Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on Influenza Virus Host Resistance in Mice

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) causes numerous immunotoxic effects including thymic involution and an immunosuppression of nonspecific as well as specific cell- and humoral-mediated immunity. TCDD administration to laboratory animals also results in a decreased resistance to numerous bacteria, viruses, and parasites. Effects on virus host resistance appear to be among the most sensitive effects of TCDD immunotoxicity. However, previous studies have not achieved a no effect level. The present studies utilized an influenza virus host resistance model in mice to quantify the sensitivity of this model to TCDD and to determine the NOAEL (no observed adverse effect level) of TCDD for influenza virus. Results indicated that a single dose of TCDD at 0.10, 0.05, or 0.01 μg/kg resulted in an increased mortality to Hong Kong influenza virus when mice were challenged 7 days after TCDD administration. Increased mortality was not correlated with increased virus titers in the lungs. TCDD at 0.005 or 0.001 μg/kg had no effect on influenza-induced mortality. TCDD alone did not affect thymus weight at any dose administered in this study. TCDD also did not alter the virus-enhanced increase in lung weight/body weight ratio nor the virus-induced decrease in thymus weight. Thus, low levels of TCDD exposure lead to enhanced mortality to influenza virus; however, the mechanism of this effect remains to be elucidated. Nonetheless, enhanced mortality to influenza virus in mice following a single dose of 10 ng TCDD/kg represents the most sensitive adverse effect yet reported for TCDD.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the prototype of the polyhalogenated aromatic hydrocarbons and has been reported to exert immunotoxic effects on the thymus, on nonspecific immunity, and on cell- and humoral-mediated immune responses (reviewed by Holsapple et al., 1991a,b; Kerkvliet and Burleson, 1994). A major aim of immunotoxicological investigations is to obtain and utilize data for risk assessment. One of the critical issues in risk assessment is the interpretation of immunotoxic effects with respect to increased susceptibility or severity of infectious disease. Animal host resistance models that are comparable to and accurately reflect human diseases are available and have been used to assess the effect of TCDD on altered host resistance. There are numerous studies indicating that TCDD enhances susceptibility to bacterial, viral, parasitic, and neoplastic challenges. Examples include Salmonella enterica (Thigpen et al., 1975), Salmonella typhimurium var. copenhagen (Hindsdill et al., 1980), endotoxin (Vos et al., 1978; Thomas and Hindsdill, 1979; Rosenthal et al., 1989), Streptococcus pneumoniae (White et al., 1986), Herpesvirus type II (Clark et al., 1983), influenza virus (House et al., 1990), Plasmodium yoelii (Tucker et al., 1986), and PYB6 tumor cells (Luster et al., 1980). TCDD (3.0 μg/kg maternal body wt) exposure at Gestational Day 19 had no effect on antibody or numbers of encysted larvae but led to a small increase in mortality of offspring of rats infected with Trichinella spiralis (Korte et al., 1990). Luebke et al. (1994) observed delayed adult parasite elimination at 10 or 30 μg TCDD/kg and suppressed proliferative response at 1.0 μg/kg in B6C3F1 mice, while no effect was observed in Fischer 344 rats at 10 or 30 μg/kg (Luebke et al., 1995). Host resistance to Herpesvirus suis.
also known as pseudorabies virus, was not altered by TCDD (Thigpen et al., 1975). Various results have been observed in host resistance studies in mice with Listeria monocytogenes (Luster et al., 1980; Hinsdill et al., 1980; Von et al., 1978; Thomas and Hinsdill, 1979; House et al., 1990). These disparate results may reflect different study designs including dose, route, single versus multiple administrations, mouse strain, age, or sex. However, it is clear that TCDD, under certain conditions, results in increased susceptibility to Listeria.

The studies with Herpes virus type II indicate that increased mortality is observed when mice are dosed with TCDD intraperitoneally once a week for 4 weeks with doses of 0.04, 0.4, or 4.0 µg/kg weekly (total dose = 0.16, 1.6, and 16.0 µg/kg) and challenged with Herpes simplex II strain 33 virus (Clark et al., 1983). House et al. (1990) reported an increased mortality of mice to influenza A/Taiwan/1/64 (H2N2) virus after administration of a single dose of TCDD at 10, 1.0, or 0.1 µg/kg. Neither the Herpes virus nor the influenza virus host resistance studies achieved a no effect level. The present study was designed (1) to validate and confirm previous results obtained by House et al. (1990) on increased susceptibility to influenza virus after administration of a single dose of TCDD, (2) to extend the dose-response to TCDD in order to determine the NOAEL (no observed adverse effect level) for this highly sensitive endpoint, and (3) to determine if the enhanced mortality was due to enhanced and or prolonged viral replication after TCDD exposure.

MATERIALS AND METHODS

Animals. Female B6C3F1 mice were purchased from Charles River Laboratories, Inc. (Raleigh, NC) and used at 8 weeks of age in all studies. Animal quarters were designed based on the clean-dirty corridor system. The animal quarters were maintained at 22 ± 2°C and a relative humidity of 50 ± 10% with a 12-hr light-dark cycle. Mice received water and food ad libitum. After receipt, randomly selected animals were placed in designated rooms as part of a sentinel program. Sentinel animals were screened for numerous pathogens, including endoparasites and ectoparasites, fecal Pseudomonas, and nasopharyngeal, tracheal, and lung washing samples for isolation of respiratory tract pathogens. Tissues were collected for histopathological evaluation. Serum samples were tested for viral antibody to reovirus type 3, pneumonia virus of mice, encephalomyelitis virus, Sendai virus, mouse adenosivirus, mouse hepatitis virus, Toolan H-1 virus, Kilham rat virus, and lymphocytic choriomeningitis virus. Results of all these tests were negative.

TCDD. TCDD (purity greater than 99%) was obtained from Radian, CIL, Inc. (San Antonio, TX). TCDD was dissolved in acetone and then diluted in corn oil (Sigma) and the acetone was removed by evaporation. The TCDD was stored as a 10 µg/ml stock solution. Dosing solutions were prepared by diluting the stock solution into an appropriate volume of corn oil. Mice (8 weeks old) were dosed with either corn oil (vehicle controls) or 0.001, 0.005, 0.01, 0.05, 0.1, or 6.0 µg TCDD/kg at a constant dose volume by gavage (all mice received 0.01 ml/kg).

Influenza A/Hong Kong/86/38 (H3N2) virus. Influenza A/Hong Kong/ 8/68 (H3N2) virus was obtained from Dr. Philip R. Wyde (The Influenza Virus Research Center, Baylor College of Medicine, Houston, TX). This influenza virus was passaged in BALB/c mice and between passages 3 and 9 the stock became highly lethal (Wyde et al., 1977). Virus stock used in this study (passage 14) of the highly virulent influenza virus was passaged in BALB/c mice by preparation of a 10% (v/v) lung homogenate 48 hr after intranasal infection. The 10% (v/v) lung homogenate was clarified by centrifugation at 10000 g for 30 min. Quantification of infectious viral titer used in challenge studies was performed utilizing Madin–Darby canine kidney (MDCK) cells (Gaush and Smith, 1968) and an overlay medium (Appleyard and Maber, 1974) as described in detail by Ehrlich and Burleson (1991) and Burleson (1995). The stock virus had an infectious titer of 1.9 × 10^6 plaque forming units (PFU) per milliliter. This method was also used to determine the virus titers in the lungs of experimental animals.

Virus Infection. Mice were lightly anesthetized with ether and infected intranasally with virus (0.05 ml) diluted at 10^{-4} (150 PFU), 10^{-5} (95 PFU), 10^{-5.5} (60 PFU), or 10^{-5} (37 PFU) in order to achieve 30% mortality in control animals. Mice were infected with virus 7 days after TCDD exposure. This regimen was chosen in order to duplicate the studies of House et al. (1990). Animals were observed for morbidity and mortality for 21 days following virus infection. All groups of animals were challenged with influenza virus intranasally at three of the challenge levels listed above for mortality studies. This was done to assure that at least one control group would have 30% or less deaths. Use of different dilutions of the virus stock was necessary because of the highly virulent nature of this passage 14 influenza virus. The ability of a small number of infectious virus particles to cause death also results in an inherent variability associated with such a virulent virus challenge. Experiments where control groups had greater than 30% deaths were not included in statistical analyses as these mice were considered to receive too large a dose of virus to be immunomodulated and not useful for host resistance studies. These animals would already be highly compromised and thus could respond differently to TCDD or any xenobiotic. Two replicate experiments were performed for each TCDD dose and each group of animals consisted of 20 mice per group. Studies for lung weight:body weight ratio and thymus weight used mice that were infected with 0.05 ml of influenza virus at a dose (10^{-5.5} and 10^{-5} dilution of influenza virus) known not to cause mortality.

Lung weight:body weight and thymus weight. Mice were randomly allocated to 12 different groups as follows (18 animals per group): (A) TCDD at 0.001, 0.01, or 0.1 µg/kg followed 7 days later by intranasal infection with virus (10^{-5.5} dilution of stock), (B) TCDD at 0.001, 0.01, or 0.1 µg/kg followed 7 days later by intranasal infection with virus (10^{-5.5} dilution of stock), (C) TCDD at 0.001, 0.01, or 0.1 µg/kg followed 7 days later by intranasal sham-infection, (D) corn oil followed 7 days later by intranasal sham-infection, (E) corn oil followed 7 days later with virus (10^{-5.5} dilution of stock), or (F) corn oil followed 7 days later with virus (10^{-5.5} dilution of stock). Animals (6) of each group were terminated at Days 5, 9, and 12 post infection. Body weights, thymus weights, and wet lung weights were recorded.

Influenza virus titers. At 2 hr, 1, 4, 6, 7, 8, 9, 10, and 11 days post infection, mice (eight mice per time point) were terminated by CO2 exposure. Lung homogenates were prepared as described by Ehrlich and Burleson (1991) and Burleson (1995). Samples were stored at −70°C until assayed for viral titer. Pulmonary viral titers were determined using MDCK cells for plaque assay as described by Ehrlich and Burleson (1991) and Burleson (1995).

Statistical analysis. The mortality data were analyzed using a categorical linear model. Overall significance was first tested and then pairwise subtesting within the original model was performed using a modified Bonferroni correction to adjust the significance levels in the presence of multiple comparisons. Each experiment utilized different doses of TCDD compared to vehicle (corn oil) controls. Each group of animals contained 20 mice. Control groups receiving virus that resulted in 70–100% survivors were included in statistical analysis. Lung weight:body weight data and thymus weight data were analyzed using a two-way analysis of variance (ANOVA). Pairwise comparisons were performed as subtests within the ANOVA, ad-
FIG. 1. Effect of TCDD on host resistance to influenza virus. B6C3F1 mice were dosed per os with 0.1, 0.05, 0.01, 0.001, or 0.005 μg/kg TCDD or corn oil and challenged intranasally 7 days later with a dose of influenza virus calculated to result in a mortality of 30% in control mice. *Significant difference (p < 0.05) from corn oil controls. Two replicate experiments were performed for each TCDD dose and each group of animals consisted of 20 mice per group. Mice exposed to TCDD at 0.1, 0.05, or 0.01 μg/kg were not statistically different from each other.

justing the significance levels of multiple comparisons using a modified Bonferroni correction.

RESULTS

Mortality

Successive passages of influenza virus in mice results in a more virulent virus. The influenza A/Hong Kong/8/68 (H3N2) virus used in the present study was obtained from Dr. P. Wyde and was described as becoming highly virulent between passages 3 and 9 (Wyde et al., 1977). As the virus becomes more virulent, smaller numbers of infectious particles are required for death in mice, and more variability is encountered in preparing dilutions of challenge virus inoculum. The influenza A/Taiwan/1/64 (H2N2) virus from the study by House et al. (1990) was used in preliminary studies to extend the dose–response of TCDD. However, additional passage in mice to prepare a stock virus resulted in a virus preparation with greater virulence — fewer infectious virus particles required to cause mouse mortality — and an unacceptable variability in control mouse mortality. The present study was then pursued using the influenza A/Hong Kong/8/68 (H3N2) virus. TCDD administration resulted in an enhanced mortality at doses of 0.1, 0.05, or 0.01 μg/kg (Fig. 1). There was no statistical difference in percentage survivors between doses of 0.1, 0.05, and 0.01 μg/kg. TCDD at 0.005 or 0.001 μg/kg did not alter mortality in the influenza virus host resistance model. These doses (0.005 or 0.001 μg/kg) corresponded to the NOAEL for influenza virus host resistance model in mice for TCDD.

Lung and Thymus Weights

Influenza virus causes a time-related increase in the wet weight of lungs in infected mice due to an increased edema that is reflected in an increased lung weight:body weight ratio (Fig. 2). Experiments were designed to determine whether TCDD might enhance this pathogenic character of influenza virus infection as a possible mechanism of enhanced mortality. While influenza virus infection did result in an increased lung weight:body weight ratio (Fig. 2), this increased lung weight:body weight ratio was not altered by TCDD and therefore the enhanced mortality observed with TCDD was not correlated to a more severe edema reflected as an enhanced lung weight:body weight ratio. Studies for lung weight:body weight ratio used mice that were infected with 0.05 ml of $10^{-5.4}$ and $10^{-5.8}$ dilution of influenza virus with similar results obtained for each dose of virus. TCDD can cause a decreased thymus weight and this strain of influenza/A/Hong Kong/8/68 virus is known to cause a decrease in thymus weight (Wyde et al., 1977). Studies were therefore performed to determine whether TCDD or TCDD plus virus had an effect on thymus weight for the low doses of TCDD considered in this study. TCDD at doses of 0.1, 0.01, or 0.001 μg/kg did not affect thymus weight (Fig. 2b) nor did TCDD affect the loss in thymic weight due to the influenza virus at doses of 0.1, 0.01, or 0.001 μg/kg (Fig. 2c). Studies for thymus weight used mice that were infected with 0.05 ml of $10^{-5.4}$ and $10^{-5.8}$ dilution of influenza virus and similar results were obtained for each dose of virus. Therefore, enhanced mortality in TCDD-treated mice was not due to an additive or synergistic increase in thymic atrophy.
FIG. 2. (a) Effect of influenza virus alone or with different doses of TCDD on lung weight:body weight ratios. Mice were dosed with TCDD or corn oil via gavage and challenged 7 days later with influenza virus at a dose ($10^{-34}$ dilution of stock virus) known not to cause mortality. Significant time differences were observed; however, no significant difference was observed due to TCDD exposure on the influenza virus-induced increase in lung weight:body weight ratio. Each data point represents the mean of eight mice ± the standard error. (b) Effect of TCDD on thymus weight. B6C3F1 mice were dosed per os with 0.1, 0.01, or 0.001 µg/kg TCDD or corn oil and sham-infected with uninfected lung homogenate 7 days later. The thymus was removed at the times indicated after sham-infection and weighed. No significant difference was found due to TCDD exposure. Each data point represents the mean of eight mice ± the standard error. (c) Effect of TCDD on influenza virus-induced decrease in thymus weight. Mice were dosed with TCDD or corn oil via gavage and challenged 7 days later with influenza virus at a dose ($10^{-34}$ dilution of stock virus) known not to cause mortality. Significant time differences were observed; however, no significant effect was observed due to TCDD exposure on the influenza virus-induced decrease in thymus weight. Each data point represents the mean of eight mice ± the standard error.

Lung Viral Titers

Enhanced mortality observed with TCDD also was not correlated with an increase in pulmonary influenza virus titers. Administration of TCDD at 0.001, 0.01, 0.1, and 1.0 µg/kg 7 days prior to infection with Hong Kong influenza virus had no effect on pulmonary viral titers assayed on Days 6, 7, and 8 post infection (data not shown). Administration of TCDD at 6 µg/kg 7 days prior to infection with virus also did not increase viral titers in the lungs (Fig. 3). TCDD at 6 µg/kg was chosen (a) to enhance the opportunity to detect an effect on pulmonary viral titers and (b) to allow a comparison of the pulmonary effects of TCDD in Fischer 344 rats by Yang et al. (1994).

DISCUSSION

TCDD is highly toxic and has been observed to exert immunotoxicity on the thymus, on nonspecific immunity, and on cell- and humoral-mediated immune responses (reviewed by Holsapple et al., 1991a,b; Kerkvliet and Burleson, 1994). TCDD has also been reported to enhance susceptibility to numerous bacterial, viral, parasitic, and tumor challenges as discussed above. The mouse influenza virus model was used to determine the effect of TCDD exposure on viral host resistance. Influenza virus reaches high pulmonary viral titers after intranasal infection from Days 1 to 7 with virus cleared in mice by Day 10 (Fig. 3). Influenza virus host resistance studies have been performed in mice (House et
FIG. 3. Effect of TCDD on Hong Kong influenza virus titers in the lungs. Mice were dosed with TCDD (6 μg/kg) or corn oil via gavage and challenged 7 days later with influenza virus (10^{-4} dilution of stock virus or 37 PFU's). No significant effect was observed due to TCDD administration. Each data point represents the mean of six mice ± the standard error.

Influenza virus causes an increase in the wet weight of lungs in infected mice. Selgrade et al. (1988) reported lung wet weight and lung weight:body weight to be a sensitive indicator of viral damage. Experiments were designed to determine whether the TCDD-enhanced mortality might be due to increased pulmonary edema occurring after viral infection. However, the increased lung weight:body weight ratio was not altered by TCDD and the enhanced mortality observed with TCDD could not be correlated with an enhanced lung weight:body weight ratio.

The effect of TCDD on thymus weight in virus-infected animals was studied since severe thymic atrophy may lead to an increased susceptibility to influenza virus infection, as T lymphocytes are known to be critical for the clearance of this virus (Wells et al., 1979). Thymic atrophy has been reported in mice exposed to much higher doses of TCDD; however, an effect on thymus weight has not been reported at doses of TCDD causing enhanced mortality to influenza virus in the present study (Fine et al., 1990; De Waal et al., 1992). Influenza/A/Hong Kong/8/68 virus is also reported to cause a decrease in thymus weight (Wyde et al., 1977). It was therefore hypothesized that the increased mortality to influenza virus may be due to an additive or synergistic thymic atrophy due to the concomitant effects of TCDD and influenza virus infection. However, the present study determined that TCDD at doses as low as 0.1, 0.01, or 0.001 μg/kg neither affects thymus weight nor enhances the loss in thymic weight due to the influenza virus infection. These studies thus indicated that the mechanism of TCDD-augmented mortality was not due to additive or synergistic effects on lung or thymic atrophy.

TCDD did not alter Hong Kong virus replication or clearance in the present study. Lebrec and Burleson (1994) investigated different influenza virus host resistance models in mice and rats and concluded that viral replication and mortality are different endpoints and are not always associated. Thus, the mechanism of TCDD-enhanced mortality in influenza-infected mice is not related to enhanced viral replication or decreased viral clearance. The lack of a dose--response for the TCDD host resistance studies suggests that TCDD may be exerting an effect via an indirect mechanism(s) such as through an effect on cytokines. Clark et al.
reported similarities in the wasting syndrome and depletion of adipose tissue observed with acute TCDD toxicity and TNFα. TCDD causes an increase in the concentration of TNFα in the serum of endotoxin-exposed mice. Taylor et al. (1992) used antisera to TNFα or the anti-inflammatory agent dexamethasone to reduce TCDD-induced toxicity 54 and 92%, respectively. These data suggest that TNFα is one of the mediators of acute TCDD toxicity and therefore suggests that the influenza virus-induced TNFα in bronchoalveolar lavage fluid (Hennet et al., 1992) may interact synergistically with the TNF associated with TCDD toxicity. Future studies should evaluate the role of TNFα as a mechanism for TCDD-enhanced mortality in influenza virus-infected mice. TCDD-enhanced mortality in influenza virus-infected mice may also be explained by studies reported by Prell et al. (1995). TCDD administered 2 days prior to anti-CD3 enhances the cytokine release syndrome with enhanced and prolonged body weight loss and lymphoid tissue atrophy (Prell et al. 1995). Interferon-γ was suppressed at 24 hr and IL-6 levels were increased at 48 hr after anti-CD3 in TCDD treated mice. Matthys et al. (1993) observed increased IL-6 by blocking interferon-γ availability, suggesting that the increased IL-6 at 48 hr reported by Prell et al. (1995) may be related to the decreased interferon-γ. Likewise, the enhanced mortality in influenza virus-infected mice may be related to the TCDD-effect on interferon-γ and the cytokine release syndrome described by Prell et al. (1995). Studies demonstrating enhanced susceptibility using host resistance models by Clark et al. (1983) with Herpes simplex II strain 33 virus and Luster et al. (1980) with PYB6 tumor cells also did not observe a TCDD dose-dependent effect.

A cascade of immune responses occurs following viral infection including interferon production, enhanced macrophage activity, augmented NK activity, cytophagic T lymphocyte activity, and antibody production (reviewed by Burleson, 1987). Each of these immune functions is involved and important in the prevention and/or control of viral disease. Suppression of any one or more of these important immune functions by TCDD may play a role in the mechanism of TCDD-enhanced susceptibility to influenza viral disease. Natural killer (NK) cell activity has been suggested to play an important role in the host defense to virus infection (reviewed by Burleson, 1987). NK cell activity is an important nonspecific immunological parameter with three important functions (1) antiviral, (2) antitumor, and (3) immunoregulatory (as reviewed by Burleson, 1987). TCDD at 0.1, 1.0, or 10.0 μg/kg was reported to exert no immunosuppressive effect in mice on spontaneous splenic NK activity (House et al., 1990). Mantovani et al. (1980) reported that a single injection of TCDD at 30 μg/kg had no effect on the percent specific NK activity at 7 and 14 days after exposure. However, these authors reported a decreased NK activity as measured by lytic units due to the decreased number of spleen cells. Recently, Yang et al. (1994) reported in Fischer 344 rats that 3.0 and 10.0 μg/kg TCDD administered by gavage resulted in a suppression of influenza virus-augmented NK activity, while the spontaneous NK activity was not affected. Exposure to 1.0 μg/kg had no effect on spontaneous or virus-augmented NK activity. While the effect of TCDD on influenza-specific cytotoxic T lymphocyte (CTL) activity has not been reported, other studies have indicated that TCDD can decrease CTL activity in mice (Clark et al., 1981, 1983). In contrast, Hanson and Smialowicz (1994) have used higher doses than those reported by Clark and not observed decreases in the in vitro or in vivo CTL response. Kerkvliet et al. (1990) and Holsapple et al. (1991a,b) have reported decreased CTL activity in mice following exposure to TCDD. De Krey and Kerkvliet (1995) demonstrated the dose-dependent 50% immunosuppressive dose (ID50) of TCDD to be 7.2 μg/kg (2.8 μg/kg when total lytic units (LU)/spleen were calculated). TCDD did not suppress in vitro CTL activity and the in vivo suppression did not involve corticosterone (De Krey and Kerkvliet, 1995). The importance of antibody responsiveness for resistance to influenza was previously suggested by Ada et al. (1983). House et al. (1990) reported a general impairment of immunoglobulin production observed in TCDD-treated mice that may explain the increased susceptibility to influenza virus challenge.

Results from host resistance studies in rodents provide unequivocal evidence that exposure to TCDD results in increased susceptibility to bacterial, viral, parasitic, and neoplastic challenge. As indicated above for influenza virus, immunological defense against infectious disease is multifaceted and not controlled entirely by only one component. The mechanism(s) of enhanced mortality may be related to one or more of the immunological functions important for defense against viral disease or the mechanism(s) may be related to altered production of cytokines resulting in a dysfunctional immunity or enhanced pathology due to hyperproduction of inflammatory or cachetic cytokines. Small decreases in several immunological functions, not statistically significant alone, may together result in significant immunosuppression that can only be detected as an increased susceptibility to infectious disease and measured by host resistance models (Burleson, 1995). However, the TCDD-enhanced mortality observed in the present study may also be due to toxic effects that are not immune related. Thus, the mechanism of this TCDD-enhanced mortality to influenza virus remains to be elucidated. Results indicated that a single dose of TCDD at 0.10, 0.05, or 0.01 μg/kg resulted in an increased mortality to influenza virus when mice were challenged 7 days after TCDD administration and that a NOAEL of 0.005 μg/kg was observed for the host resistance model. Nonetheless, enhanced susceptibility to influenza virus following exposure to TCDD appears to be the most sensitive adverse
effect yet reported. The present investigation confirms and extends the results of House et al. (1990) with influenza virus. The results are also consistent with the enhanced mortality in mice dosed with a total dose of TCDD at 0.16, 1.6, or 16 μg/kg and infected with Herpes simplex II strain 33 virus (Clark et al., 1983). Comparable adverse effects in humans cannot be evaluated and may or may not occur. Suppression of the primary antibody response occurs following a dose of approximately 300 ng TCDD/kg (Smialowicz et al., 1994). Demasculinization of male offspring has been reported after treatment of the pregnant rat at 64 ng TCDD/kg (Mably et al., 1992). Induction of CYP1A1 mRNA can be detected following a single dose of 1 ng TCDD/kg in the rat (Vanden Heuvel et al., 1994). Doses of 1.5 ng TCDD/kg/day result in increases in CYP1A1 and CYP1A2 enzymatic activity in mice (DeVito et al., 1994). It is not clear, however, whether enzymatic changes are actually deleterious or merely adaptive responses. However, increases in influenza-induced mortality in an animal model is clearly a negative consequence of TCDD exposure.

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