulation of AhR expression and activity. Several mechanisms have been identified including regulation of the AhR gene, regulation of AhR protein levels, ligand-dependent regulation of AhR activity, and ligand-independent regulation of AhR activity. Genomic cloning of the mouse and human AhR gene failed to identify TATA boxes in the promoter region of the AhR gene, but did identify several GC boxes and Sp1 transcription factor binding sites in the promoter region (Gasiewicz et al. 2009; Harper et al. 2006). Several regions on the AhR gene containing regulatory motifs, including numerous transcriptor factor binding sites for the hepatic nuclear factor family (HNFs), BRN3, PIT1, DLX3, NKX3, LHX3, STAT6, and TCF/Lef , have been identified on the AhR gene (Gasiewicz et al. 2008; Harper et al. 2006). These binding motifs may play an important role in maintaining tissue-specific AhR levels. Additionally, potential AhR:Arnt binding sites clustered around the mouse and human AhR promoter region have been identified; however, it is not known whether these sites are responsible for upregulation of AhR expression by AhR ligands (Harper et al. 2006).

Exposure to AhR agonists elicits a depletion of AhR protein without an effect of AhR mRNA (Gasiewicz et al. 2008) and the current literature supports the loss of AhR protein as a component of the AhR signal transduction cascade (Pollenz et al. 2002). AhR is exported from the nucleus and ubiquitinated in the cytoplasm followed by degradation through the 26S proteasome. Blocking the degradation of the AhR

could result in an increase in the duration and magnitude of the AhR-responsive gene induction (Pollenz et al. 2002). Both ligand-dependent and -independent degradation of AhR have been found in rodents and humans. A distinct time course for AhR degradation was observed in the various mammalian cell lines that have been studied; the AhR showed a maximal level of degradation of 65–95% (Pollenz and Buggy 2006). Ligand-independent degradation of the Ahr is thought to occur due to changes in the conformation of the AhR:hsp90:XAP2 complex (Pollenz and Buggy 2006).

The AhR must undergo ligand-dependent transformation to become transcriptionally active, and a number of endogenous and exogenous ligands have been identified. No high affinity endogenous ligands have been identified, and it is hypothesized that the endogenous ligands are rapidly degraded by the coordinately induced detoxification enzymes and that they would act as transient inducers of AhR-depended gene expression (Denison and Nagy 2003). Flavonoids, including flavones, flavanols, flavanones, and isonflavones, are the largest group of endogenous ligands. Other dietary plan compounds that act as AhR ligands include 7,8-dihydrorutacarpine, dibenzoylmethanes, I3C, curcumin, and the carotinoids, canthaxanthin and astaxanthin (Denison and Nagy 2003). A number of exogenous AhR ligands have been identified (Denison and Nagy 2003), including planar, hydrophobic halogenated aromatic hydrocarbons such as CDDs, CDFs, dibenzofurans, and biphenyl and polyaromatic hydrocarbons such as 3-methylcholanthrene, benzo(a)pyrene, benzthracene, and benzoflavones. Of the exogenous ligands, 2,3,7,8-TCDD has the highest binding capacity.

Ligand-independent mechanisms for regulation of AhR activity have also been identified. It has been suggested that an AhR repressor (AhRR) protein blocks AhR activity by dimerizing with Arnt and competing with the AhR:Arnt complexes for binding to AhRE sites; the AhRR:Arnt complex functions as a transcriptor repressor (Mimura and Fujii-Kuriyama 2003). Although the AhRR gene is directly upregulated by activated AhR, AhRR expression does not appear to be directly related to a change in AhR responsiveness to ligands (Gasiewicz et al. 2008). Additionally, Arnt concentration does not appear to be a rate-limiting factor in AhR signaling, suggesting that the competition between AhR and AhRR for Arnt may not be an important regulator of AhR activity (Carlson and Perdew 2002). Phosphorylation can influence the ability of the Arnt complex to induce AhR-responsive genes. The formation of the AhR:Arnt heterodimer requires the phosphorylation of Arnt, and the binding of the AhR:Arnt:ligand complex to DNA requires phosphorylation of AhR and Arnt (Swanson 2002). There are also data suggesting that the responsiveness to AhR ligands may be modified by a combination of specific factors and interacting pathways (Gasiewicz et al. 2008). Activation of the protein kinase C pathway enhances the ability of the AhR:Arnt heterodimer to regulate genes by increasing the transactivation potential of the

AhR:Arnt heterodimer, which may increase the ability of co-activator proteins to interact with both an unidentified region within Arnt and the PAS domain of the AhR (Swanson 2002). However, there is conflicting evidence as to whether DNA binding by AhR with the protein kinase pathway is blocked (Carlson and Perdew 2002). Other interactions (cross talk) between signal transduction pathways and the AhR involve the estrogen receptor, hypoxia, tyrosine kinases/phosphatases, *c-myc*, AP-1, CK2, transforming growth factor β (TGFβ), p27 (Kip1), NF-1, and C2 ceramide (Carlson and Perdew 2002).

2.4.3 Animal-To-Human Extrapolations

Several factors need to be considered when understanding species differences in CDD toxicity, in particular 2,3,7,8-TCDD toxicity. One of the primary factors is binding affinity, and quantitative measures of binding affinity can serve as a preliminary indicator of species susceptibility to 2,3,7,8-TCDD. Events in the AhR signaling pathway, such as binding of cofactors (e.g., Arnt) or chaperone proteins, translocation to the nucleus, and interaction with transcriptional control elements on DNA, and transcriptional cofactors could also affect 2,3,7,8-TCDD responsiveness. However, the limited available data on species differences in these downstream AhR signaling events have not found marked differences and none of the available data suggest that a species with a low binding affinity would have a high responsiveness to 2,3,7,8-TCDD (Connor and Aylward 2006). The binding affinities (expressed as the dissociation constant, K_d) from several species are presented in Table 2-16 (note the dissociation constant is inversely proportional to the binding affinity). The dissociation constants in humans is approximately 10-fold higher than most laboratory species, indicating approximately one-tenth of the binding capacity. Molecular genetic studies have compared the human AhR to the AhRs in C57BL/6 mice (2,3,7,8-TCDD responsive strain) and DBA/2 mice (2,3,7,8-TCDD nonresponsive strain). Two single nucleotide changes are believed to be responsible for the differences in the binding affinity and function between the AhR in C57BL/6 mice and DBA/2 mice (Connor and Aylward 2006; Harper et al. 2002). In DBA/2 mice, the single nucleotide change in the ligand binding domain of the receptor causes valine to be substituted for alanine, resulting in decreased binding affinity (Harper et al. 2002). The second nucleotide change (leucine to proline) in the DBA/2 mouse converts a stop codon into an arginine, resulting in a longer transcript and extending the C-terminal portion of the AhR protein (Harper et al. 2002). Humans have both "DBA-type" mutations, whereas Sprague-Dawley rats, Golden Syrian hamsters, and domestic guinea pigs have the leucine and proline mutations (Connor and Aylward 2006).

Using *in vitro* and *in vivo* data, Connor and Aylward (2006) compared the biological responses of humans and laboratory animals to assess whether differences in binding affinity and molecular structure of the

AhR translate to differences in biological responsiveness (as assessed by induction of CYP1A1 and CYP1A2). The ratios of human EC_{50} to rat EC_{50} values for ethoxyresorufin-O-deethylase (EROD) activity, measured i*n vitro*, ranged from 8 to 34, suggesting that approximately 10-fold or higher 2,3,7,8-TCDD levels were needed in human cells to elicit the same response as rat cells, which is consistent with the comparison of ligand binding affinities. Comparisons were also made using *in vivo* data by measuring gene expression of CYP1A1 (as mRNA) in Seveso residents and German herbicide manufacturing workers. No detectable increases in gene expression of CYP1A1 were found at body burdens of ≤250 ng TEQ/kg; at body burdens of ≥750 ng TEQ/kg, increases in gene expression were observed (no studies examined body burdens between 250 and 750 ng TEQ/kg). In contrast, longer-term studies in B6C3F1 mice and Sprague-Dawley rats have found ≥4-fold increases in EROD activity at ≥100 ng TEQ/kg. These findings suggest that human do not respond with detectable induction of enzyme activity at the same dioxin body burden as laboratory rodents (Connor and Aylward 2006).

2.5 RELEVANCE TO PUBLIC HEALTH

Toxic Equivalency Factors (TEFs) and Toxic Equivalents (TEQs). As discussed in the Toxicological Profile for CDDs (Agency for Toxic Substances and Disease Registry 1998), TEFs have been developed, which allows for the comparison of the relative effect potencies of individual CDDs and other dioxin-like compounds (CDFs and some PCBs) to the reference compound, 2,3,7,8-TCDD. In 1993, the WHO established TEFs for 7 CDDs, 10 CDFs, and 12 PCB congeners; these values were updated in 1998. In 2005 (Van den Berg et al. 2006), WHO re-evaluated their TEF approach and used the Haws et al. (2006) TEF database as its starting point. A summary of the WHO 2005 TEF values (as well as the 1998 TEF values) is presented in Table 2-17.

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

As reviewed by Connor and Aylward (2006), a number of AhR polymorphisms (defined as an allelic frequency of >1% in a given population) have been identified. However, correlations between the observed human AhR genotype and CYP1A/B inducibility have not been established. Connor and Aylward (2006) note that because the AhR has been shown to have a critical role in development and homeostasis, there is little tolerance for genetic variations, other than those that are inconsequential to AhR function. Human polymorphisms frequently occur in exon 10, a region that encodes a major portion of the transactivation domain of the AhR that is responsible for regulating the expression of other genes (e.g., CYP1A1) (Harper et al. 2002). Variation that is confined to the transactivation domain may permit finely tuned modulation in gene regulation without abolishing the AhR's critical roles in development and

homeostasis (Harper et al. 2002). Most of the 'defective' phenotypes that have been identified in human cells or tissues are in the direction of non-responsiveness or low inducibility. Only one pair of human polymorphisms, those at codons 517 and 570, has been shown to have a clear-cut and strong effect on the phenotype of an AhR-mediated response.

A wide range of AhR binding capacities has been measured in humans, and a number of investigators have interpreted this range of dissociation constants as indicating a heterogeneous human AhR with functionally important polymorphisms (as reviewed by Connor and Aylward 2006). However, some of the observed variation may be due to experimental factors (differences in composition/cellular makeup of the samples) and environmental and dietary influences. Studies on human placental tissues have found at least a 10-fold range of AhR binding affinities for 2,3,7,8-TCDD. However, sequencing the AhR cDNA from a few individuals with the highest and lowest affinities did not reveal any polymorphisms that would explain the variation in ligand binding (Harper et al. 2002). Although polymorphisms on the AhR that would influence normal receptor function are unlikely, genetic variations might exist in non-AhR components such as Arnt, AhRR, co-activators, or co-repressors, which may affect AhR-mediated events. However, the possible variations have not been fully explored (Harper et al. 2002).

3. CHEMICAL AND PHYSICAL INFORMATION

No updated data.

4. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

No updated data.

5. POTENTIAL FOR HUMAN EXPOSURE

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.4 Other Environmental Media

The predominant source of CDDs in the food supply is the lipid component of animal products. The NAS (2003) estimated dioxin (CDD and CDF congeners) intake from meat, poultry, and fish for various age groups using data from the FDA's Total Diet Study; these estimates, for consumers of high and low amounts of animal products, are presented in Table 5-1.

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The levels of select CDD congeners were measured in blood samples collected as part of the NHANES. 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, OCDD, 1,2,3,7,8-PeCDD, and 2,3,7,8-TCDD levels for survey years 1999–2000, 2001–2002, and 2003–2004 are presented in the latest National Report on Human Exposures to Environmental Chemicals (CDC 2009) and are summarized in Tables 5-2–5-9. Patterson et al. (2008, 2009) reported the TEQs for dioxin-like compounds (CDDs, CDFs, coplanar PCBs, and mono-ortho-substituted PCBs) for survey years 2001–2002 and 2003–2004; these values are presented in Table 5-9. The blood TEQs of adults for the 2003–2004 monitoring period appear to be lower than levels in 2001–2002. LaKind et al. (2009) examined the temporal changes in serum CDD/CDF in adults for NHANES survey years 1999–2000, 2001–2002, and 2003–2004 (data summarized in Table 5-10) and found no significant change in median (50^{th} percentile) serum CDD/CDF levels from 1999–2000 to 2001–2002; however, there was a significant decrease in CDD/CDF serum concentration in the 2003–2004 survey year. When the participants were divided by age, 56 and 38% decreases in serum CDD/CDF levels were observed for the 2003–2004 survey year in the 12–19 and 20–39-year-olds, respectively, as compared to the 1999–2000 survey year. A slight nonsignificant decrease (6%) was observed for 40–59-year-olds and a slight increase (13%) was observed for 60+-year-olds.

6. ANALYTICAL METHODS

No updated data.

7. REGULATIONS AND ADVISORIES

ATSDR derived a chronic oral Minimal Risk Level (MRL) of 1 pg TCDD/kg/day in 1998. The MRL was based on neurobehavioral developmental changes in monkeys exposed perinatally to TCDD (for more details, see Agency for Toxic Substances and Disease Registry 1998). The MRL was also intended to serve as a health-based guidance for total TEQs of dioxins and dioxin-like chemicals. ATSDR's chronic MRL of 1 pg/kg/day is in accordance with other guidance values used around the world.

The table below summarizes the tolerable daily intake (TDI) values recommended by various international organizations and provides information that was located for nations that follow the TDI

approach for evaluating dioxin toxicity. A majority of nations have chosen to adopt TDI values recommended by WHO.

Risk Assessments Around the World

Organization	TDI	Countries
Van Leeuwen and Younes (1998)	1–4 pg TEQ/kg/day	France Germany Netherlands New Zealand
JECFA (2001)	2.3 pg TEQ/kg/day i.e.,70 pg TEQ/kg/month	Australia Canada

JECFA = Joint (FAO/WHO) Expert Committee on Food Additives

However, several nations have performed their own reassessment of the available toxicity data for dioxin to derive a TDI, rather than adopting TDI values derived by others. Japan derived a TDI of 4 pg/kg/day (Japanese Environmental Agency 1999), which is equivalent to the maximum TDI established by Van Leeuwen and Younes (1998). For the United Kingdom, the Government's independent advisory Committee on the Toxicity of Chemicals in Food (COT) recommended a TDI of 2 pg/kg/day, which is equivalent to the TDI identified by the European Commission Scientific Committee on Food (range 1–3 pg/kg/day) (EC-SCF 2001). In August 2000, several countries (Denmark, Finland, Sweden) considered revising the Nordic Council TDI value of 5 pg/kg/day to a value of 4 pg/kg-day in accordance with Van Leeuwen and Younes (1998), but it was determined that no change was appropriate (Johansson and Hanberg 2000).

In 1998, ATSDR published in the Toxicological Profile for Chlorinated Dibenzo-p-Dioxins its Policy Guideline for Dioxin and Dioxin-like Compounds in Soil (ATSDR 1998). In November 2008, this policy was updated and can be found on ATSDR's website at www.atsdr.cdc.gov/substances/dioxin/policy. The environmental media evaluation guide (EMEG) is 50 ppt of TCDD or total TEQs in soil. The 50 ppt is a screening level and serves as an initial comparison value for health assessments. The EMEG value is based on the MRL of 1 pg/kg/day. Currently, the U.S. Environmental Protection Agency (EPA) Office of Solid Waste and Emergency Response (OSWER) proposed a preliminary remediation goal (PRG) for dioxin and dioxin in soil at Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and Resource Conservation and Recovery Act (RCRA) sites based on the ATSDR's MRL of 1 pg/kg/day. For residential areas, the interim PRG is 72 ppt TEQs. The numerical difference between ATSDR and EPA/OSWER is because different body weight defaults for a child were used in the calculations of the respective guidance values (OSWER 2010).

On February 17, 2012, EPA released the dioxin health hazard (re)assessment for noncarcinogenic effects (IRIS 2012). The chronic oral RfD is 0.7 pg/kg/day. The RfD is based on two studies using the cohorts exposed in Seveso during the industrial accident. Decreased sperm count and mobility was found in men exposed to TCDD as boys (Mocarelli et al. 2008) and increased TSH was reported in neonates exposed in utero (Baccarelli et al. 2008). The LOAELs of 0.02 ng/kg/day were modeled from internal doses, and an uncertainty factor of 30 was used in the RfD derivation.

Major developments occurred in cancer classification of TCDD. In 1997, the International Agency for Research on Cancer (IARC) classified TCDD as a group 1 human carcinogen. IARC gave consideration to supporting evidence such as TCDD being a multi-site carcinogen in experimental animals; its acting through a mechanism involving Ah receptor, which functions the same way in humans as in experimental animals; and similar tissue concentrations both in heavily exposed human populations and in rats exposed to carcinogenic dosages. IARC's overall evaluation for TCDD is that it is carcinogenic to humans. Subsequently, the U.S. Department of Human Services lists 2,3,7,8-TCDD as known to be a human carcinogen (NTP 2011).

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Table 2-1. Cardiovascular Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Pelclova et al. 2002	2001 follow-up examination of 12 workers exposed 35 years earlier to TCDD in an industrial setting in herbicide production plant	1996 mean plasma level was 256 pg TCDD/g lipid (range=14–760 pg/g lipid)	Atherosclerosis in seven individuals; one had 80% stenosis of the carotid artery
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 TCDD/g lipid (range=14–760 pg/g lipid); estimated range at the time of exposure (1965–1968) was 3,300–74,000 pg 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	SMRs for heart disease showed a weak increasing trend with higher exposure (p=0.14)
‘t Mannetje et al. 2005	A total of 813 producers and 699 sprayers were classified as exposed to dioxin and phenoxy herbicides in a New Zealand study; the two cohorts were followed-up from 1 January 1969 and 1 January 1973, respectively, to 31 December 2000	Job codes were used for exposure evaluation	RRs for mortality from ischemic heart disease and all cardiovascular disease in production workers were 1.04 and 0.96, respectively

CDD = chlorinated dibenzo-*p*-dioxin; NIOSH = National Institute for Occupational Safety and Health; RR = relative risk; SMR = standardized mortality ratio; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

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

Reference	Design and population	TCDD/CDD concentrations	Effects
Lee et al. 2006	Case-control study; 52 residents living in the vicinity of a closed pentachlorophenol manufacturing factory and 33 residents in a nearby control area	Average levels of serum CDFs were 80.1±50.9 pg WHO-TEQ/g lipid for those residing in exposure area and 25.5±18.2 in control area	Statistically higher ORs for fatty liver and γ-GGT activity were found in subjects with higher serum CDFs levels and high body mass index
Neuberger et al. 1999	Case-control study; originally, 159 cases of chloracne reported during 1969–1975 in a production of the herbicide 2,4,5-T that was contaminated with TCDD; only 50 survivors participated in the follow-up until 1996 (i.e., current study cohort)	Comparisons within the exposed group; higher exposure (mean 801 pg TCDD/g blood lipid) and lower exposure group (mean 407 pg/g)	Increased chronic liver disease history associated with higher exposure; abnormal coprophorphyrins in urine; compared to controls, γ-GGT, SGOT, and SGPT were significantly higher in exposed
Pelclova et al. 2001	Follow-up examination of 13 workers exposed 30 years before to TCDD in an industrial setting in herbicide production plant	Current mean plasma level was 256 pg TCDD/g lipid (range=14–760 pg/g lipid)	Significant positive correlations between TCDD levels and levels of beta-lipoproteins, cholesterol, and triglycerides were reported; no changes in bilirubin or ALT

ALT = aminotransferase; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; γ-GGT = γ-glutamyltransferase; OR = odds ratio; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalent; WHO = World Health Organization

Table 2-3. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Calvert et al. 1999	A cross-sectional study in workers exposed >15 years before in the production of 2,4,5-trichlorophenol in the United States; the workers had individual TCDD measurements and were originally part of a much larger retrospective cohort study 281 exposed, 260 controls	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	The prevalence of diabetes was not significantly different between the groups. According to the authors, the highest exposures correlated with increased serum glucose (treated diabetics excluded) and higher FT4 index. However, the inverted U shape did not provide a convincing association, with lowest worker TCDD category having about the same glucose level and the middle two quartile levels similar to referents. Table 4 (in the original paper) with measured TCDD provided little support for Table 5 conclusion reached by the authors.
Cranmer et al. 2000	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 correlated with higher blood TCDD levels
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 associated with higher dioxins, coplanar PCBs and 12 PCB markers; results can be considered suggestive or hypothesis generating
Foster et al. 2005	Cross-sectional examination; pregnant women (n=150) attending a prenatal diagnosis clinic	Serum dioxin-like activity measured in 145 of 150 maternal serum samples; the mean serum lipid-adjusted dioxin-like activity TEQs was 0.34 pg/g	Multiple regression analysis failed to demonstrate a relationship between maternal serum dioxin-like activity and serum thyroid hormone levels

Table 2-3. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Kang et al. 2006	A health survey of 1,499 Vietnam veterans and 1,428 non-Vietnam veterans assigned to chemical operations jobs was 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	Among those Vietnam veterans who sprayed herbicides: diabetes, OR=1.50 (1.15–1.95); heart disease, OR=1.52 (1.18–1.94); hypertension, OR=1.32 (1.08–1.61); and chronic respiratory condition, OR=1.62 (1.28–2.05)
Longnecker and Michalek 2000	1,197 veterans in the Air Force Health Study who never had contact with dioxin-contaminated herbicides	Selected group TCDD levels up to the background U.S. level of 10 ng/kg lipid; four quartiles: the lowest <2.8 ng/kg lipid; the highest ≥5.2 ng/kg lipid	The multivariate-adjusted OR of diabetes among those in the highest quartile compared to the lowest one was 1.71; lower odds found after adjustments for serum triglycerides
Michalek et al. 1999a	Air Force Ranch Hand (RH) veterans exposed to TCDD in Vietnam (1962–1971) and veteran controls not exposed; 1992 follow-up High RH exposure Diabetics (n=43) Nondiabetics (n=205) Low RH exposure Diabetics (n=36) Nondiabetics (n=211) Background RH exposure Diabetics (n=32) Nondiabetics (n=344) Controls Diabetics (n=125) Nondiabetics (n=996)	Three exposure categories (current serum TCDD in ppt [median value]): 5.7, 15, and 45.8	Tested: insulin, fasting glucose, and SHBG. In nondiabetic veterans, the mean of the logarithm of insulin significantly increased in the high dioxin category. In addition, among nondiabetic veterans ≤53 years old with 25% body fat or less in the high category, the slope relating the logarithm of SHBG and the logarithm of insulin was significantly decreased.

Table 2-3. Endocrine Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Pavuk et al. 2003	U.S. Air Force veterans of Operation Ranch Hand (RH) (n=1,009) and veteran controls (n=1,429); cross-sectional analysis	Groups: high (>94 ppt), low (>10 and <94 ppt), background (<10 ppt), controls	Increased TSH means at the 1985 and 1987 examinations for the high RH group; a trend for increased TSH across all exposed groups and examinations

CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; FT4 = free thyroxine; OR = odds ratio; PCB = polychlorinated biphenyl; RH = Operation Ranch Hand; SHBG = sex hormone-binding globulin; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalent; TSH = thyroid stimulating hormone

Table 2-4. Immunological Effects in Humans Exposed to 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	TCDD in plasma (lipid adjusted) ranged from 3.5 to 90 ng/kg (ppt)	TCDD levels negatively correlated with plasma IgG concentrations
Halperin et al. 1998	A cross-sectional medical survey; the exposed (n=259) worked between 1951 and 1972 in two chemical plants manufacturing 2,4,5-trichloro-phenate and its derivatives; the controls (n=243) had no occupational exposure to phenoxy herbicides and lived within the same communities	The workers had a lipid adjusted mean serum TCDD concentration of 229 ppt compared with a mean of 6 ppt in the control group	Workers divided into categories based on their serum TCDD concentration. For all categories except the lowest (comparable with controls), there were increased odds of having lower counts of CD26 cells (activated T cells) OR 1.0, for TCDD <20 ppt; OR 1.6 for TCDD 20–51 ppt; OR 2.7 for TCDD 52–125 ppt; OR 2.6 for TCDD 125–297 ppt; OR 2.4 for TCDD 298–3,389 ppt. A less consistent finding was decreased spontaneous proliferation of cultured lymphocytes. The results are not of clinical importance.
Kim et al. 2003	Veteran patients (n=24) (50% had cardiovascular diseases, 21% neurological problems, 17% dermal problems); veterans healthy (n=27) (i.e., no chronic diseases); controls (n=36)	Exposure to Agent Orange during the war; no specific TCDD measurements	Red blood cells, hemoglobin, and hematocrit decreased in the veteran patients group; significant decrease in the IgG1 levels in veterans; IFNgamma, (cytokine mediating host resistance against infection or tumoregenesis) was lowered while IL-4 and IL-10 (cytokines involved with hypersensitivity induction) were increased in the veterans patient group

Table 2-4. Immunological Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Michalek et al. 1999b	Air Force veterans exposed to TCDD in Vietnam (1962–1971) and veteran controls not exposed; see Michalek et al. 1999a for details on the exposure groups	Three exposure categories (current serum TCDD in ppt [median value]): 5.7, 15, 45.8	Tested: delayed-type hypersensitivity skin tests; total lymphocyte counts; T-cell, B-cell, and NK-cell subsets; and expression of the activation antigen CD25 on CD3 T cells; serum concentrations of immunoglobulin (Ig)A, IgG, and IgM; M proteins; and wide-range of autoantibodies; no evidence of a consistent relation between dioxin exposure category and immune system alteration

CDD = chlorinated dibenzo-*p*-dioxin; OR = odds ratio; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Table 2-5. Neurological Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Neuberger et al. 1999	Case-control study; originally,159 cases of chloracne reported during 1969–1975 in production of the herbicide 2,4,5-T contaminated with TCDD; followed-up to 1996; only 50 survivors participated in 1996 (i.e., current study cohort)	Comparisons within the exposed group; higher exposure (mean 801 pg TCDD/g blood lipid) and lower exposure group (mean 407 pg/g)	Neurological symptoms were reported frequently (44% sleep disturbance, 32% headache, 30% neuralgia); statistically significant compared to controls except for neuralgia, which was not investigated in controls
Pelclova et al. 2001	1996 follow-up examination of 13 workers exposed 30 years ago to TCDD in an industrial setting in an herbicide production plant	Current mean plasma level was 256 pg TCDD/g lipid (range=14–760 pg/g lipid)	Abnormal EMG, EEG, and visual evoked potentials were observed in 23, 54, and 31% workers, respectively; abnormal EEG strongly correlated with plasma levels >200 pg/g
Pelclova et al. 2002	2001 follow-up examination of 12 workers exposed 35 years earlier to TCDD in an industrial setting in herbicide production plant	1996 mean plasma level was 256 pg TCDD/g lipid (range=14–760 pg/g lipid)	Neuropsychological findings were normal only in three individuals with lower TCDD levels; organic psychosyndrome and pseudo-neurasthenic syndrome were reported in those affected
Thömke et al. 1999	Herbicide production workers exposed in the period between 1950 and 1984 (some for <1 year); 35 had chloracne, 86 did not; chloracne occurred 2–20 years prior the current examination	Workers with chloracne had back-calculated median CDD/CDF level of 871 pg TEQs/g lipids and workers without chloracne 330 pg TEQs/g; TCDD median levels were 203 and 113, respectively	Workers with chloracne had significantly more frequent clinical signs of a sensory neuropathy restricted to the legs (17.1% compared to 1.2%), more frequent neurophysiologic abnormalities (34.3% compared to 14.0%), and lower mean amplitudes of the motor compound muscle potential of the peroneal nerve
Thömke et al. 2002	Herbicide production workers (some exposed for <1 year); 35 had chloracne, 86 did not; exposure occurred 2–36 years prior the current examination	Workers with chloracne had back-calculated median CDD/CDF level of 871 pg TEQs/g lipids and workers without chloracne 330 pg TEQs/g; TCDDs median levels were 203 and 113, respectively	Brainstem auditory evoked potentials more frequent in workers with chloracne (but not statistically significant); severe exposure to CDDs/CDFs is not followed by clinical signs of cranial nerve dysfunction

Table 2-5. Neurological Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Urban et al. 2007	Between 1965 and 1968, about 350 workers were exposed to TCDD in an herbicide production plant; about 80 workers developed signs of poisoning; 15 subjects from the original cohort remained available for the follow-up in 2004	The estimated mean concentration of TCDD at the time of exposure was about 5,000 pg/g of plasma lipids; the mean current TCDD plasma concentration was 128 pg/g	Neurological examination showed some central nervous system impairment in eight subjects. Signs of polyneuropathy were found in nine subjects, confirmed by nerve conduction velocity studies in three cases. EEG was abnormal in three cases; visual-evoked potential in five cases. Acquired dyschromatopsia was detected in six patients. SPECT showed focal reduction of perfusion in various brain locations in all but one patient.

CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; EEG = electroencephalography; EMG = electromyography; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalent

Table 2-6. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
De Felip et al. 2004	A pilot case-control study in 22 Italian and 18 Belgian women with and without endometriosis	Total TEQs in women without endometriosis were 18 pg/g (Italian) and 45 pg/g (Belgium)	No correlation found between TEQ levels and endometriosis
Eskenazi et al. 2003	20 years after the Seveso, Italy (1976) incident, a group of 510 exposed women (888 total pregnancies) investigated; stored serum	The median maternal TCDD serum level at the time of the incident was 46.6 ppt	Nonsignificant results for spontaneous abortions (97 pregnancies ended), low birth weight, and small for gestational age; possibly indicative of a stronger association for the last two end points during the first 8 years after the incident
Eskenazi et al. 2002a	20 years after the Seveso, Italy (1976) incident, a group of 601 women who were ≤30 years at the time of the incident; stored serum	Three groups TCDD serum levels ≤20 ppt 20.1–100 ppt >100 ppt	19 cases with endometriosis identified (confirmed surgically or by the ultrasound); relative risk ratios (to the low exposure group) were 1.2 and 2.1 for the higher exposure groups; doubled nonsignificant risk for the highest exposure; no dose-response trend
Eskenazi et al. 2002b	20 years after the Seveso, Italy (1976) incident, a group of 301 women who were ≤40 years old in 1976; stored serum	Median TCDD level 67.5 ppt	In women who were premenarcheal in 1976, a 10-fold increase in TCDD levels associated with lengthening of menstrual cycle; no link observed in women who were postmenarcheal in 1976; in both groups, decreased risk of irregular menstrual flow
Eskenazi et al. 2005	20 years after the Seveso, Italy (1976) incident, a group of 616 women investigated; stored serum	Five groups TCDD serum levels <20.4 ppt 20.4–34.2 ppt 34.3–54.1 ppt 54.2–118 ppt >118 ppt	Suggested a dose-related association between the TCDD levels and increasing risk of early menopause up to 100 ppt, but not above

Table 2-6. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Eskenazi et al. 2007	20 years after the Seveso, Italy (1976) incident, a group of 956 eligible women; stored serum	Three groups TCDD levels ≤20 ppt 20.1–75 ppt >75 ppt	251 had fibroids (uterine leiomyomata); age adjusted hazard-ratios were 0.58 and 0.62, respectively, relative to the ≤20 ppt group; antiestrogenic effects on myometrium
Fierens et al. 2003	Volunteer-case study; environmental exposure to CDDs, CDFs, PCBs (Belgium)	Total TEQs (geometric mean) Cases (n=10) 34.6 pg/g Controls (n=132) 34.5 pg/g	No association of TCDD and endometriosis
Heilier et al. 2005	Case-control study; patients with peritoneal endometriosis (n=25) or deep endometriotic (adenomyotic) nodules (n=25) and controls (n=21)	CDDs, CDFs, and dioxin-like PCB serum concentrations; the mean TEQ levels were 24.21 (controls), 30.62 (peritoneal endometriosis), and 37.60 (deep endometriotic nodules) pg TEQ/g lipids	A significantly increased risk of deep endometriotic (adenomyotic) nodules (OR=3.3) for an increment of 10 pg in total TEQ levels/g lipids. An increased risk was also found for peritoneal endometriosis (OR=1.9; 95% CI=0.9–3.8) for total TEQ levels and for dioxins alone (OR=3.2; 95% CI=1.0–9.9).
Lawson et al. 2004	A total of 1,117 live singleton births of 217 referent wives and 176 wives of workers exposed in chemical factories	Fathers TCDD levels: low 20–255 pg/g; high ≥255 pg/g; controls <20 pg/g lipids	No statistically significant differences after adjustment for confounders; TCDD is unlikely to increase the risk of low birth weight or preterm delivery through a paternal mechanism
Lin et al. 2006	Ambient exposure to incinerator generated CDDs and CDFs; 1991 cohort 1 year before the incinerator operated and 1997 cohort 5 years after the operation started; Taiwan's birth registry data; 6,697 and 6,282 neonates, respectively	Lower exposure 0.03–0.05 pg TEQ/m^3 and higher exposure >0.05 pg TEQ/m^3 groups	Incinerator-generated dioxin has little effect on birth weight and female birth, but may have a marginal effect on gestational age (OR=1.12/1.22) (for 1991/1997)
Mocarelli et al. 2000	Seveso, Italy cohort; serum from 239 men and 296 women collected in 1976–1977; births 1977–1996	The exposure/effect association starts at concentrations <20 ng TCDD per kg body weight	Exposure of men to TCDD is linked to a lowered male/female sex ratio in their offspring, which may persist for years after exposure

Table 2-6. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Mocarelli et al. 2008	Seveso, Italy cohort; serum (from 1976) from 135 exposed men and 184 controls; semen quality and reproductive hormones tested in samples collected 22 years later	Groups according to maturity at the time of exposure: A. infancy/prepuberty (1–9 years), B. puberty (10–17 years), and C. adulthood (18–26 years). A group: n=71, median serum TCDD, 210 ppt; B group: n=44, median serum TCDD, 164 ppt; C group: n=20, median serum TCDD, 123 ppt.	No effects observed in group C (i.e., exposed as adults). Reductions in sperm concentration, percent progressive motility, total motile sperm count, estradiol; and an increase in FSH found in group A. Increased total sperm count, total motile sperm count, FSH, and reduced estradiol reported in group B.
Pauwels et al. 2001	Case control study; 42 endometriosis infertile women and 27 mechanical infertile controls	Investigated total dioxin-like chemicals (in TEQs) and PCBs; higher exposure considered as 100 pg TEQs/g serum lipids	No association found between dioxin-like chemicals and endometriosis in infertile women
Ryan et al. 2002	A Russian cohort of workers (150 males and 48 females) exposed during pesticide production in 1961–1988; 227 offspring	84 blood samples measured for TCDD; mean level 240 ng TEQs/kg	Total 0.40 sex ratio for males vs. 0.512 for the background; decreased number of boys associated with paternal exposure, but not maternal
Schnorr et al. 2001	Interviews of wives (n=245) of workers exposed previously to TCDD during Agent Orange production and their controls (n=215)	Estimated TCDD levels of workers during or after exposure were: median=254 ppt (range, 3–16,340 ppt) compared to referent levels: median=6 ppt (range, 2–19 ppt)	No differences in spontaneous abortions between four exposure groups and controls; no changes in the sex ratio
Thömke et al. 1999	Herbicide production workers exposed in the period between 1950 and 1984 (some for <1 year); 35 had chloracne, 86 did not; chloracne occurred 2–20 years prior the current examination	Workers with chloracne had back-calculated median CDD/CDF level of 871 pg TEQs/g lipids and workers without chloracne 330 pg TEQs/g; TCDD median levels were 203 and 113, respectively	Workers with chloracne complained significantly more often of sexual impotence (28.6% compared to 5.8%)
Warner et al. 2007	A Seveso, Italy cohort of 363 women who were infants to 40 years old in 1976 at the time of the industrial accident and resided in the TCDD exposed area; the women eligible for this study were 20–40 years of age (by 1998) and nonusers of oral contraceptives	The median serum TCDD level was 77.3 ppt, lipid-adjusted (1976 values)	Ovarian function (ovarian cysts, ovarian follicles, ovulation rate, serum hormones) tested; no clear evidence that exposure was associated with ovarian function 20 years later in women exposed to relatively high TCDD levels

Table 2-6. Reproductive Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Warner et al. 2004	A Seveso, Italy cohort of 282 women who were premenarcheal at the time of explosion	The median serum TCDD level was 140.3 ppt (range 3.6–56,000 ppt); for those who were <8 years of age (n=158) at the time of explosion, the median level was 205.0 ppt TCDD	The individual TCDD serum levels were not related to the age at menarche; no change in the risk of onset for menarche

CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; CI = confidence interval; FSH = follicle-stimulating hormone; OR = odds ratio; PCB = polychlorinated biphenyl; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalent

Table 2-7. Developmental Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
Baccarelli et al. 2008	Seveso, Italy; a residence-based population 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 means of plasma TCDD were 6.1 ppt for the control; 17.6 and 60.5 ppt in zones B and A, respectively.	Mean neonatal blood TSH was 0.98 µU/mL (95% CI 0.90–1.08) in the reference area (n=533), 1.35 µU/mL (95% CI 1.22–1.49) in zone B (n=425), and 1.66 µU/mL (95% CI 1.19–2.31) in zone A (n=56) (p<0.001)
Cao et al. 2008	104 mother-infant pairs in Duisburg, Germany; linear regression analysis used to evaluate association between CDDs/CDFs or PCBs in maternal blood or milk with sex steroids in cord blood of newborns	CDDs/CDFs maternal blood fat=15.3 pg/g TEQs; PCBs=149 ng/g CDDs/CDFs in milk fat=13.1 pg/g TEQs; PCBs=177 ng/g	Testosterone levels decreased in cord serum of female and estradiol reduction in male newborns; reduction of hormone levels was more pronounced for dioxins than for indicator PCBs
Eskenazi et al. 2003	20 years after the Seveso, Italy (1976) incident, a group of 510 exposed women (888 total pregnancies) were investigated	The median maternal TCDD serum level at the time of the incident was 46.6 ppt, with interquartile range of 24.3–104 ppt	No association of TCDD with low birth weight or small for gestational age for all pregnancies; statistically nonsignificant increased odds for preterm delivery
Wilhelm et al. 2008	Duisburg, Germany cohort; 189 mother-infant pairs included in the final statistical analysis; measured CDDs/CDFs and PCBs in maternal blood and milk; TSH and thyroid hormones in maternal and cord serum samples; neurological examination at 2 weeks and 18 months; developmental tests at 12 and 24 months	Blood levels (n=182) of CDDs/CDFs and PCBs ranged 3.8–58.4 pg/g TEQs; breast milk levels (n=149) of CDDs/CDFs and PCBs ranged 2.6–52.4 pg/g TEQs	No decrease of thyroid hormone levels reported in association with exposure to persistent organic pollutants; no changes in the neurological or mental and motor development
Wang et al. 2005	A part of a prospective study in the general population of Taiwan; 17 CDDs and CDFs, 12 dioxin-like PCBs, and 6 indicator PCBs analyzed in placentas and thyroid and related growth hormones measured in cord serum; female (n=62) and male (n=57) newborns in the cohort	Two groups: low exposure <15.1 (n=59) and higher exposure >15.1 (n=60) of dioxin/PCB TEQs (in pg/g lipid)	Increased TSH levels associated with increased CDDs and dioxin-like PCBs; similarly, positive association between increased T4 levels and CDDs/CDFs; FT4xTSH levels were negatively correlated with concentrations of PCBs 138, 153, and 180 independently of maternal age and other congeners

CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; CI = confidence interval; FT4 = free thyroxine; PCB = polychlorinated biphenyl; T4 = thyroxine; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TEQ = toxic equivalent; TSH = thyroid-stimulating hormone

Table 2-8. Genotoxic Effects in Humans Exposed to 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	TCDD in plasma (lipid adjusted) ranged from 3.5 to 90 ng/kg (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's 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 vs. 8.18)
Valic et al. 2004	A case-control study; 2 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	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).

Table 2-8. Genotoxic Effects in Humans Exposed to TCDD/CDDs

Reference	Design and population	TCDD/CDD concentrations	Effects
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	The mean CDD, CDF, and coplanar-PCB levels were 12.9, 12.4, and 13.6 pg TEQs/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 = arylhydrocarbon 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 equivalent

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

Reference	Design and population	TCDD/CDD concentrations	Effects
Bertazzi et al. 2001	Seveso, Italy; a follow-up mortality study; zone A: 414 females and 390 males, zone B: 2,924 females and 3,017 males; zone R 19,424 females and 19, 200 males; reference zone: 118,775 females and 113,970 males	Zone A: median lipid-adjusted plasma concentration 447 ppt (296 people tested in 1976–1977); zone B: 94 ppt (80 people tested in the same year)	All cause and all cancer mortality was similar to that in the reference population; mortality from colon cancer was increased in zone B (RR=1.9)
Floret et al. 2003	A population-based, case-control study in France; incident cases (n=222); general population (n=114,00) living around a municipal incinerator; cancer registry data	Dioxin ground concentrations modeled with a second-generation Gaussian-type dispersion model; four zones: <0.0001, 0.0001–0.0002, 0.0002–0.0004, and 0.0004–0.0016 pg/m^3	The risk of developing non-Hodgkin lymphoma was 2.3 times higher for individuals living in the highest exposure area compared with the lowest one
Giri et al. 2004	A case-control study in veterans (hospital records); 47 cases with prostate cancer versus 142 controls	Exposure to Agent Orange assessed from medical records; no TCDD measurements were performed It should be noted that a Ranch Hand study analyses previously showed that the self-reported exposure was unlikely to correspond to actual exposures (Michalek et al. 1995)	After adjusting for age and race, men with prostate cancer were approximately 2 times more likely to report previous exposure to Agent Orange (OR 2.06; 95% CI 0.81–5.23)
McBride et al. 2009a	New Zealand cohort of 1,599 workers employed between 1969 and1988 in a factory that manufactured trichlorophenol	Current TCDD levels averaged 9.9 ppt in the exposed group and 9.4 ppt in the control group; the back-calculated cumulative exposures made up four groups: 0–68.3, 68.4–475.0, 475.1–2,085.7, and >2,085.8 ppt-mo	In the exposed group, deaths from total cancer (SMR=1.1), non-Hodgkin lymphoma (SMR=1.6), lung cancer (SMR=0.8), and other causes were evaluated

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

Reference	Design and population	TCDD/CDD concentrations	Effects
Pavuk et al. 2006	Vietnam veterans study; 59 and 81 prostate cancers were identified in the Ranch Hand veterans and their comparisons, respectively, between 1 January 1982 and 31 December 2003		No overall increase in the risk of prostate cancer in Ranch Hand veterans; possible increased risk in higher exposure group (RR=2.27; small numbers n=15); also, a longer service in South East Asia associated with increased risk of prostate cancer in comparison veterans
Steenland et al. 1999	A 6-year extended follow-up of the large NIOSH cohort of male workers (n=5,132) from 12 U.S. factories exposed in 1942–1984 during the production of TCDD-contaminated products	Blood samples from workers (n=253) suggested estimated mean serum level of 2,000 ppt in lipids at the time of exposure; exposure categories created based on points obtained by attributed job-exposure matrix	The SMR for all cancers combined was 1.13 (95% CI=1.02–1.25); statistically significant positive linear trends were observed in SMRs with increasing exposure for all cancers combined and for lung cancer
‘t Mannetje et al. 2005	New Zealand phenoxy herbicide producers (164 died) and sprayers (91 died); follow-up from 1969/1973 to 2000	Job classification categories; no actual data on exposure levels	Multiple myeloma increased (SMR=5.51) in production workers; colon cancer increased (SMR=1.94) in sprayers
Viel et al. 2008	General population living in the vicinity of municipal solid waste incinerators; a case (n=434) control (n=2,170) study	High, intermediate, low, and very low exposure categories; dioxin exposure assessments were GIS-based and described in Floret et al. (2003, 2006)	Among women <60 years old, no increased or decreased risk of breast cancer was found for any dioxin exposure category; for older women, a decreased risk was reported in the high exposure category (may reflect some undetermined confounders)
Warner et al. 2002	A Seveso, Italy cohort of 981 women who were infants to 40 years old in 1976 at the time of the industrial accident and resided in the exposed area	Serum TCDD levels for cases ranged from 13 to 1,960 ppt after the accident (1976–1981)	15 women (1.5%) had been diagnosed with breast cancer (by 1998); the hazard ratio was significantly increased to 2.1

CDD = chlorinated dibenzo-*p*-dioxin; CI = confidence interval; GIS = geographic information system; NIOSH = National Institute for Occupational Safety and Health; OR = odds ratio; RR = relative risk; SMR = standardized mortality ratio; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Table 2-10. Summary of Select Animal Systemic Effects Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Respiratory effects					
Rat (Sprague-Dawley), 5–8 females/group	0 or 0.125 µg/kg/day for 14, 30, or 60 weeks	NA	0.125 µg/kg/day: alveolar epithelial metaplasia (60 weeks)		Tritscher et al. 2000
Rat (Sprague-Dawley), 50 females/group	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/week for 105 weeks	NA	0.003 µg/kg: bronchiolar metaplasia of alveolar epithelium	≥0.022 µg/kg: histiocytic infiltration	NTP 2006
Cardiovascular effects					
Rat (Sprague-Dawley), 50 females/group	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/week for 105 weeks	0.003 µg/kg	0.01 µg/kg: cardiomyopathy		NTP 2006
Mouse (C57BL/6) males/group	0 or 0.18 µg/kg capsule, 5 days/week for 35 days	NA	0.18 µg/kg: ↓ acetylcholine-dependent vasorelaxation of aortic rings; ↑ mean arterial blood pressure	No effect on angiotensin II, plasma angiotensin converting enzyme activity, or plasma rennin activity	Kopf et al. 2010
Gastrointestinal effects					
Rat (Sprague-Dawley), 50 females/group	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/week for 105 weeks	0.046 µg/kg	0.1 µg/kg: squamous hyperplasia of forestomach		NTP 2006
Rat (Sprague-Dawley), 50 females/group	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/week for 105 weeks	NA	0.003 µg/kg: squamous hyperplasia of gingival oral mucosa		NTP 2006
Mouse (C57BL/6J), 5 males/group	0 or 100 µg/kg once		100 µg/kg: severe spaces in villi were observed in proximal segment of intestine (examined 10 days postexposure); ↑ serum glucose following oral glucose challenge	No effect on length or weight of intestine	Ishida et al. 2005

Table 2-10. Summary of Select Animal Systemic Effects Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Hematological effects					
Rat (Sprague-Dawley), 5 males/group	0, 0.4, or 40 µg/kg once		0.4 µg/kg: ↑ hemoglobin (6 hours only)	Animals were sacrificed 6 hours, 24 hours, or 7 days after exposure 40 µg/kg: ↑ hemoglobin (1 and 7 days); ↑ red blood cells (7 days), ↓ reticulocytes (7 days)	Fletcher et al. 2005
Rat (WAG/Rij) 4/gender/group	0.00096, 0.0096, 0.096, 0.96, or 9.6 µg/kg, once	0.0096 µg/kg	0.096 µg/kg: ↓ relative factor VII plasma levels (females only)	Rats were maintained on a vitamin-K_3 deficient diet and blood samples were collected 6, 10, 14, or 21 days after exposure No effects on factor VII were observed in males	Bouwman et al. 1999
Musculoskeletal effects					
Rat (Han/Wistar or Long-Evans) 5 females/ strain/group	0, 0.0012, 0.012, 0.12, or 1.2 (Han/Wistar only) µg/kg/day	0.0012 µg/ kg/day	0.012 µg/kg/day: discoloration, pulp chamber open to lingual dental surface and pulpal perforation, larger-than-normal pulp chamber, pulpal cell death, and arrested dentin formation in lower incisor teeth	Initial loading dose of 0, 0.035, 0.35, 2.5, or 35 (Han/Wistar only) µg/kg followed by weekly doses of 0, 0.007, 0.07, 0.7, or 7 (Han/Wistar only) µg/kg for 19 weeks; respective cumulative doses were 0.17, 1.7, 17, or 170 µg/kg or 0.0012, 0.012, 0.12, or 1.2 µg/kg/day	Kiukkonen et al. 2002
Hepatic effects					
Mouse (C57BL/6) 8 females/group	0 or 30 µg/kg once		30 µg/kg: ↑ relative liver weight after ≥24 hours; cytoplasmic vacuolization in periportal and midzonal regions ≥18 hours; ↑ serum alanine aminotransferase, free fatty acids (≥24 hours), and triglyceride levels (≥24 hours); ↓ serum cholesterol (≥72 hours)	Ovariectomized mice were used; mice were killed 2, 4, 8, 12, 18, 24, 72, or 168 hours after dosing No effect on serum BUN, creatinine, glucose, or total bilirubin	Boverhof et al. 2005

Table 2-10. Summary of Select Animal Systemic Effects Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (C57BL/6) 8 females/group	0, 0.001, 0.01, 0.1, 1, 10, 100, or 300 µg/kg once	0.1 µg/kg	1 µg/kg: minimal to moderate cytoplasmic vacuolization in periportal and midzonal regions of liver	Ovariectomized mice were used; mice were killed 24 hours after dosing	Boverhof et al. 2005
				100 µg/kg: ↑ relative liver weight	
Mouse (C57BL/6) 4 females/group	0, 0.001, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, or 300 µg/kg once		30 µg/kg: hepatocellular vacuolization, mixed cell infiltration	Ovariectomized mice were used; mice were killed 24 hours after dosing	Kopec et al. 2010
				300 µg/kg: ↑ relative liver weight, hepatocellular single cell necrosis	
Mouse (C57BL/6) 5 females/group	0, 30, or 100 µg/kg once		30 µg/kg: ↑ relative liver weight; cytoplasmic vacuolization in periportal and midzonal regions and hepatocellular necrosis	Ovariectomized mice were used; mice were killed 72 hours after dosing	Kopec et al. 2008
				100 µg/kg: mixed cell infiltration (neutrophils and mononuclear cells)	
Rat (Sprague-Dawley) 5 females/group	0 or 10 µg/kg once	NA	10 µg/kg: ↑ relative liver weight after ≥72 hours and absolute liver weight after 168 hours; minimal to moderate hepatocellular hypertrophy in cetnriacinar region (≥24 hours); ↑ serum free fatty acids, triglyceride levels and serum cholesterol at 24 hours	Ovariectomized rats were used; rats were killed 2, 4, 8, 12, 18, 24, 72, or 168 hours after dosing	Boverhof et al. 2006
				No effect on serum alanine aminotransferase, BUN, or total bilirubin	
Rat (Sprague-Dawley) 5 females/group	0, 0.001, 0.01, 0.1, 1, 10, 30, or 100 µg/kg once	10 µg/kg	30 µg/kg: minimal to mild hepatocellular hypertrophy	Ovariectomized rats were used; rats were killed 24 hours after dosing	Boverhof et al. 2006

Table 2-10. Summary of Select Animal Systemic Effects Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Sprague-Dawley), 5 males/group	0, 0.4, or 40 µg/kg once		40 µg/kg: ↓ cholesterol at 6 hours and ↑ cholesterol at 1 and 7 days; ↑ triglyceride levels (1 and 7 days); centrilobular hypertrophy (7 days)	Animals were sacrificed 6 hours, 24 hours, or 7 days after exposure	Fletcher et al. 2005
Rat (Sprague-Dawley), 5 males/group	0 or 10 µg/kg/day for 6 days	NA	10 µg/kg/day: ↑ relative liver weight, ↑ serum cholesterol and total bilirubin; ↓ serum alkaline phosphatase activity and serum triglyceride level; mild hepatocellular swelling and vacuolization; inflammatory cell infiltration	No effect on serum alanine or aspartate aminotransferase activities	Lu et al. 2010
Rat (Sprague-Dawley), 10 females/ group/period	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/ week for 14 or 31 weeks	0.01 µg/kg	0.022 µg/kg: hepatocyte hypertrophy	≥0.01 µg/kg: ↑ pigmentation 0.1 µg/kg: diffuse fatty changes	NTP 2006
Rat (Sprague-Dawley), 50 females/ group	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/ week for 105 weeks	NA	0.003 µg/kg: hepatocyte hypertrophy and inflammation	≥0.01 µg/kg: pigmentation, diffuse fatty changes, necrosis ≥0.022 µg/kg: bile duct hyperplasia ≥0.046 µg/kg: portal fibrosis 0.1 µg/kg bile duct cysts	NTP 2006
Guinea pig (Hartley), 5 males/group	0, 0.012, 0.047, 0.18, 0.66, or 2.5 µg/kg once	0.012 µg/kg	0.047 µg/kg: ↑ relative liver weight	Animals were sacrificed 28 days after dosing ≥0.18 µg/kg: ↓ hepatic vitamin A levels and gain 2.5 µg/kg: 60% mortality	Fletcher et al. 2001
Rat (Sprague-Dawley), 5 males/group	0, 0.12, 0.66, 3.5, 19, or 100 µg/kg once	NA	0.12 µg/kg: ↑ relative liver weight; ↓ hepatic vitamin A levels and gain	Animals were sacrificed 28 days after dosing	Fletcher et al. 2001

Table 2-10. Summary of Select Animal Systemic Effects Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (C57BL/6), 5 males/group	0, 1.6, 8, 40, 200, or 1000 µg/kg once	NA	1.6 µg/kg: ↓ hepatic vitamin A levels and gain ≥8 µg/kg: ↑ relative liver weight	Animals were sacrificed 28 days after dosing	Fletcher et al. 2001
Hamster (Golden Syrian), 5 males/group	0, 1.6, 8, 40, 200, or 1000 µg/kg once	NA	1.6 µg/kg: ↑ relative liver weight; ↓ hepatic vitamin A levels and gain	Animals were sacrificed 28 days after dosing	Fletcher et al. 2001
Rat (Sprague-Dawley), 6 males/group	0, 0.1, 1.0, 10, or 100 µg/kg once	NA	10 µg/kg: ↓ hepatic retinyl esters and ↑ all trans-retinoic acid levels	Animals were sacrificed 3 days after dosing or after 1 or 28 days (only 0 and 10 µg/kg tested)	Hoegberg et al. 2003
Mouse (C57BL/6), 5 males/group	0 or 0.1 µg/kg/day for 14, 28, or 42 days	NA	0.1 µg/kg/day: ↓ all-trans-retinol, ↑ all-trans retinoic acid levels, and ↑ retinal oxidase activity in liver		Yang et al. 2005
Renal effects					
Rat (Sprague-Dawley), 5 males/group	0 or 10 µg/kg/day for 12 days	NA	10 µg/kg/day: ↓ absolute kidney weight and ↑ relative kidney weight, ↑ serum creatinine and BUN levels; damage to renal tubular cells moderate swelling and flattening of proximal tubular epithelial cells, tubular deformation and diminution, tubulinterstitial congestion	↓ growth and food consumption	Lu et al. 2009
Rat (Sprague-Dawley), 50 females/ group	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/ week for 105 weeks	0.022 µg/kg	0.046 µg/kg: hyperplasia of transitional renal epithelium	0.1 µg/kg: nephropathy	NTP 2006
Rat (Sprague-Dawley), 6 males/group	0, 0.1, 1.0, 10, or 100 µg/kg once	NA	10 µg/kg: ↑ renal all trans-retinoic acid levels and renal retinyl esters levels (only 28 days postexposure); ↑ lecithin:retinol acyltransferase mRNA levels	Animals were sacrificed 3 days after dosing or after 1 or 28 days (only 0 and 10 µg/kg tested)	Hoegberg et al. 2003

Table 2-10. Summary of Select Animal Systemic Effects Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Endocrine effects					
Rat (Long-Evans), 12 males/group	0, 10, 20, or 40 µg/kg once	NA	10 µg/kg: ↓ serum total T4 levels, ↓ liver type I iodothyronine diodinase activity, ↑ brown adipose tissue type II iodothyronine diodinase activity (10 and 20 µg/kg only)	Animals were examined 4 days after dosing ↓ body weight gain observed at all dose levels No effect on serum T3 levels	Raasmaja et al. 1996
Rat (Sprague-Dawley), 5 males/group	0, 0.1, 1, 5, 15, 30, or 60 µg/kg once	1 µg/kg	5 µg/kg: ↓ serum total T4 levels, ↓ liver type I iodothyronine diodinase activity	Animals were examined 8 days after dosing No effect on serum T3 levels	Viluksela et al. 2004
Rat (Sprague-Dawley), 10 females/ group/duration	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/ week for 14 or 31 weeks	0.010 µg/kg	0.022 µg/kg: ↓ total and free T4; follicular cell hypertrophy of thyroid	≥0.046 µg/kg: ↑ total T3 and TSH (14 weeks only)	NTP 2006
Rat (Sprague-Dawley), 10 females/ group/duration	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/ week for 53 weeks	0.022 µg/kg	0.046 µg/kg: ↓ total T4; follicular cell hypertrophy of thyroid	≥0.1 µg/kg: ↑ total T3	NTP 2006
Rat (Sprague-Dawley), 50 females/ group	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/ week for 2 years	0.022 µg/kg	0.046 µg/kg: hyperplasia of adrenal gland cortex	0.1 µg/kg: cortical atrophy in adrenal gland	NTP 2006
Rat (Sprague-Dawley), 18 males/group	0 or 10 µg/kg once	NA	10 µg/kg: ↓ ratio of plasma ACTH to corticosterone	Rats were examined 10 days after 2,3,7,8-TCDD exposure No effect on pituitary or plasma ACTH levels, pituitary weight, adrenal or plasma corticosterone levels, adrenal gland weight, corticotrophin releasing hormone-stimulated ACTH secretion, or ACTH-stimulated corticosterone secretion	Pitt et al. 2000

Table 2-10. Summary of Select Animal Systemic Effects Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Dermal effects					
Mouse (hairless) 10 males/group	0, 0.00003 or 0.0001 µg/kg/day for 54 days		0.00003 µg/kg/day: increased scratching behavior at 54 days in mice receiving water or acetone/olive oil skin application	Distilled water or acetone/olive oil was applied to the rostral part of the back every 2 days for 54 days No changes in scratching behavior in mice not receiving water or acetone skin treatment	Ono et al. 2010
Metabolic effects					
Rat (Sprague-Dawley), 5 males/group	0, 0.4, or 40 µg/kg once	0.4 µg/kg	40 µg/kg: ↓ serum glucose levels (7 days only)	Animals were sacrificed 6 hours, 24 hours, or 7 days after exposure	Fletcher et al. 2005
Rat (Long-Evans), 4–6/gender/group	0, 2.5 (females only), 5, 10, 15, 20 (males only), or 50 µg/kg once	2.5 µg/kg	5 µg/kg: ↓ plasma glucose and liver glycogen content (females only)	Ad libitum and pair fed control groups used	Viluksela et al. 1999
Other systemic effects					
Rat (Sprague-Dawley), 10 females/ group/duration	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/ week for 14 or 31 weeks	0.046 µg/kg	0.01 µg/kg: acinar cytoplasmic vacuolization in the pancreas		NTP 2006
Rat (Sprague-Dawley), 50 females/ group	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg, 5 days/ week for 2 years	0.022 µg/kg	0.046 µg/kg: acinar cytoplasmic vacuolization in the pancreas	0.1 µg/kg: chronic active inflammation and acinar atrophy in pancreas	NTP 2006

[a]Doses were administered via gavage in oil, unless otherwise specified.

ACTH = adrenocorticotropin; BUN = blood urea nitrogen; LOAEL = lowest-observed-adverse-effect level; mRNA = messenger ribonucleic acid; NA = not applicable; NOAEL = no-observed-adverse-effect level; NTP = National Toxicology Program; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

Table 2-11. Summary of Select Animal Immunotoxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Effects of 2,3,7,8-TCDD on primary and secondary immune organs					
Mouse (C57BL/6) 5 females/group	0, 1.0, 3.0, or 10 µg/kg (in corn oil) once	1.0 µg/kg	3 µg/kg: ↓ thymocyte and splenocyte number and relative thymus and spleen weights	Mice immunized with 100 µg ovalbumin (intraperitoneal) immediately after CDD exposure and sacrificed on day 7	Ao et al. 2009
Mouse (C57BL/6) 5 females/group	0 or 20 µg/kg (in corn oil) once	NA	20 µg/kg: ↓ thymocyte and splenocyte number and relative thymus weight	Mice immunized with 100 µg ovalbumin (intraperitoneal) 2 weeks immediately after CDD exposure and sacrificed 1 week later	Ao et al. 2009
Mouse (C57BL/6), 3 or 6 weeks of age, 5–6 females/age/group	0, 0.3, 1.0, or 3.0 µg/kg (in corn oil) once	0.3 µg/kg	1.0 µg/kg: ↓ thymus weight, ↓ thymocyte number(3 weeks old only)	Mice immunized with 100 µg ovalbumin (intraperitoneal) immediately after CDD exposure No effect on splenocyte numbers	Inouye et al. 2005
Rat (F344) 5 males/group (74–78 weeks of age)	0, 0.1, 10, or 30 µg/kg (in corn oil) once	10 µg/kg	30 µg/kg: ↓ spleen weight, ↓ splenocytes and thymocytes	7 days after TCDD exposure, rats were infected with *Trichinella spiralis* larvae	Luebke et al. 1999
Mouse (B6C3F1) 8 females/group	0, 0.1, 1.0, or 10.0 µg/kg (in corn oil) once	0.1 µg/kg	1.0 µg/kg: ↓ spleen and thymus weights		Smialowicz et al. 1997
Rat (Sprague-Dawley) 5–6 females/group	0, 1, or 2 µg/kg (in corn oil)	NA	1 µg/kg: ↓ thymus weight	2 µg/kg: ↑ percent $CD8^+$ thymocytes No effect on thymus cellularity	Nohara et al. 2000
Mouse (B6C3F1) 7 females/group (12 weeks of age)	0, 0.1, 1, 10, or 30 µg/kg (in corn oil) once	1.0 µg/kg	10 µg/kg: ↓ spleen and thymus weight, ↓ splenocytes and thymoctyes	7 days after TCDD exposure, mice were infected with *T. spiralis* larvae	Luebke et al. 1999

Table 2-11. Summary of Select Animal Immunotoxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (B6C3F1) 7 females/group (76 weeks of age)	0, 0.1, 1, or 10 µg/kg (in corn oil) once	10 µg/kg	NA	7 days after TCDD exposure, mice were infected with *T. spiralis* larvae No effect on spleen or thymus weights or on number of splenoctyes or thymocytes	Luebke et al. 1999
Rat (Long-Evans) 4–6 females/group	0 or 25 µg/kg (in peanut oil) once	NA	25 µg/kg: thinning of thymic cortex	Groups of rats were also exposed to SEB (intraperitoneal) 2 days after CDD exposure	Huang and Koller 1998
Rat (Long-Evans) 4 females/group	0 or 25 µg/kg (in peanut oil) once	NA	25 µg/kg: dramatic thinning of thymic cortex	↑ absolute spleen weight likely due to ↓ body weight	Huang and Koller 1999
Rat (Long-Evans) 4 females/group	0 or 5 µg/kg/day (in peanut oil) for 5 days	NA	5 µg/kg/day: ↓ percent $CD4^+$ splenic T cells; thinning of thymic cortex	↑ absolute spleen weight likely due to ↓ body weight No significant alteration in splenic $CD8^+$ T cell subpopulations	Huang and Koller 1999
Effects of 2,3,7,8-TCDD on adaptive immune function					
Mouse (C57BL/6) 5 females/group	0, 1.0, 3.0, or 10 µg/kg (in corn oil) once	NA	1 µg/kg: ↓ IL-5 levels in spleen	Mice immunized with 100 µg ovalbumin (intraperitoneal) immediately after CDD exposure and sacrificed on day 7	Ao et al. 2009
Mouse (C57BL/6) 5 females/group	0 or 20 µg/kg (in corn oil) once	NA	20 µg/kg: ↓ IL-5 levels in spleen	Mice immunized with 100 µg ovalbumin (intraperitoneal) 2 weeks immediately after CDD exposure and sacrificed 1 week later	Ao et al. 2009

Table 2-11. Summary of Select Animal Immunotoxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (C57BL/6) 5–8 females/ group	0 or 20 µg/kg (in corn oil) once	NA	20 µg/kg: ↓ IgM and IgG1 levels alterations in percent of B cells in spleen, ↓ germinal center B cells in spleen	Mice immunized with 100 µg ovalbumin (intraperitoneal) immediately after CDD exposure Immunization with alum-precipitated (4-hydroxy-3-nitrophenyl) acetyl linked to chicken γ-globulin resulted in ↓ antibody-forming cells in the spleen	Inouye et al. 2003
Mouse (C57BL/6) 4–5 females/ group	0, 0.2, 1, 5, or 20 µg/kg (in corn oil) once	0.2 µg/kg	≥1 µg/kg: ↓, spleen $CD3^+$ cells and IL-5 production	Mice immunized with 100 µg ovalbumin (intraperitoneal) immediately after CDD exposure ≥5 µg/kg: ↓ IgG1 production	Ito et al. 2002
Mouse (C57BL/6) 4–5 females/ group	0 or 20 µg/kg (in corn oil) once	NA	20 µg/kg: ↓ $CD3^+$ T cells, $CD4^+$ T cells, and $CD45R/B220^+$ B cells; ↓ spleen IL-2, IL-4, IL-5, and IL-6 levels; ↑ percent $CD3^+$ T cells in spleen	Mice immunized with 100 µg ovalbumin (intraperitoneal) immediately after CDD exposure	Ito et al. 2002
Rat (Long-Evans) 4–6 females/group	0 or 25 µg/kg (in peanut oil) once	NA	25 µg/kg (TCDD-SEB group): ↑ serum and spleen IL-2 levels (as compared to controls, TCDD-only group, and SEB-only group), serum IL-6 (as compared to control and TCDD-only group)	Groups of rats were also exposed to SEB (intraperitoneal) 2 days after CDD exposure	Huang and Koller 1998
Rat (Long-Evans) 4 females/group	0 or 25 µg/kg (in peanut oil) once	25 µg/kg	NA	No significant alteration in splenic $CD4^+$ or $CD8^+$ subpopulations	Huang and Koller 1999
Rat (Long-Evans) 4 females/group	0 or 5 µg/kg/day (in peanut oil) for 5 days	NA	5 µg/kg/day: ↓ percent $CD4^+$ splenic T cells	No significant alteration in splenic $CD8^+$ T cell subpopulations	Huang and Koller 1999

Table 2-11. Summary of Select Animal Immunotoxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Monkey (Rhesus) 3–6 females/group	0, 0.00012, or 0.00064 µg/kg/day in diet for 3.5–4 years	NA	0.00012 µg/kg/day: ↑ tumor necrosis factor-α in peripheral blood mononuclear cells in response to stimulation by T-cell mitogen, PHA	Estimate of dietary intake from Rier et al. 1993; animals examined 13 years after exposure termination	Rier et al. 2001
Mouse (NC/Nga), 6 males/group	0, 5, or 20 µg/kg (in corn oil) once	NA	5 µg/kg: ↑ IFN-γ production (not observed at 20 µg/kg)	NC/Nga mice are a model of atopic dermatitis; mice were dermally sensitized with picryl chloride followed by exposure to TCDD 20 µg/kg: ↑ spleen B cells, ↓ spleen $CD3^+$, $CD4^+$, and $CD8^+$ cells No effect on IgE or IL-4 production	Ito et al. 2008
Mouse (C57BL/6 or C57B1/10) 5–6 females/group	0 or 10 µg/kg (in peanut oil) once	NA	10 µg/kg: ↓ $CD8^+$ T cell response to influenza virus	1 day after dosing, mice were infected with influenza virus	Mitchell and Lawrence 2003
Mouse (C57BL/6) 7–12 females/ group	0, 1, 2.5, 5, 7.5, or 10 µg/kg (in peanut oil) once	NA	1.0 µg/kg: ↓ IgG2a levels, ↑ IgA levels	Mice received an intranasal infection of influenza A/HKx31 virus 1 day after TCDD exposure 2.5 µg/kg: ↑ mortality, ↓ number of lymphocytes and macrophages in BAL fluid, ↓ IgG1 and IgG2b levels 5.0 µg/kg: ↓ number of $CD4^+$ cells in mesenteric lymph nodes, ↓ percent lymphocytes in BAL fluid, ↑ percent neutrophils in BAL fluid 7.5 µg/kg: ↓ number of mesenteric lymph node cells and $CD8^+$ cells 10 µg/kg: ↓interferon-γ levels in BAL fluid No effect on IL-12 or IgM levels	Vorderstrasse et al. 2003

Table 2-11. Summary of Select Animal Immunotoxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (C57Bl/6) 6–10 females/ group	0, 1.5, or 10 µg/kg (in peanut oil) once		10 µg/kg: ↓ $CD4^+$ and $CD8^+$ T cells, IL-2 and interferon-γ levels, and cytotoxic T lymphocyte activity in mediastinal lymph nodes; ↓ IgM, IgG1, IgG2a, and IgG2b plasma levels and ↑ IgA plasma levels; ↓ IL-12 and ↑ interferon-γ levels and percent $CD8^+$ cells in BAL fluid	Mice were immunized with influenza A/HKx31 virus 1 day after TCDD exposure Investigators reported increased mortality, but statistical analyses of these data were not conducted; NOAEL cannot be determined because most tests were only conducted at 10 µg/kg	Warren et al. 2000
Rat (Sprague-Dawley) 5–6 females/group	0, 1, or 2 µg/kg (in corn oil)	NA	1 µg/kg: ↓ thymus weight, ↓ ratio of $CD4^+/CD8^+$ T cells in thymus, ↓ ratio of $CD4^+/CD8^+$ T cells in mesenteric lymph nodes (not significant at 2 µg/kg)	2 µg/kg: ↑ percent $CD8^+$ thymocytes No effect on percent $CD4^+CD8^+$, $CD4^-$ $CD8^-$, or $CD4^+$ T cell subpopulations in thymus, or ratio of $CD4^+/CD8^+$ in blood lymphocytes	Nohara et al. 2000
Mouse (C57BL/6), 3 or 6 weeks of age, 5–6 females/age/ group	0, 0.3, 1.0, or 3.0 µg/kg (in corn oil) once	NA	0.3 µg/kg: ↓ IL-5 production by splenocytes	Mice immunized with 100 µg ovalbumin (intraperitoneal) immediately after CDD exposure No effect on plasma IgM levels or percent T cells and B cells	Inouye et al. 2005
Mouse (B6C3F1) female/group	0, 4.2, 14, or 42 µg/kg (in corn oil) once	NA	4.2 µg/kg: ↓ response to sheep red blood cells	14 µg/kg: ↓ total IgM antibody forming cells	Matulka et al. 1997
Mouse (B6C3F1) female/group	0, 0.3, 1.0 or 3.0 µg/kg/day (in corn oil) for 14 days	0.3 µg/kg/ day	1.0 µg/kg: ↓ total IgM antibody forming cells		Matulka et al. 1997
Mouse (DBA/2) female/group	0, 4.2, 14, or 42 µg/kg (in corn oil) once	14 µg/kg	42 µg/kg: ↓ total IgM antibody forming cells		Matulka et al. 1997
Mouse (DBA/2) female/group	0, 0.3, 1.0, or 3.0 µg/kg/day (in corn oil) for 14 days	0.3 µg/kg/ day	1.0 µg/kg: ↓ total IgM antibody forming cells		Matulka et al. 1997

Table 2-11. Summary of Select Animal Immunotoxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (B6C3F1) 8 females/group	0, 0.1, 1.0, or 10.0 µg/kg (in corn oil) once	0.1 µg/kg	1.0 µg/kg: ↓ antibody plaque forming cell response to SRBCs		Smialowicz et al. 1997
Mouse (B6C3F1) 8–14 females/ group	0, 0.0015, 0.015, 0.150, or 0.450 µg/kg (in corn oil) 5 days/week for 13 weeks	NA	0.0015 µg/kg: ↓ antibody plaque forming cell response to SRBCs	Mice were immunized with SRBC 3 days after final exposure	Smialowicz et al. 2008
Mouse (B6C3F1) 7 females/group (12 weeks of age)	0, 0.1, 1, 10, or 30 µg/kg (in corn oil) once	0.1 µg/kg	1.0 µg/kg: ↓ response to parasite antigens in spleen	7 days after TCDD exposure mice were infected with *T. spiralis* larvae 10 µg/kg: ↑ parasite count, ↓ response to parasite antigens in mesenteric lymph nodes, ↓ response to LPS in spleen	Luebke et al. 1999
Mouse (B6C3F1) 7 females/group (76 weeks of age)	0, 0.1, 1, or 10 µg/kg (in corn oil) once	0.1 µg/kg	1.0 µg/kg: ↓ splenic response to parasite antigens	7 days after TCDD exposure, mice were infected with *T. spiralis* larvae No effect on response to Con A or LPS in spleen or mesenteric lymph nodes	Luebke et al. 1999
Rats (F344) 5 males/group (74–78 weeks of age)	0, 0.1, 10, or 30 µg/kg (in corn oil) once	10 µg/kg	30 µg/kg: ↑ response to *Salmonella typhimurium* mitogen in spleen	7 days after TCDD exposure, rats were infected with *T.spiralis* larvae No effect on response to parasite antigen or Con A in spleen or mesenteric lymph nodes	Luebke et al. 1999
Hamster (Siberian) 2–5/gender/group	0, 0.1, or 2 µg/kg (in sesame oil) once	1 µg/kg	2 µg/kg: ↓ lymphocyte proliferation	Animals maintained in long days (16 hour light cycle); immunotoxicity testing conducted 7 days postexposure No effect on test of mixed lymphocyte reaction to allogeneic stimulator cells	Yellon et al. 2000

Table 2-11. Summary of Select Animal Immunotoxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (B6C2F1, BALB/c, C57BL/6N, DBA/2) 10/gender/strain/ group	0, 0.005, 0.020, 0.10, or 0.50 µg/kg (in corn oil) once	0.5 µg/kg	NA	Mice infected with influenza virus A/PR/34/8 (H1N1) 7 days after TCDD exposure No significant effect on survival rate	Nohara et al. 2002
Mouse (C57BL/6) 5–8 females/ group	0, 5, or 10 µg/kg (in peanut oil) once	NA	5 µg/kg: ↓ plasma IgM, IgG2a, and IgG2b levels and ↑ plasma IgA levels 10 and 40 days after infection 10 µg/kg: ↓ $CD8^+$ memory T cell pool	1 day after dosing, mice were infected with influenza virus	Lawrence and Vorderstrasse 2004
Other congeners					
Mouse (C57BL/6) 5 females/group	0, 1.0, 3.0, or 10 µg/kg CDD (in corn oil) once	NA	1 µg/kg: ↓ IL-5 levels in spleen	Mice immunized with 100 µg ovalbumin (intraperitoneal) immediately after CDD exposure and sacrificed on day 7 ≥3 µg/kg: ↓ thymocyte number 10 µg/kg: ↓ relative thymus spleen weights	Ao et al. 2009
Mouse (C57BL/6) 5 females/group	0 or 20 µg/kg CDD (in corn oil) once	NA	20 µg/kg: ↓ relative thymus weight and IL-5 levels in spleen	Mice immunized with 100 µg ovalbumin (intraperitoneal) 2 weeks immediately after CDD exposure and sacrificed 1 week later	Ao et al. 2009

[a]2,3,7,8-TCDD doses were administered via gavage, unless otherwise specified.

BAL = bronchoalveolar lavage; CDD = chlorinated dibenzo-*p*-dioxin; LOAEL = lowest-observed-adverse-effect level; LPS = lipopolysaccharide; NA = not applicable; NOAEL = no-observed-adverse-effect level; CDD = chlorinated dibenzo-*p*-dioxins; PHA = phytohemagglutinin; SEB = *Staphylococcal enterotoxin* B; SRBC = sheep red blood cell; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Table 2-12. Summary of Select Animal Reproductive Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Line C), 42 males/group	0, 0.1, 0.3, 1, 3, 10, or 30 µg/kg once	3 µg/kg	10 µg/kg: ↓ daily sperm production and cauda epididymal sperm reserve	30 µg/kg: ↓ serum testosterone levels No effect on relative ventral prostate weight; relative or absolute testes, seminal vesicle, epididymides, or cauda epididymides weights; number of Sertoli cells or spermatogenic cells	Simanainen et al. 2004a
Rat (Wistar), 24 males/group	0, 0.001, 0.01, or 0.1 µg/kg/day for 45 days	NA	0.001 µg/kg/day: ↓ epididymal sperm count		Latchoumy-candane et al. 2002a
Rat (Wistar), 24 males/group	0 or 0.1 µg/kg/day for 15 days	NA	0.1 µg/kg: ↓ epididymal and ventral prostate weight; ↓ sperm count, viability, and motility; ↓ serum testosterone levels		Dhanabalan et al. 2010
Rat (Wistar), 24 males/group	0, 0.001, 0.01, or 0.1 µg/kg/day for 45 days	0.1 µg/kg/day	NA	↓ absolute and relative testes, epididymides, seminal vesicle, and ventral prostate weights at all doses, but weights were within 10% of control organ weights	Latchoumy-candane et al. 2002b
Rat (Sprague-Dawley), 32 males/group	0, 0.02, 0.05, or 0.125 µg/kg/day 1 time/week for 29 weeks	0.02 µg/kg/day	0.05 µg/kg/day: ↓ sperm count, ↓ serum testosterone levels	0.125 µg/kg/day: ↓ relative testes weight, ↓ FSH and luteinizing hormone levels	Ma et al. 2010

Table 2-12. Summary of Select Animal Reproductive Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Sprague-Dawley), 10 males/group	0,0.05, 0.1, or 0.2 µg/kg/day for 60 days	NA	0.05 µg/kg/day: ↓ relative testis, epididymis, seminal vesicles, and prostate weights; ↓ sperm count and motility; ↑ sperm mortality and abnormalities		El Tawil and Elsaieed 2005
Rat (Sprague-Dawley), 8 females/group	0, 0.02, 0.05, or 0.125 µg/kg/day, 1 time/week for 29 weeks	NA	0.02 µg/kg/day: ↓ absolute and relative ovary weight; ↓ serum estradiol levels		Chen et al. 2009
Monkey (Cynomolgus), 3–4 females/ group	0, 1, 2, or 4 µg/kg once	2 µg/kg	4 µg/kg: ↓ serum progesterone, ↑ uterine antral follicle size, anovulation, lack of menstrual cycle	Monkeys were dosed during early pregnancy; ovarian function evaluated 443–625 days following dosing	Morán et al. 2001
Rat (Sprague-Dawley), 61–93 females/ group	0 or 32 µg/kg once	NA	32 µg/kg: inhibition of ovulation		Jung et al. 2010
Rat (Sprague-Dawley), 6–10 females/ group	0 or 10 µg/kg once	NA	10 µg/kg: ↓ ovarian weight ↓ ova shed	No effect on serum estradiol or progesterone levels	Petroff et al. 2000
Rat (Sprague-Dawley), 4 females/group	0 or 32 µg/kg once	NA	32 µg/kg: inhibition of ovulation		Petroff et al. 2002

Table 2-12. Summary of Select Animal Reproductive Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Sprague-Dawley), 42 females/ group	0 or 20 µg/kg once	NA	20 µg/kg: ↓ ovarian weight, ↓ ovulations	Ovarian function studied in 23-day-old hypophysectomized females administered eCG 24 hours after TCDD exposure and human chorionic gonadotropin 52 hours later No effect on serum luteinizing hormone and serum or ovarian concentrations of progesterone, estradiol, or androstenedione	Son et al. 1999
Rat (Sprague-Dawley), 32 females/ group	0 or 32 µg/kg once	NA	32 µg/kg: ↓ ovarian weight, delayed ovulation, ↑ serum estradiol levels	Ovarian function studied in 23-day-old rats; eCG administered day after TCDD exposure No effect on serum progesterone or androstenedione levels	Ushinohama et al. 2001
Rat (Sprague-Dawley), 81–82 females/ group	0, 0.003, 0.010, 0.022, 0.046, or 0.1 µg/kg/day, 5 days/week for 105 weeks	0.01 µg/kg/ day	0.022 µg/kg/day: dilation of clitoral gland ducts		NTP 2006
Monkey (Cynomolgus) 3–5 females/ group	0, 1, 2, or 4 µg/kg once	NA	1 µg/kg: squamous metaplasia in endocervix	Monkeys were examined 1.2–2.7 years after exposure	Scott et al. 2001
Rat (Sprague-Dawley), 12 females/ group	0 or 1 µg/kg once	1 µg/kg		21 days after dosing, animals underwent surgery to induce endometriosis No effect on endometriotic lesion size	Cummings et al. 1999

Table 2-12. Summary of Select Animal Reproductive Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (C57BL/6), 12 females/ group	0 or 3 µg/kg once	3 µg/kg		21 days after dosing, animals underwent surgery to induce endometriosis No effect on endometriotic lesion size; ↑ endometriotic lesion size observed when mice were exposed prenatally and dosed with 3 or 10 µg/kg 2,3,7,8-TCDD on PND 77	Cummings et al. 1999
Mouse (C57BL/6J), 6–8 females/group	0 or 1 µg/kg/day on GDs 0, 7, and 14	NA	1 µg/kg/day: ↓ mammary gland weight on GDs 17 and 19; suppression of mammary gland differentiation	Mice were administered 5 µg/kg on GDs 0, 7, and 14; time-weighted average dose is 1 µg/kg/day	Vorderstrasse et al. 2004
Hamster (Siberian) 16–43 males and females/group	0, 0.1, 2, or 100 µg/kg once	0.1 µg/kg	2 µg/kg: ↑ mortality, ↑ time to pregnancy	Animals were mated 10 days after dosing	Yellon et al. 2000
Mouse (NIH), 10 females/ group	0, 0.002, 0.05, or 0.1 µg/kg/day on GDs 1–3, 1–8, or 4–8	NA	0.002 µg/kg/day: ↓ serum progesterone levels	0.05 µg/kg/day: ↓ implantation sites, ↓ uterine weight gain (GDs 1–3 and 1–8) No effect on serum estradiol levels	Li et al. 2006
Monkey (Cynomolgus) 3–7 females/ group	0, 1, 2, or 4 µg/kg on GD 12	NA	1 µg/kg: early fetal loss		Guo et al. 1999, 2000
Mink (Standard dark), 12 females/ group	0, 0.00003, 0.0008, 0.0003, 0.007, or 0.07 µg/kg/day in diet for 132 days	0.00003 µg/ kg/day	0.008 µg/kg/day: no females whelped	Female mink were exposed to 2,3,7,8-TCDD for 35 days prior to mating	Hochstein et al. 2001

Table 2-12. Summary of Select Animal Reproductive Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Holtzman), 6–12 dams/ group	0, 0.8, or 1.6 µg/kg on GD 15	NA	0.8 µg/kg: altered placental histology (↑ glycogen cells and cysts filled with eosinophilic material) at GD 20	Groups were divided into two subgroups and sacrificed on GD 16 or 20	Ishimura et al. 2002b
			1.6 µg/kg: ↑ interuterine deaths at GD 20	No effect on fetal weight	
Mouse (ICR), 40 males/group	0, 0.0001, or 0.1 µg/kg/day weekly for 5 weeks	0.0001 µg/ kg/day	0.1 µg/kg/day: ↓ male/female ratio	Mice were given an initial loading dose of 0.002 or 2 µg/kg and then weekly doses of 0.0004 or 0.4 µg/kg for 5 weeks; time-weighted average dose were 0.0001 or 0.1 µg/kg/day	Ishihara et al. 2007
				No effect on fertility index; morphology of germ cells, Sertoli cells, or Leydig cells; epididymal sperm count	
Mouse (ICR), 49–59 males/ group	0 or 0.1 µg/kg/day weekly for 5 weeks	NA	0.1 µg/kg/day: ↓ male/female ratio	Mice were given an initial loading dose of 2 µg/kg and then weekly doses of 0.4 µg/kg for 5 weeks; time-weighted average dose was 0.1 µg/kg/day	Ishihara et al. 2010
				No effect on epididymal sperm count; sperm motility	

[a]Doses were administered via gavage in oil, unless otherwise specified.

eCG = equine chlorionic gonadotropin; FSH = follicle-stimulating hormone; GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NA = not applicable; NOAEL = no-observed-adverse-effect level; NTP = National Toxicology Program; PND = postnatal day; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Fetal/pup mortality					
Rat (Han/Wistar), 55–75 dams/group	0, 0.05, 0.2, or 1 µg/kg on GD 15	0.2 µg/kg	1 µg/kg: ↓ number of live pups	Male pups were examined on PND 70 or 120	Bell et al. 2007a
Rat (Holtzman), 6–12 dams/ group	0, 0.8, or 1.6 µg/kg on GD 15	0.8 µg/kg	1.6 µg/kg: ↑ interuterine deaths at GD 20		Ishimura et al. 2002b
Rat (Holtzman) 11–18 dams/ group	0, 1.5, or 6 µg/kg on GD 10	NA	1.5 µg/kg: ↑ pup mortality	Offspring were examined on GD 20 and PND 7	Kransler et al. 2009
Mouse (C57BL/6) 12 dams/group	0, 2.5, 5, or 10 µg/kg on GD 12	5 µg/kg	10 µg/kg: 90% pup lethality by PND 28		Mustafa et al. 2008
Guinea pig (Hartley) 7–15 dams/group	0, 0.15, or 1.5 µg/kg on GD 14	0.15 µg/kg	1.5 µg/kg: ↑ percent fetal mortality		Kransler et al. 2007
Rat (Han/Wistar), 65–75 females/ group	0, 0.0024, 0.008, or 0.046 µg/kg/day in diet for 15 weeks	0.008 µg/ kg/day	0.046 µg/kg/day: ↑ total litter loss, ↓ surviving pups	Female rats exposed to 2,3,7,8-TCDD in the diet for 12 weeks prior to mating and during mating and gestation; F1 males were mated with untreated females during postnatal week 16	Bell et al. 2007b
Mouse (C57BL/6J)[b]	0, 0.04, 0.1, or 0.5 µg/kg/day on GDs 0, 7, and 14 and PND 2	0.1 µg/ kg/day	0.5 µg/kg/day: 4/6 litters had 100% mortality within 1 week of birth	Pregnant mice were administered 0 or 0.25 µg/kg on GDs 0 and 7 followed by a single dose of 0.25, 1, or 5 µg/kg on GD 14 and PND 2; time-weighted doses were 0.04, 0.1, or 0.5 µg/kg/day	Vorderstrasse et al. 2006
Mink (Standard dark), 12 females/ group	0, 0.00003, 0.0008, 0.0003, 0.007, or 0.07 µg/kg/day in diet for 132 days	NA	0.003 µg/kg/day: ↓ pup survival in first 3 weeks	Female mink were exposed to 2,3,7,8-TCDD for 35 days prior to mating	Hochstein et al. 2001

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Structural malformations and anomalies					
Mouse (Wild-type)[b]	0 or 24 µg/kg on GD 12	NA	24 µg/kg: cleft palate, hydronephrosis		Bryant et al. 2001
Rat (Holtzman) 7–15 dams/ group	0, 1.5, 3, 6, or 18 µg/kg on GD 10	6 µg/kg	18 µg/kg: cleft palate	≥3 µg/kg: ↑ percent fetal mortality/litter, ↓ fetal body weight	Kransler et al. 2007
Mouse (C57BL/6J) 4–6 dams/group	0 or 40 µg/kg on GD 12.5	NA	40 µg/kg: cleft palate and hydronephrosis		Mimura et al. 1997
Mouse (C57BL/6J, A/J, and ICR)[b]	0, 10, 20, or 40 µg/kg	NA	10 µg/kg: cleft palate	Increased incidence of cleft palate occurred in all three strains of mice	Yamada et al. 2006
Mouse (C57BL/6J) 23–24 dams/group	0 or 6 µg/kg on GD 14.5		6 µg/kg: ↑ relative weight of left ventricle plus septum, hydronephrosis, ↓ plasma volume, altered systolic blood pressure response to angiotensin II	Male offspring were examined at 3.5 months of age Assessment of kidney function showed normal filtration function	Aragon et al. 2008a, 2008b
Hamster (Golden Syrian) 9–12 dams/ group	0, 1.5, 3, 6, or 18 µg/kg on GD 10	NA	1.5 µg/kg: kidney congestion	≥3 µg/kg: hydronephrosis 18 µg/kg: ↓ viable fetuses/dam, ↑ late fetal deaths and percent mortality	Kransler et al. 2007
Mouse (EGFR+/-)[b]	0, 1.5, 4.4, 19.1, 29.6, 30.1, 42, 55.6, or 106 µg/kg on GD 10	1.5	4.4 µg/kg: hydronephrosis	≥29.6 µg/kg: cleft palate	Miettinen et al. 2004
Rat (Holtzman) 6 dams/group	0 or 1 µg/kg on GD 15	NA	1 µg/kg: ↓ fetal body weight, hydronephrosis (lactation only and gestation/lactation pups)	On PND 1, litters were culled to 4 pups/gender and some pups were cross-fostered	Nishimura et al. 2006
Guinea pig (Hartley) 7–15 dams/group	0, 0.15, or 1.5 µg/kg on GD 14	0.15 µg/kg	1.5 µg/kg: ↑ percent fetal mortality	No effect on fetal body weight or length or alterations in incidence of malformations or anomalies	Kransler et al. 2007

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Effects on growth					
Rat (Holtzman) 6 dams/group	0 or 1 µg/kg on GD 15	NA	1 µg/kg: ↓ fetal body weight	On PND 1, litters were culled to four pups/gender and some pups were cross-fostered	Nishimura et al. 2006
Rat (Holtzman), 3–9 dams/group	0, 0.2 or 0.8 µg/kg on GD 15	0.8 µg/kg	NA	No effect on body weight	Ikeda et al. 2005a
Rat (Holtzman), 6 dams/group	0, 0.2, or 0.8 µg/kg on GD 15	NA	0.2 µg/kg: ↓ serum T4 levels (PND 21 only), ↑ serum T3 levels (PND 21 only)	Pups were examined on PNDs 21 and 49 0.8 µg/kg: ↓ litter size, ↑ T4 levels (PND 49 only), ↑ TSH levels; hyperplasia of follicular cells in thyroid No effect on sex ratio, pup body weight, thyroid weight	Nishimura et al. 2003
Rat (Holtzman) 7–15 dams/group	0, 1.5, 3, 6, or 18 µg/kg on GD 10	NA	1.5 µg/kg: ↓ fetal length	≥3 µg/kg: ↓ fetal body weight	Kransler et al. 2007
Rat (Sprague-Dawley)[b]	0, 0.25, 0.5, or 1 µg/kg on GD 15	0.5 µg/kg	1 µg/kg: ↓ pup body weight on PNDs 3, 5, and 10		Nayyar et al. 2002
Rat (Wistar)	0 or 0.1 µg/kg/day on GDs 9–19	NA	0.1 µg/kg: ↓ fetal body weight on GD 19,		Nishijo et al. 2007
Mouse (C57BL/6), 10 dams/group	0 or 1 µg/kg/day for 4 days postpartum	NA	1 µg/kg/day: ↓ pup body weight on PNDs 7, 21, 30, and 60	No additional information on exposure days was provided.	Jin et al. 2010
Mouse (EGFR+/-)[b]	0, 1.5, 4.4, 19.1, 29.6, 30.1, 42, 55.6, or 106 µg/kg on GD 10	106 µg/kg	NA	No effect on fetal body weight	Miettinen et al. 2004
Guinea pig (Hartley) 7–15 dams/group	0, 0.15, or 1.5 µg/kg on GD 14	1.5 µg/kg	NA	No effect on fetal body weight or length	Kransler et al. 2007

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Impaired development of respiratory, cardiovascular, skeletal, and gastrointestinal systems					
Rat (Holtzman) 11–18 dams/ group	0, 1.5, or 6 µg/kg on GD 10	NA	1.5 µg/kg: ↑ pup mortality, ↓ absolute and relative lung weight, lung changes indicative of immaturity and hypoplasia, altered lung function	Offspring were examined on GD 20 and PND 7 6 µg/kg: ↓ pup body weight on GD 20	Kransler et al. 2009
Mouse (C57BL/6J) 4–21 dams/group	0, 1.5, 3, 6, 12, or 24 µg/kg on GD 14.5	1.5 µg/kg	3 µg/kg: ↓ fetal relative heart weight	≥6 µg/kg: ↓ cardiocyte proliferation, ↓ heart rate No evidence of cardiac arrhythmias detected on EKGs from 21-day-old pups	Thackaberry et al. 2005a
Mouse (C57BL/6J) 23–24 dams/group	0 or 6 µg/kg on GD 14.5		6 µg/kg: ↑ relative weight of left ventricle plus septum, ↓ plasma volume, altered systolic blood pressure response to angiotensin II	Male offspring were examined at 3.5 months of age	Aragon et al. 2008a, 2008b
Rat (Line C), 5–8 dams/group	0, 0.03, 0.1, 0.3, or 1 µg/kg on GD 15	0.3 µg/kg	1 µg/kg: ↓ length, cortical bone mineral density, cross-sectional area, periosteal and endosteal circumference in tibia and/or femur; ↓ mechanical strength	Line C rats have no TCDD resistance alleles Only female pups were examined	Miettinen et al. 2005
Rat (Line C), 5–8 dams/group	0 or 1 µg/kg on GDs 11, 13, and 19, and PNDs 0, 2, and 4	NA	1 µg/kg: ↓ length, cortical bone mineral density (GDs 11, 13, and 19 only), cross-sectional area, periosteal and endosteal circumference in tibia and/or femur	Line C rats have no TCDD resistance alleles In general, earlier exposures caused more severe effects	Miettinen et al. 2005

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Sprague-Dawley)[b]	0 or 1 µg/kg of GD 11	NA	1 µg/kg: ↓ bone mineralization (cortical bone mineral content and mineral density), geometry (length, cortical cross-sectional area, cortical thickness, periosteal and endosteal circumference, cross sectional moment of inertia, moment of resistance), and strength (maximum force, stiffness) on PNDs 35 and 70.		Finnilä et al. 2010
Rat (Line C),4–8 dams/group	0, 0.03, 0.1, 0.3, or 1 µg/kg on GD 15	NA	0.03 µg/kg: ↓ size of third molar (females only)	Line C rats have no TCDD resistance alleles; female offspring were examined on PND 35 and male offspring were examined on PND 70 ≥0.3 µg/kg: ↓ size of third molar (males and females) 1 µg/kg: prevented the development of the third lower molar in 50% of females and 60% of males; ↓ size of lower first and second molars	Kattainen et al. 2001
Rat (Line C), 5–7 dams/group	0 or 1 µg/kg on GDs 11, 13, and 19, and PNDs 0, 2, and 4	NA	1 µg/kg: ↑ stillborn pups in GD 11 group; ↑ pup mortality in GD 11 and 13 groups; ↓ pup body weight, ↑ pups with missing third molar in GD 11, 13, and 19 groups, ↓ length of lower molars; accelerated eye opening	Line C rats have no TCDD resistance alleles	Miettinen et al. 2002

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (C57BL/6J, BALB/cByJ, CBA/J, C3H/HeJ, or C57BL/10J)[b]	0, 0.01, 0.1, or 1 µg/kg on GD 13	NA	0.01 µg/kg: altered mandible shaped in C3H/HeJ males	≥0.1 µg/kg: altered mandible shape in C57BL/6J males 1 µg/kg: altered mandible shape in C57BL/10J males No effect in BALB/cByJ or CBA/J strains	Keller et al. 2008
Rat (Holtzman) 7–15 dams/ group	0, 1.5, 3, 6, or 18 µg/kg on GD 10	NA	1.5 µg/kg: intestinal hemorrhage	≥3 µg/kg: ↑ percent fetal mortality/litter, ↓ fetal body weight	Kransler et al. 2007
Impaired thyroid function and metabolic effects					
Rat (Long-Evans), 10 dams/group	0 or 1 µg/kg on GDs 15 and 20, and PNDs 1, 3, 5, and 10	NA	1 µg/kg: ↑ TSH (GD 20 and all PND groups)	No effect on T3 or T4 levels	Fenton et al. 2002
Rat (Holtzman), 6 dams/group	0, 0.2, or 0.8 µg/kg on GD 15	NA	0.2 µg/kg: ↓ serum T4 levels (PND 21 only), ↑ serum T3 levels (PND 21 only)	Pups were examined on PNDs 21 and 49 0.8 µg/kg: ↓ litter size, ↑ T4 levels (PND 49 only), ↑ TSH levels; hyperplasia of follicular cells in thyroid No effect on sex ratio, pup body weight, thyroid weight	Nishimura et al. 2003
Rat (Holtzman), 12 dams/group	0 or 1 µg/kg on GD 15	NA	1 µg/kg: ↓ serum total T4 and free T4 levels (*in utero*/lactation and lactation only groups); ↑ serum TSH level (*in utero*/lactation and lactation only groups), hyperplasia of follicular cells in thyroid (*in utero*/lactation and lactation only groups)	At birth, pups were cross fostered No effect on serum T3 levels	Nishimura et al. 2005b

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Holtzman), 12 dams/group	0 or 1 µg/kg on GD 15	NA	1 µg/kg: ↓ serum total T4 and free T4 levels (*in utero*/lactation and lactation only groups); ↑ serum TSH level (*in utero*/lactation and lactation only groups), hyperplasia of follicular cells in thyroid (*in utero*/lactation and lactation only groups)	At birth, pups were cross fostered No effect on serum T3 levels	Nishimura et al. 2005b
Impaired development and functional alterations of the immune system					
Mouse (C57BL/6) 12 dams/group	0, 2.5, 5, or 10 µg/kg on GD 12	NA	2.5 µg/kg: ↓ pup body weight, ↓ thymus weight, ↓ thymic cellularity	Offspring were examined at 24 weeks of age 5 µg/kg: ↓ $CD4^+CD8^+$ T cells in thymus and lymph nodes, ↓ $CD4^-CD8^+$ T cells in spleen, ↓ $CD24^+B220$ B cells in spleen, splenocytes, ↓ bone marrow derived non-B cells, and ↑ B cell progenitors in bone marrow 10 µg/kg: 90% pup lethality by PND 28 Investigators also reported ↑ IgG and C3 deposition in kidneys, disruption of architecture in mesenteric lymph nodes, and infiltration of lymphocytes and moderate small foci of necrosis with inflammation in the liver in the 2.5 and 5 µg/kg groups; however, statistical analyses were not conducted and incidence data were not reported	Mustafa et al. 2008

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Holtzman)[b]	0, 0.0125, 0.05, 0.2, or 0.8 µg/kg on GD 15	0.8 µg/kg	NA	No effect on spleen or thymus weights in male offspring on PND 5, 21, 49, or 120	Nohara et al. 2000
Mouse (C57BL/6NCji) 8/group	0, 0.001, or 0.011 µg/kg in drinking water during lactation days 1–17	0.001 µg/kg	0.011 µg/kg: ↓ spleen weight, ↑ $CD4^+$ thymocytes, ↑ tumor necrosis factor-α and interferon-γ levels, ↓ clearance of *Listeria* from spleen	On PND 21, offspring were infected with *Listeria monocytogenes* (intraperitoneal)	Sugita-Konishi et al. 2003
Mouse (C57BL/6J)[b]	0, 0.04, 0.1, or 0.5 µg/kg/day on GDs 0, 7, 14, and PND 2	0.04 µg/kg/day	0.1 µg/kg/day: ↓ IgG1, IgG2a, and IgG2b levels (females only), ↓ total cellularity and number of cytotoxic T lymphocytes in mediastinal lymph nodes (females only), ↑ pulmonary neutrophilia and pulmonary interferon-γ levels	Pregnant mice were administered 0 or 0.25 µg/kg on GD 0 and 7 followed by single dose of 0.25, 1, or 5 µg/kg on GD 14 and PND 2; time-weighted doses were 0.04, 0.1, or 0.5 µg/kg/day; adult offspring were infected with influenza A/HKx31 virus 0.5 µg/kg/day: 4/6 litters had 100% mortality within 1 week of birth	Vorderstrasse et al. 2006

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (C57BL/6)[b]	0 or 0.2 µg/kg/day on GDs 0, 7, and 14, and PND 2	NA	0.2 µg/kg/day: ↓ virus-specific $CD8^+$ T cells in influenza A infected group, ↓ antibodies in ovalbumin immunized group	Pregnant mice were administered 0 or 1 µg/kg on GDs 0, 7, and 14, and PND 2; time-weighted dose was 0.2 µg/kg/day; at age 6–12 weeks, offspring were infected with influenza A/HKx31 virus or immunized with ovalbumin Another group of offspring were cross fostered; ↓ $CD8^+$ T cells in gestation and lactation group and lactation only group and ↑ neutrophils and interferon-γ levels in gestation and lactation group	Hogaboam et al. 2008
Impaired development and functional alterations of the nervous system					
Monkey (Cynomolgus) 11/group	0 or 4 µg/kg on GD 15 or 20	NA	4 µg/kg: altered morphology of developing neural tube		Moran et al. 2004
Rat (Dark-Agouti)[b]	0 or 0.7 µg/kg on GD 18	NA	0.7 µg/kg: delayed myelination in brain	Offspring were examined on PNDs 2/3, 14, 30, and 135	Fernández et al. 2010
Rat (Sprague-Dawley)[b]	0 or 0.180 µg/kg on GD 8	NA	0.18 µg/kg: reversal of hemispheric dominance based on morphological changes		Hojo et al. 2006
Mouse (C57BL/6N)[b]	0 or 20 µg/kg on GD 7	NA	20 µg/kg: ↓ thickness of sensory cortex	Offspring were examined on PND 21	Mitsuhashi et al. 2010

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Han/Wistar), 55–75 dams/group	0, 0.05, 0.2, or 1 µg/kg on GD 15	0.2 µg/kg	1 µg/kg: ↓ number of live pups, delayed preputial separation, ↓ sperm path velocity and straight line velocity, ↑ mean percent abnormal sperm, ↓ testes weight	Male pups were examined on PND 70 or 120; on PNDs 84–91, males were tested for learning ability, motor activity, and a FOB; during postnatal week 16, males were mated with untreated females No TCDD-related changes in learning, motor behavior, or FOB; sperm count, no effects on pre-coital time, fertility index, or mating index in F1	Bell et al. 2007a
Rat (Han/Wistar), 65–75 females/ group	0, 0.0024, 0.008, or 0.046 µg/kg/day in diet for 15 weeks	NA	0.0024 µg/kg/day: delayed preputial separation	Female rats exposed to 2,3,7,8-TCDD in the diet for 12 weeks prior to mating and during mating and gestation; F1 males were mated with untreated females during postnatal week 16 0.046 µg/kg/day: ↑ total litter loss, ↓ surviving pups; ↓ motor activity, ↑ proportion of abnormal sperm, and ↓ mean number of spermatids (observed at PND 70 but not at PND 120) No effect on learning and memory or FOB tests (males offspring tested during postnatal weeks 12–13); or mating performance in F1 males	Bell et al. 2007b

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Long-Evans) 7–9 dams/group	0, 0.2, or 0.8 µg/kg on GD 15	0.2 µg/kg	0.8 µg/kg: altered sexual behavior in males	No effect on spontaneous activity	Kakeyama et al. 2003
Rat (Holtzman), 7–13 dams/ group	0, 0.06, 0.18, or 0.54 µg/kg on GD 15	0.06 µg/kg	0.18 µg/kg: altered performance on operant behavior test	Operant behavior training began on PND 100	Markowski et al. 2002
Rat (Sprague-Dawley), 28 dams/ group	0 or 0.1 µg/kg/day on GDs 10–16	NA	0.1 µg/kg/day: impaired learning on the visual reversal learning task	Male and female offspring were tested on three spatial learning and memory tasks (radial arm maze, Morris water maze, and spatial discrimination reversal learning and visual reversal learning test) Improved performance on the radial arm maze (exposed males made fewer errors than controls)	Seo et al. 1999
Rat (Sprague-Dawley), 21 dams/ group	0, 0.1, or 0.2 µg/kg/day on GDs 10–16	0.1 µg/ kg/day	0.2 µg/kg/day: ↓ litter size	Male and female offspring were tested on 8- and 12-arm radial arm mazes Improved performance on both radial arm mazes (exposed males made fewer errors than controls) at 0.1 µg/kg/day but not at 0.2 µg/kg/day	Seo et al. 2000
Rat (Sprague-Dawley), 10 dams/ group	0 or 0.1 µg/kg/day on GDs 10–16	NA	0.1 µg/kg/day: altered performance on spatial and visual reversal learning tasks	Male and female offspring were tested beginning on PND 100	Widholm et al. 2003

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Long-Evans), 6–8 dams/group	0, 0.05, 0.2, or 0.8 µg/kg on GD 15	0.05 µg/kg	0.2 µg/kg: ↑ response rate in females indicative of hyperactive behavior	Starting at postnatal week 11, learning behavior was evaluated in schedule-controlled operant behavior tests in male and female offspring No effect on litter size or sex ratio at birth; no changes in response rate were observed at 0.8 µg/kg	Hojo et al. 2008
Rat (Long-Evans)[b]	0, 0.05, 0.2, or 0.8 µg/kg on GD 15	NA	0.05 µg/kg: ↓ response rate	Starting at postnatal week 11, learning behavior was evaluated in schedule-controlled operant behavior tests in male and female offspring 0.2 µg/kg: ↑ response rate No changes in response rate were observed at 0.8 µg/kg	Hojo et al. 2004
Mouse (C57BL/6J), 8–9 dams/group	0 or 3.0 µg/kg on GD 12.5	NA	3.0 µg/kg: impaired acquisition and retention of fear memory	Male offspring underwent behavioral testing at 25–28 weeks of age	Haijima et al. 2010
Rat (Wistar)[b]	0 or 1 µg/kg on GD 15	NA	1 µg/kg: ↓ freezing time in fear conditioning tests (males only)	At 11 weeks of age, offspring underwent gonadectomy and tested for fear conditioning responses at week 12	Mitsui et al. 2006

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Wistar)	0 or 0.1 µg/kg/day on GDs 9–19	NA	0.1 µg/kg: ↓ fetal body weight on GD 19, ↑ latency on righting test on inclined plane; impaired active avoidance learning, ↓ locomotor activity in males on PNDs 31–44	Results of the righting test on the inclined plane showed significantly increased latency in treated rats, specially in males; in the tests conducted postweaning, active avoidance learning was longer and locomotor activity was reduced in males in the 31–44 PND period	Nishijo et al. 2007
Rat (Long-Evans)[b]	0, 0.1, or 0.7 µg/kg on GD 15	0.1 µg/kg	0.7 µg/kg: ↓ mean spontaneous and evoked activity in sensory cortex	Male offspring were examined on PND 45	Hood et al. 2006
Impaired development and functional alterations of the reproductive system					
Rat (Holtzman) 4–5 dams/group	0 or 1 µg/kg on GD 15	NA	1 µg/kg: altered vaginal morphogenesis as early as GD 19 (↑ thickness of mesenchymal tissue between the caudal Mullerian ducts; failure of the Mullerian ducts to fuse; impaired regression of Wolffian ducts by ↑ size of interductal mesenchyme and by preventing fusion of the Mullerian ducts), which resulted in persistent vaginal thread defect	4–5 dams/group were sacrificed on GD 17, 18, 19, 20, or 21	Dienhart et al. 2000
Rat (Long-Evans)[b]	0 or 1 µg/kg on GD 15	NA	1 µg/kg: altered vaginal morphology (↑ width of mesenchyme separating the Mullerian ducts in GD 18 and 19 fetuses and ↑ zone of unfused Mullerian ducts in GD 19 and 21 fetuses)	Fetuses were examined on GDs 17, 18, 19, and 21	Hurst et al. 2002

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Long-Evans), 16–18 dams/group	0, 0.2, or 0.8 µg/kg on GD 15	NA	0.2 µg/kg: accelerated appearance of corpora lutea and ovulation and premature maturation of the hypothalamus-pituitary axis	Offspring were killed up to PND 64 to examine ovaries and uteri 0.8 µg/kg: delayed vaginal opening, ↓ time to first estrous No effect on estrous cyclicity	Kakeyama et al. 2008
Rat (Sprague-Dawley) 4–5 dams/group	0, 1, or 2.5 µg/kg on GD 15	NA	1 µg/kg: ↓ number of days spent in estrous 2.5 µg/kg: ↓ ovulation rate, ↓ serum levels of estradiol on PND 25; ↓ large preovulatory follicles following subcutaneous injection of eCG	Estrous cycles were monitored on PNDs 45–66 and ovulation was evaluated on PNDs 125–165 No effect on the number of primordial, primary, secondary, or preantral follicles in immature rat ovaries on PND 23	Salisbury and Marcinkiewicz 2002
Rat (Sprague-Dawley), 50 dams/group	0 or 1 µg/kg on GD 8	1 µg/kg		On PND 98, animals underwent surgery to induce endometriosis No effect on endometriotic lesion size	Cummings et al. 1999
Mouse (C57BL/6), 40 dams/group	0 or 3 µg/kg on GD 8	3 µg/kg		On PND 98, animals underwent surgery to induce endometriosis No effect on endometriotic lesion size; an increase in endometriotic lesion size was observed when mice were also dosed with 3 or 10 µg/kg 2,3,7,8-TCDD on PND 77	Cummings et al. 1999

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Hamster (Syrian), 9–10 dams/group	0 or 2 µg/kg on GD 11.5	NA	2 µg/kg: delayed vaginal and eye opening and abnormal estrous cycles in F1, ↓ pregnancy rate, and ↑ maternal mortality in F1, ↓ pup survival in F2, ↓ ovarian weight in F1, malformations of external genitalia (hypospadias, cleft phallus, and ↓ urethral-vaginal distance) in F1	At PNDs 152–166, female offspring were mated with untreated males	Wolf et al. 1999
Mouse (C57BL/6), 15–16 dams/group	0 or 10 µg/kg on GD 15.5	NA	10 µg/kg: ↓ pregnancy rate in *in utero* group (43–55%), *in utero* plus prepubital (29–75%), and *in utero* plus prepubital plus pubital (0%) groups compared to controls (100%); ↓ gestation lengths in F1 groups resulting in ↑ fetal mortality	At 10–12 weeks of age, female F1 mice were mated with untreated males; F2, and F3 females were also mated to unexposed males; groups of F1, F2, and F3 females were also exposed to 10 µg/kg at 4 weeks of age (*in utero* and prepubertal exposure) and a third set of F1 females were exposed to 10 µg/kg at 4 weeks of age and 9 weeks of age (*in utero* plus prepubertal plus pubertal exposure)	Bruner-Tan and Osteen 2010
Rat (Sprague-Dawley), 6 females/group	0 or 10 µg/kg on PND 29	NA	10 µg/kg: accelerated onset of reproductive senescenence		Franczak et al. 2006
Rat (Sprague-Dawley) 4 dams/ group	0, 0.007, or 0.029 µg/kg/day during gestation, lactation, and 8-month post-lactation exposure (see comment)	NA	0.007 µg/kg/day: ↓ normal estrous cycles at 8 months, ↓ serum estradiol levels, ↑ serum FSH	Female rats received a single dose of 0, 0.05, or 0.2 µg/kg on GDs 14 and 21, and PNDs 7 and 14; after lactation, the female offspring were directly exposed weekly for 8 months; daily dose was estimated for direct exposure to the offspring	Franczak et al. 2006

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Sprague-Dawley) 4 dams/ group	0, 0.00014, 0.0007, 0.007, or 0.0209 µg/kg/day on GDs 14 and 21, PNDs 7 and 14, and weekly during PNDs 21–270	0.00014 µg/ kg/day	0.0007 µg/kg/day: ↓ serum estradiol levels	Female rats received a single dose of 0, 0.001, 0.005, 0.05, or 0.2 µg/kg on GDs 14 and 21, and PNDs 7 and 14 and weekly during PNDs 21–270; daily dose was estimated for direct exposure to the offspring ≥0.007 µg/kg/day: accelerated reproductive senescence 0.029 µg/kg/day: delayed vaginal opening	Shi et al. 2007
Rat (Sprague-Dawley) 4 dams/ group	0, 0.00014, 0.0007, 0.007, or 0.0209 µg/kg/day on GDs 14 and 21, PNDs 7 and 14, and weekly during PNDs 21–330	0.00014 µg/ kg/day	0.0007 µg/kg/day: ↓ proestrous serum estradiol levels	Female rats received a single dose of 0, 0.001, 0.005, 0.05, or 0.2 µg/kg on GDs 14 and 21, and PNDs 7 and 14, and weekly during PNDs 21–330; daily dose was estimated for direct exposure to the offspring	Valdez et al. 2009
Rat (Lewis-Furth), 24 dams/group	0 or 0.007 µg/kg/day on GDs 14 and 21, PNDs 7 and 14, and weekly during PNDs 21–330	NA	0.007 µg/kg/day: accelerated onset of acyclicity	Female rats received a single dose of 0 or 0.05 µg/kg on GDs 14 and 21, and PNDs 7 and 14, and weekly during PNDs 21–240; daily dose was estimated for direct exposure to the offspring	Jablonska et al. 2010

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Han/Wistar), 2–8 dams/group	0, 0.05, 0.1, 0.5, or 1.0 µg/kg on GD 13.5	0.1 µg/kg	0.5 µg/kg: ↑ plasma testosterone levels	Male fetuses were examined on GD 19.5 1.0 µg/kg: ↓ male fetal body weight, ↑ pituitary luteinizing hormone No effect on testicular testosterone levels	Haavisto et al. 2001
Rat (Long-Evans), 2–5 dams/group	0, 0.05, 0.1, 0.5, or 1.0 µg/kg on GD 13.5	1.0 µg/kg	NA	Male fetuses were examined on GD 19.5 No significant changes in male fetal body weight, testicular or plasma testosterone levels, or pituitary luteinizing hormone levels	Haavisto et al. 2001
Rat (Sprague-Dawley), 10 dams/ group	0, 0.04, 0.2, or 1 µg/kg on GD 13	1 µg/kg	NA	Male pups were examined on PND 14 No effect on testes weight and no changes in plasma testosterone, luteinizing hormone, or FSH levels; microscopic examination of the testes showed increased diameter of the developing seminiferous cords only at 0.04 µg/kg and did not affect the average number of primary spermatocytes	Haavisto et al. 2006

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Sprague-Dawley), 3–9 dams/group	0, 0.01, 0.2, or 0.2 µg/kg on GD 15	0.2 µg/kg	NA	No effect on litter size, gender ratio, or pup body weight, testicular levels of testosterone or 4-androstenedione, testicular or epididymal weights, testicular histology, sperm count in the caput, corpus, or cauda epidiymis or daily sperm production (measured on PNDs 67 and 145) Mating of 15-week-old males with untreated females showed no effect on fertility, litter size, or sex ratio; F2 males had normal sperm counts and daily sperm production	Rebourcet et al. 2010
Mouse (C57BL/6), 10 dams/group	0 or 1 µg/kg/day for 4 days postpartum	NA	1 µg/kg/day: ↓ pup body weight on PNDs 7, 21, 30, and 60; ↓ testes and epididymides (PND 60 only) weights; ↓ anogenital distance at PNDs 30 and 60; ↓ sperm count at PND 60	No additional information on exposure days was provided; male offspring were examined on PNDs 30 and 60	Jin et al. 2010

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Holtzman), 6 dams/group	0, 0.0125, 0.05, 0.2, or 0.8 µg/kg on GD 15	0.0125 µg/kg	0.05 µg/kg: ↓ anogenital distance (PND 120)	Male pups were examined on PNDs 49 and 120 No effect on testicular weight or histology, daily sperm production or sperm reserve, relative epididymal weight, cauda epididymal weight ≥0.2 µg/kg: ↓ relative ventral prostate weight (PND 120), ↓ urogenital complex weight (PND 120) 0.8 µg/kg: ↓ relative ventral prostate weight (PND 49)	Ohsako et al. 2001
Rat (Sprague-Dawley), 5 dams/group	0 or 1 µg/kg on GD 15 or 18	NA	1 µg/kg: ↓ testes, epididymal, cauda epididymal, ventral prostate weights (GD 15 only), ↓ anogenital distance, ↓ cauda sperm reserve (GD 15 only), urogenital-glans penis length (GD 15 only)	Male pups were examined on PND 70; no effect on daily sperm production	Ohsako et al. 2002
Rat (Line C), 5–8 dams/group	0, 0.03, 0.1, 0.3, or 1 µg/kg on GD 15	0.3 µg/kg	1 µg/kg: ↓ pup body weight, ↓ anogenital distance (PND 1 or 4), ↓ ventral, anterior, and dorsolateral prostate weights (PNDs 14–49), ↓ daily sperm production and cauda epididymal sperm reserves (PND 70)	Male pups were examined on PND 70; in a separate study, male pups were examined at various times on PNDs 14–49 No effect on serum testosterone levels or seminal vesicle, testes, or epididymis weights	Simanainen et al. 2004b

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (C57BL/6J)[b]	0 or 5 µg/kg on GD 13	NA	5 µg/kg: ↓ ventral (PNDs 35 and 90), dorsolateral (PND 35), and anterior prostate (PNDs 35 and 90) growth; inhibition of branching morpho-genesis in all prostate lobes; ventral prostate never developed any ductal structure; ↓ number of ductal tips and main ducts in dorsal and lateral prostate	Prostates from male pups were examined on PNDs 1, 7, 14, 21, 35, and 90 No effect on testicular or plasma testosterone or 3α-diol levels	Ko et al. 2002
Mouse (C57BL/6J)[b]	0 or 5 µg/kg on GD 13 or 16	NA	5 µg/kg: ↓ relative ventral prostate weight (all groups; greatest effect in *in utero* [GD 13] plus lactation and *in utero* [GD 13] only groups), ↓ dorsolateral prostate weight (all groups; greatest effect in *in utero* [GDs 13 and 16] plus lactation groups), ↓ anterior prostate weight (all groups; greatest effect in *in utero* [GD 13] plus lactation and *in utero* [GD 13] only groups), ↓ seminal vesicle weight (*in utero* [GDs 13 and 16] plus lactation groups)	At birth, pups from dams exposed on GD 13 were cross-fostered; pups from dams dosed on GD 16 remained with the dams at birth; all measurements were made on male pups on PND 35	Lin et al. 2002b
Rat (Long-Evans), 14 dams/group	0 or 1 µg/kg on GD 15	NA	1 µg/kg: ↓ body weight (PND 120), ↓ seminal vesicle weight (PNDs 49, 63, 120), altered histology of seminal vesicles (reduced epithelium with little branching) examined on PND 32	Male pups were examined on PND 15, 25, 32, 49, 63, or 120	Hamm et al. 2000
Rat (Sprague-Dawley), 6–8 dams/group	0, 0.3, or 1 µg/kg on GD 11	NA	0.3 µg/kg: ↓ testicular testosterone levels	1 µg/kg: ↓ plasma luteinizing hormone levels No effect on litter size, fetus sex ratio, male fetus body weight, or plasma corticosterone levels	Adamsson et al. 2008

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Mouse (C57BL/6J)[b]	0, 0.2, 1, or 5 µg/kg on GD 12	0.2 µg/kg	1 µg/kg: ↓ number of anterior-dorsolateral and ventral prostatic buds	Male fetuses examined on GD 17.5	Abbott et al. 2003
Mouse (Wild type)[b]	0 or 5 µg/kg on GD 13.5	NA	5 µg/kg: inhibition of ventral prostatic bud formation, ↓ number of dorsolateral prostatic buds	Male fetuses examined on GD 18.5	Allgeier et al. 2009
Rat (Han/Wistar), 55–75 dams/group	0, 0.05, 0.2, or 1 µg/kg on GD 15	0.2 µg/kg	1 µg/kg: ↓ number of live pups, delayed preputial separation, ↓ sperm path velocity and straight line velocity, ↑ mean percent abnormal sperm, ↓ testes weight	Male pups were examined on PND 70 or 120; on PNDs 84–91, males were tested for learning ability, motor activity, and a FOB; during postnatal week 16, males were mated with untreated females No TCDD-related changes in learning, motor behavior, or FOB or sperm count; no effects on pre-coital time, fertility index, or mating index in F1	Bell et al. 2007a
Rat (Holtzman), 12 dams/group	0 or 0.02 µg/kg/day for 9 weeks	NA	0.02 µg/kg/day: ↓ male body weight, ↓ ventral prostate weight, ↓ male/female ratio in F2	Female rats received a single loading dose of 0.4 µg/kg 2 weeks prior to mating followed by weekly doses of 0.08 µg/kg during mating, gestation, and lactation; time-weighted average dose was 0.02 µg/kg/day; F1 males were mated with untreated females No effect on litter size, sex ratio, or anogenital distance	Ikeda et al. 2005b

Table 2-13. Summary of Select Animal Developmental Toxicity Studies

Species (strain)	Dose[a], exposure duration/frequency	NOAEL	LOAEL	Comments	Reference
Rat (Holtzman), 3–9 dams/group	0, 0.2, or 0.8 µg/kg on GD 15	NA	0.2 µg/kg: ↓ male/female ratio of brain aromatase activity, ↑ saccharin consumption in males (both effects indicative of demasculinization)	0.8 µg/kg: ↓ male/female sex ratio, ↓ anogenital distance No effect on serum testosterone (0.2 µg/kg only examined)	Ikeda et al. 2005a
Rat (Sprague-Dawley)[b]	0, 0.025, or 0.1 µg/kg/day on GDs 10–16	NA	0.025 µg/kg/day: ↓ saccharin consumption and preference in females (indicative of masculinized behavior)	No effect on litter size, pup body weight	Amin et al. 2000
Rat (Holtzman), 9–12 dams/ group	0 or 1 µg/kg on GD 15	NA	1 µg/kg: impaired structural differentiation of mammary gland	Female pups were ovariectomized at 9 weeks of age	Lewis et al. 2001
Rat (Long-Evans), 10 dams/group	0 or 1 µg/kg on GD 15	NA	1 µg/kg: ↓ primary branching from the collecting duct, delayed migration from the epithelium through the fat pad, and ↓ terminal branches and alveolar buds in mammary gland first observed on PND 4; ↓ female pup body weight; delayed vaginal opening; ↑ TSH levels, ↓ T4 levels	Female pups were examined on PNDs 4, 33, 37, 45, 68, and 110; vaginal opening was examined beginning on PND 29 and estrous cycling monitoring was done on PNDs 42–60 No effect on estrous cyclicity	Fenton et al. 2002

[a]Unless otherwise noted, 2,3,7,8-TCDD was administered via gavage in oil.
[b]Number of dams/group was not specified.

eCG = equine chlorionic gonadotropin; EKG = electrocardiogram; FSH = follicle-stimulating hormone; GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NA = not applicable; NOAEL = no-observed-adverse-effect level; PND = postnatal day; T3 = triiodothronine; T4 = thyroxine; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TSH = thyroid-stimulating hormone

Table 2-14. Summary of Select Animal Carcinogenicity Studies

Species (strain)	Dose[a], exposure duration/frequency	No effect	CEL	Comments	Reference
2,3,7,8-TCDD					
Rat (Sprague-Dawley) 81–82 females/group	0, 0.003, 0.010, 0.022, 0.046, or 0.100 µg/kg 2,3,7,8-TCDD (in corn oil), 5 days/week for 105 weeks	0.046 µg/kg	0.10 µg/kg; increased incidence of liver tumors (hepatocellular adenomas, cholangiocarcinomas), cystic keratinizing epithelioma in lungs, gingival squamous cell carcinoma in oral mucosa, c-cell adenoma in thyroid gland	Increased incidence of squamous cell carcinoma of the uterus was observed at 0.046 µg/kg, but not at 0.10 µg/kg	NTP 2006; Yoshizawa et al. 2005
Rat (Sprague-Dawley) 50 females/group	0 or 0.100 µg/kg 2,3,7,8-TCDD (in corn oil), 5 days/week for 30 weeks followed by a 70-week recovery period	0.10 µg/kg			NTP 2006
Rat (Sprague-Dawley), 6–14 females/group	0 or 1.25 µg/kg 2,3,7,8-TCDD (in corn oil) biweekly (0.125 µg/kg/day) for 14, 30, or 60 weeks	0.125 µg/kg/day		Increased incidence of ovarian tumors (6/14 compared to 0/11 in controls) in rats initiated with diethylnitrosamine (175 mg/kg administered intraperitoneally) 2 weeks prior to TCDD exposure	Davis et al. 2000
Rat (Sprague-Dawley) 5–15 females/group	0 or 1.75 µg/kg 2,3,7,8-TCDD (in corn oil) biweekly (0.125 µg/kg/day) for 14, 30, or 60 weeks	125 µg/kg/day		Nonsignificant increase in liver tumors in rats initiated with diethylnitrosamine (175 mg/kg administered intraperitoneally) 2 weeks prior to TCDD exposure	Walker et al. 2000
Mouse (Tg.AC) 20 females/group	0, 0.105, 0.450, or 1.250 µg/kg 5 days/week for 26 weeks (0, 0.075, 0.321, or 0.893 µg/kg/day)			Tg.AC transgenic mouse is a genetically initiated, tumor promoter-sensitive epidermal tumorigenesis model	Wyde et al. 2004

Table 2-14. Summary of Select Animal Carcinogenicity Studies

Species (strain)	Dose[a], exposure duration/frequency	No effect	CEL	Comments	Reference
Other CDD congeners					
Rat (Sprague-Dawley) 30–60 females/group	0, 1.0, 2.8, 3.1, 3.4, 3.8, or 4.1 mg/kg 1,2,3,4,6,7,8-HpCDD (in corn oil) once	1.0 mg/kg	2.8 mg/kg Increased incidence of lung cancer (squamous cell carcinoma)	Animals allowed to live out their natural lifespan; decreased survival at ≥2.8 mg/kg ≥3.4 mg/kg increased incidence of liver cancer (hepatocellular carcinoma and cholangiocarcinoma)	Rozman et al. 2005
Rat (Sprague-Dawley) 30 females/group	0 or 1.0+0.03 mg/kg biweekly, 2.5+0.63 mg/kg on day 150; or 2.8+0.086 mg/kg biweekly 1,2,3,4,6,7,8-HpCDD (in corn oil) every other week for lifespan	NA	0.0065 mg/kg/day (time-weighted average) Increased incidence of lung cancer (squamous cell carcinoma)	Rats received an initial loading dose followed by every other week exposure to second dose listed; cumulative doses were 2.1, 3.1, and 4.6 mg/kg, respectively, and estimated time-weighted average doses were 0.0065, 0.012, and 0.038 mg/kg/day[b] Decreased survival at ≥0.012 mg/kg/day (time-weighted average) ≥0.012 mg/kg increased incidence of liver cancer (hepatocellular carcinoma and cholangiocarcinoma)	Rozman et al. 2005

[a]Doses were administered via gavage, unless otherwise specified.
[b]Time-weighted average doses estimated using the following equation: cumulative dose/[average time to death – age at start of study (187 days)]. Cumulative doses were 2.1, 3.1, and 4.6 mg/kg, respectively, and average times to death were 510, 440, and 308 days, respectively.

CDD = chlorinated dibenzo-*p*-dioxin; CEL = cancer effect level; FOB = functional observational battery; HpCDD = heptachlorodibenzo-*p*-dioxin; NA = not applicable; NTP = National Toxicology Program; TCDD = 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

Table 2-15. Genes with functional AhRE

Aldehyde dehydrogenase 3A1
Aryl hydrocarbon repressor (AhRR)
Bax
c-jun
c-myc
Cathepsin D
Cyclooxygenase-2
CYP1A1
CYP1A2
CYP1B1
CYP2A5
CYP2S1
Epiregulin
Gluthathione-S-transferase Ya
Filaggrin
HES-1
Hsp27
Insulin-like growth factor binding protein
IgM μ gene
Interleukin-2
junD
NAD(P)H-quinone oxidoreductase-1
NF-E2 p45 –related factor (NRF2)
$p21^{CIP1}$
$p27^{KIP1}$
pS2
Slug
Suppressor of cytokine signaling 2 (Socs2)
UDP glucuronosyltransferase 1A1
UDP glucuronosyltransferase 1A6

Source: Gasiewicz et al. 2008

Table 2-16. AhR-TCDD Equilibrium Dissociation Constants (K_d) in various Laboratory Species and Human Tissues

Species/strain	Dissociation constant[a] ($K_{d,}$ nM)
Hartely guinea pig	0.06; 0.16
Gerbil	0.20
Syrian hamster	0.22
Sprague-Dawley rat	0.12 , 0.22, 2.4
C56BL/6 (N or 1) mouse	0.29, 0.27, 0.52, 0.5–1.6, 1.1, 1.8
B6D2F1 mouse	0.42
Long-Evans rat	3.4
Han/Wistar (Kuopio) rat	3.9
Wistar rat	5.4
Cynomolgus monkey	0.26, 16.5
Swine	17.5
Beagle dog	17.1
DBA/2 mouse	16
Human liver	18.6
Human lymphoblastoid cells	4.6
Human tonsils	17.6
Human placenta	9.6

[a]For some species, values were taken from multiple studies.

Source: Connor and Aylward (2006)

Table 2-17. World Health Organization (WHO) Toxic Equivalency Factor (TEF) Values

Compound	WHO 1998 TEF	WHO 2005 TEF[a]
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.0001	**0.0003**
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	**0.03**
2,3,4,7,8-PeCDF	0.5	**0.3**
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	**0.0003**
3,3',4,4'-tetraCB (PCB 77)	0.0001	0.0001
3,4,4',5-tetraCB (PCB 81)	0.0001	**0.0003**
3,3',4,4',5-pentaCB (PCB 126)	0.1	0.1
3,3'4,4'5,5'-hexaCD (PCB 169)	0.01	**0.03**
2,3,3',4,4'-pentaCB (PCB 105)	0.0001	**0.00003**
2,3,4,4',5-pentaCB (PCB 114)	0.0005	**0.00003**
2,3',4,4',5-pentaCB (PCB 118)	0.0001	**0.00003**
2'.3.4.4'.5-pentaCB (PCB 123)	0.0001	**0.00003**
2,3,3',4,4',5-hexaCB (PCB 156)	0.0005	**0.00003**
2,3,3',4,4',5'-hexaCB (PCB 157)	0.0005	**0.00003**
2,3',4,4',5,5'-hexaCB (PCB 167)	0.00001	**0.00003**
2,3,3',4,4',5,5'-heptaCB (PCB 189)	0.0001	**0.00003**

[a]Bold values indicate a change in TEF value from 1998 assessment.

heptaCB = heptachlorobiphenyl; HpCDD = heptachlorodibenzo-*p*-dioxin; HpCDF = heptachlorodibenzofuran; hexaCB = heptachlorobiphenyl; HxCDD = hexachlorodibenzo-*p*-dioxin; HpCDF = hepatchlorodibenzofuran; OCDD = octachlorodibenzo-*p*-dioxin; OCDF = octachlorodibenzofuran; PCB = polychlorinated biphenyl; pentaCB = pentachlorobiphenyl; PeCDD = pentachlorodibenzo-*p*-dioxin; PeCDF = pentachlorodibenzofuran; tetraCB = tetrachlorobiphenyl; TCDD = tetrachlorodibenzo-*p*-dioxin; TCDF = tetrachlorodibenzofuran

Source: Van den Berg et al. 2006

Table 5-1. Estimated Intake of CDD and CDF from Meat, Poultry, and Fish

	TEQ intake[a] (pg/kg body weight/day)
Males and females, 1–5 years of age (not breastfeeding)	1.76–1.26
Males and females, 6–11 years of age	1.14–0.77
Males, 12–19 years of age	0.89–0.47
Males, ≥20 years of age	0.69–0.40
Females, 12–19 years of age, not pregnant or lactating	0.69–0.47
Females, ≥20 years of age, not pregnant or lactating	0.59–0.38
Females, pregnant or lactating	0.65–0.54
Males and females, ≥1 years of age, including pregnant or lactating	0.78–0.64

[a]Includes CDD and CDF congeners only; range represents average intake for consumers of high and low (< 3 oz) intakes of meat, poultry, and fish. TEQs calculated using 0.5 (LOD) for nondetects.

CDD = chlorinated dibenzo-p-dioxin; CDF = chlorinated dibenzofuran; TEQ = toxic equivalents

Source: NAS (2003)

Table 5-2. Serum 1,2,3,4,6,7,8-HpCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Total					
1999–2000	NC	<LOD	58.2 (<LOD–63.6)	86.0 (75.5–96.7)	112 (101–131)
2001–2002	39.0 (33.7–45.0)	40.2 (34.9–46.9)	68.7 (56.7–82.2)	115 (88.2–138)	147 (126–181)
2003–2004	25.3 (23.4–27.3)	24.9 (22.8–26.9)	42.5 (38.8–48.1)	70.4 (62.7–80.1)	91.3 (73.5–117)
Age group					
12–19 years					
1999–2000	NC	<LOD	<LOD	<LOD	63.6 (<LOD–75.6)
2001–2002	ND	ND	ND	ND	ND
2003–2004	16.7 (15.1–18.4)	16.4 (15.1–18.3)	23.6 (21.5–25.8)	33.4 (28.6–36.8)	46.7 (34.5–78.1)
≥20 years					
1999–2000	NC	<LOD	62.0 (57.1–66.7)	92.9 (81.2–101)	120 (102–139)
2001–2002	39.0 (33.7–45.0)	40.2 (34.9–46.9)	68.7 (56.7–82.2)	115 (88.2–138)	147 (126–181)
2003–2004	26.8 (24.6–29.2)	27.3 (24.6–29.0)	45.6 (41.3–53.2)	73.7 (64.1–88.6)	95.0 (76.1–126)
Gender					
Males 1999–2000	NC	<LOD	<LOD	73.6 (69.0–80.8)	94.7 (83.1–103)
2001–2002	36.6 (31.7–42.3)	39.0 (33.3–42.6)	62.1 (49.7–75.0)	102 (75.8–132)	138 (103–169)
2003–2004	24.2 (21.7–27.0)	23.2 (21.1–25.6)	40.6 (35.3–46.9)	64.2 (58.8–73.7)	85.0 (65.8–113)
Females					
1999–2000	NC	<LOD	62.7 (<LOD–69.1)	102 (86.0–118)	131 (111–164)
2001–2002	41.2 (34.9–48.7)	43.6 (35.3–52.4)	76.0 (59.5–90.1)	125 (93.4–150)	158 (130–191)
2003–2004	26.3 (24.4–28.3)	26.8 (24.3–28.3)	44.4 (41.1–50.2)	76.1 (65.3–89.1)	95.7 (80.7–128)
Race/ethnicity					
Mexican Americans					
1999–2000	NC	<LOD	61.4 (<LOD–69.0)	97.7 (82.8–111)	132 (108–159)
2001–2002	39.6 (35.7–43.9)	39.7 (33.6–47.4)	64.0 (55.8–74.7)	107 (82.4–128)	149 (111–171)
2003–2004	25.8 (22.6–29.4)	26.1 (20.9–30.9)	41.9 (36.7–44.7)	61.0 (49.7–71.9)	80.1 (65.0–89.1)

Table 5-2. Serum 1,2,3,4,6,7,8-HpCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Non-Hispanic blacks					
1999–2000	NC	<LOD	58.1 (<LOD–71.1)	95.0 (75.1–110)	125 (102–183)
2001–2002	43.7 (35.4–54.0)	42.8 (32.2–59.8)	80.6 (60.9–106)	134 (101–166))	167 (130–230
2003–2004	25.8 (22.6–29.4)	23.7 (20.7–27.1)	41.2 (32.6–56.4)	69.2 (54.6–115)	115 (67.1–164)
Non-Hispanic whites					
1999–2000	NC	<LOD	59.0 (<LOD–64.8)	84.9 (72.0–97.0)	106 (96.7–122)
2001–2002	39.3 (33.0–46.8)	40.5 (34.0–50.1)	71.0 (56.3–87.5)	117 (87.1–147)	147 (125–186)
2003–2004	25.0 (22.6–27.7)	24.6 (22.3–27.4)	42.6 (39.4–48.5)	73.5 (60.4–86.7	93.7 (71.6–127)

HpCDD = heptachlorodibenzo-*p*-dioxin; LOD = limit of detection for Survey years 1999–2000, 2001–2002, and 2003–2004 were 55.9, 10.3, and 13.0 pg/g lipid, respectively; NC = not calculated; proportion of results below limit of detection was too high to provide a valid result; ND = data not collected for this age group for survey years 2001–2002.

Source: CDC 2009

Table 5-3. Serum 1,2,3,4,7,8-HxCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Total					
2001–2002	NC	<LOD	<LOD	10.7 (<LOD–13.9)	14.9 (11.7–20.0)
2003–2004		<LOD	<LOD	<LOD	<LOD
Age group					
12–19 years					
2001–2002	ND	ND	ND	ND	ND
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
≥20 years					
2001–2002	NC	<LOD	<LOD	10.7 (<LOD–13.9)	14.9 (11.7–20.0)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Gender					
Males 2001–2002	NC	<LOD	<LOD	10.9 (<LOD–14.3)	14.7 (11.5–17.6)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Females					
2001–2002	NC	<LOD	<LOD	10.7 (<LOD–14.1)	15.6 (11.1–23.0)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Race/ethnicity					
Mexican Americans					
2001–2002	NC	<LOD	<LOD	<LOD	9.20 (<LOD–11.8
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Non-Hispanic blacks					
2001–2002	NC	<LOD	<LOD	13.9 (<LOD–17.6)	18.3 (13.9–23.0)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD

Table 5-3. Serum 1,2,3,4,7,8-HxCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Non-Hispanic whites					
2001–2002	NC	<LOD	<LOD	11.3 (<LOD–14.4)	15.1 (12.0–20.5)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD

HxCDD = hexachlorodibenzo-*p*-dioxin; LOD = limit of detection for Survey years 2001–2002 and 2003–2004 were 9.0 and 11.9 pg/g lipid, respectively; NC = not calculated; proportion of results below limit of detection was too high to provide a valid result; ND = data not collected for this age group for survey years 2001–2002.

Source: CDC 2009

Table 5-4. Serum 1,2,3,6,7,8-HxCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Total					
1999–2000	NC	<LOD	32.6 (28.3–38.2)	56.9 (47.4–67.3)	74.0 (68.3–82.4)
2001–2002	34.6 (29.6–40.6)	39.2 (32.7–44.7)	60.8 (50.3–74.2)	95.2 (76.2–120)	128 (99.4–153)
2003–2004	17.2 (15.7–18.9)	20.0 (17.8–22.9)	36.5 (32.2–40.0)	53.0 (48.1–59.6)	68.5 (59.6–74.9)
Age group					
12–19 years					
1999–2000	NC	<LOD	<LOD	<LOD	26.7 (20.2–29.6)
2001–2002	ND	ND	ND	ND	ND
2003–2004	NC	<LOD	<LOD	16.1 (14.3–18.1)	19.4 (16.4–27.7)
≥20 years					
1999–2000	NC	<LOD	36.2 (31.5–40.7)	62.8 (53.6–69.1)	75.6 (70.5–84.2)
2001–2002	34.6 (29.6–40.6)	39.2 (32.7–44.7)	60.8 (50.3–74.2)	95.2 (76.2–120))	128 (99.4–153
2003–2004	19.7 (17.8–21.8)	23.8 (20.7–26.4)	39.3 (35.4–42.2)	56.6 (49.7–63.8)	70.8 (60.7–82.2)
Gender					
Males 1999–2000	NC	<LOD	31.5 (23.7–38.2)	55.0 (45.7–64.2)	71.3 (59.4–79.4)
2001–2002	34.1 (28.3–41.1)	38.9 (32.1–44.7)	61.9 (50.0–79.5)	94.9 (70.8–131)	130 (88.5–181)
2003–2004	17.5 (15.5–19.8)	19.8 (17.8–21.6)	35.5 (29.8–40.3)	52.9 (45.4–63.2)	70.2 (57.5–88.7)
Females					
1999–2000	NC	<LOD	34.9 (29.1–39.7)	61.2 (51.0–69.2)	74.9 (68.4–92.2)
2001–2002	35.1 (29.9–41.2)	40.1 (32.4–46.3)	59.8 (49.8–72.3)	97.6 (77.1–114))	126 (108–142
2003–2004	16.9 (15.3–18.6)	20.5 (17.8–24.6)	36.9 (33.2–41.0)	53.6 (48.3–59.6)	65.6 (60.0–73.4)
Race/ethnicity					
Mexican Americans					
1999–2000	NC	<LOD	21.3 (<LOD–27.6)	43.3 (34.1–52.3)	58.0 (49.5–64.8)
2001–2002	18.3 (15.6–21.4)	21.2 (19.4–25.0)	31.9 (27.5–40.3)	51.5 (40.3–69.9)	68.3 (48.0–111)

Table 5-4. Serum 1,2,3,6,7,8-HxCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
2003–2004			21.1 (16.3–26.5)	32.2 (24.5–47.4)	43.0 (31.5–65.3)
Non-Hispanic blacks					
1999–2000	NC	<LOD	31.9 (26.6–41.2)	56.7 (44.9–74.6)	81.6 (72.2–91.7)
2001–2002	38.9 (33.6–45.0)	40.3 (33.5–47.3)	63.5 (54.6–76.9)	93.9 (78.7–133))	136 (92.6–185
2003–2004	16.2 (12.9–20.4)	18.1 (14.4–21.6)	34.9 (28.4–42.9)	54.5 (44.4–69.4)	74.0 (54.3–122)
Non-Hispanic whites					
1999–2000	NC	<LOD	35.5 (29.7–40.0)	60.9 (51.4–68.3)	74.3 (68.3–83.0)
2001–2002	37.8 (31.5–45.4)	42.8 (33.9–51.2)	65.0 (52.3–82.9)	99.6 (78.4–130)	131 (103–165)
2003–2004	18.7 (17.0–20.6)	22.9 (19.9–26.2)	38.0 (35.2–41.5)	56.6 (48.7–63.8)	69.0 (60.6–74.9)

HxCDD = hexachlorodibenzo-*p*-dioxin; LOD = limit of detection for Survey years 1999–2000, 2001–2002, and 2003–2004 were 20.1, 9.1, and 12.3 pg/g lipid, respectively; NC = not calculated; proportion of results below limit of detection was too high to provide a valid result; ND = data not collected for this age group for survey years 2001–2002

Source: CDC 2009

Table 5-5. Serum 1,2,3,7,8,9-HxCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Total					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	12.5 (10.5–15.3)	17.0 (14.3–20.0)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Age group					
12–19 years					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	ND	ND	ND	ND	ND
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
≥20 years					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	12.5 (10.5–15.3)	17.0 (14.3–20.0)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Gender					
Males 1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002				12.1 (<LOD–14.8)	15.1 (12.9–18.5)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Females					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002				13.0 (10.7–16.8)	18.3 (15.7–21.1)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Race/ethnicity					
Mexican Americans					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	9.60 (<LOD–11.6)	12.2 (<LOD–20.6)

Table 5-5. Serum 1,2,3,7,8,9-HxCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Non-Hispanic blacks					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	14.6 (11.2–20.0)	19.9 (14.6–23.9)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
Non-Hispanic whites					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	12.9 (9.90–15.9)	17.3 (14.7–20.6)
2003–2004	NC	<LOD	<LOD	<LOD	<LOD

HxCDD = hexachlorodibenzo-*p*-dioxin; LOD = limit of detection for Survey years 1999–2000, 2001–2002, and 2003–2004 were 20.3, 9.3, and 12.3 pg/g lipid, respectively; NC = not calculated; proportion of results below limit of detection was too high to provide a valid result; ND = data not collected for this age group for survey years 2001–2002

Source: CDC 2009

Table 5-6. Serum 1,2,3,4,6,7,8,9-OCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Total					
1999–2000	NC	<LOD	406 (359–453)	674 (597–767)	913 (787–1,010)
2001–2002	346 (<LOD–394)	333 (<LOD–402)	573 (498–668)	944 (780–1,090)	1,260 (998–1,610)
2003–2004	NC	<LOD	336 (283–389)	582 (490–658)	767 (645–913)
Age group					
12–19 years					
1999–2000	NC	<LOD	<LOD	<LOD	421 (363–517)
2001–2002	ND	ND	ND	ND	ND
2003–2004	NC	<LOD	<LOD	244 (<LOD–330)	352 (264–458)
≥20 years					
1999–2000	NC	<LOD	445 (389–496)	710 (624–802))	948 (822–1,080
2001–2002	346 (<LOD–394)	333 (<LOD–402)	573 (498–668)	944 (780–1,090)	1260 (998–1,610)
2003–2004	220 (<LOD–244)	223 (<LOD–243)	358 (297–421)	597 (502–719)	794 (665–978)
Gender					
Males 1999–2000	NC	<LOD	<LOD	517 (447–580)	704 (563–838)
2001–2002	NC	<LOD	442 (346–579)	767 (593–968)	1,030 (837–1,240)
2003–2004	NC	<LOD	270 (244–320)	457 (377–559)	668 (501–856)
Females					
1999–2000	NC	<LOD	504 (422–579)	802 (674–928)	1,010 (928–1,180)
2001–2002	410 (356–472)	405 (335–502)	647 (574–751)	1,020 (858–1,360)	1,450 (1,060–1,780)
2003–2004	235 (<LOD–256)	238 (225–248)	402 (321–486)	640 (551–749)	829 (675–1,020)
Race/ethnicity					
Mexican Americans					
1999–2000	NC	<LOD	418 (365–502)	703 (610–873)	940 (737–1,230)
2001–2002	NC	<LOD	432 (394–545)	755 (578–1,220)	1,150 (696–1,640)

Table 5-6. Serum 1,2,3,4,6,7,8,9-OCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
2003–2004	NC	<LOD	296 (225–356)	452 (363–540)	588 (417–861)
Non-Hispanic blacks					
1999–2000	NC	<LOD	444 (371–519)	741 (566–983)	1,120 (799–1,560)
2001–2002	421 (352–503)	420 (339–509)	682 (537–907)	1,110 (956–1,520)	1,640 (1,130–1,900)
2003–2004	NC	<LOD	345 (276–455)	642 (513–883)	926 (636–1,310)
Non-Hispanic whites					
1999–2000	NC	<LOD	391 (333–452)	625 (562–754)	861 (676–1,010)
2001–2002	349 (<LOD–409)	335 (<LOD–421)	574 (496–679)	945 (764–1,170)	1,290 (972–1,660)
2003–2004	NC	<LOD	343 (282–403)	585 (464–674)	758 (635–922)

LOD = limit of detection for Survey years 1999–2000, 2001–2002, and 2003–2004 were 329.0, 319.0, and 218.0 pg/g lipid, respectively; NC = not calculated; proportion of results below limit of detection was too high to provide a valid result; ND = data not collected for this age group for survey years 2001–2002; OCDD = octachlorodibenzo-*p*-dioxin

Source: CDC 2009

Table 5-7. Serum 1,2,3,7,8-PeCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Total					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	11.3 (9.30–13.6)	15.8 (13.3–19.8)
2003–2004	NC	<LOD	6.10 (5.50–6.80)	9.00 (8.30–9.70)	11.0 (9.90–12.2)
Age group					
12–19 years					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	ND	ND	ND	ND	ND
2003–2004	NC	<LOD	<LOD	<LOD	4.80 (<LOD–5.90)
≥20 years					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	11.3 (9.30–13.6)	15.8 (13.3–19.8)
2003–2004	NC	<LOD	6.60 (5.90–7.20)	9.30 (8.60–10.1)	11.3 (10.1–12.7)
Gender					
Males 1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	10.8 (9.10–13.3)	14.5 (11.7–19.4)
2003–2004	NC	<LOD	5.90 (5.30–6.40)	8.90 (7.90–9.60)	11.0 (9.60–12.7)
Females					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	6.10 (<LOD–7.80)	11.8 (9.40–14.3)	16.6 (13.7–20.8)
2003–2004	NC	<LOD	6.50 (5.70–7.20)	9.10 (8.30–10.1)	11.0 (10.0–12.2)
Race/ethnicity					
Mexican Americans					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	<LOD	8.70 (<LOD–12.7)
2003–2004	NC	<LOD	<LOD	6.50 (5.20–7.90)	7.80 (6.70–9.20)

Table 5-7. Serum 1,2,3,7,8-PeCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Non-Hispanic blacks					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	7.70 (<LOD–9.30)	13.9 (9.60–18.4)	18.4 (14.2–24.0)
2003–2004	NC	<LOD	6.40 (5.30–8.20)	9.90 (8.50–13.4)	14.4 (9.60–20.1)
Non-Hispanic whites					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	11.7 (9.50–14.3)	16.7 (13.6–20.2)
2003–2004	NC	<LOD	6.50 (5.80–7.10)	9.30 (8.60–10.0)	11.1 (10.1–12.2)

LOD = limit of detection for Survey years 1999–2000, 2001–2002, and 2003–2004 were 14.2, 6.0, and 4.5 pg/g lipid, respectively; NC = not calculated; proportion of results below limit of detection was too high to provide a valid result; ND = data not collected for this age group for survey years 2001–2002; PeCDD = pentachlorodibenzo-*p*-dioxin

Source: CDC 2009

Table 5-8. Serum 2,3,7,8-TCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
Total					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	<LOD	<LOD
2003–2004	NC	<LOD	<LOD	4.10 (<LOD–4.40)	5.20 (4.30–5.80)
Age group					
12–19 years					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	ND	ND	ND	ND	ND
2003–2004	NC	<LOD	<LOD	<LOD	<LOD
≥20 years					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	<LOD	<LOD
2003–2004	NC	<LOD	<LOD	4.30 (3.90–4.60)	5.30 (4.50–6.10)
Gender					
Males 1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	<LOD	<LOD
2003–2004	NC	<LOD	<LOD	<LOD	4.60 (3.80–5.30)
Females					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	<LOD	6.40 (<LOD–9.20)
2003–2004	NC	<LOD	<LOD	4.40 (4.00–4.90)	5.50 (4.50–6.60)
Race/ethnicity					
Mexican Americans					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	<LOD	<LOD

Table 5-8. Serum 2,3,7,8-TCDD (Lipid Adjusted) Concentrations (pg/g Lipid) for the U.S. Population

Survey years	Geometric mean (95% confidence limit)	Selected percentiles (95% confidence limit) 50th	75th	90th	95th
2003–2004	NC	<LOD	<LOD	<LOD	3.80 (<LOD–5.50)
Non-Hispanic blacks					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	<LOD	7.50 (<LOD–10.0)
2003–2004	NC	<LOD	<LOD	4.50 (<LOD–6.10)	6.20 (4.40–10.3)
Non-Hispanic whites					
1999–2000	NC	<LOD	<LOD	<LOD	<LOD
2001–2002	NC	<LOD	<LOD	<LOD	<LOD
2003–2004	NC	<LOD	<LOD	4.10 (<LOD–4.50)	5.20 (4.30–5.90)

LOD = limit of detection for Survey years 1999–2000, 2001–2002, and 2003–2004 were 112.1, 5.8, and 3.8 pg/g lipid, respectively; NC = not calculated; proportion of results below limit of detection was too high to provide a valid result; ND = data not collected for this age group for survey years 2001–2002; TCDD = tetrachlorodibenzo-*p*-dioxin

Source: CDC 2009

Table 5-9. Blood TEQ levels[a] for Dioxin-Like Compounds (CDDs, CDFs, and select PCBs) Levels (pg/g Lipid) at 90th and 95th Percentiles by Age Group in NHANES 2001–2002 and 2003–2004 Survey Years

	TEQ for 2001–2002 survey years		TEQ for 2003–2004 survey years	
	90th percentile	95th percentile	90th percentile	95th percentile
Total, ≥12 years	No data[b]	No data	30.9 (28.2–33.9)[c]	37.8 (35.3–43.4)
Total, ≥20 years	41.0 (35.8–47.1)	56.1 (47.6–65.4)	32.5 (29.2–35.7	39.9 (36.6–45.7)
Age group				
12–29 years	No data	No data	12.1 (10.9–13.0)	14.0 (12.4–15.9)
20–39 years	23.0 (19.7–25.2)	26.2 (23.7–32.5)	16.2 (14.5–17.7)	18.7 (16.9–20.1)
40–49 years	35.4 (29.7–44.8)	46.9 (36.4–66.1)	28.2 (23.7–32.6)	32.0 (28.0–45.3)
≥60 years	67.7 (56.4–79.7)	79.7 (68.2–96.3)	49.7 (41.5–58.6)	63.2 (50.9–75.1)

[a]Toxic Equivents calculated using WHO 2005 toxic equivalency factors (TEFs).
[b]Data were not collected for this age group in the 2001–2002 survey.
[c]95% confidence interval.

CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; NHANES = National Health and Nutrition Examination Survey; PCB = polychlorinated biphenyl; TEQ = toxic equivalent

Source: Patterson et al. 2008, 2009

Table 5-10. Serum CDD/CDF Concentrations for Mean and Selected Percentiles for the NHANES 1999-2000, 2001-2002, and 2003–2004 Survey Years

	Serum CDD/CDF concentrations (pg TEQ/g lipid)		
Percentile	1999–2000	2001–2002	2003–2004
10	8.36 (8.03–8.71)[a]	7.76 (7.07–8.07)	5.17 (4.92–5.50)
25	10.52 (10.17–10.99)	10.38 (10.17–10.80)	7.29 (6.95–7.68)
50	13.46 (12.92–13.81)	13.98 (13.4214.59)	11.39 (10.60–12.15)
75	17.68 (17.04–18.30)	20.88 (19.57–22.12)	17.71 (16.61–18.65)
95	27.68 (24.90–29.65)	44.45 (40.11–48.79)	30.62 (28.51–32.34)
Arthimetic mean	15.4 (14.68–15.94)	18.05 (17.25–18.78)	13.90 (13.35–14.42)

[a]95% confidence interval.

Source: LaKind et al. 2009