Immunotoxic Effects of Trichloroethylene and Tetrachloroethylene

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Allergic diseases have been increasing worldwide in industrialized countries. The interplay of genetic and environmental factors is involved in the induction and progression of several types of allergic diseases. Recently, there have been many reports that the increase and spread of allergic diseases are related to chronic exposure to several environmental pollutants, such as diesel exhaust particles and formaldehyde. Trichloroethylene (TCE) and tetrachloroethylene (PCE) are categorized into chlorinated organic compounds, and are the most widely used extensively in various industrial processes. As a consequence of their widespread use, TCE and PCE are becoming environmental pollutants. Generally, TCE and PCE exposure causes organ toxicological effects on the liver, kidney, and the central nervous system; however, studies of TCE and PCE exposure-induced immune modulations are limited. In this review, we summarize research into immunotoxic effects, such as allergy hypersensitivity, autoimmune disease, and immunosuppression, of TCE and PCE from experimental animal studies and human epidemiological studies.

Key words —— trichloroethylene, tetrachloroethylene, immunotoxic effect, allergy, autoimmune disease

INTRODUCTION

Allergic diseases have been increasing in westernized countries[1] and are caused by both genetic and environmental factors.[2, 3] Recently, there have been many reports that environmental pollutants, such as diesel exhaust particles and formaldehyde, interact with the immune system and, in humans and experimental animals, have also been shown to cause immunological changes and to increase several allergic diseases.[4–10] There may be several reasons for this, including increased allergen exposure levels and exposure to allergy-promoting (adjuvant) chemicals and environmental pollutants. Therefore, to increase allergic sensitization, an increase in allergen-specific IgE in humans and in animal studies may be used for hazard identification of an adjuvant chemical effect.

Several chlorinated organic compounds are well known to disturb the central nervous system and induce changes in the parenchymal organs, especially the liver and kidneys.[11, 12] High levels of exposure to chlorinated organic compounds are mainly responsible for toxic effects,[13] whereas moderate exposure induces inflammatory responses.[14]

Among all of the chlorinated organic compounds, trichloroethylene (TCE) and tetrachloroethylene (perchloroethylene; PCE) are the most widely used extensively in various industries, such as metal degreasing processes, dry cleaning agents, paint removers, and components of adhesives and lubricants. As a consequence of their widespread use, TCE and PCE are becoming environmental contaminants and are found in groundwater, drinking water, soil, and indoor/outdoor air in the U.S.A.,[15–18] and inappropriate waste disposal has been associated with linkages and emissions that are potentially harmful to human health and the natural environment.[19, 20] The main exposure routes of TCE and PCE are inhalation (*e.g.*, indoors and outdoors), ingestion

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Table 1. Animal Studies of Allergy and Hypersensitivity

Authors	Speicies	Experimental method	Results
Seo *et al*. (2008)	rat	Intraperitoneal treatment with TCE and PCE	Enhancement of histamine release, increase of IL-4 and TNF-alpha secretion, enhancement of PCA reaction
Seo *et al*. (2008)	rat	TCE (0.3, 3 mg/ml) and PCE (0.1, 1 mg/ml) exposure from drinking water for 2 and 4 weeks	Enhancement of PCA reaction, increase of infiltration of mononuclear leukocytes into dermal skin lesion, increase of mast cell accumulation perivascularly
Kobayashi *et al*. (2010)	mice	TCE (0.3, 3 mg/ml) exposure from drinking water for 2 and 4 weeks	Enhancement ACA reaction, enhancement of antigen-stimulated splenocyte proliferation
Tang *et al*. (2002, 2008)	guinea pig	TCE and TCE metabolize intradermal injection	Skin edma and erythema, skin sensitazation rate 66–71%
Chen *et al*. (2005)	swine	TCE and PCE exposure for swine trachea directly	Increasing prostaglandin E release from swine trachea

(*e.g.*, contaminated drinking water, breast milk, and daily products), and dermal absorption (*e.g.*, bathing in contaminated water). Chronic exposure to TCE and PCE is accompanied by many severe toxicological and pathological problems. The general toxic effects of their exposure are associated with disturbances in the central nervous system and changes in the parenchymal organs in the liver and kidneys.[21–23] Recently, there has been increasing epidemiological and clinical evidence that chronic exposure to several environmental pollutants at levels too low to be overtly toxic can harm human health by altering immune function. Adverse effects on the immune system resulting from environmental pollutants exposure fall within the following immunotoxic effects: allergy hypersensitivity, autoimmunity, and immunosuppression (host resistance). In this review, we summarize the research into the immunotoxic effects of TCE and PCE from experimental animal studies and human epidemiological studies.

ALLERGY AND HYPERSENSITIVITY

Animal Studies

Evidence of the increasing effect of allergy and hypersensitivity by TCE and PCE exposure is limited (Table 1). Seo *et al*. examined the effect of several chlorinated organic compounds on histamine release and inflammatory cytokines production from antigen-stimulated rat mast cells.[24] TCE and PCE exposure significantly increased histamine release and inflammatory cytokines, interleukin (IL)-4 and tumor necrosis factor (TNF)-α, secretion from antigen-stimulated mast cells; moreover, both TCE and PCE administration intraperitoneally enhanced passive cutaneous anaphylaxis

(PCA) reaction in rats. In two other *in vivo* studies, Seo *et al*. examined the enhancing effect of low concentrations of TCE (0.03 or 3 mg/l) or PCE (0.01 or 1 mg/l) ingestion from drinking water on antigen-stimulated allergic responses.[25, 26] TCE or PCE ingestion from drinking water increased the infiltration of mononuclear leukocytes into dermal skin lesions and increased mast-cell accumulation perivascularly, and then enhanced PCA reactions. This was a result of T helper (Th) 1/Th2 cell imbalance and the increase of total IgE in serum. In addition to these adjuvant-like effects of TCE, TCE ingestion from drinking water increases antigen-specific T cell proliferation, and alters antigen-specific IgE and cytokine production from antigen-specific T cells.[27] In the development of immune responses, activated local B cells can act as antigen-presenting cells for CD^{4+} and CD^{8+} T cells.[28] CD^{4+} T cells that recognize major histocompatibility complex (MHC) class II molecules are of two functional types: Th1 and Th2. Th1 cells are specialized to activate macrophages that are promoted by, or have ingested, pathogens. Th2 cells are specialized for B cell activation.[29] There are two main components of the immune response leading to IgE production. The first consists of signals that favor the differentiation of naive Th0 cells to the Th2 phenotype. The second involves the action of cytokines and co-stimulatory signals from Th2 cells that stimulate B cells to switch to producing IgE antibodies. Th2 cells produce IL-4, IL-5, IL-10, and IL-13, and these responses are down-regulated by Th1 cytokines, interferon-γ (IFN-γ), TNF-α, and IL-2.[30–32] IL-4 plays crucial role as an inflammatory mediator in allergic asthma by inducing Th2 inflammation and IgE synthesis.

Tang *et al*. examined TCE-induced delayed hypersensitivity using the guinea pig maximization

Table 2. Human Studies of Allergy and Hypersensitivity

Authors	Location	Source of data (period, sample size, age *etc.*)	Results
Lehmann *et al.* (2002)	Germany	85 neonates (4 weeks of age), indoor air samples (including TCE and PCE), cytokine-secreting CD^{3+} cord-blood T-cells	TCE: Decrease of IL-4 secreting T-cells, increase of IFN-γ secreting T-cells PCE: Decrease of IFN-γ secreting T-cells
Lehmann *et al.* (2001)	Germany	28 children (3 years of age), indoor air samples (including TCE and PCE), cytokine-secreting CD^{3+} and CD^{8+} T-cell populations from blood samples	PCE: No relation between PCE exposure and allergic sensitazation to egg white
Iavicoli *et al.* (2005)	Italy	35 male workers (mean ~33 years of age), workplace TCE measures, serum cytokine levels	TCE: Decrease of IL-4 secretion, increase of IFN-γ secretion
Tanios *et al.* (2004)	U.S.A.	Female worker (42 years of age), historical and physical examination	PCE: Hypersensitivity pneumonitis
Kimijima *et al.* (2007)	Asia	260 Asian patients, TCE concentraiotion	Skin disorder

test. In this test, sensitization rates of 66–71% and skin edema and erythema were observed.[33, 34] Inflammatory cell infiltration was found with an acute intradermal dose of 4500 mg/kg TCE.[34]

In addition to the increase of histamine release from mast cells, TCE and PCE increased prostaglandin release from the tracheal epithelium, particular prostaglandin E2 from swine tracheal epithelium, by modulating the influence of enzyme, cyclooxygenase, or prostaglandin biosynthesis.[35] Thus, TCE and PCE appear to enhance the release of arachidonic acid metabolites, promoting inflammation. Further study is required to elucidate whether prostaglandin production from airway epithelia exposure to TCE and PCE results in airway inflammation.

Human Studies

Allergy and hypersensitivity have not been extensively studied with respect to the effects of TCE or PCE exposure. The available data from studies in humans suggested an association between TCE or PCE exposure and inflammatory cytokine secretion, which represents immune function (Table 2).

Lehmann *et al.* examined cord blood samples from 85 healthy, full-term neonates (4 weeks of age) for cytokine-secreting CD3^{+} (or CD3^{+} and CD8^{+}) T-cells and associated them with indoor air samples (including TCE and PCE). TCE exposure data showed a decrease of IL-4-secreting T-cells and an increase of IFN-γ-secreting T-cells, whereas PCE exposure data showed a significant decrease of IFN-γ-secreting T-cells.[36] Another study examining indoor air samples (including TCE and PCE) and allergic sensitization and cytokine secretion in 28 children (3 year of age) at high risk for the development of allergic disease (*e.g.*, low birth weight, high cord blood IgE, family history of atopy) found no association between PCE exposure and allergic sensitization to egg whites, and an elevated but non-significant association between PCE exposure and allergic sensitization to milk.[37] Iavicoli *et al.* measured cytokine levels in 35 male workers exposed to TCE from a painting area in a factory. Occupational exposure to TCE resulted in significantly increased serum levels of the T cell-derived proinflammatory cytokine IFN-γ.[38] These studies provide weak evidence of an effect of TCE or PCE exposure during childhood on allergic sensitization or exacerbation of asthma; however, the observation of an association between increased TCE or PCE exposure and the decrease of IFN-γ in cord blood samples may reflect a sensitive period of development, and points to the current lack of understanding of the potential immunotoxic effects of prenatal exposure.

Occupational exposure to TCE or PCE has been associated with severe, generalized skin disorder that is distinct from contact dermatitis. PCE exposure may result in immune-mediated organ-specific or systemic effects, as decribed in a case report of hypersensitivity pneumonitis in a 42-year-old female dry cleaning worker.[39] Recently, an increasing number of cases TCE-induced hypersensitivity dermatitis in TCE-exposed workers have been reported. Kamijima *et al.* reviewed case reports describing 260 Asian patients with TCE-induced generalized skin disorders.[40] The measured concentration of TCE ranged from 50 to 4000 mg/m^3, and

Table 3. Animal Studies of Autoimmune Disease

Authors	Strains	Experimantal method	Results
Gilbert *et al.* (1999)	MRL$^{+/+}$ mice	TCE (2.5, 5 mg/ml) exposure from drinking water for 4, 8, and 22 weeks	Increase of ANA and IgG level in serum at 4 and 8 weeks
Griffin *et al.* (2000)	MRL$^{+/+}$ mice	TCE (2.5, 5 mg/ml) exposure from drinking water for 4, 8, and 22 weeks	Enhancement of activation of CD^{4+} cells and increase of IFN-γ secretion at 4 weeks
Griffin *et al.* (2000)	MRL$^{+/+}$ mice	TCE (0.1, 0.5, 2.5 mg/ml) exposure from drinking water for 4 and 32 weeks	Increase of IFN-γ secretion at 4 weeks, enhancement of activation of CD^{4+} cells and increase of ANA at 32 weeks
Khan *et al.* (1995, 2001)	MRL$^{+/+}$ mice	TCE and TCE metabolizes exposure from drinking water	Increase of ANA level and inflammatory cytokine secretion, enhancement of T cell activation
Blossom *et al.* (2004, 2006)	MRL$^{+/+}$ mice	TCE and TCE metabolizes exposure from drinking water	Increase of ANA level and inflammatory cytokine secretion, enhancement of T cell activation
Cai *et al.* (2008)	MRL$^{+/+}$ mice	TCE (0.5 mg/ml) exposure from drinking water for 48 weeks	Infiltration of leukocytes in the portal vein of the liver
Green *et al.* (2001)	MRL-lpr/lpr and MRL$^{+/+}$ mice	PCE (5 mmol/kg) for 24 hr or every fourth day for 6 weeks	Detecting trichloroacetylated protein adducts formed in the liver

exposure scenarios included inhalation only and inhalation with dermal exposure. Disease manifestation generally occurred with 2–5 weeks of initial exposure, with some intervals of up to 3 months.

AUTOIMMUNE DISEASE

Animal Studies

Several mouse strains spontaneously develop conditions resembling systemic lupus erythematosus seen in humans. MRL$^{+/+}$ mice have been extensively used in mechanistic research pertaining to disease pathogenesis and are also most often used in experimental studies of TCE exposure (Table 3).

The initial drinking water study used relatively high TCE concentrations of 2.5 and 5.0 mg/ml, with serologic measurements of antinuclear antibodies (ANA) and IgG levels and assays of activation of CD^{4+} T cells from splenocytes.[41, 42] Another study examined lower TCE exposure levels (0.1, 0.5, and 2.5 mg/ml) and extended the observation to 32 weeks.[43] These studies demonstrated an acceleration of the autoimmune response. T cell activation peaked at 4–8 weeks in the high-dose experiment and at 32 weeks in the lower dose experiment. The higher dose study showed evidence of a reversal of the effects at 22 weeks, with lower levels of CD^{4+} T cell expression and IFN-γ

in exposed mice compared with controls. Moreover, several studies demonstrated the involvement of one or more potential metabolites of TCE, such as dichloroacethyl chloride, trichloroacetaldehyde hydrate, and trichloroacetic acid, in the autoimmune response seen in MRL$^{+/+}$ mice: acceleration of ANA expression, T cell activation, and secretion of inflammatory cytokines.[44–47] Chronic TCE exposure was shown to induce autoimmune hepatitis, characterization by lymphocytic infiltration around the portal vein, in MRL$^{+/+}$ mice.[43, 48]

This evidence of immunological alterations following TCE exposure provides suggestive evidence against PCE, a halogenated solvent that shares some common metabolites with TCE. To date, similar studies in MRL$^{+/+}$ mice have not been conducted with other solvents, so the extent to which these findings pertain to PCE is limited. Only one report has shown the detection of trichloroacetylated protein adducts formed in the liver of MRL-lpr/lpr and MRL$^{+/+}$ mice treated with tetrachloroethylene.[49]

Human Studies

Exposure to organic solvents or chlorinated solvents has been associated with a 2- to 3-fold increased risk of systemic sclerosis (scleroderma) in epidemiological studies summarized in a recent meta-analysis[50] and in subsequent studies (Table 4).[51, 52]

Table 4. Human Studies of Autoimmune Disease

Authors	Location	Source of data (period, sample size, age *etc.*)	Results
Aryal *et al.* (2001)	U.S.A.	Case control study and one cohort study, period 1989–1998	2–3 fold increased risk of scleroderma
Garabrant *et al.* (2003)	U.S.A.	Structured interview, 660 cases and 2227 frequency-matched controls, period 1980–1992	2–3 fold increased risk of scleroderma
Maitre *et al.* (2004)	France	10 men and 83 women diagnosed with scleroderma and 206 age- and sex-matched controls, period 1995–1999	2–3 fold increased risk of scleroderma
Byers *et al.* (1988)	U.K.	23 family members of the patients with leukemia with particular emphasis	Altered ratios of T lymphocyte subpopulations, increased incidence of autoantibodies
Kilburn *et al.* (1992)	U.S.A.	362 subjects exposed toTCE and PCE *etc.* in water obtained from wells	Increased symptoms of lupus erythematosus as perceived by the ARA score and the increased ANA titers
Nietert *et al.* (1998)	U.S.A.	Structured interview, 178 cases mean age 45.2 years, period 1995–1997	Incidence of scleroderma associated with occupational solvent exposure
Lacey *et al.* (1999)	U.S.A.	Structured interview, 205 cases and 2095 population controls, period 1980–1992	Increase of the risk of developing undifferentiated connective tissue disease
Beaudreuil *et al.* (2005)	France	Structured interview, 60 cases of ANCA-positive patients, period 1999–2000	ANCA in plasma partially be related with occupational exposure

A number of epidemiological reports and case studies have suggested that chronic exposure to low-level TCE relates to a variety of autoimmune diseases in humans, including lupus, scleroderma, bullous pemphigoid, diabetes, and immune-mediated hepatitis.[53−55] Byers *et al.* collected serum samples from 23 family members of leukemia patients in Woburn[53] to assess the presence of several autoantibodies, such as ANA, anti-smooth muscle, anti-ovarian, anti-thyroglobulin, and anti-microsomal antibodies, in the family member samples and compared the results with laboratory reference values. None of the serum samples from family members contained anti-thyroglobulin or anti-microsomal antibodies, but ANA was detected in samples from 10 family members (compared with $< 5\%$ expected based on the reference value).

Three case-control studies of undifferentiated connective tissue disease,[56] scleroderma,[51] and antineutrophil cytoplasmic antibody (ANCA)-related diseases[57] provide data concerning dry cleaning work or PCE exposure. As expected in population-based studies, the exposure prevalence is low, with approximately 4% of controls reporting work in dry cleaning and 1% reporting exposure to PCE. The observed associations are generally weak (odds ratios for dry cleaning around 1.5 in 3 large studies of women) and none of the individual studies were statistically significant. The results seen for exposure to PCE in the three studies that attempted this type of assessment were more varied.

IMMUNOSUPPRESSION

Animal Studies

A number of animal studies have examined immunosuppression and host resistance against infection in relation to TCE or PCE exposure by various routes and concentrations. Immunological parameters ware altered in B6C3F1 mice administered a chemical mixture of 25 groundwater contaminants. After 14 or 90 days of exposure, immunosuppressive evidence was observed in immunological parameters with a dose-related decrease in antibody response to sheep red blood cells and decreased host resistance to subsequent challenge to *Plasmodium yoeli*. There was no change in lymphocyte number, T cell subpopulations, natural killer cell activity, or in challenge listeria monocytgens.[58] CD1 mice exhibited increased susceptibility to infectious agents (*e.g.*, *Streptococcus zooepidimicus*, *Klebsiella pneumoniae*) with short-term inhalation of TCE ranging from 2.6 to 48 ppm.[59] In another study, after 3 days of single intraperitoneal injections of TCE in Sprague-Dawley (SD) rats at 0.05, 0.5, or 5 mmol/kg per day and B6C3F mice at 10 mmol/kg per day, natural killer cell activity in the liver was depressed in rats given the highest dose (22% lower than control levels, $p < 0.05$); in mice,

a smaller decrease was reported (14% lower than control levels, no significant difference).[60]

Human Studies

Data on the immunosuppressive effects in humans are very limited. In 1982, Lagakos et al.[11] conducted a telephone survey of Woburn residents to collect information on the residential history and history of 14 medically diagnosed conditions. This survey included 4978 children born since 1960 who had lived in Woburn before they reached 19 years of age. The exposure information was based on estimates of the contribution of water from contaminated wells (including TCE and PCE) in various zones within the town, and this information was used to estimate cumulative exposure based on each child's length of residence in Woburn. A higher cumulative exposure measure was associated with a history of kidney and urinary tract disorders (primarily kidney or urinary infection) and with lung and respiratory disorders (asthma, chronic bronchitis, or pneumonia).

CONCLUSION

Several animal models have been tested to identify chemical allergy;[61] however, although environmental chemical allergens tend to have weak, minimal immunogenicity, these methods have focused on the detection of strong allergic reactions. Although allergic effects of environmental chemical exposure have been studied extensively, allergic effects at low doses remain unknown;[62–64] therefore, there is a need for protocols for the treatment and detection of weakly immunogenic and low doses allergic reactions. A low level of environmental chemical exposure causes breathing problems such as asthma and other allergic reactions.[65, 66] Recently, environmental chemical exposure was found to induce significantly altered expression in genes regulated through the Toll-like receptor signaling pathway, T cell receptor signaling pathway cytokine-cytokine receptor interaction, and natural killer cell-mediated cytotoxicity.[67] This genome-wide expression profile approach will provide a novel mechanism of environmental chemical-induced immunotoxic effects in the future.

In this review, we have discussed several reports on the immunotoxicity of chlorinated organic compounds, especially TCE and PCE; however additional data from inhalation, oral, and dermal exposure of different durations are needed to assess the potential immunotoxicity of TCE and PCE from multiple viewpoints, including immunosuppression, autoimmunity, and allergic sensitization. The lack of data together with the concern that other structurally related solvents are associated with immunotoxicity contributes to uncertainty in the database for TCE and PCE.

Furthermore, many environmental pollutants generally coexist with other chemicals and are exposed to human and animals. Recently, there was a report that coexposure to methylmercury aggravates TCE-induced autoimmune hepatitis in MRL$^{+/+}$ mice compared with single exposure with TCE;[68] therefore, further studies are needed to clarify the immunotoxic evaluations about not only single exposure to TCE or PCE but also coexposure to other environmental pollutants.

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