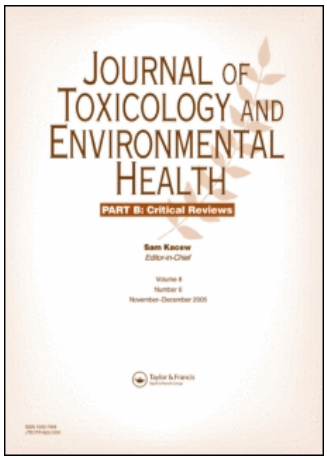

Journal of Toxicology and Environmental Health, Part B
Critical Reviews
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713667286

The Comparative Immunotoxicity of Five Selected Compounds Following Developmental or Adult Exposure

Robert W. Luebke [a]; David H. Chen [b]; Rodney Dietert [c]; Yung Yang [d]; Marquea King [e]; Michael I. Luster [f]
[a] Immunotoxicology Branch, Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency. Research Triangle Park, North Carolina. USA
[b] Office of Children's Health Protection, U.S. Environmental Protection Agency. Washington, DC. USA
[c] Department of Microbiology and Immunology, Institute of Comparative and Environmental Toxicology, Cornell University. Ithaca, New York. USA
[d] Office of Pesticides Program, U.S. Environmental Protection Agency. Washington, DC. USA
[e] Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency. Washington, DC. USA
[f] Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. Morgantown, West Virginia. USA

Online Publication Date: 01 January 2006
To cite this Article: Luebke, Robert W., Chen, David H., Dietert, Rodney, Yang, Yung, King, Marquea and Luster, Michael I. , (2006) 'The Comparative Immunotoxicity of Five Selected Compounds Following Developmental or Adult Exposure', Journal of Toxicology and Environmental Health, Part B, 9:1, 1 - 26
To link to this article: DOI: 10.1080/15287390500194326
URL: http://dx.doi.org/10.1080/15287390500194326

Journal of Toxicology and Environmental Health, Part B, 9:1–26, 2006

ISSN: 1093–7404 print / 1521–6950 online
DOI: 10.1080/15287390500194326

THE COMPARATIVE IMMUNOTOXICITY OF FIVE SELECTED COMPOUNDS FOLLOWING DEVELOPMENTAL OR ADULT EXPOSURE

Robert W. Luebke[1], David H. Chen[2], Rodney Dietert[3], Yung Yang[4], Marquea King[5], Michael I. Luster

[1]Immunotoxicology Branch, Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, [2]Office of Children's Health Protection, U.S. Environmental Protection Agency, Washington, DC, [3]Department of Microbiology and Immunology and Institute of Comparative and Environmental Toxicology, Cornell University, Ithaca, New York, [4]Office of Pesticides Program, U.S. Environmental Protection Agency, Washington, DC, [5]Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, DC, and [6]Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, West Virginia, USA

It is well established that human diseases associated with abnormal immune function, including some common infectious diseases and asthma, are considerably more prevalent at younger ages. Although not established absolutely, it is generally believed that development constitutes a period of increased immune system susceptibility to xenobiotics, since adverse effects may occur at lower doses and/or immunomodulation may be more persistent, thus increasing the relative risk of xenobiotic exposure to the immunologically immature organism. To address this issue, a brief overview of immune maturation in humans is provided to demonstrate that functional immaturity alone predisposes the young to infection. Age-dependent differences in the immunotoxic effects of five diverse compounds, diethylstilbestrol (DES), diazepam (DZP), lead (Pb), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and tributyltin oxide (TBTO), which have undergone adult and developmental immunotoxicity testing in rodents, are then reviewed, as are human data when available. For all five chemicals, the developing immune system was found to be at greater risk than that of the adult, either because lower doses produced immunotoxicity, adverse effects were more persistent, or both.

It is well established that diseases associated with abnormal immune function, including common infectious diseases and asthma, are considerably more prevalent at younger ages. Several factors are thought to account for this increased susceptibility, including functional immaturity of the immune system and age-related differences in the integrity of the host's anatomical and functional barriers. Although not established absolutely, it is generally believed that the immature immune system is also more susceptible to xenobiotics than the fully mature system, and that sequela of developmental immunotoxicant exposure may be particularly persistent, in contrast to effects observed following adult exposure, which generally occur at higher doses and are expected to resolve soon after exposure ends. Based on experimental animal studies, perturbations of the developing immune system may be manifested as a qualitative (i.e., affecting the developing immune system without affecting the adult immune system) or a quantitative (i.e., affecting the developing immune system at lower doses than in adults) difference. Immune maturation may simply be delayed by

This review was prepared by members of the Immunotoxicology Workgroup, under Interagency Agreement DW75991101 between the U.S. Environmental Protection Agency's Office of Children's Health Protection and the Health Effects Laboratory Division of the National Institute for Occupational Safety and Health (NIOSH). Members of the workgroup not included as authors are Drs. Dori Germolec (National Institute of Environmental Health Sciences, National Toxicology Program), Christine Park, Michael Kashon, and Laura Blanciforti (NIOSH, Health Effects Laboratory Division). Special thanks to Drs. Katherine Anitole, Linda Birnbaum, Ralph Smialowicz, Ron Ward, Mike Holsapple, and John Armstrong for reviewing the manuscript, and to Dr. Bob Sonawane (EPA/ORD/NCEA) for helping to organize this effort.

This report has been reviewed by the U.S. Environmental Protection Agency and the National Institute of Occupational Safety and Health and approved for publication. Approval does not signify that the contents reflect the views of the agencies.

Address correspondence to Robert Luebke, Immunotoxicology Branch, Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, office of Research and Development, U.S. EPA, Research Triangle Park, NC 27711, USA. E-mail: luebke.robert@epi.gov

xenobiotic exposure and recover to normal adult levels over time or, if exposure interferes with a critical step in the maturational process, lifelong defects in immune function may follow. These defects may be expressed as immunosuppression or as dysregulation of the immune system, resulting in decreased resistance to infection or development of a functional phenotype that is normally associated with allergy and asthma. Experimental evidence indicates that development can be hindered or delayed to such an extent that certain effector mechanisms either are absent or do not function properly for essentially the lifetime of the individual (see DES and TCDD examples, below). In humans, the clinical effects of immunotoxicant exposure during development may be expressed immediately or later in life, presenting as either increased infectious or neoplastic diseases, or increased incidences/severity of allergic or autoimmune disease. Although not extensively evaluated in humans, several studies in children have shown increased infectious disease following perinatal exposure to environmental agents (Luster et al., 2003). An important study in this area was conducted by Weisglas-Kuperus et al. (2000), who demonstrated that exposure to levels of halogenated aromatic hydrocarbons normally found in highly industrialized countries is associated with increases in childhood infections and lower vaccination responses. Likewise, Karmaus et al. (2001) found that children with elevated levels of the DDT metabolite DDE and polychlorinated biphenyls or DDE and hexachlorobenzene had more cases of inner ear infection than expected. Higher burdens of DDE alone increased the odds ratio for the development of asthma and elevated immunoglobulin (Ig) E levels. Limited human immune function data are available for offspring of women taking therapeutic doses of immunosuppressants during pregnancy. Although sample sizes in the few reported studies are small, significant effects on the developing immune system have been described. For example, bone-marrow function, thymus size, and immunoglobulin levels of infants born to females given azathioprine during gestation are reported to be suppressed for up to 1 yr of age (Price et al., 1976). Cyclosporin A use during pregnancy has been associated with delayed development or maturation of T and B cells, decreased expression of the major histocompatibility complex (MHC) antigens, and decreased immunoglobulin levels for up to 1 yr of age, which led to a recommendation that vaccination be delayed until infants are more than 1 yr old (Tendron et al., 2002), particularly if vaccines contain live virus (Schena et al., 2002). In contrast to the effects of environmental chemical exposure, immunosuppressive drug use was not reported to increase the incidence of infections in offspring, perhaps because the half-life of therapeutics is significantly shorter than that of the lipophilic xenobiotics associated with human developmental immunotoxicity or because of the relatively small sample size in the case studies.

From a risk assessment standpoint, the issue at hand is whether exposure to xenobiotics during development or maturation of the immune system produces more severe or persistent health effects than exposure to the same chemicals after immune system maturation. In 1993, a landmark report was prepared by the National Research Council (NRC), entitled “Pesticides in the Diets of Infants and Children” (NRC, 1993). Following publication of the report, federal regulatory agencies in the United States and abroad began expressing an interest in protecting children’s health from the effects of agents that may potentially damage the immune system. This report was followed in 1996 by the Food Quality Protection Act (FQPA), which required the U. S. Environmental Protection Agency (EPA) and other regulatory agencies that deal with pesticides to specifically consider children’s health risks. Amendments to the Safe Drinking Water Act (SWDA) in the same year (1996) also emphasized children’s health in setting health advisories for drinking water. To account for potential greater sensitivity in this group, the risk assessment process provides for an additional safety factor of up to 10× to be applied to avoid possible adverse health effects in the very young.

During the last 3–5 yr, experts have convened a number of workshops to present research on the role of environmental factors in childhood asthma as well as developmental immunotoxicity (immunosuppression). These conferences have been useful, as they have allowed identification of additional research needs and have been instrumental in spurring interest in the area. Regarding developmental immunotoxicity, a workshop was convened by ILSI/HESI in June 2001 that not only provided an opportunity for experts to present their findings but also allowed discussions to address specific questions that relate to developmental immunotoxicity tests and their interpretation for risk assessment (Sandler, 2002). This workshop was followed by a “consensus” workshop sponsored by

NIEHS/NIH and NIOSH/CDC on the most appropriate screening tests to evaluate developmental immunotoxicity (Luster et al., 2003). ILSI/HESI recently held a roundtable discussion on additional methodological details for conducting developmental immunotoxicology screening tests, including under what conditions such testing might be required (Holsapple, 2003). This information should be useful to investigators in the area of developmental immunotoxicology, to scientists involved in toxicity testing, and to risk assessors in interpreting experimental and epidemiological findings. To date, the integration of parameters that address the immune system in developmental toxicology studies has been quite minimal, as is best illustrated by the fact that immune organs are still not routinely included as potential target organs in most developmental toxicity protocols.

The purpose of this document is to compare the relative sensitivity of the developing and mature immune systems to immunotoxic xenobiotics, particularly, although not exclusively, when exposure produces suppression of the immune response. A brief overview of immune system maturation in human neonates and children is provided in the second section as an aid in understanding how functional immaturity alone predisposes the young to infection. In the third section, effects of five diverse compounds, which have undergone extensive adult and developmental rodent immunotoxicity testing, are reviewed. The chemicals were chosen based on the fact that each had been studied fairly extensively, resulting in a significant number of peer-reviewed publications. This review is not exhaustive; rather, representative data are presented that exemplify and contrast potential effects of the chemical on the developing and mature immune systems. Human data, when available, are included to provide a comparison of effects in rodent models, which are only intended to serve as surrogates of potential human effects. It should be noted that at birth, humans are more immunologically mature than are rodents (Holsapple, 2003). Nevertheless, the steps involved in human and rodent immune system maturation are remarkably similar, and there is no compelling evidence to suggest that effects observed in rodents are not representative of what might be expected to occur in humans. Thus, effects of rodent exposure shortly after birth are likely to reflect what may happen in humans exposed during late gestation, assuming that the xenobiotic crosses the placenta. This concept was reviewed in detail by Holladay and Smialowicz (2000) and by Holsapple (2003).

OVERVIEW OF HUMAN IMMUNE FUNCTION AND RESISTANCE TO INFECTION IN THE VERY YOUNG

Common infectious diseases occur often, and are usually more severe, in the very young. Age-related physical or physiological differences in tissues or organs may increase susceptibility to infection, although in most cases it is the relative immaturity of the immune system in the young that prevents the host from making an adequate response to microorganisms. Reduced ability to resist infection is of concern to immunotoxicologists because the combined lack of immune system maturity and chemical perturbation of function may act in an additive or synergistic manner, so that even minor chemically-induced suppression may have a greater effect in the young.

Antibody Production

Although antibody synthesis in neonates is roughly 30% that of adults, maternally derived IgG, which is actively and passively transported across the placenta, provides good protection against infection if the mother has high circulating levels of specific antibodies. However, this form of passive protection wanes as the maternal antibody is catabolized, and within 1 to 3 mo following birth, the neonate has only 30% of total adult IgG levels (Stiehm & Fudenberg, 1966). IgM and IgG levels present in 7 to 12-mo-old infants are approximately half that of healthy adults, but IgA does not reach the 50% level until 3–5 yr old (Stiehm & Fudenberg, 1966).

Cell-Mediated Immunity

Development and maturation of the T-cell response begin early in gestation. The thymus appears at about 6 wk, and lymphoid cells are detectable at about 8–9 wk of age. At 10 wk of gestation, T lymphocytes in the thymus respond to stimulation with nonspecific mitogens, and by 12–14 wk

of age T cells are able to respond to foreign antigens. Neonates have a higher percentage of total lymphocytes in the circulation than adults, although approximately 90% of circulating thymus-derived lymphocytes are naive (i.e., have not encountered antigen), compared to approximately 50% in adults (Ciccimarra, 1994). These cells are incapable of making certain cytokines that are necessary for mounting effective immune responses, and, most importantly, of generating a population of long-lived "memory cells." The balance of Th1 and Th2 cytokine production also differs between neonates and adults (Upham et al., 2002). The predominance of Th2 responses, even in children up to 12 yr of age, decreases the efficiency of many host-protective responses, particularly to intracellular bacteria.

Nonspecific Immunity

Natural killer (NK) cells are important in limiting the spread of certain types of tumors, particularly those of lymphoid origin, and also have a role in killing certain infectious agents. The percentage of NK cells in umbilical cord blood is significantly lower than in peripheral blood of adults. Furthermore, cord blood NK cells bind fewer tumor cells than those of adults and kill fewer tumor cells than adult NK cells (Baley & Schacter, 1985).

Polymorphonuclear leukocytes (PMNs) are the first cells to arrive at sites of infection or tissue damage and are central in resistance to certain types of bacteria (see later example). Bacteria that are engulfed by PMNs are killed by a variety of lytic enzymes contained in cytoplasmic granules. However, PMNs from newborns contain significantly lower quantities of several enzymes (Ambruso et al., 1984; Levy et al., 1999). In addition to functional deficits, there is a relatively low rate of PMN production by the neonatal bone marrow; thus, the supply of PMNs can be exhausted during infection (Wilson, 1986).

Complement is a collective term for a family of proteins that are critical to defense against certain bacteria. Complement components coat (opsonize) bacteria as part of the innate immune response and subsequently bind to receptors on the surface of PMNs, facilitating phagocytosis of the pathogen. Wolach et al. (1994) reported that preterm infants and newborns have only about 80% of adult serum levels of complement activity, and only 60% of C3, the main opsonizing complement component. Decreased killing of certain bacteria by newborns is attributable, at least in part, to decreased complement levels.

Resistance to Infection

Neonates are very susceptible to certain types of infections that require adultlike production of antibodies and other proteins (e.g., complement) that lead to engulfment and destruction of the bacteria. For example, poor antibody responses to antigens present on encapsulated bacterial (e.g., group B *Streptococcus* and *Haemophilus*), combined with decreased innate immune function, lead to inefficient bacterial killing and the subsequent development of sepsis. Bacteria that are commonly associated with neonatal sepsis are initially controlled by PMNs that home to the site of infection. Once on site, PMNs engulf bacteria that have complement and/or antibodies coating the surface; phagocytosed bacteria are then destroyed by internal enzymes and proteins. This initial innate response is critical to recovery because bacteria multiply so rapidly (some as quickly as once every 20 min) that failure to control the early phase of bacterial growth can result in overwhelming infection before the adaptive immune system can respond. As noted earlier, functional immaturity leads to poor accumulation of PMNs at the site of infection, decreased phagocytosis, and poor intracellular killing. These age-related defects are predisposing factors in repeated inner ear infections that commonly occur in young children: Faden (2001) noted that 5–10% of children experience 4 or more inner ear infections within the first year of life, particularly with *H. influenzae*. Antibody (IgG) responses to a conserved capsular protein do not increase rapidly after 2 yr of age in the infection-prone group, and T-cell responses to the same antigen are also reduced, suggesting that repeated infections may be caused by "subtle immunologic abnormality" (Faden, 2001). Weisglas-Kuperus et al. (1995, 2000) reported an increased incidence of inner ear infections of children exposed to elevated levels of organochlorines in breast milk; whether these clinical observations are related to immunosuppression has yet to be conclusively proven, but the increased rate of repeated infections suggests a link.

LABORATORY MODELS OF DEVELOPMENTAL IMMUNOTOXICITY

Diethylstilbestrol (DES)

Background

Between 5 and 10 million pregnant women were given diethylstilbestrol (DES), a potent synthetic nonsteroidal estrogen, between 1938 and 1971 to prevent premature delivery or pregnancy loss. Its use was terminated when a rare form of reproductive system cancer was found in female offspring of DES-exposed mothers. Male and female reproductive systems malformations have been reported in children of treated women, as has anecdotal evidence of immune system dysfunction. DES was also used to increase weight gain in livestock, although this use is no longer permitted in most countries.

Effects on the Immune System in Humans Both female and male children of DES-exposed mothers report a higher incidence of autoimmune diseases and asthma (Baird et al., 1996). In general, these diseases are considered to be the result of inappropriate immune system responses, or possible loss of homeostatic control, instead of immune system suppression.

Effects on the Immune System in Rodents

In utero exposure Luster et al. (1978b) reported that a single injection of 0.1 mg DES/kg body weight on gestational day (GD) 16 did not affect the antibody response to the T-cell-dependent antigen, sheep red blood cells (SRBC), when evaluated in 7-wk-old male and female offspring of Swiss-Webster mice. The T-independent IgM response of female offspring to bacterial lipopolysaccharide (LPS) was suppressed by DES, but was similar to control responses when females were immunized for a second time. In marked contrast, the male offspring response to LPS immunization was enhanced after both first and second immunizations, an effect attributed to the stimulating effect of estrogen on the antibody response to LPS. Delayed-type hypersensitivity responses (DTH) were suppressed in female, but not in male, offspring, even though thymus weights and T-cell responses to polyclonal stimulation were suppressed in both genders (Luster et al., 1979). Further studies suggest that DES targets early precursors of T lymphocytes in the fetal liver, accounting for thymic atrophy and suppression of DTH (Holladay et al., 1993), but not for defects in T-independent responses to LPS of female offspring.

Neonatal exposure Nonspecific T- and B-cell proliferation was reported to be suppressed in 6-wk-old female NMRI mice given 5 μg DES/d (roughly 2.2 mg DES/kg/d) over postnatal days (PND) 1–5 (Kalland et al., 1979); suppression was still evident at 17 mo of age (normal life span ~24 mo). It is noteworthy that neither estradiol nor corticosterone exposure over PND 1–5 produced long-term suppression, and that lymphocyte proliferation was comparable to control values at 6 wk of age in females exposed to DES over PND 6–10. Lower doses (approximately 4.4, 44, or 440 μg/kg/d) had no effect on proliferative response. The 5-μg DES/d exposure regimen also decreased NK cell activity in 6- to 8-wk-old female inbred C57Bl/6 (75%↓) and BALB/*c* (53%↓) mice and in outbred NMRI (28%↓) mice (Kalland, 1980a). NMRI or AKR/J female mice, exposed to 5 μg DES/d over PND 1–5, were also more likely to develop tumors after low dose injection of a known carcinogen (Kalland & Forsberg, 1981). A subsequent paper (Kalland, 1984) reported that, on a per cell basis, NK cells from DES mice were as active as cells from the control group, but that exposure reduced the number of NK cell precursors in the bone marrow. In other words, NK cells from experimental animals were as efficient as those from controls, but a deficiency in NK cell precursors produced functional suppression of NK activity at the whole animal level. The same postnatal exposure regimen (Kalland, 1980a) reduced the T-lymphocyte-dependent antibody response to SRBC by ~60%, and the T-independent response to bacterial LPS by ~40% when examined in 16- to 18-wk-old NMRI mice. Suppression of the T-dependent response was reportedly due to a defect in T-helper cells. DTH responses were likewise suppressed in 6- and 9-mo-old NMRI females exposed to approximately 2.2 mg/kg/d over PND 1–5 (Kalland & Forsberg, 1978). Kalland (1980b) also reported a persistent (at least 6.5 mo postpartum) decrease in the proportion of T cells in the spleens of DES-exposed mice.

Adult exposure Luster et al. (1980) reported suppression of the antibody response to SRBC or LPS, and the DTH to keyhole limpet hemocyanin (KLH), in adult female mice exposed to 2 or 8 mg

DES/kg/d × 5 d. The DTH was decreased in mice dosed with DES after, but not before, sensitization with KLH, suggesting that the suppressive effects of DES on DTH were not persistent. Using the same exposure regimen, resistance to bacterial or parasite infection was decreased and tumor incidence in animals challenged with tumor cells was increased at ≥2 mg DES/kg/d (Dean et al., 1980). T-cell-mediated resistance to a nematode infection was suppressed by 5 d of exposure to 0.2 mg DES/kg/d if exposure began on the day of infection; if exposure commenced 5 d before or 3 or 8 d after infection, decreased resistance was only observed at the highest dose (8 mg/kg/d) (Luebke et al., 1984).

Mode(s) of Action DES is a potent estrogen, and likely affects immune function via the estrogen receptor (ER). Evidence includes similar effects of known estrogens (17β-estradiol) on the immune system of adult and neonatal rodents, blockade of certain immunotoxic effects by pharmacologic antagonism of the ER (Luster et al., 1984), and antagonism of estrogen-mediated immune system effects in mice lacking ERα (Staples et al., 1999). DES appears to target precursor cells in the bone marrow (adults and neonates) and fetal liver (neonates), producing a long-lasting or perhaps permanent reduction in numbers of precursor cells. This defect explains a significant portion of long-lived immunosuppressive effects (e.g., Kalland's 1984 paper on suppressed NK activity), although the effects of adult exposure also includes damage to the thymic epithelium (Luster et al., 1984). The underlying mechanism of long-term suppression following exposure of the developing immune system to DES is not known, but the default assumption is that a critical cell population is lost to developmental exposure; either this purported population is refractory to estrogen-mediated ablation in adults or repair and recovery mechanisms are present in adults that are lacking in the developing immune system.

Data Gaps There has been no systematic evaluation of persistent DES-mediated immunosuppression in adult animals. Dose-response data are not available for many of the of the developmental exposure studies that revealed persistent effects.

Summary In utero exposure to 0.1 mg DES/kg during the last trimester of pregnancy suppressed T-cell- and B-cell-mediated responses only in female offspring. The gender dependence of effects was remarkable in that T-independent responses in male offspring were enhanced, yet suppressed in females. Exposure during gestation produced effects that persisted into the equivalent of young adulthood. In neonates there appears to be a critical developmental window during PND 1–5, during which exposure to DES produces persistent immune system defects that last well into adulthood or persist for most of the normal life span of the mouse. These effects are among the most persistent reported for any chemical. In adults, immunosuppression occurs at doses similar to those that produce immunotoxicity in developing animals. However, the immune system-related endpoints that have been evaluated over time in exposed adult animals (bone marrow cellularity, thymus weights) recover relatively quickly (Forsberg, 1984). In adults, recovery may occur so quickly that suppression of cell function or resistance to infection may require ongoing exposure to maintain suppression.

Conclusions Immunotoxicity has been reported at similar doses when exposure occurs during late gestation, early postpartum, or as adults. However, the distinguishing feature of developmental exposure to DES is the persistence of effects, some of which are still apparent in very old mice. In contrast, immune system-related endpoints that have been evaluated (bone marrow cellularity, thymus, weights) suggest that adults recover relatively quickly (Forsberg, 1984).

Diazepam (DZP)

Background

Diazepam (DZP, e.g., Valium) is a prescription drug in the benzodiazepine (BDZP) family. It binds with equal affinity to both central (i.e., within the CNS) and peripheral BDZP receptors. DZP freely crosses the placenta; studies in rats have shown that brain levels of DZP are essentially the same in the dam as in the offspring from birth until PND 10, following maternal exposure on GD 13–20 (Simmons et al., 1983). In humans, DZP is used for the short-term relief of anxiety symptoms and in the treatment of seizures and muscle spasms in certain neurological diseases. Typical human

adult doses are in the range of 0.1–0.3 mg/kg/d, depending on the intended therapeutic effect. DZP is absorbed rapidly and extensively and metabolized initially to *N*-desmethyldiazepam and temazepam. The mean elimination half-life is about 32 h. In the past, DZP was prescribed for use during late pregnancy to control signs of early uterine contractions and given to neonates prior to intubation as a tranquilizer and muscle relaxant. However, current warnings often advise against its use during pregnancy or lactation, and it is not recommended for use by children under 1 mo of age.

Effects on the Immune System in Humans No studies were found that describe the in vivo effects of DZP on human immune function, possibly because the drug is prescribed for patients with disorders that alone are known to alter immune function (Covelli et al., 1998). However, a study of patients admitted to an intensive care unit with tetanus found that the use of high doses of DZP was a major risk factor for developing pneumonia (Cavalcante et al., 2001).

Effects on the Immune System in Rodents

In utero exposure Schlumpf et al. (1989) reported that the T-lymphocyte proliferative response to foreign antigen was suppressed by at least 50% in both male and female offspring of Long-Evans rats given 1.25 mg DZP/kg/d from GD 14 to 20. This dose resulted in plasma levels that were comparable to human plasma levels following a single therapeutic dose. Suppression was evident for the first 2 mo of life, but returned to control levels by approximately 79 d of age. The authors determined that exposure during late gestation (GD 16–20) was critical to suppression of the response, as earlier exposure (d 12–16) was without effect. They postulated that effects were mediated by binding to the peripheral BDZP receptors, which cannot be detected until GD 16. Similar results were obtained when rats were given clonazepam, a BDZP with high affinity for central BDZP receptors, and Ro 5–4864, a selective peripheral BDZP receptor agonist (Schlumpf et al., 1990). Furthermore, production of tumor necrosis factor-alpha (TNF-α) in response to bacterial lipopolysaccharide (LPS) (Schreiber et al., 1993a) and interleukin (IL)-6 (Schreiber et al., 1993b) was suppressed in leukocytes of 2- or 8-wk-old offspring of dams given 1.25 mg DZP/kg/d from GD 14 to 20. Immunotoxic effects of prenatal DZP exposure are not limited to ex vivo cellular responses. Convincing evidence of significant immunosuppression was reported by Schlumpf et al. (1994) in adult offspring of dams given 1.25 mg DZP/kg/d from GD 14 to 20. Exposed offspring challenged with the nematode parasite *Trichinella spiralis* had approximately twice as many encysted parasite larvae (the pathological phase of infection) and decreased titers of IgG antibodies directed against the parasite. Resistance to the parasite is mediated by both cellular and humoral responses. In a separate study, Livezey et al. (1986) reported that 13 of 52 offspring of dams given 6 mg DZP/kg for the last 5 d of gestation, compared to none of the controls, developed tumors over 20 mo of observation, and that total IgG levels were only 45% that of controls in 6-mo-old exposed offspring. They also reported increased incidence and severity of spontaneous infections in exposed offspring, including bacterial infections of the uterus, kidney, skin, and salivary glands. In yet another study, offspring of golden hamsters treated with 1 or 1.5 mg DZP/kg between GD 9 and 15 were infected twice (on 75 and 107 d of age) with *Mycobacterium bovis* (Ugaz et al., 1999), an organism closely related to the bacterium that produces human tuberculosis. The adult offspring of dams given the higher dose of DZP experienced greater weight loss and mortality as well as increased size of granulomas and greater burdens of *M. bovis* in the liver, lung, and spleen than control and lower dose treatment groups. Resistance to this organism depends on intact macrophage and T-cell function, indicating that DZP exposure during gestation significantly suppressed one or both defense mechanisms in adult animals.

Neonatal exposure Dostal et al. (1995) exposed 7-d-old male and female Wistar rat pups to a single injection of 10 mg DZP/kg, or to 5 mg/kg/d on d 5, 6, and 7 after birth. Animals were sensitized to assess T-cell-mediated delayed hypersensitivity responses and immunized to evaluate antibody production at 6, 12, and 24 mo of age. A single exposure to 10 mg/kg/d decreased the DTH in 6-mo-old animals and suppressed both IgM and IgG antibody responses, essentially for the lifetime (24 mo) of the animals. Pups given 3 doses of DZP were only evaluated at 7 mo of age; both IgM and IgG responses were also suppressed in these animals.

Adult exposure Descotes et al. (1982) reported that the primary antibody and delayed-type hypersensitivity (DTH) responses to SRBC were suppressed in outbred adult Swiss mice given

8 mg DZP/kg/d ip when exposure spanned the 3 d before or after sensitization. A subsequent study (Descotes et al., 1982–1983) established that the DTH response was suppressed by a single dose of 4 or 8 mg DZP/kg on or 1 d after sensitization. Lower doses (0.5, 1, or 2 mg/kg/d) were without effect. To determine whether resistance to infection was compromised in exposed mice given 1, 2, 4, or 8 mg DZP/kg/d for 3 d, animals were challenged with bacteria (*Klebsiella pneumoniae*). The number of bacteria required to produce an LD_{50} was reduced 40% by 1-, 2-, or 4-mg DZP/kg/d doses and by 72% at the highest DZP dose (Laschi et al., 1983).

Mode(s) of Action DZP binds to both central and peripheral BDZP receptors, and while it is likely that the immunotoxic effects of prenatal DZP administration depends on binding to a receptor, it has not been absolutely established whether effects are mediated by interaction with the peripheral or central receptors, as developmental exposure to clonazepam, a BDZP with high affinity for central receptors, also resulted in immunosuppression (Schlumpf et al., 1989). Schlumpf et al. (1989) reported that DZP exposure from GD 16 to GD 20, but not from GD 12 to GD 16, decreased the T-cell response to foreign antigens, and suggested that developmental immunotoxicity may depend on expression of peripheral type BDZP receptors, which are present at GD 16 in both thymus and spleen, before central receptors are present. Later studies by this group (Schlumpf et al., 1992, 1993) specifically identified a persistent (2-mo) decreased binding capacity of peripheral BDZP receptors on lymphocytes and macrophages following late-gestation exposure, and suggested a relationship between ligation of BDZP receptors and decreased production of macrophage-derived IL-1, and T-lymphocyte-derived IL-2. On the other hand, central BDZP receptors bind to a site on type A gamma-aminobutyric acid ($GABA_A$) receptors, and it has been hypothesized that DZP binding during CNS development may alter the maturation or function of $GABA_A$ receptors, which may explain the long-lasting effects on behavior, the endocrine system, and immune function (Kellogg, 1999).

Data Gaps Studies that evaluated immune function following developmental DZP exposure were conducted in rats or hamsters, whereas adult DZP immunotoxicology studies were conducted in mice. A lack of dose-response data in neonatal rats precludes drawing conclusions on the relative sensitivity of the two age groups. Adult exposure studies suggest that induction of immunotoxicity requires higher DZP doses following immune system maturation and that effects persist for only a short time, when compared to the effects of late-gestation exposure in rats. However, it is not known whether the apparent differences are dependent on the species tested or the age at exposure. To clarify the relative sensitivity of the immature and mature immune system to DZP exposure, adult studies, including establishment of no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) doses should be conducted in rats.

Summary Maternal exposure of rats to 1.25 mg DZP/kg during the last trimester of pregnancy decreased production of critical lymphocyte and monocyte cytokines by offspring. These effects persisted until young adulthood (8 wk of age), and decreased resistance of young adult offspring to a parasite infection. Spontaneous tumor development occurred in offspring of exposed, but not control dams, as did increased rate and severity of infection. Reduced resistance to infection was also observed in offspring of exposed hamsters. Exposure of rat pups to 10 mg DZP/kg within 1 wk of birth suppressed cellular and humoral immune responses for 6 or 24 mo, respectively. Humoral and cellular immune responses were also suppressed in adult mice given 3 injections of 8 mg DZP/kg, as was resistance to bacterial infection.

Conclusions Maternal exposure to DZP in the range of 1 mg DZP/kg during the third trimester suppressed both functional immune system endpoints and resistance to infection that persisted until early adulthood in rats. Direct dosing of neonates also suppressed functional and host resistance endpoints, but at higher doses (5 or 10 mg/kg as 3 or 1 injection, respectively). However, suppression persisted for at least 6 mo and in some cases for 24 mo, the average lifetime of mice. Adult mice exposure to ≥4 mg DZP/kg for 3 consecutive days also suppressed cellular and humoral immunity, but suppression of functional endpoints only occurred when exposure and challenge occurred over a short span of time. These results suggest that the immature immune system is more sensitive to DZP exposure, and that adverse effects persist over a longer period of time than effects following adult exposure to DZP, although it has yet to be determined whether species, rather than increased developmental sensitivity, is responsible for the apparent differences.

Lead (Pb)

Background

The heavy metal lead (Pb) is a naturally occurring element that has been utilized for numerous industrial applications particularly during the 20th century and continues to be used extensively in certain developing countries. Lead was used as a major "anti-knock" additive of gasoline, is present in certain types of batteries, was added to many paint products, is found in certain tableware (crystal and pottery), and is still used in some ammunition and hair dye products. As a result of heavy past use, environmental deposition of lead is extensive despite efforts to restrict use in the United States. Safety limits have been readjusted several times between the 1970s and the present based on concern over the capacity of low-level lead exposure to alter neurobehavioral capabilities (e.g., IQ) in children. Such effects in early childhood would require embryonic or neonatal exposure. Other physiological systems readily affected by lead include the immune, renal, hepatic, and respiratory systems. It should be noted that based upon experimental animal studies, pulsed exposure to lead (i.e., spanning different portions of embryonic development) can produce different immune outcomes in the juvenile and adult offspring.

Effects on the Immune System in Humans

Neonatal/juvenile exposure Lutz et al. (1999) examined an urban population of 279 children, ranging from 9 mo to 6 yr of age, with blood lead levels between 1 and 45 μg/dl. A positive correlation was found between blood lead levels (BLL) and serum IgE levels, suggesting an immune system dysfunction consistent with an increased risk of allergy.

Adult exposure A large number of immunotoxicology studies have been conducted in humans using occupationally exposed male workers. The predominant immunological findings included moderately reduced immunoglobulin levels, B- and T-lymphocyte numbers, and/or T-cell mitogenic responses (Ewers et al., 1982; Coscia et al., 1987; Fischbein et al., 1993; Undeger et al., 1996; Anetor & Adeniyi, 1998; Sata et al., 1998; Misra et al., 2003), although in two studies no differences in immune responses were found (Kimber et al., 1986; Queiroz et al., 1994). Valentino et al. (1991) reported reduced chemotactic activity for PMNs in lead exposed male workers. Pinkerton et al. (1998) found only subtle immune effects among 145 moderately exposed workers, including elevated numbers of B cells, elevated numbers of CD4+/CD45RA+(naive) T cells, and reduced serum IgG concentrations.

Effects on the Immune System in Rodents

In utero exposure Bunn et al. (2001a) and Miller et al. (1998) administered lead in the drinking water at concentrations of 50, 100, 250, or 500 ppm to female F344 rats from either d 2 to 21 of gestation or beginning 2 wk preceding mating throughout pregnancy. Pups were assessed at 5 and/or 13 wk after the birth. Numerous immune alterations were observed, particularly in the female pups, including a pronounced reduction in the DTH response and IFN-γ production. In contrast, production of IL-4 and total serum IgE were elevated. Changes in the number of circulating leukocytes as well as relative lymphoid organ weights were noted. The LOAEL was 100 ppm, which included changes in spleen cellularity and weight as well as IgE concentrations, while the NOAEL was 50 ppm. DTH responses were reduced in female offspring of dams exposed to 250 ppm. In the Bunn et al. (2001a) study, BLL for the 100 ppm dose in females at birth was 7.6 μg/dl. At this same dose, Miller et al. (1998) reported an elevation in serum IgE concentrations in offspring when assessed as adults. Suppressed DTH response was associated with a BLL (immediately post exposure) of 38 μg/dl. Bunn et al. (2001b) found a similar effect in an acute dose study. In both of these studies, BLLs at the time of immune assessment were at background levels. Bunn et al. (2001c) also used a pulsed exposure to lead acetate in the drinking water of CD rats between d 3 and 9 of gestation. The BLL at birth in male offspring was 5.3 μg/dl, and macrophage nitric oxide production capacity in these animals was reduced when tested as adults. Snyder et al. (2000) administered lead acetate in water (0.1 m*M*, 40 ppm) to BALB/*c* mice from GD 15 to 20. They reported effects similar to those observed in rats, with significant increases in serum IgE levels, and a reduction in splenic T-cell and NK-cell numbers at 2 wk postpartum. BLLs, both at 1 wk of age

and at the time of assessment, were similar to controls (approximately 5 μg/dl). Results of this study suggested that very low BLLs at birth may be associated with immunotoxicity, and elevated levels of Pb do not need to be maintained postnatally to produce adverse effects. Diet can have an impact on the risk of lead exposure in early life. Maternal and/or early postnatal diet appears to influence blood lead levels and potential immunotoxic outcomes. Chen et al. (2004) reported that in the rat, reduced protein intake among lead-exposed dams lowered their blood lead levels during pregnancy and lactation and also altered the spectrum of immune alterations in the offspring. In a study of Albany County, NY, mother–infant pairs, Schell et al. (2004) reported that increased intake of iron was associated with reduced blood lead levels in the neonates. However, the impact of dietary protein intake on blood lead levels depended specifically on the window of early postnatal development. These findings suggest the impact of some dietary factors on blood lead levels and immune outcomes may vary with age.

Combined pre- and postnatal exposure Luster et al. (1978a) and Faith et al. (1979) examined the effects of lead acetate on female CD rats following exposure from preconception through lactation, with the offspring also receiving lead until 40–50 d of age at doses of 0, 25, or 50 ppm administered in the drinking water. They reported decreases in thymus weights, antibody responses, DTH responses, and serum total IgG levels. The LOAEL was estimated to be 25 ppm, corresponding to a BLL at the time of sacrifice of 29 μg/dl. In another study, BALB/*c* mice were exposed to 40 ppm (0.1 m*M*) lead acetate from d 15 of gestation through PND 14 (Snyder et al., 2000). BLLs in the offspring were approximately 15 and 18 μg/dl, measured at 1 and 2 wk after birth, respectively. Pre-/postnatally treated offspring had significantly elevated serum IgE levels compared with controls when measured at 2 wk of age.

Neonatal exposure Villagra et al. (1997) exposed Sprague-Dawley (SD) rats to 172 mg/kg body weight lead acetate by subcutaneous injections on postnatal d 14, 16, 18, and 20. They reported altered blood neutrophil and eosinophil levels as well as eosinophil degranulation associated with a BLL of 47 μg/dl. Snyder et al. (2000) exposed BALB/*c* mice to approximately 40 ppm lead acetate in drinking water from birth through PND14 (lactational exposure only). For this single dosage (producing a BLL of approximately 14 μg/dl at 2 wk) they reported a significant increase in serum IgE. Dyatlov and Lawrence (2002) reported Pb-induced alterations in IL-1 and IL-6 cytokine levels and among thymocyte populations in Pb-exposed offspring challenged with a bacterial infection immediately after weaning. In these studies, female BALB/*c* mice were given drinking water that contained 0.5 m*M* lead acetate from PND 0 to PND 21 (weaning); offspring were then directly exposed to the same concentration. BLLs at the time of sacrifice were 17.4 μg/dl.

Adult exposure Several studies in adult rodents measured Pb-induced changes in lymphoid populations, cytokine production, IgE levels, and the DTH reaction. Following a 10-wk exposure of CBA mice to 0, 13, 130, or 1300 ppm lead in the drinking water, Koller and Brauner (1977) reported a LOAEL of 130 ppm and a NOAEL of 13 ppm, based on decreased splenic B-cell numbers. Neilan et al. (1980) reported altered mitogen responses in adult C57Bl/6 male mice associated with a BLL of 70 μg/dl. Blakley and Archer (1981) reported lead-induced reduction in some T-cell parameters among BD F1 female mice given lead acetate for 3 wk at dose levels as low as 50 ppm. Koller et al. (1983) noted decreased antibody responses in male SD rats after 6 wk of exposure to 10 ppm lead acetate and a NOAEL at 1 ppm. Heo et al. (1996) administered 6 injections (sc) of lead chloride for 2 wk to adult female and male BALB/*c* mice, producing a BLL of 38 μg/dl. While plasma IL-4 levels were elevated, there was no significant change in IgE following lead exposure. This contrasts with the in utero exposure results from the same research group, using the same strain of mice, where a BLL of approximately 5 μg/dl (not significantly different from the background/control group BLL) was associated with a significant IgE increase as well as cellular changes. Muller et al. (1977), after administering lead acetate ip daily to BALB/*c* mice for 30 d, reported a LOAEL of 0.025 mg lead acetate (~0.83 mg/kg/d) using the DTH to SRBCs. BLLs were not reported. In a study using BALB/c mice exposed to lead acetate in drinking water for 3 wk, McCabe et al. (1999) reported that the DTH response was decreased, with a NOAEL of 128 ppm and a LOAEL of 512 ppm. These exposures corresponded to BLLs of 49 μg/dl and 87 μg/dl, respectively.

Mode(s) of Action Lead is known to displace certain ions (namely, calcium and zinc) that play critical roles as both enzyme cofactors and regulatory components in signal transduction and gene regulation. Given this general capacity, it is not surprising that lead can modulate numerous physiological systems to varying extents depending on the concentration and timing of exposure. For the immune system, lead targets macrophages and T lymphocytes, preferentially but not exclusively. Vulnerability of these cells differs with age of exposure. Macrophages are more vulnerable early in development, when Pb exposure effects may include altered metabolism (e.g., nitric oxide production). T cells are more vulnerable after thymus development, when thymocyte maturation occurs. The major effect on T cells may be an increased skewing of Th2 cells at the expense of Th1 cells. Interferon-gamma production is depressed (producing a decreased DTH response), while IL-4 and IgE production are increased in parallel with changes in T-helper cell type.

Data Gaps While there is a wealth of lead exposure data in the literature, much of it is not appropriate for age-related comparisons. For example, host resistance studies have largely been confined to exposures involving adult rodents. Age-related dose sensitivity comparisons are available for DTH, IgE, lymphoid organ, macrophage, and cytokine measurements following in utero versus adult exposure. However, studies conducted to date have not provided a direct dose sensitivity comparison of immunotoxicity following prenatal and pre–postnatal exposure, and studies that addressed different developmental stages have used only single-dose exposures.

Summary Elevated BLLs (up to 45 μg/dl) in urban children were found to correlate with elevated IgE levels. Adult human exposure to lead produces subtle immune alterations, generally characterized by altered T cell and T-cell-dependent responses. Additionally, some inflammatory cell changes have been noted. Most studies demonstrated BLLs between 50 and 80 μg/dl in the affected populations. As was observed in children with elevated BLL, in utero exposure of rodents to Pb increased serum IgE levels (Lutz et al., 1999). Changes in T-cell responses (DTH) and altered production of cytokines controlling antibody synthesis suggest that lead exposure may persistently skew the response toward "allergic" (Th2) responses. Single-digit BLLs at or near birth were associated with juvenile and/adult immunotoxicity in both the rat and the mouse. Few pre–postnatal exposure studies have been performed, but the data suggest that lead exposure resulting in neonatal blood lead concentrations below 20 μg/dl can produce immunotoxicity and appear to result from late gestational exposure to lead. While these studies do not enable determination of a NOAEL for this combined exposure window, the results suggest that the perinatal immune system is very sensitive to lead-induced alteration. Lead exposure of neonatal rodents producing BLLs in the 15–20 μg/dl range increased IgE levels and altered production of inflammatory cytokines. Studies in adult rodents indicate lead exposure may alter macrophage and T-cell function, antibody responses, cytokine profiles, T-cell subpopulations, and disease resistance to tumors, viruses, and bacteria. The NOAEL in adult rodents occurs at a BLL of 49 μg/dl and LOAEL of 87 μg/dl. Incorporating a 13-wk recovery period before immune assessment, Miller et al. (1998), found no change in DTH, IgE levels, or macrophage parameters in adult female rats that had experienced a BLL of 112 μg/dl prior to mating and during gestation. Taken together, these results suggest that adult rodents are unlikely to exhibit significant changes in DTH or IgE levels when BLLs are below 40–50 μg/dl. The adult BLLs associated with immunotoxicity would appear to be similar among rodents and humans.

Conclusions Adult animal exposure studies suggest that immune effects can be noted at BLLs similar to those that have been detected in certain workers (i.e., >50 μg/dl range), with some reports indicating alterations at lower levels (38 μg/dl), depending on the endpoint examined (e.g. cytokines) and the species examined. Rodents exposed during gestation to quantities of lead producing BLLs less than 10 μg/dl exhibited juvenile and/or adult immune alterations, although lead-immunosuppressed animals had control blood lead levels at the time of immune assessment. This would appear to place the developing immune system on par with the developing neurological system for lead sensitivity. The results from neonatal exposures also suggest a greater sensitivity to lead-induced immunotoxicity than occurs in adults. BLLs in the range of 10–20 μg/dl following pre–postnatal Pb exposure are associated with immunotoxicity compared to BLLs of 40–50 μg/dl, or even greater, required to produce immune changes in adults. Additionally, while exposure of the

developing immune system to lead seems to produce persistent alterations, the duration of changes following adult exposure is not well established. Finally, results obtained using pulsed exposure to lead during narrow windows of embryonic development (Bunn et al., 2001c) suggest that the spectrum of persistent immunotoxic changes (e.g., macrophage function vs. T-helper balance) can be qualitatively different. This appears to be associated with the status of the developing immune system at the time of exposure.

2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD)

Background

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the prototype of a class of halogenated aromatic hydrocarbons that are formed during combustion processes such as waste incineration, forest fires, and backyard trash burning. Historically, significant quantities were also produced during the manufacture of certain herbicides (e.g., Agent Orange) and bleaching of paper. These unwanted by-products have been found in coffee filters, milk cartons, and diapers. TCDD is extremely toxic to some animal species and has been demonstrated to produce tumors, and to produce developmental, reproductive, endocrinologic, and immunologic toxicities (Birnbaum & Tuomisto, 2000; Dragan & Schrenk, 2000). Immunotoxicity is one of the more sensitive manifestations of TCDD toxicity in rodents and is characterized by suppressed humoral and cell-mediated immunity and increased susceptibility to infections (Holsapple et al., 1991). Well over 150 manuscripts describing the effects of TCDD on the immune system have been published. The following summarizes the most appropriate studies that allow for comparison of age-related sensitivity to TCDD immunotoxicity.

Effects on the Immune System in Humans A limited number of human studies examined cohorts exposed to TCDD either occupationally or as a result of residing in a TCDD-contaminated area. The most informative studies evaluated individuals exposed to TCDD following an explosion at an herbicide factory in Seveso, Italy. An epidemiological study of 44 children (3–7 yr of age, 20 of whom had chloracne), conducted within 2 yr of the accident, showed no abnormalities in concentrations of plasma immunoglobulin, levels of circulating complement, or lymphoproliferative responses (Mocarelli et al., 1986; Pocchiari et al., 1979). Another study conducted 6 yr after the explosion, with different cohorts of TCDD-exposed children, found a significant increase in complement levels, which correlated with the incidence of chloracne, as well as increased numbers of peripheral blood lymphocytes and increased lymphoproliferative responses (Tognoni & Bonaccorsi, 1982). Twenty years after the Seveso accident, Baccarelli et al. (2002) measured plasma immunoglobulin and complement levels in a random sample of the population in the most highly exposed zone (TCDD plasma levels as high as 89.9 parts per trillion [ppt]) and in the surrounding area (TCDD plasma levels as low as 1.2 ppt). They concluded that there was a strong inverse relationship between plasma IgG and increasing TCDD levels. The association remained significant in multiple regression analysis after adjusting for age, gender, smoking, and consumption of domestic livestock and poultry and persisted after excluding subjects with inflammatory diseases and those currently using antibiotics or nonsteroidal anti-inflammatory drugs. However, the plasma levels of TCDD did not exhibit a consistent correlation with IgM, IgA, or complement component levels. A review of the literature by these authors, summarizing all studies published from 1966 through 2001 on human subjects exposed to TCDD, showed that the evidence for effects of TCDD on humoral immunity is sparse, although variability in methods and failure to control for confounding factors were cited as possible explanations for a failure to detect consistent changes in immune endpoints. The clinical significance of decreased IgG levels is unknown; however, the authors speculated that the finding may reflect a broad alteration of the immune system that could be revealed with more sensitive markers. Geyer et al. (1990) studied the correlation between the acute toxicity of TCDD and total body fat content in mammals and concluded that there was a significant inverse relationship between total body fat and the acute toxicity of TCDD, because increased total body fat represents an enhanced capacity to remove TCDD from the systemic circulation, thus decreasing the acute toxicity of TCDD. The authors speculated that newborns with 13.6% body fat would be 10

times more sensitive than adults with 21% body fat, and the human fetus with only 6.7% body fat would be even more sensitive to acute toxicity of TCDD (Geyer et al., 2002).

Rodent studies suggest that TCDD may be a human developmental immunotoxicant (discussed later). Human data to support this assumption are few, perhaps because evaluation of immune function in populations exposed exclusively or primarily to dioxins is rare. However, if exposure to dioxin-like PCB congeners, expressed as toxic equivalents of TCDD (i.e., a decimal equivalent of 2,3,7,8-TCDD potency, with TCDD = 1), is considered, several studies have found altered immune function and increased susceptibility to infection in breastfed offspring of exposed mothers. For example, in Dutch preschool children, PCB levels in mothers' breast milk were associated with increased recurrent otitis media and other symptoms of respiratory infection (Weisglass-Kuperus et al., 2000). Likewise, offspring of mothers in China and Japan that had been exposed to rice oil contaminated with dioxin-like compounds had lower levels of serum IgA and IgM and higher frequencies of respiratory infections and otitis media compared to matched, unexposed controls (Lu & Wu, 1985; Nakanishi et al., 1985).

Effects on the Immune System in Rodents

Pre-/postnatal exposure In an in utero study (Holladay et al., 1991), C57Bl/6N mice were dosed via gavage with 0, 1.5, or 3 μg TCDD/kg/d over GD 6–14; pups were then cross-fostered to unexposed lactating mice. Significant thymic atrophy was reported on GD18 and PND 6 in offspring of dams in the highest dose group. There was a significant decrease in the percentage of immature $CD4^+CD8^+$ thymocytes and significant increases in $CD4^-CD8^-$ and $CD4^-CD8^+$ thymocytes in the 3-μg/kg/d group. However, the thymic effects were no longer apparent by PND 14, indicating that the depression of thymic cellularity was reversible after TCDD exposure was terminated after birth. Proliferative responses to mitogens and antibody responses were similar in control and treatment groups at 7–8 wk of age. However, the cytotoxic T lymphocyte (CTL) response was significantly suppressed compared to controls at 8 wk of age. In a follow-up study, Blaylock et al. (1992) found that TCDD inhibited thymocyte maturation at a very early stage, and may be responsible for defects in the CTL response. Gehrs et al. (1997a) reported similar phenotypic changes in the offspring of F344 rats given a single dose of TCDD (0, 1, or 3 μg/kg) on GD 14. Fetuses from the 3-μg/kg group exhibited thymic atrophy on GD 19. The percentage of immature $CD3^-CD4^+CD8^+$ thymocytes was decreased and the percentage of $CD3^-CD4^-CD8^+$ cells was increased in these fetuses. It was suggested that TCDD mediates its effects by blocking maturation from $CD3^-CD4^-CD8^+$ to $CD3^-CD4^+CD8^+$ or by selectively eliminating $CD3^-CD4^+CD8^+$ cells. No effect was seen in the 1-μg TCDD/kg group. On GD 22/PND1, thymic atrophy was no longer present in the 3-μg/kg group. The apparently short-lived effects on thymocyte phenotypic markers notwithstanding, exposure during the perinatal period has been shown to induce severe suppression of cell-mediated immunity in rats and mice. A dose of 5 μg/kg in mice and rats on various days of gestation and/or lactation suppresses a variety of cell-mediated immune parameters including DTH (Vos & Moore, 1974; Faith & Moore, 1977), skin graft rejection, and graft versus host reactivity (Vos & Moore, 1974). The effects were more severe and persistent when TCDD was administered during the pre- (GD 18) and postnatal period (PND 0, 7, and 14) than if administered solely in postnatal life (i.e., PND 0, 7, and 14) (Faith & Moore, 1977). Host resistance to the bacterium *Listeria monocytogenes* and to PYB6 tumor cells (Luster et al., 1980) and DTH response to bovine serum albumin were also suppressed (Gehrs et al., 1997b). Suppression of the DTH response persisted through late adulthood in the offspring of rat dams receiving TCDD on GD 14 (Gehrs & Smialowicz, 1999). Suppression occurred at lower maternal doses in males (maternal dose 0.1 μg/kg) than in females (maternal dose 0.3-μg/kg). Phenotypic analysis in subsets of thymocytes and lymph node cells did not show clear differences between control and TCDD exposed offspring.

Postnatal exposure Female mice (C57BL/6NCji) were provided with drinking water, beginning on the day they delivered litters, containing 1.8 or 18 ng TCDD/L. Offspring were weaned and infected with *Listeria monocytogenes* on PND 21 (i.e., before reaching immunological maturity). The higher concentration of TCDD increased the number of organisms present in the spleens of male and female offspring on the day of peak organism numbers (Sugita-Konishi et al., 2003).

Adult exposure The antibody response is the immune function endpoint most sensitive to TCDD exposure in adult mice: Smialowicz et al. (1994) reported an ED50 (the dose that suppressed antibody production by 50%) for a single-dose exposure of 0.7 μg TCDD/kg. In contrast, TCDD failed to suppress, and in fact enhanced, the antibody response to SRBC in rats at doses as high as 30 μg TCDD/kg. The authors suggested that species differences in the effect of TCDD on the antibody response to SRBC were unrelated to hepatic CYP1A1 or CYP1A2 induction but related to differences in the effect of TCDD exposure on populations of $CD4^{-}CD8^{+}$ splenocytes in mice and rats.

Mode(s) of Action The majority of experimental animal data suggests that the toxicity of TCDD is dependent on the activation of the aryl hydrocarbon receptor (AhR) (review by Kerkvliet, 2002). However, it is possible that the immunotoxicity of TCDD might be induced indirectly through nonlymphoid tissues, apart from its direct transcriptional activation of drug metabolizing genes. The author also proposed a new paradigm for the mechanism of immunotoxic action of TCDD, moving from one focused on the suppression of immune functions to one focused on the inappropriate activation of cells, leading to anergy or death, and the consequent premature termination of the immune response.

Data Gaps Although long-lived immunosuppression has been documented following developmental TCDD exposure in laboratory rodents, the persistence of immune system effects following adult exposure has not been systematically evaluated.

Summary Human exposure to relatively low levels of dioxin or dioxin-like molecules during immune system maturation is correlated with decreased immunocompetence and increased susceptibility to infections. In rodent studies, cellular maturation, particularly of T cells, appears to be especially sensitive to developmental TCDD exposure, and exposure during both gestation and lactation produces the greatest immunotoxicity. While phenotypic evidence for altered maturation may be short-lived in mice, functional suppression persists into young adulthood and may last essentially for a lifetime. As adults, immune function is quite sensitive to TCDD exposure in mice, whereas rats appear to lose their sensitivity when fully mature.

Conclusions Exposure to TCDD during immune system maturation is associated with maturational defects in T cells and cell-mediated immunity while exposure in adults tends to be directed against humoral immunity. Although evaluation of rodent T-cell subsets over time has shown that phenotypic changes are not particularly persistent, it is evident that suppressed T-cell function persists at least until young adulthood in mice and essentially for a lifetime in rats. Male rat offspring may be more sensitive than females to TCDD-mediated suppression of T cell activity (DTH), in that the maternal LOAEL for suppressed DTH response was reported to be 0.1 μg TCDD/kg, versus 0.3 μg TCDD/kg for females. The rat data are particularly noteworthy in that adult exposure enhances, rather than suppresses immune function, whereas doses 100 times lower produced sustained suppression of T-cell function in offspring when exposure occurs during the last trimester.

Tributyltin (TBT)

Background

Tributyltin compounds, including bis(tri-*n*-butyltin) oxide (TBTO) and tributyltin chloride (TBTC), are widely used as industrial and agricultural biocides. This includes their use as a preservative in wood, plastic, paper, textiles, leather, and glass, as a disinfectant, and as antifouling agents in paint. TBTs contaminate human food, including shellfish, dairy products, and water, mainly through contributions from industrial effluents. TBTs are highly toxic to marine species and have endocrine disruptive effects in some marine organisms, which develop ambiguous genitalia when grown in contaminated waters. Trialkyltins are also toxic to mammalian species, producing liver and cutaneous toxicity as well as immunotoxicity, with the latter first noted by its propensity to induce thymic atrophy, specifically depletion of cortical thymocytes, almost 30 yr ago (Ishaaya et al., 1976). Since then, numerous immunotoxicity studies have been conducted. The following summarizes the most appropriate studies that allow for comparing age-related sensitivity to TBT immunotoxicity.

Effects on the Immune System in Humans No reports were found on the assessment of immune function in humans exposed to TBT. However, exposure to relatively low (nanomolar) concentrations of TBT in vitro has been shown to rapidly (1 h) and markedly (80–90%) suppress the cytotoxic activity of human NK cells (Whalen et al., 1999, 2002). Significant suppression was detected at concentrations approaching those found in the blood of some heavily exposed humans.

Effects on the Immune System in Rodents

Pre- and postnatal exposure Smialowicz et al. (1989) exposed preweanling rats to TBTO by oral gavage to 2.5, 5, or 10 mg/kg per dose 3 times/week for 10 doses, which allowed direct comparison to the adult studies employing intermittent exposure (see Adult Exposure subsection, next). At 3 wk of age, thymic involution and suppressed lymphoproliferative responses were observed at 5 mg/kg. The mixed lymphocyte response (MLR) and NK cell activity were suppressed in the 10 mg/kg treatment group. Only lymphoproliferative responses were evaluated at various ages after weaning; the response to both T- and B-cell mitogens was suppressed at 10 but not at 13 wk of age. SD rats were exposed to tributyltin chloride (TBTC) from GD 8 through lactation (PND 21) through maternal exposure (gavage) to daily doses of 0.025, 0.25, or 2.5 mg/kg body weight (Tryphonas et al., 2004). On PND 21, the rats were weaned and groups exposed directly by gavage at the same doses until the age of 30, 60, or 90 d. Note that these doses were similar to doses of TBTO used by Vos et al. (1990) and that, once ingested, TBTO is immediately converted to TBTC in the acid environment of the stomach. A broad spectrum of immune tests was conducted and multiple effects were seen at the high dose (2.5 mg/kg) as well as significant dose-response trends (either increased or decreased), including IgM concentrations, NK cell numbers, percentages of $CD8^+$ (T suppressor/cytotoxic) cells, $CD5^+$ (all T) cells, $CD4^+8^+$ double-positive (immature) cells, delayed hypersensitivity responses, NK cell activity, and thymus cortical atrophy. Statistically significant effects detected in the low and/or medium dose groups included elevated serum IgM concentrations (0.025 and 0.25 treatment groups) in female rats treated up to 60 d of age and decreased IgA levels (0.25 dose). Clearance of bacteria (*Listeria monocytogenes*) was decreased 2 or 3 d after a challenge infection in offspring exposed to 0.25 or 2.5 mg TBTO/kg until they were 60 or 90 d old, respectively. In males exposed for 90 d, serum levels of IgA were also decreased and IgM levels were increased, as was NK cell activity and delayed hypersensitivity responses. Thymic atrophy was observed at the highest doses at all ages. It should be noted that the experimental design employed by Tryphonas et al. (2004) (i.e., continued exposure beginning in utero and continuing until immune system evaluation) does not allow conclusions to be drawn on the effect of TBT exposure exclusively during immune system development, except for the noted increase in NK cell numbers observed at 30 d of age.

Adult exposure Studies have been conducted in adult Wistar rats fed concentrations of 0, 0.5, 5, or 50 ppm TBTO in the diet for up to 18 mo (Vos et al., 1990). At these dietary concentrations, estimated daily doses were 0.025, 0.25, or 2.5 mg/kg body weight/d. An interim examination at 4.5 mo determined that several immune parameters were affected, including thymus weight reduction at 2.5 mg/kg and resistance to *Trichinella spiralis* infection at both the 0.25- and 2.5-mg/kg dose levels. The authors estimated a NOAEL of 0.025 mg/kg body weight. Although NK cell activity was reduced at all dose levels after 16 mo of exposure, these responses were not consistent. Van Loveren et al. (1998) modeled these data using dose-response curves based on numbers of muscle larvae after infection with *T. spiralis* and estimated that a TBTO dose of 0.0195 mg/kg body weight/d would increase the larvae burden by 10%, which in their view represents a clinically significant change. An oral reference dose (RfD) for TBTO was established by the U.S. EPA in September 1997 (TBTO, IRIS; CASRN 56–35–9; BMD10 = 0.03mg/kg-d [0.68 ppm diet]; UF 11; MF 1; RfD 3E-4 mg/kg-d) that was based on immunotoxicology studies in adult rats exposed to TBTO for up to 18 mo (Vos et al., 1990). Smialowicz et al. (1989) exposed adult rats orally to TBTO by gavage using two different dosing regimens. In the first design, rats were administered 1.25, 2.5, 5, or 10 mg/kg body weight daily for 10 consecutive days. The most sensitive parameters affected were thymus atrophy and enhanced antibody responses, which were observed at the 2.5-mg/kg dose and above. In the second experimental design rats were administered 5, 10, or 20 mg/kg body weight TBTO

intermittently, 3 times per week for a total of 10 doses. Again, thymus atrophy was the most sensitive endpoint, being observed at the 5-mg/kg treatment group. Since this was the lowest dose tested for the intermittent exposure, a no-effect level was not ascertained. Lymphocyte proliferation was suppressed in the 10-mg/kg treatment group. The MLR was suppressed in the 20-mg/kg treatment group, while NK cell activity was not affected. Verdier et al. (1991) observed thymic atrophy and decreased resistance to *L. monocytogenes* in rats exposed to 5 or 50 mg/kg-d of TBTO in the diet. Lower doses were not examined. Vandebriel et al. (1998) fed rats TBTO in the diet for 6 wk at 5, 20, or 80 ppm (equivalent to 0.25, 1, and 4 mg/kg body weight/d). Effects were again seen at the lowest dose, 4 mg/kg-d, and included changes in T-cell cytokines involved in T-cell maturation. Krajnc et al. (1984) reported changes in serum immunoglobulin levels and leukopenia in rats administered TBTO at levels of 3.5 mg/kg-d for 28 d.

Mode(s) of Action Organotins are neurotoxic and, based on their reported ability to alter thyroid hormones, have also been considered as endocrine disruptors. There is no evidence, however, that these effects are associated with TBT immunotoxicity. TBT appears to directly target the thymus and either affects the influx of thymocyte precursor cells from the periphery or their normal maturation once within the thymus. The latter may result from hydrophobic-dependent intracellular distribution, resulting in inhibition of phospholipid metabolism. One effect of blocking phospholipid metabolism would be inhibition of intracellular phospholipid transport between organelles and disruption of membrane-mediated signal transduction (Penninks & Seinen, 1983).

Data Gaps There are few data on gestational and lactational transfer of TBT from the dam to her offspring. Further study is required to determine the persistence of effects in adult animals after exposure ceases.

Summary Exposure to TBTO 3 times/wk over the first 3 wk of life suppressed immune function and induced thymic atrophy. Suppression of lymphocyte proliferative responses persisted until 10 wk of age. Thymus atrophy and reduced host resistance occurred after 4.5 mo of exposure to 0.25 mg TBTO/kg in Wistar-derived rats, in contrast to increased antibody responses after daily exposure to 2.5 mg/kg in F344 rats, suggesting either strain-dependent or length of exposure-dependent effects in adult animals.

Conclusions Since TBTO is converted to TBTC in the stomach, it may be assumed that oral exposure to TBTC will produce similar effects as TBTO. Evaluation of perinatal versus adult immune sensitivity provides reasonably good evidence to indicate that the immune system is more sensitive to TBTO during the perinatal than adult period. This is based on adult rat immunotoxicity studies that indicated an oral no-effect level at 0.025 mg/kg/d and only minimal effects at 0.25 mg/kg compared to observable effects at >0.025 mg/kg/d when exposure was initiated prenatally. More importantly, a much broader spectrum of immune effects, particularly functional changes, were evident at lower doses in animals exposed exclusively during the perinatal than adult period. Based on a recent review by the U.S. EPA to establish cumulative risk estimates from exposure to chemical mixtures, triphenyltin hydroxide (TPTH) would be presumed to show similar characteristics based on a similar mode of action as TBTO (U.S. EPA, in review).

COMPOSITE CONCLUSIONS

Animal data presented in Tables 1–5 illustrate that exposure of the developing immune system to xenobiotics may produce a variety of immune system defects. These include slowed maturation of function and alteration of normal function, potentially for the lifetime of the animal. In some cases, immunotoxicity in exposed offspring occurred at doses that are not immunotoxic in adults. Early postnatal exposure of mice to DES provides a clear example of increased sensitivity in the developing immune system. Similar doses of DES suppress immune function in immature and mature animals, particularly in females; however, adult animals quickly recover, whereas neonatal exposure suppresses both innate and acquired cellular and humoral immune responses well into adulthood. In this case, the age of the test animal would not affect the outcome of function assessment, but immunotoxicologic evaluation in adults would greatly underestimate the relative risk of immature exposure. TCDD provides a compelling example of age sensitivity: A single injection of

TABLE 1. Summary of key studies used to compare age-associated exposure differences in DES immunotoxicity

Exposure period	Dose	Species, strain, and gender	Reported effects	NOAEL and LOAEL (mg/kg/d)	Persistence	Reference
GD16	0.1 mg/kg	Mouse; CD1; ♂ and ♀ offspring	↓IgM to T-independent Ag in ♀, ↑IgM in♂	Single dose level study	7–9 wk	Luster et al., 1978b, 1979
PND 1≈5	0, ≈2.2 mg DES/kg/d, sc injection	Mouse; BALB/*c*, NMRI; ♀	↓IgM to both T-dependent and -independent Ag	Single dose level study	16–18 wk	Kalland, 1980a
PND1≈5	0, ≈2.2 mg DES/kg/d, sc injection	Mouse; BALB/*c*, NMRI, C57Bl/6; ♀	↓ NK cell activity	Single dose study	11 mo, NK 6 mo, PFC	Kalland, 1980a
Adult, 5 d	0, 0.2, 2, 8 mg DES/kg/d	Mouse; B6C3F1; ♀	↓IgM to both T-dependent and -independent Ag, ↓ DTH at 2 and 8 mg/kg/d	NOAEL = 0.2 LOAEL = 2.0	Not evaluated	Luster et al., 1980
Adult, 5 d	0, 0.2, 2, 8 mg DES/kg/d	Mouse; B6C3F1; ♀	↓ Mortality after tumor cell or bacterial challenge, ↓resistance to nematode infection at ≥0.2 mg/kg/d	LOAEL = 0.2	Not evaluated	Dean et al., 1980

TABLE 2. Summary of key studies used to compare age-associated exposure differences in diazepam immunotoxicity

Exposure period	Dose	Species, strain, and gender	Reported effects	NOAEL and LOAEL	Persistence	Reference
GD16–20	0, 1.5 mg/kg	Rat; Long-Evans; ♂ and ♀ offspring	↓T-cell response to foreign Ag, production of TNFα, IL6, IL1, IL2, resistance to nematode (*T. spiralis*) infection	Single dose level study	8 wk	Schlumpf et al., 1989,1992, 1993, 1994, Schreiber et al., 1993a, 1993b
GD17–21	0, 6 mg/kg	Rat; Sprague-Dawley; ♂ and ♀ offspring	↓Total IgG, ↑ tumor and infection incidence	Single dose level study	20 mo	Livezey et al., 1986
GD 9–15	0, 1.0, 1.5 mg/kg/d	Golden hamster; ♂ offspring	↓ Resistance to bacterial (*M. bovis*) infection	LOAEL = 1.0 mg/kg/d	Infected on PND 75	Ugaz et al., 1999
PND7	0, 10 mg/kg	Rat; Wistar; ♂ and ♀	↓IgM and IgG response, DTH response	Single dose level study	↓Antibody, 24 mo; ↓DTH, 7 mo	Dostal et al., 1995
Adult, 3 d	0, 0.5, 1, 2, 4, 8 mg/kg/d	Mouse; Swiss; ♀	↓ Antibody and DTH	LOAEL = 4.0 mg/kg/d × 3 d NOAEL = 2 .0 mg/kg/d × 3 d	Not evaluated	Descotes et al., 1982, 1982–1983
Adult, 3 d	0, 1, 2, 4, 8 mg/kg/d	Mouse; IOPS OF1; ♂ and ♀	↓LD_{50} dose of bacteria (*Klebsiella pneumoniae*)	LOAEL = 1.0 mg/kg/d × 3 d	Not evaluated	Laschi et al., 1983

TABLE 3. Summary of key studies used to compare age-associated exposure differences in Pb immunotoxicity

Exposure period	Dose	Species, strain and gender	Reported effects	NOAEL and LOAEL	Persistence	Reference
GD 2–21	0, 50, 100, 250 ppm Pb in drinking water	Rat; F344; ♂ and ♀ offspring	↓DTH ↑Relative spleen weight Altered cell distribution	NOAEL = 50 ppm LOAEL = 100 ppm	13 wk	Bunn et al., 2001a
GD 15–20	40 ppm Pb in drinking water	Mouse; BALB/*c*; ♂ and ♀ offspring	↑IgE ↓Spleen cellularity	Single dose level; BLL ≈ 5.0 µg/dl at 1 wk	2 wk	Snyder et al., 2000
GD –14 to GD21	0, 100, 250, 500 ppm lead in drinking water	Rat; F344; ♀ offspring	↓DTH ↑IgE	LOAEL = 100 ppm for ↑ IgE; 250 ppm for ↓ DTH	13 wk	Miller et al., 1998
PND 0–21 (maternal)	40 ppm Pb acetate in drinking water	Mouse; BALB/*c*; ♂ and ♀ offspring	↑IgE	Single dose level; BLL ≈ 15 µg/dl	Not determined	Snyder et al., 2000
GD 15–PND 14	40 ppm Pb in drinking water	Mouse; BALB/*c*; ♂ and ♀ offspring	↑IgE ↓Spleen cellularity	Single dose level; BLL (14 d) ≈ 18 µg/dl	Not determined	Snyder et al., 2000
GD–49 to PND 40–50	0, 25, 50 ppm Pb	Rat; CD; ♂ and ♀ offspring	↓Thymus weight, PFC, IgG, DTH, thymocyte PHA response	LOAEL = 25 ppm BLL = 29 µg/dl	Not determined	Luster et al., 1978a; Faith et al., 1979
Adults, 3 wk	0, 32, 128, 512, 2048 ppm Pb in drinking water	Mouse; BALB/*c*; ♀	↓DTH	NOAEL = 128 ppm; LOAEL = 512 ppm	Not determined	McCabe et al., 1999
Adults, 3 wk	1, 50, 200, 1000 ppm in drinking water	Mouse; BDF1; ♀	↓T-dependent responses	LOAEL = 50 ppm	Not determined	Blakley and Archer, 1981
Adults, 30 d	0–0.5 mg Pb acetate, ip	Mouse; BALB/*c*; gender not reported	↓DTH	LOAEL = 0.025 mg/d	Not determined	Muller et al., 1977

TABLE 4. Summary of key studies used to compare age-associated exposure differences in TCDD immunotoxicity

Exposure period	Dose	Species, strain and gender	Reported effects	NOAEL and LOAEL (μg/kg/d)	Persistence	Reference
GD 6–14	0, 1.5, 3 μg/kg/d	Mouse; C57Bl/6N; ♂ and ♀ offspring	Thymic atrophy (GD 18) ↑Immature T cells ↓ CTL (7–8 wk)	NOAEL = 1.5 LOAEL = 3.0	No phenotype effects on PND 14	Holladay et al., 1991
GD 14	0, 1, 3 μg/kg	Rat, F344; ♂ and ♀ offspring	Thymic atrophy (GD 19) ↓ $CD3^-CD4^+CD8^+$ ↑$CD3^-CD4^-CD8^+$ ↓DTH	NOAEL = 1 LOAEL = 3	No phenotype effects, GD 22/ PND 1	Gehrs et al., 1997a
GD 14	0, 3 μg/kg or 0, 1 μg/kg, cross-fostered	Rat, F344; ♂ and ♀ offspring; Rat, F344; ♂ and ♀ offspring	↑DTH	Only 3 μg/kg dose at 17 wk	↓DTH, 17 wk ↑DTH, 5 wk	Gehrs et al., 1997b
GD 14	0, 0.1, 0.3, 1, 3 μg/kg	Rat, F344; ♂ and ♀ offspring	↓DTH; ♂ offspring affected at lower dose than ♀	Only 3 μg/kg tested at 19 mo LOAEL ♂ 0.1 μg/kg ♀ 0.3 μg/kg LOAEL	↓DTH, 19 mo, ♂, 4 mo, ♀ ↓DTH 14 mo	Gehrs and Smialowicz, 1999
PND 0–21	0, 1.8, 18 ng TCDD/L drinking water	Mouse; C57Bl/6; ♂ and ♀ offspring	Decreased clearance of bacteria by immunologically immature offspring	NOAEL = 1.8 ng/L LOAEL = 18 ng/L	Not evaluated	Sugita-Konishi et al., 2003
Adult, one exposure	0, 1, 3, 10, 30 μg TCDD/kg	Mouse; B6C3F1; ♀ Rat; F344; ♂ and ♀ Rat; Long-Evans; ♀	Mouse: ED_{50} for ↓ antibody = 0.7 μg kg Rat: Increased antibody	Mouse LOAEL = 1 Rat (enhancement) NOAEL = 1; LOAEL = 3	Not evaluated	Smialowicz et al., 1994

TABLE 5. Summary of key studies used to compare age-associated . exposure differences in tributyltins (TBTs) immunotoxicity

Exposure period	Dose	Species; strain; gender	Reported effects	NOAEL and LOAEL (mg/kg/d)	Persistence	Reference
Pre-weanling, 3 X/wk, 10 doses	TBTO 2.5, 5, 10 mg/kg bw/d	Rat; F344; ♂	Thymus atrophy, ↓ lymphocyte proliferation and NK cell activity	LOAEL = 5.0	≥10 wk	Smialowicz et al., 1989
GD 8 –PND 90	TBTC 0.025, 0.25, 2.5 mg/kg bw/d	Rat; Sprague-Dawley; ♂ and ♀ offspring	Thymus atrophy, ↓ or ↑ T cell numbers, non-dose-responsive NK cell activity, DTH, immunoglobulin levels	LOAEL = 0.025	Not evaluated	Tryphonas et al., 2004
Adult, 4–6 or 15 months	TBTO dietary concentrations of 0.5, 5.0, or 50 mgTBTO/kg feed (~.025, .25, 2.5 mg/kg bw/d)	Rat; Wistar; ♂	Thymus atrophy, ↓ host resistance, NK cell activity, altered T- and B-cell numbers	NOAEL = 0.025 LOAEL = 0.25	Not evaluated	Vos et al., 1990
Adult (10 consecutive days)	TBTO 1.25–10.0 mg/kg/d	Rat; F344; ♂	Thymus atrophy, ↑antibody synthesis	NOAEL = 1.25 LOAEL = 2.5	1 wk, antibody	Smialowicz et al., 1989
Adult, 3 × /wk, 10 doses	TBTO 5.0, 10, 20 mg/kg/d	Rat; F344; ♂	Thymus atrophy, ↓ T cell proliferation	LOAEL = 5.0	<3 wk	Smialowicz et al., 1989

≤30 μg TCDD/kg has either no effect or enhances the immune response of adult rats, whereas a single maternal dose of 0.1 to 0.3 μg TCDD/kg body weight during the third trimester suppresses T-cell responses, essentially for the lifetime of the animal. Considering that adults were exposed directly, and developing animals only were exposed to a fraction of the maternal dose, it is clear that the effective dose for adverse effects is dramatically different in offspring, as are the consequences of exposure.

The most often identified data gap in the reviewed studies is the lack of data on persistence of effects in animals exposed as adults. Existing data generally indicate that immune function recovers quickly in both laboratory animals and humans once exposure stops, unless exposure severely compromises or destroys the hematopoietic system. Nevertheless, a systematic comparison of persistence following developmental or adult exposure to representative immunotoxicants would provide formal validation of this concept. Until data to the contrary are available, it appears prudent to err on the side of caution in hazard identification and risk assessment, and to assume that development and maturation of the immune system constitutes a period of greater sensitivity to xenobiotic exposure. This approach is supported by the limited existing human data from offspring of women treated with immunosuppressive drugs during pregnancy that experienced decreased immune function for up to 1 yr after birth. Current toxicity risk assessment guidelines address this issue to an extent by imposing an additional safety factor of up to 10× to protect the developing organism, which may or may not be sufficient, depending on a variety of factors.

Although this review has focused primarily on immunosuppression, it should be noted that other forms of dysfunction may occur, including the development of allergy, hypersensitivity, and autoimmunity (see review by Holladay & Smialowicz, 2000). As recently reviewed by van Loveren et al. (2003), assessment of immune function during routine toxicity testing is possible under the exposure/evaluation scenarios of several sets of test guidelines. Given the heightened sensitivity of the developing immune system to chemical exposure from both a quantitative standpoint and persistence of effect, it appears reasonable to assume that testing only in adults would not provide a sufficient level of sensitivity to identify immunotoxicity in the neonate. Gender-dependent sensitivity of the developing immune system has been described for some chemicals (e.g., DES in females; heptachlor, methoxychlor, atrazine, and TCDD in males), a fact that may cloud interpretation of results if not taken into account.

REFERENCES

Ambruso, D. R., Bentwood, B., Henson, P. R., and Johnston, R. B. 1984. Oxidative metabolism of cord blood neutrophils: relationship to content and degranulation of cytoplasmic granules. *Pediatr. Res.* 18:1148–1153.

Anetor, J. L., and Adeniyi, F. A. 1998. Decreased immune status in Nigerian workers occupationally exposed to lead. *Afr. J. Med. Med. Sci.* 27:169–172.

Baccarelli, A., Mocarelli, P., Patterson, D. G., Bonzini, M., Pesatori, A. C., Caporaso, N., and Landi, M. T. 2002. Immunologic effects of dioxin: New results from Seveso and comparison with other studies. *Environ. Health Perspect.* 110:1169–1173.

Baird, D. D., Wilcox, A. J., and Herbst, A. L. 1996. Self-reported allergy, infection, and autoimmune diseases among men and women exposed in utero to diethylstilbestrol. *J. Clin. Epidemiol.* 49:263–266.

Baley, J. E., and Schacter, B. Z. 1985. Mechanisms of diminished natural killer cell activity in pregnant women and neonates. *J. Immunol.* 134:3042–3048.

Birnbaum, L. S., and Tuomisto, J. 2000. Non-carcinogenic effects of TCDD in animals. *Food Addit. Contam.* 17:275–288.

Blakley, B. R., and Archer, D. L. 1981. The effect of lead acetate on the immune response in mice. *Toxicol. Appl. Pharmacol.* 61:18–26.

Blaylock, B. L., Holladay, S. D., Comment, C. E., Heindel, J. J., and Luster, M. I. 1992. Exposure to tetrachlorodibenzo-*p*-dioxin (TCDD) alters fetal thymocyte maturation. *Toxicol. Appl. Pharmacol.* 112:207–213.

Bunn, T. L., Parsons, P. J., Kao, E., and Dietert, R. R. 2001a. Gender-based profiles of developmental immunotoxicity to lead in the rat: Assessment in juveniles and adults. *J. Toxicol. Environ. Health A.* 64:101–118.

Bunn, T. L., Ladics, G. S., Holsapple, M. P., and Dietert, R. R. 2001b. Developmental immunotoxicology assessment in the rat: Age, gender and strain comparisons after exposure to Pb. *Toxicol. Methods.* 11:41–58.

Bunn, T., Parsons, L., Kao, P. J. E., and Dietert, R. R. 2001c. Exposure to lead during critical windows of embryonic development: Differential immunotoxic outcome based on stage of exposure and gender. *Toxicol. Sci.* 64:57–66.

Cavalcante, N. J., Sandeville, M. L., and Medeiros, E. A. 2001. Incidence of and risk factors for nosocomial pneumonia in patients with tetanus. *Clin. Infect. Dis.* 33:1842–1846.

Chen, S., Golemboski, K. A., Piepenbrink, M., and Dietert, R. R. 2004. Developmental immunotoxicity of lead in the rat: Influence of maternal diet. *J. Toxicol. Environ. Health A.* 67:495–511.

Ciccimarra, F. 1994. Fetal and neonatal immunology. *J. Perinat. Med.* 22(suppl. 1):84–87.

Coscia, G. C., Discalzi, G., and Ponzetti, C. 1987. Immunological aspects of occupational lead exposure. *Med. Lav.* 78:360–364.

Covelli, V., Maffione, A. B., Nacci, C., Tato, E., and Jirillo, E. 1998. Stress, neuropsychiatric disorders and immunological effects exerted by benzodiazepines. *Immunopharmacol. Immunotoxicol.* 20:199–209.

Dean, J. H., Luster, M. I., Boorman, G. A., Luebke, R. W., and Lauer, L. D. 1980. The effect of adult exposure to diethylstilbestrol in the mouse: Alterations in tumor susceptibility and host resistance parameters. *J. Reticuloendothel. Soc.* 28:571–583.

Descotes, J., Tedone, R., and Evreux, J. C. 1982. Suppression of humoral and cellular immunity in normal mice by diazepam. *Immunol. Lett.* 5:41–43.

Descotes, J., Laschi, A., Tachon, P., Tedone, R., and Evreux, J. C. 1982–1983. Effects of variations in time and dose of diazepam injection on delayed hypersensitivity. *J. Immunopharmacol.* 4:279–284.

Dostal, M., Benesova, O., Tejkalova, H., and Soukupova, D. 1995. Immune response of adult rats is altered by administration of diazepam in the first postnatal week. *Reprod. Toxicol.* 9:115–121.

Dragan, Y. P., and Schrenk, D. 2000. Animal studies addressing the carcinogenicity of TCDD (or related compounds) with an emphasis on tumor promotion. *Food Addit. Contam.* 17:289–302.

Dyatlov, V. A., and Lawrence, D. A. 2002. Neonatal exposure to lead potentiates sickness behavior induced by *Listeria monocytogenes* infection in mice. *Brain Behav. Immun.* 16:477–492.

Ewers, U., Stiller-Winkler, R., and Idel, H. 1982. Serum immunoglobulin, complement C3 and salivary IgA levels in lead workers. *Environ. Res.* 29:352–357.

Faden, H. 2001. The microbiologic and immunologic basis for recurrent otitis media in children. *Eur. J. Pediatr.* 160:407–413.

Faith, R. E., and Moore, J. A. 1977. Impairment of thymus-dependent immune functions by exposure of the developing immune system to 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD). *J. Toxicol. Environ. Health.* 3:451–464.

Faith, R. E., Luster, M. I., and Kimmel, C. A. 1979. Effect of chronic developmental lead exposure on cell-mediated immune functions. *Clin. Exp. Immunol.* 35:413–420.

Fischbein, A., Tsang, P., Luo, J. -C. J., Roboz, J. P., Jiang, J. D., and Bekesi, J. G. 1993. Phenotypic aberrations of lymphocytes at low-level occupational exposure to lead. *Clin. Immunol. Immunopathol.* 66:163–168.

Forsberg, J. -G. 1984. Short-term and long-term effects of estrogen on lymphoid tissues and lymphoid cells with some remarks on the significance for carcinogenesis. *Arch. Toxicol.* 55:7–90.

Gehrs, B. C., and Smialowicz, R. J. 1999. Persistent suppression of delayed-type hypersensitivity in adult F344 rats after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicology* 134:79–88.

Gehrs, B. C., Riddle, M. M., Williams, W. C., and Smialowicz, R. J. 1997a. Alterations in the developing immune system of the F344 rat after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. I. Effects on the fetus and the neonate. *Toxicology* 122:219–228.

Gehrs, B. C., Riddle, M. M., Williams, W. C., and Smialowicz, R. J. 1997b. Alterations in the developing immune system of the F344 rat after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. II. Effects on the pup and the adult. *Toxicology* 122:229–240.

Geyer, H. J., Scheuntert, I., Rapp, K., Kettrup, A., Korte, F., Greim, H., and Rozman, K. 1990. Correlation between acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and total body fat content in mammals. *Toxicology* 65:97–107.

Geyer, H. J., Schranmm, K. W., Feicht, E. A., Bechechti, A., Steinberg, C., Bruggemann, R., Poiger, H., Henkelmann, B., and Kettrup, A. 2002. Half-lives of tetra-, penta-, hexa-, hepat-, and octachlorodibenzo-*p*-dioxin in rats, monkeys, and humans—A critical review. *Chemosphere* 48:631–644.

Heo, Y., Parsons, P. J., and Lawrence, D. A. 1996. Lead differentially modifies cytokine production in vitro and in vivo. *Toxicol. Appl. Pharmacol.* 138:149–157.

Holladay, S. D., and Smialowicz, R. J. 2000. Development of the murine and human immune system: Differential effects of immunotoxicants depend on time of exposure. *Environ. Health Perspect.* 108(suppl. 3):463–473.

Holladay, S. D., Lindstrom, P., Blaylock, B. L., Comment, C. E., Germolec, D. R., Heindell, J. J., and Luster, M. I. 1991. Perinatal thymocyte antigen expression and postnatal immune development altered by gestational exposure to tetrachlorodibenzo-*p*-dioxin (TCDD). *Teratology* 44:385–393.

Holladay, S. D., Blaylock, B. L., Comment, C. E., Heindel, J. J., Fox, W. M., Korach, K. S., and Luster, M. I. 1993. Selective prothymocyte targeting by prenatal diethylstilbestrol exposure. *Cell. Immunol.* 152:131–142.

Holsapple, M. P. 2003. Developmental immunotoxicity testing: A review. *Toxicology* 185:193–203.

Holsapple, M. P., Snyder, N. K., Wood, S. C., and Morris, D. L. 1991. A review of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced changes in immunocompetence: 1991 Update. *Toxicology* 69:219–255.

Ishaaya, I., Engel, J. L., and Casida, J. E. 1976. Dietary triorganotins affect lymphoid tissues and blood composition of mice. *Pestic. Biochem. Physiol.* 6:270–279.

Kalland, T. 1980a. Alterations of antibody responses in female mice after neonatal exposure to diethylstilbestrol. *J. Immunol.* 124:194–198.

Kalland, T. 1980b. Decreased and disproportionate T-cell population in adult mice after neonatal exposure to diethylstilbestrol. *Cell. Immunol.* 51:55–63.

Kalland, T. 1984. Exposure of neonatal female mice to diethylstilbestrol persistently impairs NK activity through reduction of effector cells at the bone marrow level. *Immunopharmacology* 7:127–134.

Kalland, T., and Forsberg, J. -G. 1978. Delayed hypersensitivity responses to oxazolone in neonatally estrogenized mice. *Cancer Lett* 4:141–146.

Kalland, T., and Forsberg, J. -G. 1981. Natural killer cell activity and tumor susceptibility in female mice treated neonatally with diethylstilbestrol. *Cancer Res* 41:5134–5140.

Kalland, T., Strand, O., and Forsberg, J. -G. 1979. Long-term effects of neonatal estrogen treatment on mitogen responsiveness of mouse spleen lymphocytes. *JNCI* 63:413–421.

Karmaus, W., Kuehr, J., and Kruse, H. 2001. Infections and atopic disorders in childhood and organochlorine exposure. *Arch. Environ. Health.* 56:485–492.

Kellogg, C. K. 1999. Sex differences in long-term consequences of prenatal diazepam exposure: Possible underlying mechanisms. *Pharmacol. Biochem. Behav.* 64:673–680.

Kerkvliet, N. 2002. Recent advances in understanding the mechanisms of TCDD immunotoxicity. *Int. Immunopharmacol.* 2:277–291.

Kimber, I., Stonard, M. D., Gidlow, D. A., and Niewola, Z. 1986. Influence of chronic low level exposure to lead on plasma immunoglobulin concentration and cellular immune function in man. *Int. Arch. Occup. Environ. Health.* 57:117–125.

Koller, L. D., and Brauner, J. A. 1977. Decreased B-lymphocyte response after exposure to lead and cadmium. *Toxicol. Appl. Pharmacol.* 42:621–624.

Koller, L. D., Exon, J. H., and Norbury, K. C. 1983. Induction of humoral immunity to protein antigen without adjuvant in rats exposed to immunosuppressive chemicals. *J. Toxicol. Environ. Health.* 12:173–181.

Krajnc, E. I., Wester, P. W., Loeber, J. G., Van Leeuwen, F. X. R., Vos, J. G., Vaessen, H. A. M. G., and Van der Heijden, C. A. 1984. Toxicity of bis(tri-*n*-butyltin) oxide in the rat. I. Short-term effects on general parameters and on the endocrine and lymphoid systems. *Toxicol. Appl. Pharmacol.* 75:363–386.

Laschi, A., Descotes, J., Tachon, P., and Evreux, J. C. 1983. Adverse influence of diazepam upon resistance to *Klebsiella pneumoniae* infection in mice. *Toxicol. Lett.* 16:281–284.

Levy, O., Martin, S., Eichenwald, E., Ganz, T., Valore, E., Carroll, S. F., Lee, K., Goldman, D., and Thorne, G. M. 1999. Impaired innate immunity in the newborn: Newborn neutrophils are deficient in bactericidal/permeability-increasing protein. *Pediatrics* 104:1327–1333.

Livezey, G. T., Marczynski, T. J., McGrew, E. A., and Beluhan, F. Z. 1986. Prenatal exposure to diazepam: Later postnatal teratogenic effect. *Neurobehav. Toxicol. Teratol.* 8:433–440.

Lu, Y. C., and Wu, Y. C. 1985. Clinical findings and immunological abnormalities in Yu-Cheng patients. *Environ. Health Perspect.* 59:17–29.

Luebke, R. W., Luster, M. I., Dean, J. H., and Hayes, H. T. 1984. Altered host resistance to *Trichinella spiralis* infection following subchronic exposure to diethylstilbestrol. *Int. J. Immunopharmacol.* 6:609–617.

Luster, M. I., Faith, R. E., and Kimmel, C. A. 1978a. Depression of humoral immunity in rats following chronic developmental lead exposure. *J. Environ. Pathol. Toxicol.* 1:397–402.

Luster, M. I., Faith, R. E., and McLachlan, J. A. 1978b. Alterations of the antibody response following *in utero* exposure to diethylstilbestrol. *Bull. Environ. Contam. Toxicol.* 20:433–437.

Luster, M. I., Faith, R. E., McLachlan, J. A., and Clark, G. C. 1979. Effect of *in utero* exposure to diethylstilbestrol on the immune response in mice. *Toxicol. Appl. Pharmacol.* 47:279–285.

Luster, M. I., Boorman, G. A., Dean, J. H., Harris, M. W., Luebke, R. W., Padarathsingh, M. L., and Moore, J. A. 1980. Examination of bone marrow, immunologic parameters and host susceptibility following pre- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Int. J. Immunopharmacol.* 2:301–310.

Luster, M. I., Hayes, H. T., Korach, K., Tucker, A. N., Dean, J. H., Greenlee, W. F., and Boorman, G. A. 1984. Estrogen immunosuppression is regulated through estrogenic responses in the thymus. *J. Immunol.* 133:110–116.

Luster, M. I., Dean, J. H., and Germolec, D. R. 2003. Consensus workshop on methods to evaluate developmental immunotoxicity. *Environ. Health Perspect.* 111:579–583.

Lutz, P. M., Wilson, T. J., Ireland, J., Jones, A. L., Gorman, J. S., Gale, N. L., Johnson, J. C., and Hewett, J. E. 1999. Elevated immunoglobulin E (IgE) levels in children with exposure to environmental lead. *Toxicology* 134:63–78.

McCabe, M. J., Jr., Singh, K. P., and Reiner, J. J., Jr. 1999. Lead intoxication impairs the generation of a delayed type hypersensitivity response. *Toxicology* 139:255–264.

Miller, T. E., Golemboski, K. A., Ha, R. S., Bunn, T. L., Sanders, F. S., and Dietert, R. R. 1998. Developmental exposure to lead causes persistent immunotoxicity in Fischer 344 rats. *Toxicol. Sci.* 42:129–135.

Misra, K. P., Singh, V. K., Rani, R., Yadav, V. S., Chandran, V., Srivastava, S. P., and Seth, P. K. 2003. Effect of lead exposure on the immune response of some occupationally exposed individuals. *Toxicology* 188:251–259.

Mocarelli, P., Marocchi, A., Brambilla, P., Gerthoux, P., Young, D. S., and Mantel, N. 1986. Clinical laboratory manifestations of exposure to dioxin in children, a six year study of the effects of an environmental disaster near Seveso, Italy. *J. Am. Med. Assoc.* 256:2687–2695.

Muller, S., Gillert, K. E., Krause, C., Gross, U., Age-Stehr, J. L., and Diamanstein, T. 1977. Suppression of delayed type hypersensitivity of mice by lead. *Experimentia* 33:667–668.

Nakanishi, Y., Shigematsu, N., Kurita, Y., Matsuba, K., Kanegae, H., Ishimaru, S., and Kawazoe, Y. 1985. Respiratory involvement and immune status in Yusho patients. *Environ. Health Perspect.* 59:31–36.

National Research Council 1993. *Pesticides in the diets of infants and children.* Washington, DC: National Academy Press.

Neilan, B. A., Taddeini, L., McJilton, C. E., and Houdweger, B. S. 1980. Decreased T cell function in mice exposed to chronic low levels of lead. *Clin. Exp. Immunol.* 39:746–749.

Penninks, A. H., and Seinen, W. 1983. The lymphocyte as target of toxicity: A biochemical approach to dialkyltin induced immunosuppression. In *Advances in immunopharmacology,* eds. J. W. Hadden, L. Chedid, P. Dukor, F. Spreafidco, and D. Willoughby, pp. 41–60. New York: Pergamon Press Vol 2.

Pinkerton, L. E., Biagini, R. E., Ward, E. M., Hull, R. D., Deadens, J. A., Boeniger, M. F., Schnorr, T. M., Mackenzie, B. A., and Luster, M. I. 1998. Immunologic findings among lead-exposed workers. *Am. J. Ind. Med.* 33:400–408.

Pocchiari, F., Silano, V., and Zampieri, A. 1979. Human health effects from accidental release of tetrachlorodibenzo-*p*-dioxin (TCDD) at Seveso, Italy. *Ann. NY Acad. Sci.* 320:311–320.

Price, H. V., Salaman, J. R., Laurence, K. M., and Langmaid, H. 1976. Immunosuppressive drugs and the fetus. *Transplantation* 21:294–298.

Queiroz, M. L. S., Perlingeiro, R. C. R., Bincoletto, C., Almeida, M., Cardoso, M. P., and Dantas, D. C. M. 1994. Immunoglobulin levels and cellular immune function in lead exposed workers. *Immunopharmacol. Immunotoxicol.* 16:115–128.

Sandler, D. 2002. Developmental immunotoxicology and risk assessment: Special issue. *Hum. Exp. Toxicol.* 21:469–572.

Sata, F., Araki, S., Tanigawa, T., Morita, Y., Sakurai, S., Nakata, A., and Katsuno, N. 1998. Changes in T cell subpopulations in lead workers. *Environ. Res.* 76:61–64.

Schell, L. M., Denham, M., Stark, A. D., Ravenscroft, J., Parsons, P., and Schulte, E. 2004. Relationship between blood lead concentration and dietary intakes of infants from 3 to 12 months of age. *Environ. Res.* 96:264–273.

Schena, F. P., Stallone, G., Schena, A., Manfredi, G., Derosa, C., Procino, A., and Di Paolo, S. 2002. Pregnancy in renal transplantation: Immunologic evaluation of neonates from mothers with transplanted kidney. *Transplant Immunol* 9:161–164.

Schlumpf, M., Ramseier, H., and Lichtensteiger, W. 1989. Prenatal diazepam induced persisting depression of cellular immune responses. *Life Sci* 44:493–501.

Schlumpf, M., Parmar, R., Ramseier, H., and Lichtensteiger, W. 1990. Prenatal benzodiazepine immunosuppression: Possible involvement of peripheral benzodiazepine site. *Dev. Pharmacol. Ther.* 15:178–185.

Schlumpf, M., Parmar, R., Schreiber, R., Ramseier, H., Butikofer, E., Abriel, H., Barth, M., Rhyner, T., and Lichtensteiger, W. 1992. Nervous and immune system as targets for developmental effects of benzodiazepines. A review of recent studies. *Dev. Pharmacol. Ther.* 18:145–158.

Schlumpf, M., Lichtensteiger, W., and Ramseier, H. 1993. Diazepam treatment of pregnant rats differentially affects interleukin-1 and interleukin-2 secretion in their offspring during different phases of postnatal development. *Pharmacol. Toxicol.* 73:335–340.

Schlumpf, M., Lichtensteiger, W., and van Loveren, H. 1994. Impaired host resistance to *Trichinella spiralis* as a consequence of prenatal treatment of rats with diazepam. *Toxicology* 94:223–230.

Schreiber, A., Frei, K., Lichtensteiger, W., and Schlumpf, M. 1993a. The effect of prenatal diazepam exposure on TNF-alpha production by rat splenocytes. *Agents Actions* 38:265–272.

Schreiber, A., Frei, K., Lichtensteiger, W., and Schlumpf, M. 1993b. Alterations in interleukin-6 production by LPS- and Con A-stimulated mixed splenocytes, spleen macrophages, and lymphocytes in prenatally diazepam-exposed rats. *Agents Actions* 39:166–173.

Simmons, R. D., Miller, R. K., and Kellogg, C. K. 1983. Prenatal diazepam: Distribution and metabolism in perinatal rats. *Teratology* 28:181–188.

Smialowicz, R. J., Riddle, M. M., Rogers, R. R., Luebke, R. W., and Copeland, C. B. 1989. Immunotoxicity of tributyltin oxide in rats exposed as adults or pre-weanlings. *Toxicology* 57:97–111.

Smialowicz, R. J., Riddle, M. M., Williams, W. C., and Diliberto, J. J. 1994. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on humoral immunity and lymphocyte subpopulations: Differences between mice and rats. *Toxicol. Appl. Pharmacol.* 124:248–256.

Snyder, J. E., Filipov, N. M., Parsons, P. J., and Lawrence, D. A. 2000. The efficiency of maternal transfer of lead and its influence on plasma IgE and splenic cellularity of mice. *Toxicol. Sci.* 57:87–94.

Staples, J. E., Gasiewicz, T. A., Fiore, N. C., Lubahn, D. B., Korach, K. S., and Silverstone, A. E. 1999. Estrogen receptor α is necessary in thymic development and estradiol-induced thymic alterations. *J. Immunol.* 163:4168–4174.

Stiehm, E. R., and Fudenberg, H. H. 1966. Serum levels of immune globulins in health and disease. *Pediatrics* 37:715–727.

Sugita-Konishi, Y., Kobaayashi, K., Naito, H., Miura, K., and Suzuki, Y. 2003. Effect of lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the susceptibility to *Listeria* infection. *Biosci. Biotechnol. Biochem.* 67:89–93.

Tendron, A., Gouyon, J. -B., and Decramer, S. 2002. In utero exposure to immunosuppressive drugs: Experimental and clinical studies. *Pediat. Nephrol.* 17:121–130.

Tognoni, G., and Bonaccorsi, A. 1982. Epidemiological problem with TCDD (A critical review). *Drug Metab. Rev.* 13:447–469.

Tryphonas, H., Cooke, G., Caldwell, D., Bondy, G., Parenteau, M., Hayward, S., and Pulido, O. 2004. Oral (gavage), in utero and postnatal exposure of Sprague-Dawley rats to low doses of tributyltin chloride. Part II: Effects on the immune system. *Food Chem. Toxicol.* 42:227–241.

Ugaz, E., Pinheiro, S., Guerra, J., and Palermo-Neto, J. 1999. Effects of prenatal diazepam treatment on *Mycobacterium bovis*-induced infection in hamsters. *Immunopharmacology* 41:209–217.

Undeger, U., Basaran, N., Canpinar, H., and Kansu, E. 1996. Immune alterations in lead-exposed workers. *Toxicology* 109:167–172.

Upham, J. W., Lee, P. T., Holt, B. J., Heaton, T., Prescott, S. L., Sharp, M. J., Sly, P. D., and Holt, P. G. 2002. Development of interleukin-12-producing capacity throughout childhood. *Infect. Immun.* 70:6583–6588.

Valentino, M., Governa, M., Marchiseppe, I., and Visona, I. 1991. Effects of lead on polymorphonuclear leukocyte (PMN) functions in occupationally exposed workers. *Arch. Toxicol.* 65:685–688.

Vandebriel, R. J., Meredith, C., Scott, M. P., Roholl, P. J., and van Loveren, H. 1998. Effects of in vivo exposure to bis(tri-*n*-butyltin)oxide, hexachlorobenzene and benzo(a)pyrene on cytokine (receptor) mRNA levels in cultured rat splenocytes and on IL-2 receptor protein levels. *Toxicol. Appl. Pharmacol.* 148:126–136.

van Loveren, H., Vos, J., Putman, E., and Piersma, A. 2003. Immunotoxicological consequences of perinatal chemical exposures: A plea for inclusion of immune parameters in reproduction studies. *Toxicology* 185:185–191.

van Loveren, H., Slob, W., Vandebriel, R. J., Hudspith, B. N., Meredith, C., and Garssen, J. 1998. Immunotoxicology: Extrapolation from animal to man—Estimation of the risk associated with TBTO exposure. *Arch. Toxicol.* 20:285–292.

Verdier, F., Virat, M., Schweinfurth, H., and Descotes, J. 1991. Immunotoxicity of bis(tri-*n*-butyltin) oxide in the rat. *J. Toxicol. Environ. Health.* 32:307–317.

Villagra, R., Tchernitchin, N. N., and Tchernitchin, A. N. 1997. Effect of subacute exposure to lead and estrogen on immature pre-weaning rat leukocytes. *Bull. Environ. Contam. Toxicol.* 58:190–197.

Vos, J. G., and Moore, J. A. 1974. Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Int. Allergy Appl. Immunol.* 47:777–794.

Vos, J. G., DeKlerk, A., Krajnc, E. I., van Loveren, H., and Rozing, J. 1990. Immunotoxicity of bis(tri-*n*-butyltin)oxide in the rat: Effects on thymus-dependent immunity and on nonspecific resistance following long-term exposure in young versus aged rats. *Toxicol. Appl. Pharmacol.* 105:144–155.

Weisglas-Kuperus, N., Sas, T. C., Koopman-Esseboom, C., van der Zwan, C. W., De Ridder, M. A., Beishuizen, A., Hooijkaas, H., and Sauer, P. J. 1995. Immunologic effects of background prenatal and postnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. *Pediatr. Res.* 38:404–410.

Weisglas-Kuperus, N., Patandin, S., Berbers, G. A., Sas, T. C., Mulder, P. G., Sauer, P. J., and Hooijkaas, H. 2000. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. *Environ. Health Perspect.* 108:1203–1207.

Whalen, M. M., Loganathan, B. G., and Kannan, K. 1999. Immunotoxicity of environmentally relevant concentrations of butyltins on human natural killer cells in vitro. *Environ. Res.* 81:108–116.

Whalen, M. M., Green, S. A., and Loganathan, B. G. 2002. Brief butyltin exposure induces irreversible inhibition of the cytotoxic function on human natural killer cells, in vitro. *Environ. Res.* 88:19–29.

Wilson, C. B. 1986. Immunologic basis for increased susceptibility of the neonate to infection. *J. Pediatr.* 108:1–12.

Wolach, B., Carmi, D., Gilboa, S., Satar, M., Segal, S., Dolfin, T., and Schlesinger, M. 1994. Some aspects of the humoral immunity and the phagocytic function in newborn infants. *Isr. J. Med. Sci.* 30:331–335.