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Journal of Toxicology and Environmental Health, Part A

ISSN: 1528-7394 (Print) 1087-2620 (Online) Journal homepage: https://www.tandfonline.com/loi/uteh20

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To cite this article: Robert W. Luebke , David H. Chen , Rodney Dietert , Yung Yang & Michael I. Luster (2006) Immune System Maturity and Sensitivity to Chemical Exposure, Journal of Toxicology and Environmental Health, Part A, 69:9, 811-825, DOI: 10.1080/15287390600591496

To link to this article: https://doi.org/10.1080/15287390600591496


Published online: 24 Feb 2007.


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Journal of Toxicology and Environmental Health, Part A, 69:811–825, 2006
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ISSN: 1528–7394 print / 1087–2620 online
DOI: 10.1080/15287390600591496

IMMUNE SYSTEM MATURITY AND SENSITIVITY TO CHEMICAL EXPOSURE

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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. The immune system continues to mature after birth, and functional immaturity accounts for much of the increased susceptibility in the young. 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. Data from published reports were compared to determine whether age and developmental stage at exposure influence the immunotoxic effects of diethylstilbestrol (DES), diazepam (DZP), lead (Pb), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD),

We thank Drs. Ralph Smialowicz, Linda Birnbaum, and Jamie DeWitt for reading the manuscript and providing helpful suggestions. 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).

This article was prepared from a talk presented at the 2005 Toxicology and Risk Assessment Conference, Fairborn, OH. A more detailed version of this article was published in the *Journal of Toxicology and Environmental Health, Part B*.

This report was 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.

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and tributyltin oxide (TBTO). These compounds were chosen for comparison based on the fact that each had been studied fairly extensively, resulting in a significant number of peer-reviewed publications. Based on lowest-observed-adverse-effect level (LOAEL) values for all five compounds, the developing immune system was found to be at greater risk than that of the adult, either because lower doses induced immunotoxicity, adverse effects were more persistent, or adverse effects were reported following developmental, but not adult, exposure.

Immunotoxicologists are well aware of studies conducted since the mid-1970s that suggest that the developing immune system has greater sensitivity to xenobiotics compared to the adult. However, a report entitled "Pesticides in the Diets of Infants and Children," published by the National Research Council (NRC, 1993), was one of the first documents directed to a wide audience to formally recognize that the immature organism may be more sensitive to chemical exposure than are adults. The document led federal regulatory agencies in the United States and abroad to express an interest in protecting children's health from the effects of agents that may damage the immune system. The NRC 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 sensitive subpopulations in setting health advisories for drinking water. A significant body of clinical literature also indicates that immune function wanes as individuals age, suggesting that the elderly constitute a second sensitive subpopulation that may be more susceptible to the immunosuppressive effects of xenobiotics than young adults. In the United States, increased sensitivity in the elderly could be significant as the "baby boom" cohort approaches the onset of immunosenescence.

This document provides a general overview of how compromised immune function, in the absence of drug or xenobiotic exposure, predisposes populations at the extremes of age to infectious diseases, and compares the relative sensitivity of the developing and mature immune systems to immunotoxic xenobiotics. Five diverse compounds were chosen for comparison of doses that cause developmental and adult immunotoxicity 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 (for a comprehensive review, see Holladay & Smialowicz, 2000); rather, representative data are presented that exemplify and contrast potential effects of chemicals on the developing and mature immune systems. Age of exposure comparisons were made based on lowest-observed-adverse-effect level (LOAEL) values to provide a point of reference for both immunotoxicologists and risk assessors.

OVERVIEW OF RESISTANCE TO INFECTION

Protection against infection is provided by physiological and immunological mechanisms. Physiological mechanisms include skin and mucus linings that

act as physical barriers to invasion, proteolytic activity in the stomach that efficiently digests proteins, including those of many pathogens, and a variety of bacteriostatic and bactericidal compounds that are present in saliva, tears, and sweat. The innate immune system, broadly conserved across a wide range of biological complexity, provides a first line of defense against a variety of infectious agents and certain tumor cells. Innate effector mechanisms rely on end-stage cells or their products; thus, clonal expansion is not required to produce an effective number of protective cells within hours of infection. For example, neutrophilic polymorphonuclear cells (a.k.a. neutrophils), a type of leukocyte that comprises 50% to 70% of the total circulating population in human adults, rapidly accumulate at the site of extracellular bacterial infections. Neutrophils engulf and destroy bacteria; if the response is compromised by disease or cellular depletion, the normal rate of bacterial replication may cause overwhelming infection before the adaptive immune response can control infection. Natural killer (NK) cells (a subpopulation of lymphocytes) kill certain types of infectious agent, and some types of tumor cells, and serve as a valuable source of immunoregulatory cytokines. Specific immune responses to structural components and products released by infectious agents involve a cascade of events that begins with recognition of the antigen, progresses to stimulation of clonal expansion and highly scripted rearrangement of specific genes, and culminates in the activity of effector molecules and cells that antagonize or prevent replication of pathogens and facilitate the destruction of pathogens or infected cells. This process is much slower than innate responses, often requiring up to 4 or 5 d until effective control of the pathogens or their products is achieved.

CONSTITUTIVE IMMUNOSUPPRESSION

Common infectious diseases (e.g., pneumonia, influenza, otitis media) occur more often, and are usually more severe, in the very young and the elderly populations, and represent significant causes of morbidity and mortality in these populations. In some cases, age-related physical or physiological differences in tissues or organs increase susceptibility to infection. However, in most cases, it is the relative immaturity of the immune system in the very young, and the age-related immunological changes in the elderly ("immunosenescence"), that prevent the host from mounting an adequate response to microorganisms. In the simplest terms, the very young lack immunologic experience, and the elderly, in spite of a wealth of experience, are no longer able to function as well as the younger population. Constitutive immunosuppression is of concern to immunotoxicologists because age-related immunosuppression and chemical perturbation of function may act in an additive or synergistic manner, so that even minor chemically induced suppression may translate into greater risk of morbidity.

Antibody Production in the Young and Aged Populations

Although antibody synthesis in neonates is roughly 30% that of adults, maternally derived immunoglobulin (IgG) is actively and passively transported

across the placenta and provides good protection against organisms to which the mother has protective 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 Ig levels (Stiehm & Fudenberg, 1966) and is thus more vulnerable to infection. IgM and IgG levels present in 7- to 12-mo-old infants are approximately half those of healthy adults, but IgA does not reach the 50% level until 3–5 yr of age (Stiehm & Fudenberg, 1966). Antibody responses are diminished in the elderly, although the total numbers of circulating B cells (precursors of plasma cells that produce antibody) do not change with age (Weksler, 2000). When evaluated at the single cell level, plasma cells in younger subjects are more plentiful and produce more antibody, on a per cell basis, than those from the elderly (Burns et al., 1995).

Cell-Mediated Immunity in the Young and Aged Populations

Development and maturation of T-cell responses begins early in gestation. The thymus appears at about 6 wk, and lymphoid cells are detectable at about 8–9 wk, of gestation. At 10 wk of gestation, T lymphocytes in the thymus respond to stimulation with nonspecific mitogens, and by 12–14 wk of gestation 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 many of the cytokines that are necessary for mounting effective immune responses, and, most importantly, of generating a population of long-lived "memory cells." The profile of cytokine production, even in children up to 12 yr of age, decreases the efficiency of many host-protective responses, particularly to intracellular bacteria. In spite of a greater percentage of specifically educated memory cells in the elderly, which should be valuable in destroying microorganisms that the host has previously encountered, a portion of the memory cells do not respond to stimulation as well as memory cells in younger individuals. As in the very young, shifts in the relative quantities of certain cytokines decrease T-cell responses that are required to clear most common infections.

Nonspecific Immunity in the Young and Aged Populations

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 the 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).

Neutrophils are the first cells to arrive at sites of infection or tissue damage and are central in resistance to bacteria that produce infections in extracellular spaces (e.g., *Streptococcus*). Bacteria that are engulfed by neutrophils are

destroyed by a variety of lytic enzymes contained in cytoplasmic granules. However, the neutrophils of newborns contain significantly lower quantities of several enzymes critical to killing ingested organisms (Ambruso et al., 1984; Levy et al., 1999). In addition to a variety of functional deficits, there is a relatively low rate of neutrophil production by the neonatal bone marrow; thus, the supply can be exhausted during infection (Wilson, 1986). Neutrophil function in the elderly was reviewed by Lord et al. (2001). The cells are present in normal numbers in the circulation, and migrate to infected tissues similarly in the elderly and young adults but appear to undergo apoptosis sooner in the aged population, perhaps reducing the numbers of protective cells present in infected tissue. Phagocytosis by neutrophils is less efficient in the elderly on a per-cell basis, with each cell ingesting fewer bacteria than those from young adults. Production of superoxide, which is critical to killing ingested bacteria, is likewise reduced in the elderly.

IMMUNOTOXICOLOGY AND LIFE STAGE: GENERAL CONSIDERATIONS

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 in adults. 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.

Although not established absolutely, it is generally believed that the immature immune system is more susceptible to xenobiotics than the fully mature system, and sequelae of developmental immunotoxicant exposure may be more persistent, in contrast to effects observed following adult exposure (Holladay & Smialowicz, 2000). Based on results obtained in various experimental animal studies, perturbations of the developing immune system may be manifested as a qualitative (i.e., affecting only the developing immune system) or a quantitative (i.e., lower doses affect the developing immune system) difference. Following developmental exposure, immune maturation may simply be delayed 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 (see DES and TCDD examples, later). The steps involved in human and rodent immune system maturation appear to be remarkably similar, and no compelling evidence exists to suggest that effects observed in rodents are not representative of what might be expected to occur in humans. It should be noted that while the process of human and rodent immune ontogeny is similar, newborn humans are more immunologically mature than are newborn rodents. 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 Holsapple (2003).

Several studies showed increased rates of certain infections in children following perinatal exposure to environmental agents (Luster et al., 2003). For example, Weisglas-Kuperus et al. (2000) demonstrated that exposure to levels of polyhalogenated aromatic hydrocarbons normally found in highly industrialized countries was associated with increases in childhood infections and lower vaccination responses. Likewise, Karmaus et al. (2001) reported a greater incidence of inner ear infection in children with elevated levels of the DDT metabolite DDE and polychlorinated biphenyls or DDE and hexachlorobenzene. In addition, the odds ratio for the development of asthma and elevated IgE levels were increased in children with elevated levels of only DDE. Limited human immune function data are available from offspring of women taking therapeutic doses of immunosuppressants during pregnancy, although maternal use of azathioprine (Price et al., 1976) or cyclosporin A (Tendron et al., 2002) during gestation was reported to suppress immune function in offspring up to 1 yr of age.

Chronic stress in elderly humans may suppress immune function, resistance to infection (Glaser & Kiecolt-Glaser, 1998), and the response to vaccination (Kiecolt-Glaser et al., 1996). However, the effects of xenobiotics on immunocompetence in aged populations of humans or laboratory animals have rarely been addressed. Although immunotoxicity data in the aged population are scarce, a few animal studies have been done. For example, while age affected functional endpoints or resistance to infection in control animals, 1 yr-old rats exposed to tributyltin oxide (Vos et al., 1990) or aged (approximately 19 mo old) mice and rats exposed to 2,3,7,8-TCDD (Luebke et al., 1999) experienced little to no additional suppression compared to unexposed, aged controls. However, these conditions did not mimic probable chronic lifelong exposures that humans are subject to, and until a variety of xenobiotics have been tested under a variety of conditions, these results should not be considered representative.

COMPARISON OF IMMUNOTOXIC EFFECTS FOLLOWING DEVELOPMENTAL AND ADULT XENOBIOTIC EXPOSURE

Five diverse compounds were chosen for comparison, based on the availability of data and differing modes of action. The chemicals, their uses, and typical conditions of human exposure are as follows: Diethylstilbestrol (DES), a potent nonsteroidal synthetic estrogen, was used to prevent miscarriage in humans. Between 5 and 10 million pregnant women were given DES between 1938 and 1971 to prevent premature delivery or pregnancy loss, but use was discontinued when adverse effects were reported in offspring. Diazepam (DZP) is used for the short-term relief of anxiety symptoms and in the treatment of seizures and muscle spasms in certain neurological diseases. DZP was formerly prescribed for use during late pregnancy to control signs of early uterine

contractions and was given to neonates prior to intubation as a tranquilizer and muscle relaxant. DZP freely crosses the placenta; brain levels of DZP are essentially the same in the dam and offspring from birth until postnatal d 10 following maternal dosing over gestational days 13–20. Lead has had wide use as an antiknock additive to gasoline, as a paint pigment, in solder used for plumbing, and in various glazes. Lead is a well-documented human developmental neurotoxicant. Human exposure data and blood lead levels indicate that immunotoxic effects in adult rodents and humans occur within a similar dose range. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is arguably the most researched, and one of the most potent, immunotoxicants in adult mice. The calculated dose that suppresses the antibody response in mice is a single dose of 0.7 μg TCDD/kg, 7 d before immunization. While adult rats are refractory to TCDD-mediated immunosuppression at relatively high doses, the developing rat immune system is sensitive to even lower doses. In addition, TCDD is one of the few compounds that has been evaluated for immunotoxicity in aged animals (Luebke et al., 1999). Tributyltin oxide (TBTO) has been used to prevent fouling of ship's hulls, to prevent growth of organisms in cooling water, and as an anti-mildew agent in paints and stains. The U.S. EPA established the reference dose for TBTO based on changes in resistance to infection in adult rats (Tributyltin oxide [TBTO], 1997). The immunotoxicity of TBTO has also been evaluated in aged (17-mo-old) rats exposed from weaning, and in 1 yr-old rats exposed for an additional 5 mo (Vos et al., 1990).

Diethylstilbestrol

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.

Developmental exposure A single maternal injection of 0.1 mg DES/kg body weight on gestational day (GD) 16 reduced T-cell-mediated delayed-type hypersensitivity (DTH) responses in female, but not in male, mouse offspring, even though thymus weights and T-cell responses to polyclonal stimulation were suppressed in both genders (Luster et al., 1979). Exposure to 5 μg DES/d (approximately 2.2 mg DES/kg/d) over postnatal days (PND) 1–5 decreased T- and B-cell proliferation responses for 17 mo (Kalland et al., 1979) and NK cell activity for 11 mo in females (Kalland, 1980). The NK cell deficit was due to a reduced number of NK cell precursors in the bone marrow, rather than reduced activity on a per-cell basis (Kalland, 1984). The same postnatal DES exposure regimen (Kalland, 1980) reduced the T lymphocyte-dependent antibody response to SRBC by approximately 60% and the T-independent response to bacterial lipopolysaceharide (LPS) by approximately 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 female mice exposed to approximately 2.2 mg/kg/d over PND 1–5 (Kalland & Forsberg, 1978).

Adult exposure Luster et al. (1980) reported suppression of the T-dependent and independent antibody responses and the DTH response in adult female mice exposed to 2 or 8 mg DES/kg/d for 5 d. DTH was decreased in mice dosed with DES after, but not before, antigen sensitization, 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 (*Trichinella spiralis*) infection was suppressed following 5 d of exposure to 0.2 mg DES/kg/d if exposure was started 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).

Summary The LOAEL for immune system effects was essentially the same in adult and newborn female mice, but the effects were far more persistent if exposure occurred during immune system development. Persistence of functional suppression was related to reduced numbers of precursor cells in the bone marrow of neonatally exposed animals.

Diazepam

A systematic evaluation of immune system effects in humans given diazepam has not been reported.

Developmental 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 2.5 mo of life, and returned to control levels by approximately 79 d of age. Exposure during late gestation (GD 16–20) was critical to suppression of the response, as exposure over GD12–16 was without effect. The authors postulated that effects were mediated by DZP binding to the peripheral benzodiazepine receptors, which cannot be detected until GD 16. Cellular and humoral resistance to infection with the nematode parasite *Trichinella spiralis* was also reported in adult offspring of dams given 1.25 mg DZP/kg/d from GD 14 to 20 (Schlumpf et al., 1994). 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 an increased incidence and severity of spontaneous infections in exposed offspring, including bacterial infections of the uterus, kidneys, skin, and salivary glands. 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 DTH 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. Both IgM and IgG responses were suppressed in pups given 3 doses of DZP when evaluated at 7 mo of age.

Adult exposure Descotes et al. (1982) reported that the IgM antibody response and DTH response to sheep red blood cells (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 DTH 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, mice given 1, 2, 4, or 8 mg DZP/kg/d for 3 d were challenged with bacteria (*Klebsiella pneumoniae*). The number of bacteria required to produce an LD_{50} was reduced by 40% in the 1, 2, or 4 mg DZP/kg/d treatment groups and by 72% at the highest DZP dose (Laschi et al., 1983).

Summary Immunosuppression was observed at a lower dose, and results were more persistent, following developmental exposure. Higher doses, which temporarily suppressed immune function in adults, produced suppression that persisted into adulthood or even for an average life span when given to neonates.

Lead

A study of urban children (Lutz et al., 1999) found elevated blood lead levels (BLL) were associated with increased circulating levels of IgE, a class of antibody associated with allergy. Results of studies in occupationally exposed adults range from no effect to decreased levels of total circulating antibody levels and decreased number of T and B lymphocytes.

Developmental exposure Bunn et al. (2001) and Miller et al. (1998) administered lead in the drinking water to female F344 rats for either d 2–21 of gestation or for the 2 wk preceding mating and throughout pregnancy. Numerous immune alterations were observed, particularly in the female pups, including a pronounced reduction in the DTH response (LOAEL = 250 ppm) and IFN-γ production, while production of IL-4 and total serum IgE were elevated (LOAEL = 100 ppm). In the Bunn et al. (2001) study, suppression of the DTH response was associated with a BLL (immediately postexposure) of 38 μg/dl; BLL for the 100 ppm dose in females at birth was 7.6 μg/dl. It is noteworthy that in both of these studies, BLLs at the time of immune assessment (5 and 13 wk of age) were at background levels.

Adult exposure Several studies in adult rodents measured Pb-induced changes in lymphoid populations, cytokine production, IgE levels and the DTH reaction. Koller et al. (1983) noted decreased antibody responses in male SD rats after 6 wk of exposure to 10 ppm lead acetate. 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 LOAEL of 512 ppm. These exposures corresponded to BLLs of 49 μg/dl and 87 μg/dl, respectively.

Summary Similar changes that suggested a shift toward an allergic phenotype and away from responses that protect against infections were reported following developmental or adult exposure to lead. LOAEL values, expressed as ppm lead in drinking water, were roughly the same for both ages. However, BLL in affected offspring generally ranged from undetectable to 10 μg/dl (the current human action level) at the time of functional testing, and effects persisted for 2–13 wk. Effects in adult animals were reported at higher BLL, ranging from 30 to 50 μg/dl.

TCDD

A 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 levels of circulating antibodies or complement, or in lymphoproliferative responses (Mocarelli et al., 1986; Pocchiari et al., 1979). 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 reported a strong inverse relationship between plasma IgG and TCDD levels after adjusting for age, gender, smoking, and consumption of domestic livestock and poultry. However, plasma levels of TCDD did not correlate consistently 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.

Rodent studies suggest that TCDD is a potent developmental immunotoxicant (discussed later), but 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 compounds, expressed as toxic equivalents (TEQ) of TCDD, is considered, several studies found altered immune function and increased susceptibility to infection in breastfed offspring of exposed mothers. For example, in Dutch preschool children, TEQ levels in mothers' breast milk were associated with increased recurrent otitis media and symptoms of respiratory infection (Weisglass-Kuperus et al., 2000). Likewise, offspring of mothers in China and Japan who were 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).

Developmental exposure Blaylock et al. (1992) found that TCDD inhibited thymocyte maturation at a very early stage in mice, and 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. These effects persisted for a very short time. However, exposure during the perinatal period was 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).

Adult exposure Humoral immunity is very sensitive to TCDD exposure in adult mice: Smialowicz et al. (1994) reported an ED_{50} (the dose that suppressed antibody production by 50%) for a single-dose exposure of 0.7 μg TCDD/kg. In marked 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.

Summary Immune system maturity has a marked influence on the immunotoxicity of TCDD in rats, the species typically used for toxicity testing. Cell-mediated immune responses were suppressed for more than half a normal life span in rats when TCDD exposure occurred during immune system development, yet were actually enhanced in TCDD-exposed adult rats.

Tributyltin Oxide

No reports were found that described testing immune function in cells derived from humans exposed to TBTO. Exposure to relatively low (nanomolar) concentrations of TBTO in vitro was shown to rapidly (1 hr) and markedly (80–90%) suppress the cytotoxic activity of human NK cells (Whalen et al., 1999, 2002). Significant suppression was detected at concentrations approximately 10-fold those found in the blood of some heavily exposed humans.

Developmental exposure Smialowicz et al. (1989) exposed preweanling F344 rats by oral gavage to 2.5, 5, or 10 mg TBTO/kg per dose, 3 times/wk for 10 doses. 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.

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 per day. 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 no-observed-adverse-effect level (NOAEL) of 0.025 mg/kg body weight. NK cell activity was reduced at all dose levels after 16 mo of exposure but the data were inconsistent. Data from this study was used by the U.S. EPA IRIS program to set an oral reference dose (RfD) for TBTO in September 1997 (Tributyltin oxide, 1997: RfD = 3×10^{-4} mg/kg-d). Smialowicz et al. (1989) exposed adult rats orally to TBTO by gavage intermittently, as described earlier for developmental exposure (3/wk for a total of 10 doses). Thymus atrophy was the most sensitive endpoint, being observed at the 5-mg/kg treatment level, and lymphocyte proliferation was suppressed in the 10-mg/kg treatment group. This group also reported thymus atrophy and enhanced antibody responses, at 2.5, 5, and 10 mg/kg in rats given daily oral exposures for 10 consecutive days.

Summary The immature immune system appears to be marginally more sensitive to TBTO exposure. However, the consequences of developmental exposure on the immune system are more persistent than those following adult exposure.

IMPLICATIONS FOR RISK ASSESSMENT

The majority of immunotoxicity data have been generated in adult animals. From a practical standpoint, it is important to understand whether data generated in adults are predictive of effects that may occur if the developing immune system is exposed to the same xenobiotic. Based on data generated in animals exposed during immune system development or as adults, reliance on testing only in adults may not always provide accurate data for risk assessment. In the case of diazepam, lead, and tributyltin oxide exposure, invoking an additional safety factor to protect susceptible subpopulations would more than likely provide protection, since immune system effects were similar in both age groups but occurred at doses that were two- to four-fold lower in the developing animal. However, data generated in adults would not predict adverse effects that persisted until offspring reached sexual maturity. Evaluation of DES and higher exposure levels of diazepam only in adults would also detect immunotoxicity, but would grossly underestimate the persistence of effects, particularly in the case of DES. Although the LOAEL for DES-induced immunotoxicity was similar (approximately 2 mg DES/kg/d for 5 d) in adults

and neonates, antibody synthesis in response to immunization was significantly decreased for 6 mo (well into young adulthood) and NK cell activity was suppressed for 11 mo (approximately half the normal life span) in female mice. The most dramatic difference in the outcome of immunotoxicity testing following adult or developmental exposure is exemplified by the effects of dioxin on immune function in rats, the species most commonly used for toxicity testing. While adult exposure to a single relatively high dose of TCDD (30 μg TCDD/kg) increased the antibody response to immunization, a single exposure to 0.1 (males) or 0.3 (females) μg TCDD/kg on gestational day 14 suppressed T-cell-mediated immunity for 14 mo (approximately half the normal life span). Based on these animal data, evidence indicates that testing xenobiotics for immunotoxicity only in adults will not always adequately predict the potential effects of the same xenobiotic on the developing immune system.

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