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Autoimmune potential of perchloroethylene: role of lipid-derived aldehydes

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Abstract

Tetrachloroethene (perchloroethylene, PCE), an ubiquitous environmental contaminant, has been implicated in inducing autoimmunity/autoimmune diseases (ADs), including systemic lupus erythematosus (SLE) and scleroderma in humans. However, experimental evidence suggesting the potential of PCE in mediating autoimmunity is lacking. This study was, therefore, undertaken to explore PCE's potential in inducing/exacerbating an autoimmune response. Six-week old female MRL+/+ mice, in groups of 6 each, were treated with PCE (0.5 mg/ml) via drinking water for 12, 18 and 24 weeks and markers of autoimmunity and oxidative stress were evaluated. PCE exposure led to significant increases in serum anti-nuclear antibodies (ANA), anti-dsDNA and anti-scleroderma-70 (anti-Scl-70) antibodies at 18 weeks and, to a greater extent at 24 weeks, suggesting that PCE exposure exacerbated autoimmunity in our animal model. The increases in autoantibodies were associated with time-dependent increases in malondialdehyde (MDA)-protein adducts and their antibodies, as well as significantly decreased levels of antioxidants GSH and SOD. The splenocytes isolated from mice treated with PCE for 18 and 24 weeks showed greater Th17 cell proliferation and increased release of IL-17 in culture supernatants following stimulation with MDA-mouse serum albumin adducts, suggesting that MDA-modified proteins may act as an immunologic trigger by activating Th17 cells and contribute to PCE-mediated autoimmunity. Our studies thus provide an experimental evidence that PCE induces/exacerbates an autoimmune response and lipid-derived aldehydes (such as MDA) contribute to this response.

Keywords

perchloroethylene; autoimmunity; autoantibody; oxidative stress; lipid-derived aldehydes; Th17 cell

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Conflict of interest statement

The authors declare that there is no conflict of interest.

Introduction

Tetrachloroethene (also known as perchloroethylene, PCE), one of the most widely used chlorinated organic solvents in dry-cleaning, fabric- finishing and metal-degreasing in the United States (US), is an ubiquitous environmental contaminant (Akita et al., 2007; ATSDR, 2014; Gold et al., 2008; Lash and Parker, 2001). Over 400 million pounds of PCE is produced in the US annually, and about 85% of used PCE is lost to the atmosphere, resulting high concentrations of PCE in air in urban or industrial areas (Gold et al., 2008; Lash and Parker, 2001). PCE has been identified in at least 945 of the 1699 hazard waste sites nationally and also in other non-national priorities list sites due to air, water and soil contamination (ATSDR, 2014; Salahudeen, 1998). PCE is also one of the most frequently identified organic contaminants in surface and ground water with up to 25% of drinking water samples in USA contaminated with PCE (Akita et al., 2007; ATSDR, 2014; Williams et al., 2004). The humans may be exposed to PCE occupationally or environmentally via air, water, food and soil (Mckone and Daniels, 1991; Lash and Parker, 2001; ATSDR, 2014). The Agency for Toxic Substances and Disease Registry (ATSDR) estimates that more than 650,000 workers are regularly exposed to PCE (ATSDR, 2014; Gold et al., 2008; Salahudeen, 1998). Therefore, a clear understanding of the potential adverse health effects of PCE, especially systemic toxic effects is warranted.

PCE, besides causing organ toxicity and cancer, has also been implicated in autoimmune diseases (ADs), including scleroderma and systemic lupus erythematosus (SLE)- like symptoms in humans (Aschengrau et al., 2015; Chaigne et al., 2015; Guyton et al., 2014; Kilburn and Warshaw, 1992; Lash and Parker, 2001; Marie et al., 2014; Mutti et al., 1992; Pralong et al., 2009; Salahudeen, 1998). Since Sparrow's first report of a Raynaud's disease along with high titers of anti-nuclear antibodies (ANA) in a subject exposed to PCE exposure (Sparrow, 1977), a number of reports as well as epidemiologic studies have provided further evidence for the involvement of PCE in the development of ADs both from environmental and occupational exposures (Chaigne et al., 2015; Kilburn and Warshaw, 1992; Marie et al., 2014; Mora, 2009; Mutti et al., 1992; Pralong et al., 2009). Evidences are also presented for an association between scleroderma and environmental and/or occupational PCE exposure (Marie et al., 2014; Mora, 2009; Pralong et al., 2009). Remarkably, signs of nephropathies have also been shown in workers occupationally exposed to PCE (Mutti et al., 1992; Salahudeen, 1998). The resident population exposed to low levels of organic solvents including PCE through contaminated water, had higher titers of ANA and greater SLE symptoms compared to referent populations (Kilburn and Warshaw, 1992). The potential of PCE to induce ADs is also supported by the significant association of Sjögren's syndrome with occupational PCE exposure (Chaigne et al., 2015).

Despite PCE's implications in the development of ADs in humans for decades (Chaigne et al., 2015; Kilburn and Warshaw, 1992; Marie et al., 2014; Mutti et al., 1992; Pralong et al., 2009; Sparrow, 1977), the experimental studies to establish the role of PCE in inducing/ exacerbating ADs are lacking and the mechanism(s) contributing to PCE- induced/ exacerbated autoimmunity remains largely unknown. Oxidative stress (including lipid peroxidation) is known to contribute to the pathogenesis of ADs (Khan et al., 2001; Iuchi et al., 2010) and increased oxidative stress is reported in ADs (Grimsrud et al., 2008; Wang et

al., 2010). Lipid-derived aldehydes (LDAs) such as malondialdehyde (MDA), bind covalently with proteins to form MDA-protein adducts (Khan et al., 2002; Ben Mansour et al., 2010), and higher levels of MDA-modified proteins have been observed in AD patients (Frostegard et al., 2005; Kurien and Scofield, 2008; Ben Mansour et al., 2010; Wang et al., 2010), suggesting a potential role of LDAs in ADs. PCE exposure has also been reported to lead to oxidative stress both from in vivo and in vitro studies (Chen et al., 2002; Green et al., 1990; Lash and Parker, 2001; Salahudeen, 1998; Toraason et al., 1999, 2003; Zhu et al., 2005). These evidences led us to hypothesize that PCE exposure could lead to an autoimmune response/disease via its ability to induce oxidative stress. To test our hypothesis, this study using an animal model evaluated, 1) the autoimmune potential of PCE and, 2) contributory role of LDAs (MDA-protein adducts) in PCE-mediated autoimmunity.

Materials and methods

Animals and treatments

MRL/MpJ (MRL+/+) mouse is a model of SLE, and spontaneously develops various autoantibodies after several months and SLE in the second year of life (Anam et al., 2009; Khan et al., 1995, 2001; Wang et al., 2012b). Since SLE develops late in the second year of life and autoantibodies appear several months after their birth, the MRL+/+ mice are an ideal model to study the role of PCE in inducing/exacerbating ADs. Female mice were chosen for this study due to higher susceptibility and prevalence of ADs in females (Danchenko et al., 2006; Frostegard et al., 2005; Margery-Muir et al., 2017; Wang et al., 2010, 2012b). Five-week old female MRL+/+ mice, purchased from The Jackson Laboratories (Bar Harbor, ME), were housed in plastic cages on a bedding of wood chips at the University of Texas Medical Branch (UTMB) animal house facility maintained at ~ 22 °C, 50–60% relative humidity, and a 12h light/dark cycle. The animals were provided standard lab chow and drinking water ad libitum and were acclimated for one week before the treatment. PCE (purity 99+ %, Sigma-Aldrich, St. Louis, MO) was dissolved in drinking water containing 1% Alkamuls EL-620 emulsifier (Rhone-Poulenc, Cranbury, NJ). The experiments were performed in accordance with the guidelines of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee (IACUC) of UTMB. The mice were divided into 6 groups of 6 each and received PCE (0.5 mg/ml) or drinking water containing 1% Alkamuls EL-620 emulsifier only (controls). The dose used in this study is occupationally relevant, based on the current 8h Permissible Exposure Limit (established by Occupational Safety and Health Administration) for PCE of 100 ppm (678 mg/m³), which is ~96 mg/kg/day (Center for Disease Control and Prevention). Also oral LD50 for PCE in mice is 7800 mg/kg (Cederberg et al., 2010). Thus, the dose used is ~1/78th of the LD50. The consumption of PCE-containing drinking water was measured and the water was changed on alternate days. The mice were weighed on a weekly basis to monitor weight changes. After 12, 24 and 36 weeks of PCE treatment, respectively, the animals were euthanized under ketamine/xylazine (120/10 mg/kg, i.p.), and blood was withdrawn. Major organs were removed and weighed. At the same time, spleens were removed immediately and splenocytes were isolated (Wang et al., 2008, 2012a). Individual sera, obtained following blood clotting and centrifugation, were stored in small aliquots at –80 °C until further analysis.

ANA, anti-dsDNA and anti- scleroderma-70 (anti-Scl-70) antibodies in the serum

Serum levels of ANA, anti-dsDNA antibodies and anti-Scl-70 antibodies were determined by using mouse-specific ELISA kits (Alpha Diagnostic Int'l, San Antonio, TX) as described earlier (Khan et al., 1995; Wang et al., 2007, 2012a, 2015).

Quantitation of MDA-protein adducts in the serum

For the quantitation of MDA-protein adducts in the sera from PCE-treated mice and controls, competitive ELISAs were performed as described earlier (Khan et al., 2002; Wang et al., 2007, 2012a, 2015). Briefly, flat bottomed 96-well plates were coated with MDA-ovalbumin adducts or ovalbumin (0.5 μ g/well) overnight at 4 °C. For the competitive ELISA, rabbit anti-MDA sera (1:2000 diluted; Alpha Diagnostics Int'l, San Antonio, TX) were incubated with test samples (standards or unknown) at 4 °C overnight. The coated plates were blocked with a blocking buffer (Sigma-Aldrich) for 2 h at room temperature (RT), then a 50 μ l aliquot of each of the above mentioned incubation mixtures was added to duplicate wells and incubated for 2 h at RT. After washing, 50 μ l of goat anti-rabbit IgG-horseradish peroxidase (HRP; 1:2000 diluted, Millipore, Billerica, MA) was added and incubated for 1 h at RT. After washing, 100 μ l of tetramethylbenzidine (TMB) peroxidase substrate (KPL, Gaithersburg, MD) was added to each well. The reaction was stopped after 10 min by adding 100 μ l 2 M H₂SO₄ and the absorbance was read at 450 nm on a Bio-Rad Benchmark plus microplate spectrophotometer (Bio-Rad Laboratories, Hercules, CA).

Anti-MDA-protein adduct specific antibodies in the serum

An ELISA to analyze anti-MDA-protein adduct-specific antibodies in the mouse serum was performed as described earlier (Khan et al., 2001; Wang et al., 2007, 2012a, 2015). Briefly, flat-bottomed 96-well plates were coated with MDA-ovalbumin adducts or ovalbumin (0.5 μ g/well) overnight at 4 °C. The plates were washed with Tris-buffered saline-Tween 20 (TBST) and the non-specific binding sites were blocked with Tris-buffered saline (TBS) containing 1% bovine serum albumin (BSA; Sigma-Aldrich) at RT for 1 h. After washing extensively with TBST, 50 μ l of 1:100 diluted mouse serum samples were added to duplicate wells of the coated plates and incubated at RT for 2 h. The plates were washed 5 times with TBST and then 50 μ l of rabbit anti-mouse IgG-HRP (1:2000 in TBS; Chemicon, Temecula, CA) was added and incubated at RT for 1 h. After washing, 100 μ l of TMB peroxidase substrate (KPL) was added to each well. The reaction was stopped after 10 min by adding 100 μ l 2 M H₂SO₄ and the OD was read at 450 nm on a Bio-Rad Benchmark plus microplate spectrophotometer (Bio-Rad Laboratories).

Serum superoxide dismutase activity

The superoxide dismutase (SOD) activity in the sera was determined using the Superoxide Dismutase Activity Assay Kit (Abcam, Cambridge, MA) following manufacturer's instruction.

Glutathione content in sera

The contents of reduced (GSH) and oxidized (GSSG) glutathione in the sera were analyzed by using Glutathione Assay kit (Cayman Chemical Co., Ann Arbor, MI). For

deproteinization, an equal volume of 10% (w/v) of metaphosphoric acid was added to the sera. After centrifugation at $2000 \times g$ for 5 min, the resulting supernatants were neutralized with 4 M of triethanolamine (50 μ l per ml supernatant) for the measurement of the GSH and GSSG levels. The quantification was done according to the procedure described in the assay kit and the values were expressed as nmol mg⁻¹ protein.

Flow cytometry analysis for splenocyte proliferation

Splenocytes isolated from spleens of control and PCE-treated mice were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) using CellTrace CFSE Cell Proliferation kit for Flow Cytometry (Molecular Probes, Eugene, OR), and then plated into 24-well flat-bottom plates at 2×10^6 /well in a total volume of 1ml. Native mouse serum albumin (MSA, 10 μ g/ml; Sigma) or MDA-MSA adducts (10 μ g/ml) were added to culture plates, respectively, to stimulate lymphocytes and incubated at 37°C with 5% CO₂ (Khan et al., 2001; Wang et al., 2008). After 72 h, the splenocytes from each well were blocked with anti- mouse CD16/CD32 (Fc γ III/II Receptor, BD Biosciences, San Jose, CA), then stained with anti-mouse IL17-PE (BD Biosciences), and analyzed using a Becton-Dickinson FACS Canto (BD Biosciences).

Determination of IL-17 in splenocyte cultures

Splenocytes isolated from the spleens of control and PCE-treated MRL^{+/+} mice were plated into 24-well flat-bottom plates at 2×10^6 /well in a total volume of 1ml. MSA (10 μ g/ml), MDA-MSA adducts (10 μ g/ml) or anti-CD3/CD28 antibodies (2.5/1.0 μ g/ml) were added, respectively, to stimulate lymphocytes in the culture and incubated at 37 °C with 5% CO₂. After 72 h, culture supernatants from each well were harvested and the release of IL-17 into the cultures was quantitated using specific ELISA kits (Biolegend Inc, San Diego, CA).

Statistical analysis

The values are means $\pm$ SD. One-way ANOVA followed by Tukey-Kramer multiple comparisons test (GraphPad Instat 3 software, La Jolla, CA) was performed for the statistical analysis. Spearman's rank correlation was used to calculate correlation coefficients between serum ANA and anti-MDA-protein antibodies. A *p* value < 0.05 was considered to be statistically significant.

Results

PCE potentiates autoantibody production in MRL^{+/+} mice

Implication of PCE exposure leading to an autoimmune response was reported as early as in 1977 (Sparrow, 1977). Epidemiologic studies following both occupational and environmental exposures to PCE further suggest an association between PCE exposure and autoimmunity in humans (Chaigne et al., 2015; Kilburn and Warshaw, 1992; Mutti et al., 1992; Pralong et al., 2009). To assess and provide an experimental evidence for autoimmune potential of PCE, autoantibodies which are important laboratory evaluation indices and biomarkers of ADs (Egner, 2000; Arbuckle et al., 2003; Reveille, 2004), were determined in the serum of MRL^{+/+} mice treated with PCE for 12, 18 or 24 weeks (Fig. 1). There were moderate but insignificant increases in serum ANA levels in mice treated with PCE for 12

weeks (12% increase) but the increases (42%) were statistically significant at 18 weeks of PCE exposure ($p < 0.05$) compared to respective controls (Fig. 1A). However, PCE-induced increases in ANA were highly remarkable (79%) following 24 weeks of PCE exposure in comparison to the controls ($p < 0.05$, Fig. 1A). Also, the ANA levels in mice treated with PCE for 24 weeks were significantly greater than those treated for 12 or 18 weeks. Similarly, serum anti-dsDNA and anti-Scl-70 antibodies also increased in a time-dependent fashion following PCE exposure. The serum anti-dsDNA and anti-Scl-70 antibodies in the mice treated with PCE for 18 or 24 weeks were significantly greater compared to their respective controls ($p < 0.05$). Furthermore, the anti-dsDNA and anti-Scl-70 levels in mice treated with PCE for 24 weeks were remarkably higher vs. mice exposed for 12 or 18 weeks (Fig. 1B, 1C). These results suggest that PCE exposure induces/exacerbates an autoimmune response in these MRL^{+/+} mice in a time-related pattern.

PCE exposure causes increased formation of MDA-protein adducts in the sera of mice

It has been reported that PCE exposure can induce oxidative stress both in vivo and in vitro (Chen et al., 2002; Green et al., 1990; Lash and Parker, 2001; Salahudeen, 1998; Toraason et al., 1999, 2003; Zhu et al., 2005). To explore the potential role of PCE-induced oxidative stress, such as LPO, in the induction/exacerbation of autoimmune response, we first determined the serum levels of MDA-protein adducts, the markers of oxidative stress, especially LDAs, in MRL^{+/+} mice treated with PCE for 12, 18 or 24 weeks (Fig. 2A). As shown in Fig. 2A, formation of MDA-protein adducts in the mice treated with PCE for 12 weeks increased significantly (38% increases, $p < 0.05$) compared to the controls. The increases in the formation of these adducts were even greater at 18 and 24 weeks (41% and 73% increases, respectively), and were statistically significant compared to their respective controls ($p < 0.05$). Moreover, increases in these adducts in mice treated with PCE for 24 weeks were also significantly greater than mice treated with PCE for 12 or 18 weeks (Fig. 2A).

Increased formation of anti-MDA-protein adduct antibodies in PCE-treated mice

To support our hypothesis that LDA formation could contribute to the PCE-mediated autoimmunity, serum anti-MDA-protein adduct antibodies were also determined (Fig. 2B). Following 12 weeks of PCE exposure, there were significant increases in serum levels of anti-MDA-protein adduct antibodies (46% increase) compared to the controls ($p < 0.05$, Fig. 2B). The increases in anti-MDA-protein adduct antibodies were much greater (53%, 115%, respectively) and significant ($p < 0.05$) after 18 and 24 weeks of PCE exposure compared to the respective controls. Interestingly, the increases in these antibodies in mice treated with PCE for 24 weeks were even greater vs. the mice treated with PCE for 12 or 18 weeks (Fig. 2B). These increases suggest that increased formation of LDA-modified protein adducts could act as immunologic triggers to contribute to PCE-mediated autoimmunity.

Relationship between autoantibodies and anti-MDA-protein antibodies

To evaluate the potential contribution of MDA-modified protein adducts to PCE-mediated autoimmune disorder, we also analyzed the possible relationship of the increased serum ANA with anti-MDA-protein antibodies. The correlation coefficient between serum ANA and anti-MDA-protein antibodies was calculated using Spearman's rank correlation and the

respective values in serum ANA and anti-MDA-protein antibodies of each sample from all three points are plotted in Fig. 3. As evident from Fig. 3, there is an existence of a significantly positive correlation between serum ANA and anti-MDA-protein antibodies, suggesting that the increased anti-MDA-protein antibodies could be multi-specific and may also contribute to increased ANA.

Serum levels of Cu/Zn SOD activity in PCE-treated mice

Increased oxidative stress and impaired antioxidant capacity have been reported in ADs including SLE (Grimsrud et al., 2008; Zaieni et al., 2015; Wang et al., 2016). To assess the redox status in mice, we measured the serum SOD, one of the major antioxidant enzymes. There were only moderate decreases in SOD levels in the mice treated with PCE for 12 weeks compared to the controls. The decreases in serum SOD were significant in the mice treated with PCE for 18 or 24 weeks compared with their respective controls. Furthermore, the SOD levels showed greater decline in mice treated with PCE for 24 weeks in comparison to mice treated with PCE for 12 or 18 weeks ($p < 0.05$, Table 1). This remarkable reduction in serum SOD provides evidence for a compromised antioxidant balance.

Effect of PCE on GSH and GSH/GSSG ratio in the sera

Glutathione (GSH) is a tripeptide antioxidant, which defends against damage to important cellular components from oxidative stress (Pompella et al., 2003; Ballatori et al., 2009). The ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) is a widely used marker for oxidative stress (Rahman et al., 2005; Ballatori et al., 2009; Owen and Butterfield, 2010). Therefore, to further assess the redox status following PCE exposure and evaluate the contribution of oxidative stress in PCE-induced autoimmunity, the glutathione levels were analyzed in the sera. As presented in Table 2, PCE exposure caused significant decreases in serum GSH at 12, 18 and 24 weeks in comparison to their respective controls, with lowest levels seen at 24 weeks which was also significantly lower than that in mice treated with PCE for 12 and 18 weeks. Similar pattern but more remarkable decreases in GSH/GSSG ratio were also observed following PCE treatment (Table 2).

Stimulatory effect of MDA-MSA on Th17 cell proliferation

Th17 cells are key effector T cells involved in the development and pathogenesis of a variety of ADs including SLE and rheumatoid arthritis (RA) (Han et al., 2015; Korn et al., 2009; Perl, 2016; Singh et al., 2014; Tabarkiewicz et al., 2015). To assess the effects of MDA-protein adducts on adaptive immune responses, splenocytes isolated from PCE-treated and control mice were labeled with CFSE and cultured with or without MDA-MSA. Seventy two hours later, splenocytes were intracellularly stained with anti-mouse IL-17 antibodies and Th17 cell proliferation was analyzed using flow cytometry. The splenocytes from PCE-treated mice for 18 or 24 weeks cultured in the presence of MDA-MSA resulted in greater Th-17 cell proliferation compared to those from respective controls (Fig. 4, data shown for 24 weeks only).

IL-17 release into splenocyte cultures following treatment with MDA-MSA

To further evaluate the contribution of MDA-modified proteins in the pathogenesis of PCE-mediated autoimmunity and provide even more compelling support to our hypothesis that oxidative stress mediates an autoimmune response via activation of Th17 cells, splenocytes isolated from PCE-treated and control mice were cultured with or without MDA-MSA or anti-mouse CD3/CD28, and the release of IL-17 in the culture supernatants was quantitated. Evidenced from Fig. 5, the splenocytes from mice treated with PCE for 18 or 24 weeks released significantly greater amount of IL-17 than those from respective control mice ($p < 0.05$) following 72h incubation without MDA-MSA. However, following the stimulation with MDA-MSA, the splenocytes from mice treated with PCE for 18 or 24 weeks not only released much larger amount of IL-17 than those from corresponding controls and the shorter term PCE exposure, but also released significantly greater amount of IL-17 than the splenocytes from the same group of mice without MDA-MSA stimulation. Moreover, after 24 weeks of PCE exposure, the amount in IL-17 release into cultures was 1.0- and 1.1-fold incubated with MSA, and 1.3- and 2.2-fold incubated with MDA-MSA for controls and PCE-treated mice compared to their UN samples, respectively. The results showed significant increases in IL-17 release following MDA-MSA incubation of splenocytes obtained from PCE-treated mice for 18 or 24 weeks, suggesting a potential role of MDA-MSA in activating Th17 cells.

Discussion

PCE exposure has been implicated in the development of ADs in humans both from environmental and occupational exposures (Chaigne et al., 2015; Kilburn and Warshaw, 1992; Marie et al., 2014; Mutti et al., 1992; Pralong et al., 2009). However, experimental studies in animals towards establishing the potential of PCE in mediating an autoimmune response are lacking. Experimental animals which represent as model of certain states of the diseases, also provide critical information on mechanistic aspects of diseases. In our studies, we chose MRL+/+ mice because they are susceptible to ADs and serve well as a model for delayed development of SLE (Anam et al., 2009; Khan et al., 1995, 2001; Wang et al., 2012b). This slow development of AD allows the evaluation of specific effects of agents in relatively young animals (Khan et al., 1995, 2001; Wang et al., 2012b). Thus, to assess the autoimmune potential of PCE and the mechanism(s) contributing to PCE-induced autoimmunity, this study was undertaken in female MRL+/+ mice by treating them with PCE. Here we provide first evidence that PCE induces/exacerbates an autoimmune response and a role of oxidative stress (LDAs) in PCE-mediated autoimmunity.

ADs include more than 80 medical disorders involving up to 5% of population (Hayter and Cook, 2012; Vojdani, 2014). The etiology of these diseases are not fully understood, however, multiple factors, including genetic, hormonal and environmental triggers are thought to contribute to the pathogenesis of these diseases. As there have been remarkable increases in the incidence of ADs worldwide, research dedicated to environmental factors such as chemicals in contributing to autoimmunity has also grown significantly over the years (Barragán-Martínez et al., 2012; Hayter and Cook, 2012; Selmi et al., 2012; Vojdani, 2014). Via this study, for the first time, we provide evidence to support that PCE exposure

induces/exacerbates an autoimmune response in MRL $+/+$ mice, as evident from increased levels of various autoantibodies, including ANA, anti-dsDNA antibodies and anti-Scl-70 antibodies, which are established biomarkers of ADs (Egner, 2000; Arbuckle et al., 2003; Reveille, 2004; Callado et al., 2007). Increases in ANA and anti-dsDNA antibodies support PCE's potential to induce/exacerbate SLE-like disease in this animal model. Our findings on increased anti-Scl-70 antibodies are remarkable as they also serve as biomarkers of scleroderma (Callado et al., 2007), one of the systemic immune disorders observed in humans following PCE exposure (Marie et al., 2014; Pralong et al., 2009). Thus, the time-related increases in ANA, anti-dsDNA antibodies and anti-Scl-70 antibodies in MRL $+/+$ mice following PCE exposure in this study provide support to reports that PCE mediates autoimmune disorders in humans (Chaigne et al., 2015; Kilburn and Warshaw, 1992; Marie et al., 2014; Mutti et al., 1992; Pralong et al., 2009). These findings are highly significant as they clearly provide experimental evidence that PCE exposure is capable of inducing/exacerbating an autoimmune disorder. Furthermore, the study also validates the use of MRL $+/+$ mice as a potential animal model to study pathogenesis and mechanisms of PCE-mediated autoimmunity.

Increasing evidence supports that oxidative stress could play a role in the pathogenesis of ADs (Hayter and Cook, 2012; Khan et al., 2001; Wang et al., 2010, 2016; Vojdani, 2014). Studies have documented that PCE exposure is associated with oxidative stress as evident from increases in lipid peroxidation and oxidative DNA damage (Chen et al., 2002; Green et al., 1990; Lash and Parker, 2001; Salahudeen, 1998; Toraason et al., 1999, 2003; Zhu et al., 2005). This led us to assess the potential association of PCE-mediated oxidative stress (lipid peroxidation) with PCE-induced/exacerbated autoimmunity. It is evident from our data that PCE exposure resulted in increased formation of MDA-modified protein adducts and anti-MDA-protein adduct antibodies in a time-related manner. Interestingly, the serum levels of MDA-modified protein adducts and anti-MDA-protein adduct antibodies were significantly increased following 12 weeks of PCE exposure, a time at which no remarkable/significant increases in other autoantibodies (ANA and anti-scl-70 antibodies) were noticed. The data on the formation of these adducts and their antibodies further suggest that oxidative stress could be an early mechanism in the pathogenesis of an autoimmune response. It remains to be verified if neoantigens generated via LDAs could be an early trigger in PCE-mediated autoimmunity. Furthermore, it could be highly interesting to see if anti-MDA antibodies have multi-specificity and, at least partially, also contribute to formation of other conventional autoantibodies. More importantly, our data show existence of positive relationship between anti-MDA-protein antibodies and increased serum ANA. Nevertheless, our findings not only suggest that PCE exposure promotes lipid peroxidation, but also provide support to the idea that increased formation of MDA-modified proteins could trigger the formation of neoantigens to elicit/drive an autoimmune response.

The adverse effects of oxidative stress are prevented by the antioxidant defense mechanisms including enzymatic and non-enzymatic antioxidant defense systems under physiological conditions (Ballatori et al., 2009; Grimsrud et al., 2008; Kemp et al., 2008; Zaieni et al., 2015). SOD and GSH serve as the major enzymatic and non-enzymatic antioxidant defense mechanisms. SOD and GSH/GSSG ratio also serve as important indicators of redox status (Ballatori et al., 2009; Grimsrud et al., 2008; Kemp et al., 2008; Zaieni et al., 2015). In view

of increased oxidative stress, it was logical to assess the redox state following PCE exposure. As evident from the results, PCE exposure led to time-related decreases in SOD as well as GSH and GSH/GSSG ratios in the sera. The decreases in SOD activity along with the decreases in GSH, particularly time-related decreases in GSH/GSSG ratio provide support to a compromised defense mechanism contributing to oxidative stress following PCE exposure and thus, a potential role in PCE-mediated autoimmunity.

Th17 cells, which produce IL-17, IL-21 and IL-22, play a critical role in the development and pathogenesis of a variety of ADs, including SLE, RA and multiple sclerosis (Han et al., 2015; Korn et al., 2009; Perl, 2016; Singh et al., 2014; Tabarkiewicz et al., 2015). Recent studies suggest the potential role of LDA-modified endogenous proteins in the ADs (Kurien and Scofield, 2008; Grimsrud et al., 2008; Wang et al., 2010, 2016; Ben Mansour et al., 2010; Otaki et al., 2010). To evaluate autoimmune potential of LDA-protein adducts, we stimulated splenocytes obtained from PCE-treated and control mice in culture with MDA-MSA and found that MDA-MSA was not only able to increase Th17 cell proliferation, but also resulted in an increased release of IL-17 from splenocytes isolated from PCE-treated mice in a time-related fashion. The Th17 cytokines, such as pro-inflammatory cytokine IL-17, have been involved in disease progression and pathogenesis of various ADs, and are considered as potential biomarkers for renal involvement in SLE (Han et al., 2015; Korn et al., 2009; Onishi and Gaffen, 2010; Perl, 2016; Singh et al., 2014; Tabarkiewicz et al., 2015). Increased IL-17 may consequently induce its target gene products including cytokines, chemokines and inflammatory effectors to promote development of ADs (Onishi and Gaffen, 2010). Therefore, our findings provide an evidence that MDA-protein adducts could contribute to autoimmunity via activating Th17 cells and increased release of IL-17. These findings thus, strongly support the potential contribution of oxidative stress in the pathogenesis of PCE-mediated autoimmunity and also provide evidence that the MDA-modified proteins may contribute to autoimmunity via activating Th17 cells.

In summary, this study, for the first time, demonstrates a link between PCE exposure and autoimmune response in an animal model. The PCE-mediated increases in autoantibodies were associated with increased oxidative stress. Our data further show that MDA-modified proteins can act as an immunologic trigger by activating Th17 cells, as evidenced by increased cell proliferation and IL-17 release. Our observations, therefore, strongly support that at occupationally relevant dose, PCE exposure can induce/exacerbate autoimmune response, and LDAs play a critical role in triggering this response via activating Th17 cells. Future studies will focus on identifying distinct pathways by which oxidative stress contributes to autoimmunity, especially the role of gene expression, knocking out/down target genes, mapping proteome and blocking/inhibiting specific signaling pathways to provide better understanding of PCE-mediated autoimmune disorders.

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References

1. Akita Y, Carter G, Serre ML. Spatiotemporal nonattainment assessment of surface water tetrachloroethylene in New Jersey. *J. Environ. Qual.* 2007; 36:508–520. [PubMed: 17332255]
2. Anam K, Amare M, Naik S, Szabo KA, Davis TA. Severe tissue trauma triggers the autoimmune state systemic lupus erythematosus in the MRL/ lupus-prone mouse. *Lupus.* 2009; 18:318–331. [PubMed: 19276300]
3. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* 2003; 349:1526–1533. [PubMed: 14561795]
4. Aschengrau A, Winter MR, Vieira VM, Webster TF, Janulewicz PA, Gallagher LG, Weinberg J, Ozonoff DM. Long-term health effects of early life exposure to tetrachloroethylene (PCE)-contaminated drinking water: a retrospective cohort study. *Environ. Health.* 2015; 14:36. [PubMed: 25889838]
5. ATSDR (the Agency for Toxic Substances and Disease Registry). Toxicological profile for tetrachloroethylene. ATSDR, US Department of Health and Human Services; Atlanta, GA: 2014.
6. Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. *Biol. Chem.* 2009; 390:191–214. [PubMed: 19166318]
7. Barragán-Martínez C, Speck-Hernández CA, Montoya-Ortiz G, Mantilla RD, Anaya JM, Rojas-Villarraga A. Organic solvents as risk factor for autoimmune diseases: a systematic review and meta-analysis. *PLoS One.* 2012; 7:e51506. [PubMed: 23284705]
8. Ben Mansour R, Lassoued S, Elgaied A, Haddouk S, Marzouk S, Bahloul Z, Masmoudi H, Attia H, Aifa MS, Fakhfakh F. Enhanced reactivity to malondialdehyde-modified proteins by systemic lupus erythematosus autoantibodies. *Scand. J. Rheumatol.* 2010; 39:247–253. [PubMed: 20429675]
9. Callado MR, Viana VS, Vendramini MB, Leon EP, Bueno C, Velosa AP, Teodoro WR, Yoshinari NH. Autoantibody profile in the experimental model of scleroderma induced by type V human collagen. *Immunology.* 2007; 122:38–46. [PubMed: 17442023]
10. CDC (Centers for Disease Control and Prevention). National Institute for Occupational Safety and Health (NIOSH) : Workplace safety and health topics for tetrachloroethylene, Occupational health guideline for tetrachloroethylene 1978. p. 1-5. <https://www.cdc.gov/niosh/topics/tetrachloro/>
11. Cederberg H, Henriksson J, Binderup ML. DNA damage detected by the alkaline comet assay in the liver of mice after oral administration of tetrachloroethylene. *Mutagenesis.* 2010; 25:133–138. [PubMed: 19892777]
12. Chaigne B, Lasfargues G, Marie I, Hüttenberger B, Lavigne C, Marchand-Adam S, Maillot F, Diot E. Primary Sjögren's syndrome and occupational risk factors: A case-control study. *J. Autoimmun.* 2015; 60:80–85. [PubMed: 25963068]
13. Chen SJ, Wang JL, Chen JH, Huang RN. Possible involvement of glutathione and p53 in trichloroethylene- and perchloroethylene- induced lipid peroxidation and apoptosis in human lung cancer cells. *Free Radic. Biol. Med.* 2002; 33:464–472. [PubMed: 12160929]
14. Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus.* 2006; 15:308–318. [PubMed: 16761508]
15. Egner W. The use of laboratory tests in the diagnosis of SLE. *J. Clin. Pathol.* 2000; 53:424–432. [PubMed: 10911799]
16. Frostegard J, Svenungsson E, Wu R, Gunnarsson I, Lundberg IE, Klareskog L, Horkko S, Witztum JL. Lipid peroxidation is enhanced in patients with systemic lupus erythematosus and is associated with arterial and renal disease manifestations. *Arthritis Rheum.* 2005; 52:192–200. [PubMed: 15641060]
17. Gold LS, De Roos AJ, Waters M, Stewart P. Systematic literature review of uses and levels of occupational exposure to tetrachloroethylene. *J. Occup. Environ. Hyg.* 2008; 5:807–839. [PubMed: 18949603]
18. Green T, Odum J, Nash JA, Foster JR. Perchloroethylene- induced rat kidney tumors: an investigation of the mechanisms involved and their relevance to humans. *Toxicol. Appl. Pharmacol.* 1990; 103:77–89. [PubMed: 1969182]

19. Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. Oxidative stress and covalent modification of protein with bioactive aldehydes. *J. Biol. Chem.* 2008; 283:21837–21841. [PubMed: 18445586]
20. Guyton KZ, Hogan KA, Scott CS, Cooper GS, Bale AS, Kopylev L, Barone S, Makris SL, Glenn B, Subramaniam RP, Gwinn MR, Dzibow RC, Chiu WA. Human health effects of tetrachloroethylene: key findings and scientific issues. *Environ. Health Perspect.* 2014; 122:325–334. [PubMed: 24531164]
21. Han L, Yang J, Wang X, Li D, Lv L, Li B. Th17 cells in autoimmune diseases. *Front Med.* 2015; 9:10–19. [PubMed: 25652649]
22. Hayter SM, Cook MC. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. *Autoimmun. Rev.* 2012; 11:754–765. [PubMed: 22387972]
23. Iuchi Y, Kibe N, Tsunoda S, Suzuki S, Mikami T, Okada F, Uchida K, Fujii J. Implication of oxidative stress as a cause of autoimmune hemolytic anemia in NZB mice. *Free Radic. Biol. Med.* 2010; 48:935–944. [PubMed: 20079426]
24. Kemp M, Go YM, Jones DP. Nonequilibrium thermodynamics of thiol/disulfide redox systems: a perspective on redox systems biology. *Free Radic. Biol. Med.* 2008; 44:921–937. [PubMed: 18155672]
25. Khan MF, Kaphalia BS, Prabhakar BS, Kanz MF, Ansari GAS. Trichloroethene- induced autoimmune response in female MRL +/+ mice. *Toxicol. Appl. Pharmacol.* 1995; 134:155–160. [PubMed: 7676450]
26. Khan MF, Wu X, Ansari GAS. Anti-malondialdehyde antibodies in MRL+/+ mice treated with trichloroethene and dichloroacetyl chloride: possible role of lipid peroxidation in autoimmunity. *Toxicol. Appl. Pharmacol.* 2001; 170:88–92. [PubMed: 11162772]
27. Khan MF, Wu X, Tipnis UR, Ansari GA, Boor PJ. Protein adducts of malondialdehyde and 4-hydroxynonenal in livers of iron loaded rats: quantitation and localization. *Toxicology.* 2002; 173:193–201. [PubMed: 11960672]
28. Kilburn KH, Warshaw RH. Prevalence of symptoms of systemic lupus erythematosus (SLE) and of fluorescent antinuclear antibodies associated with chronic exposure to trichloroethylene and other chemicals in well water. *Environ. Res.* 1992; 57:1–9. [PubMed: 1740091]
29. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu. Rev. Immunol.* 2009; 27:485–517. [PubMed: 19132915]
30. Kurien BT, Scofield RH. Autoimmunity and oxidatively modified autoantigens. *Autoimmun. Rev.* 2008; 7:567–573. [PubMed: 18625446]
31. Lash LH, Parker JC. Hepatic and renal toxicities associated with perchloroethylene. *Pharmacol. Rev.* 2001; 53:177–208. [PubMed: 11356983]
32. Margery-Muir AA, Bundell C, Nelson D, Groth DM, Wetherall JD. Gender balance in patients with systemic lupus erythematosus. *Autoimmun. Rev.* 2017; 16:258–268. [PubMed: 28137478]
33. Marie I, Gehanno JF, Bubenheim M, Duval-Modeste AB, Joly P, Dominique S, Bravard P, Noël D, Cailleux AF, Weber J, Lagoutte P, Benichou J, Levesque H. Prospective study to evaluate the association between systemic sclerosis and occupational exposure and review of the literature. *Autoimmun. Rev.* 2014; 13:151–156. [PubMed: 24129037]
34. McKone TE, Daniels JI. Estimating human exposure through multiple pathways from air, water, and soil. *Regul. Toxicol. Pharmacol.* 1991; 13:36–61. [PubMed: 2024045]
35. Mora GF. Systemic sclerosis: environmental factors. *J. Rheumatol.* 2009; 36:2383–2396. [PubMed: 19797508]
36. Mutti A, Alinovi R, Bergamaschi E, Biagini C, Cavazzini S, Franchini I, et al. Nephropathies and exposure to perchloroethylene in dry-cleaners. *Lancet.* 1992; 340:189–193. [PubMed: 1353133]
37. Onishi RM, Gaffen SL. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. *Immunology.* 2010; 129:311–321. [PubMed: 20409152]
38. Otaki N, Chikazawa M, Nagae R, Shimozu Y, Shibata T, Ito S, Takasaki Y, Fujii J, Uchida K. Identification of a lipid peroxidation product as the source of oxidation-specific epitopes recognized by anti-DNA autoantibodies. *J. Biol. Chem.* 2010; 285:33834–33842. [PubMed: 20736172]
39. Owen JB, Butterfield DA. Measurement of oxidized/reduced glutathione ratio. *Methods Mol. Biol.* 2010; 648:269–277. [PubMed: 20700719]

40. Perl A. Activation of mTOR (mechanistic target of rapamycin) in rheumatic diseases. *Nat. Rev. Rheumatol.* 2016; 12:169–182. [PubMed: 26698023]
41. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. The changing faces of glutathione, a cellular protagonist. *Biochem. Pharmacol.* 2003; 66:1499–1503. [PubMed: 14555227]
42. Pralong P, Cavailles A, Balme B, Cottin V, Skowron F. Diffuse systemic sclerosis after occupational exposure to trichloroethylene and perchloroethylene. *Ann. Dermatol. Venereol.* 2009; 136:713–717. [PubMed: 19801256]
43. Rahman I, Biswas SK, Jimenez LA, Torres M, Forman HJ. Glutathione, stress responses, and redox signaling in lung inflammation. *Antioxid. Redox Signal.* 2005; 7:42–59. [PubMed: 15650395]
44. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus.* 2004; 13:290–297. [PubMed: 15230281]
45. Salahudeen AK. Perchloroethylene- induced nephrotoxicity in dry-cleaning workers: is there a role for free radicals? *Nephrol. Dial. Transplant.* 1998; 13:1122–1124. [PubMed: 9623539]
46. Schmidt T, Paust HJ, Krebs CF, Turner JE, Kaffke A, Bennstein SB, et al. Function of the Th17/interleukin-17A immune response in murine lupus nephritis. *Arthritis Rheumatol.* 2015; 67:475–487. [PubMed: 25385550]
47. Selmi C, Leung PS, Sherr DH, Diaz M, Nyland JF, Monestier M, Rose NR, Gershwin ME. Mechanisms of environmental influence on human autoimmunity: a National Institute of Environmental Health Sciences expert panel workshop. *J. Autoimmun.* 2012; 39:272–84. [PubMed: 22749494]
48. Singh RP, Hasan S, Sharma S, Nagra S, Yamaguchi DT, Wong DT, Hahn BH, Hossain A. Th17 cells in inflammation and autoimmunity. *Autoimmun. Rev.* 2014; 13:1174–81. [PubMed: 25151974]
49. Sparrow GP. A connective tissue disorder similar to vinyl chloride disease in a patient exposed to perchlorethylene. *Clin. Exp. Dermatol.* 1977; 2:17–22. [PubMed: 862217]
50. Tabarkiewicz J, Pogoda K, Karczmarczyk A, Pozarowski P, Giannopoulos K. The Role of IL-17 and Th17 Lymphocytes in Autoimmune Diseases. *Arch. Immunol. Ther. Exp.* 2015; 63:435–449.
51. Toraason M, Clark J, Dankovic D, Mathias P, Skaggs S, Walker C, Werren D. Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene. *Toxicology.* 1999; 138:43–53. [PubMed: 10566590]
52. Toraason M, Butler MA, Ruder A, Forrester C, Taylor L, Ashley DL, Mathias P, Marlow KL, Cheever KL, Krieg E, Wey H. Effect of perchloroethylene, smoking, and race on oxidative DNA damage in female dry cleaners. *Mutat. Res.* 2003; 539:9–18. [PubMed: 12948810]
53. Vojdani A. A Potential Link between Environmental Triggers and Autoimmunity. *Autoimmune Dis.* 2014; 2014:437231. [PubMed: 24688790]
54. Wang G, Ansari GA, Khan MF. Involvement of lipid peroxidation-derived aldehyde-protein adducts in autoimmunity mediated by trichloroethene. *J. Toxicol. Environ. Health A.* 2007; 70:1977–1985. [PubMed: 17966069]
55. Wang G, König R, Ansari GAS, Khan MF. Lipid peroxidation-derived aldehyde-protein adducts contribute to trichloroethene-mediated autoimmunity via activation of CD4+ T cells. *Free Radic. Biol. Med.* 2008; 44:1475–1482. [PubMed: 18267128]
56. Wang G, Pierangeli SS, Papalardo E, Ansari GA, Khan MF. Markers of oxidative and nitrosative stress in systemic lupus erythematosus: correlation with disease activity. *Arthritis Rheum.* 2010; 62:2064–2072. [PubMed: 20201076]
57. Wang G, Wang J, Fan X, Ansari GA, Khan MF. Protein adducts of malondialdehyde and 4-hydroxynonenal contribute to trichloroethene-mediated autoimmunity via activating Th17 cells: dose- and time-response studies in female MRL+/+ mice. *Toxicology.* 2012a; 292:113–122. [PubMed: 22178267]
58. Wang G, Li H, Firoze Khan M. Differential oxidative modification of proteins in MRL+/+ and MRL/lpr mice: Increased formation of lipid peroxidation-derived aldehyde-protein adducts may contribute to accelerated onset of autoimmune response. *Free Radic. Res.* 2012b; 46:1472–1481. [PubMed: 22950782]

59. Wang G, Wakamiya M, Wang J, Ansari GA, Khan FM. iNOS null MRL^{+/+} mice show attenuation of trichloroethene-mediated autoimmunity: contribution of reactive nitrogen species and lipid-derived reactive aldehydes. *Free Radic. Biol. Med.* 2015; 89:770–776. [PubMed: 26472195]
60. Wang G, Pierangeli SS, Willis R, Gonzalez EB, Petri M, Khan MF. Significance of Lipid-Derived Reactive Aldehyde-Specific Immune Complexes in Systemic Lupus Erythematosus. *PLoS One*. 2016; 11:e0164739. [PubMed: 27749917]
61. Williams PR, Benton L, Sheehan PJ. The risk of MTBE relative to other VOCs in public drinking water in California. *Risk Anal.* 2004; 24:621–634. [PubMed: 15209934]
62. Zaieni SH, Derakhshan Z, Sariri R. Alternations of salivary antioxidant enzymes in systemic lupus erythematosus. *Lupus*. 2015; 24:1400–1405. [PubMed: 26113360]
63. Zhu QX, Shen T, Ding R, Liang ZZ, Zhang XJ. Cytotoxicity of trichloroethylene and perchloroethylene on normal human epidermal keratinocytes and protective role of vitamin E. *Toxicology*. 2005; 209:55–67. [PubMed: 15725514]

Highlights

- PCE induced/exacerbated an autoimmune response in MRL^{+/+} mice.
- PCE led to increases in lipid-derived aldehyde (LDA)-protein adducts in MRL^{+/+} mice.
- LDAs contribute to PCE-mediated autoimmunity via activating Th17 cells.

Fig. 1. Serum levels of ANA (A), anti-dsDNA antibodies (B) and anti-Scl-70 antibodies (C) in MRL^{+/+} mice treated with PCE for 12, 18 or 24 weeks. The values are means $\pm$ SD. * $p < 0.05$ vs. controls; # $p < 0.05$ vs. PCE-exposed for 12 and/or 18 weeks.

Fig. 2. MDA-protein adducts (A) and anti-MDA-protein adduct antibodies (B) in the sera of MRL +/+ mice treated with PCE for 12, 18 or 24 weeks. The values are means $\pm$ SD. * $p < 0.05$ vs. controls; # $p < 0.05$ vs. PCE-exposed for 12 and/or 18 weeks.

Fig. 3.
Correlation of serum ANA and anti-MDA-protein antibodies.

Fig. 4.

Th-17 cell proliferation following 24 weeks of PCE exposure. CFSE- labeled splenocytes were cultured with or without MDA-MSA adducts. After 72 h, cells were stained and analyzed by flow cytometry. The data is representative of samples from mice treated with PCE for 24 weeks. The values are means $\pm$ SD. * $p < 0.05$ vs. controls; # $p < 0.05$ vs. without MDA-MSA.

Fig. 5.

IL-17 release in the culture supernatants of splenocytes from control and PCE-treated mice following 12, 18 or 24 weeks of PCE exposure. Splenocytes were stimulated with MSA alone, MDA-MSA or anti-CD3/CD28 antibodies for 72 h. US: un-stimulated cells. *p < 0.05 vs. respective controls; #p < 0.05 vs. stimulated cells from mice exposed to PCE for 12 and/or 18 weeks; +p < 0.05 vs. US and MSA alone in the same group.

Table 1

The serum levels of SOD in control and PCE-treated mice

	12 Weeks	18 Weeks	24 Weeks
Controls	41.6±2.7	40.7±2.5	38.5±2.8
PCE-treated	38.5±3.6	34.2±3.3 *	29.6±3.1 *#

The SOD levels are expressed as U/ml and are means ± SD.

* p < 0.05 vs. controls;

p < 0.05 vs. PCE-exposed for 12 and 18 weeks.

Table 2

GSH and GSSG in the kidneys of control and PCE-treated mice

	GSH	GSSG	GSH/GSSG
12 Weeks			
Controls	1.88±0.12	0.12±0.01	15.93±1.04
PCE-treated	1.42±0.15 *	0.12±0.02	11.52±1.39 *
18 Weeks			
Controls	1.83±0.12	0.13±0.01	14.12±1.26
PCE-treated	1.36±0.13 *	0.15±0.03	9.46±1.31 *#
24 Weeks			
Controls	1.63±0.12	0.13±0.02	12.46± 1.27
PCE-treated	0.96±0.11 *#	0.17±0.03 *#	5.85± 1.2 *#

GSH and GSSG values are expressed as nmol/mg protein and are means ± SD.

* p < 0.05 vs. controls;

p < 0.05 vs. PCE-treated mice for 12 and/or 18 weeks.