

## Short report

## Occupational trichloroethylene exposure and antinuclear antibodies: a cross-sectional study in China

Mark Purdue <sup>1</sup>, Luoping Zhang <sup>2</sup>, Roel Vermeulen <sup>3</sup>, Martyn T Smith,<sup>2</sup> Wei Hu,<sup>1</sup> Jongeun Rhee,<sup>1</sup> Cuiju Wen,<sup>4</sup> Yongshun Huang,<sup>4</sup> Xiaojiang Tang,<sup>4,5</sup> Sonja I Berndt,<sup>1</sup> Ashley A Frazer-Abel,<sup>6</sup> Kevin D Deane,<sup>6</sup> Nathaniel Rothman,<sup>1</sup> Qing Lan<sup>1</sup>

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/oemed-2022-108266>).

<sup>1</sup>Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland, USA

<sup>2</sup>School of Public Health, University of California Berkeley, Berkeley, California, USA

<sup>3</sup>Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

<sup>4</sup>Guangdong Province Hospital for Occupational Disease Prevention and Treatment, Guangzhou, Guangdong, China

<sup>5</sup>Zhuhai BesTest Biotechnology Co. Ltd, Zhuhai, Guangdong, China

<sup>6</sup>Department of Medicine, University of Colorado Anschutz Medical Campus, Denver, Colorado, USA

## Correspondence to

Dr Mark Purdue, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, USA; [purdue@mail.nih.gov](mailto:purdue@mail.nih.gov)

KDD, NR and QL contributed equally.

Received 2 February 2022

Accepted 16 April 2022

Published Online First

3 May 2022



Check for updates

© Author(s) (or their employer(s)) 2022. No commercial re-use. See rights and permissions. Published by BMJ.

**To cite:** Purdue M, Zhang L, Vermeulen R, et al. *Occup Environ Med* 2022;**79**:717–720.

## ABSTRACT

**Objectives** There has been concern over the possible risk of autoimmune diseases from exposure to trichloroethylene (TCE), an industrial solvent and common pollutant near hazardous waste sites. Studies of TCE-exposed lupus-prone mouse strains have reported increases in serum antinuclear antibodies (ANAs), a marker of autoimmunity, and autoimmune pathologic changes, while epidemiologic studies have provided limited support for an association between TCE exposure and scleroderma. To investigate exposure-related biologic evidence of autoimmunity in humans, we measured ANA levels in sera from a cross-sectional study of TCE-exposed (n=80) and TCE-unexposed (n=96) workers in Guangdong, China.

**Methods** Full-shift personal air exposure measurements for TCE were taken prior to blood collection. Serum ANAs were detected by immunofluorescence on HEp-2 cells. We calculated ORs and 95% CI relating levels of TCE exposure (categorised using tertiles as cut-points) and ANA positivity (1+ intensity at 1:320 dilution) using multivariable logistic regression.

**Results** Samples from 16 of 176 participants were ANA-positive. We found higher levels of TCE exposure (concentrations > 17.27 ppm) to be associated with an elevated odds of ANA positivity (OR 4.7, 95% CI 1.3 to 16.8) compared with unexposed controls. This association remained after excluding two subjects with diagnosed autoimmune disease (OR 4.5, 95% CI 1.2 to 16.2). We did not observe an association with ANAs at lower exposure levels.

**Conclusions** Our findings, to our knowledge the first direct human evidence of an association between TCE exposure and systemic autoimmunity, provide biologic plausibility to epidemiologic evidence relating TCE and autoimmune disease.

## INTRODUCTION

Trichloroethylene (TCE) is a synthetic chlorinated hydrocarbon that has been used since the early 20th century as an industrial solvent for cleaning metal parts, dry cleaning clothes and other applications. TCE is also a common soil and water pollutant, released into the environment primarily through industrial wastewater and leaching from hazardous waste sites.

## Key Messages

## What is already known on this topic?

⇒ Epidemiologic studies have suggested an association between exposure to trichloroethylene (TCE) and autoimmune diseases. Human mechanistic evidence relating TCE exposure to molecular markers of autoimmunity could help clarify whether a causal relationship exists.

## What this study adds?

⇒ In a cross-sectional study conducted in China, workers with high TCE exposures (concentrations > 17.27 ppm) measured from personal monitoring badges were significantly more likely than unexposed workers to have serum antinuclear antibodies, a marker of autoimmunity.

## How this study might affect this research, practice and/or policy?

⇒ As the exposure levels at which we observed this association are substantially lower than the current US Occupational Safety and Health Administration permissible exposure limit for TCE (100 ppm 8-hour time-weighted average), stricter limits may be warranted.

There has been longstanding concern over the risk of autoimmune disorders from TCE, originating with case reports of exposed workers diagnosed with scleroderma.<sup>1</sup> Later epidemiologic studies have provided limited support for an association between TCE exposure and scleroderma among men, but inconsistent evidence for women.<sup>1–4</sup> Experimental studies of lupus-prone mouse strains have found consistent evidence that TCE exposure induces an accelerated onset of autoimmune effects, with most studies observing exposure-related increases in levels of serum antinuclear antibodies (ANAs), a marker of systemic autoimmunity, and autoimmune hepatitis or other histopathologic changes.<sup>1</sup>

Molecular epidemiologic investigations of autoimmunity markers among TCE-exposed workers could be informative for inferring a causal

relationship with autoimmune disease in humans. At present, no study to date has directly investigated the relationship between TCE exposure and molecular markers of autoimmunity. To address this research gap, we used serum samples from a cross-sectional study of TCE-exposed and unexposed Chinese workers to investigate the relationship between TCE exposure and serum ANAs.

## METHODS

For this cross-sectional molecular epidemiologic study conducted in Guangdong, China, 80 workers who were being exposed to TCE in 6 study factories with TCE cleaning operations were enrolled, as well as 96 unexposed controls.<sup>5</sup> The controls were enrolled from two clothing manufacturing factories, one food production factory, and a hospital that did not use TCE; selected controls were frequency matched to exposed workers on the basis of sex and age ( $\pm 5$  years). A questionnaire-based interview that assessed demographic information, medical history and lifestyle characteristics as well as an occupational history was administered to all subjects. Blood samples were delivered on ice to the laboratory within 6 hours of being collected and centrifuged, and serum samples were preserved at  $-80^{\circ}\text{C}$ .

Full-shift personal air exposure measurements were taken in the factories using 3M organic vapour monitoring (OVM) badges before blood collection. For a subset of control workers ( $n=45$ ), a single OVM badge was collected before biological sample collection. TCE air levels in parts per million (ppm) were based on the arithmetic mean of an average of two to three measurements per subject. TCE was non-detectable in the samples from control workers.

ANAs in study serum samples were detected using HEp-2 cell slides (Kallestad, Bio-Rad Laboratories, Hercules, California, USA), incubated with a 1:80 dilution of sera, washed and incubated with the burro anti-human polyvalent immunoglobulin fluorescein isothiocyanate conjugate (Kallestad) and read using fluorescent microscopy (Leitz Fluorescence Scope, 50/1.0 magnification). Using fluorescent microscopy, technicians who were unaware of subject TCE exposure status scored each subject's sample for ANA fluorescence intensity on a 0–4 scale at increasing dilutions (eg, 1:160, 1:320). We used a cut-off for ANA positivity of 1+ intensity at 1:320 dilution and tested for antibodies to extractable nuclear antigen (anti-ENAs) for samples positive at this threshold, including antiribonuclear protein, anti-Smith, anti-Sjogren's syndrome related antigen A (anti-SSA, combined 52 and 60 kDa), anti-SSB, and antidouble-stranded deoxyribonucleic acid. All ANAs and ENAs were tested with positive and negative controls; in addition, a random subsample of participant sera was retested for ANA quality control and we observed 91% concordance in assay results.

The statistical analysis was conducted using SAS V.9.4 (SAS Institute). We compared subject characteristics between the unexposed and exposed workers (categorised into three groups using concentration tertiles as cut-points:  $\leq 7.23$ , 7.24–17.27,  $> 17.27$  ppm, [table 1](#)) using the Kruskal-Wallis test for continuous variables and  $\chi^2$  test for categorical variables. We fit unconditional logistic regression models to compute ORs and 95% CIs relating ANA positivity (the model dependent variable) and TCE exposure categories (vs the control group), with adjustment for age, sex, body mass index (BMI), current smoking status, current alcohol use and recent infection. In addition to evaluating the TCE exposure categories defined by tertiles, we conducted analyses further subdividing the highest exposure category using the intracategory median (35.23 ppm) to investigate associations

**Table 1** ORs and 95% CIs relating trichloroethylene exposure and antinuclear antibody positivity

Exposure	All	
	N <sub>ANA+</sub> /N <sub>Total</sub> (%)	OR* (95% CI)
Controls	7/96 (7.3)	1.0
Exposed (ppm)	9/80 (11.3)	1.7 (0.6 to 4.9)
$\leq 7.23$	1/27 (3.7)	0.4 (0.1 to 3.7)
7.24–17.27	2/27 (7.4)	0.9 (0.2 to 4.9)
$> 17.27$	6/26 (23.1)	4.7 (1.3 to 16.8)
17.28–35.23†	2/13 (15.4)	2.8 (0.5 to 16.2)
$> 35.23$ †	4/13 (30.8)	7.2 (1.6 to 33.6)
		P <sub>trend</sub> =0.008
*ORs adjusted for age, sex, smoking, alcohol, infection, body mass index.		
†Upper category ( $> 17.27$ ppm) subdivided into two subcategories using intracategory median (35.23 ppm). The test of trend p value is 0.008 including these subcategories and 0.02 using the original upper category.		
ppm, parts per million.		

across a wider range of exposure levels. We calculated tests of trend across TCE exposure categories by modelling the intracategory medians as a continuous covariate. We also explored the association with ANA for exposure categories defined using federal exposure standards; we evaluated upper cut-points of 10 ppm (the American Conference of Governmental Industrial Hygienists (ACGIH) time-weighted average threshold limit value (TLV)) and 25 ppm (the National Institute of Occupational Safety and Health (NIOSH) 10-hour time-weighted average recommended exposure limit (REL)) and conducted analyses restricting to subjects with exposures  $< 100$  ppm (the US Occupational Safety and Health Administration (OSHA) 8-hour time-weighted average permissible exposure limit (PEL)).<sup>6</sup> We also repeated these analyses stratifying on sex and excluding subjects reporting a history of autoimmune disease. Statistical analyses of ENAs were not performed due to the negligible number of positive subjects.

## RESULTS

Selected characteristics of control and TCE-exposed participants, with the latter group categorised by exposure level, are summarised in online supplemental table 1. Statistically significant differences across groups were observed for age, with control and high-exposed participants older than those with TCE exposures  $\leq 17.27$  ppm, and sex, with men most prevalent in the group with exposures  $> 17.27$  ppm. Age and sex comparisons restricted to the high-exposed and control groups did not reach statistical significance. The participant groups did not significantly differ in relation to BMI, smoking, alcohol consumption or recent infection history.

Serum ANAs were positive in 16 of 176 participants (9%). Among individuals with positive ANAs, tests for autoantibodies against ENAs were generally negative across all samples with the exception of SSA, which was positive in two samples (1 TCE exposed, 1 control). In comparisons across exposure groups, the prevalence of ANA positivity among those with exposures  $> 17.27$  ppm was significantly higher than that of the unexposed control workers (23.1% vs 7.3%,  $p=0.02$ ; online supplemental figure 1). A Fisher's exact test of the same two exposure groups was also statistically significant ( $p=0.03$ ). Similarly, in multivariable logistic regression analyses, we found a significantly higher odds of ANA positivity among workers with TCE exposure concentrations  $> 17.27$  ppm versus controls (OR 4.7, 95% CI 1.3 to 16.8; [table 1](#)), but not among those with

lower levels of exposure. When we split the upper exposure category into two using the intracategory median, the ORs for 17.27–35.23 ppm and >35.23 ppm were 2.8 (95% CI 0.5 to 16.2) and 7.2 (95% CI 1.6 to 33.6), respectively. In sex-stratified analyses, the association with TCE exposure >17.27 ppm was evident among men (OR 4.8, 95% CI 1.1 to 21.2), while the result for women was too statistically unstable to interpret given the small number of exposed subjects (online supplemental table 2). No statistically significant association with ANA positivity was observed for exposures below the ACGIH TLV of 10 ppm (OR 0.7, 95% CI 0.1 to 3.9) or between 10 ppm and the NIOSH REL of 25 ppm (OR 1.4, 95% CI 0.3 to 5.8). Our findings did not materially change when we repeated our analysis excluding subjects with TCE exposure concentrations above 100 ppm (n=3; OR 5.8 95% CI 1.5 to 21.5 for >17.27 ppm vs controls) and subjects with diagnosed autoimmune disease (two controls with rheumatoid arthritis; OR 4.5, 95% CI 1.2 to 16.2).

## DISCUSSION

In this cross-sectional study of workers in Guangdong, China, we observed a significantly higher prevalence of ANA positivity with high (>17.27 ppm) occupational TCE exposure concentrations compared with a control group of unexposed workers, with this association persisting even after exclusion of individuals with known autoimmune disease. We did not observe an association with ANAs at lower levels of exposure. It is notable that the association was present at exposure levels below the OSHA PEL of 100 ppm and absent below the ACGIH TLV of 10 ppm, while a weak, statistically non-significant association was observed for exposures between 10 ppm and the NIOSH REL of 25 ppm.

The presence of ANAs at high levels is characteristic of a variety of clinical autoimmune disorders, and can precede disease onset by several years.<sup>7,8</sup> However, ANAs are also detected in individuals without autoimmune disease, with prevalence estimates varying widely (1%–20%) due to differences in measurement methodology, cut-off levels for positivity and the sex and age distributions of the study populations.<sup>9</sup> Analyses in different cycles of the US National Health and Nutrition Examination Survey have yielded prevalence estimates of ANA positivity ranging between 11% and 16%, while a prevalence of 5.9% was observed in a large cross-sectional study in China.<sup>9–11</sup> Given the higher prevalence of ANA positivity in the general population relative to the comparatively low prevalence of diagnosed autoimmune disorders (≈3%),<sup>12</sup> systemic autoimmunity as measured by ANAs may in some cases represent a subclinical precursor state that can progress in a subgroup of individuals, for reasons still unclear, to autoimmune disease.<sup>8</sup> Of note, while ANA positivity may be considered a relatively non-specific marker of immune dysregulation, the level for ANA positivity used in this study (≥1:320 with 1+ fluorescence) is higher than an ANA-positive cut-off of ≥1:80 that is established as an inclusion factor for SLE classification.<sup>13</sup> Furthermore, the cut-off we used is more specific for clinically diagnosed connective tissue disease.<sup>14,15</sup> As such, in this context, our observed association with ANA positivity (even with removal of the two subjects with diagnosed autoimmune disease) suggests that TCE exposure may be associated with substantial immune dysregulation and autoimmunity.

The evidence to date suggesting a relationship between TCE and autoimmunity and/or autoimmune disease has been generated from two approaches: experimental toxicologic investigations conducted predominantly in lupus-prone mouse models

and epidemiologic case–control studies, mainly of scleroderma, using questionnaire-based approaches to assess potential occupational exposure.<sup>1</sup> Our findings address a unique space between these two sources of evidence, providing human mechanistic data relating TCE exposure to autoimmunity using high-quality exposure assessment based on exposure monitoring in the workplace. Our study, however, has some limitations. The relatively small sample size limited our ability to explore potential differences by sex (with a paucity of highly exposed women in particular) and detect weaker associations with ANA positivity in low-exposed subjects. As a consequence, the generalisability of our findings to women and workers experiencing lower exposures is unclear. Additionally, although several efforts were made in the design of our study to avoid the potential for coexposures to other solvents or potential hydrocarbons in the study workplaces, we cannot entirely rule out the possibility of confounding from other occupational exposures. Lastly, given the cross-sectional nature of this study, we are unable to assess the temporal relationship between TCE exposure and the onset of ANA positivity, limiting inferences of causality.

In conclusion, our findings provide the first human evidence directly relating TCE exposure and an elevated prevalence of ANA positivity. There is a need for additional research incorporating molecular epidemiologic methods and high-quality exposure assessment to provide further mechanistic insight into the autoimmune effects of TCE and, more broadly, other suspected occupational risk factors for autoimmune diseases.

**Contributors** MP conceptualised the antinuclear antibody (ANA) investigation, conducted the data analysis and wrote the first draft of the paper. QL, NR, LZ and MTS designed the cross-sectional study. RV, CW, YH and XT conducted the study fieldwork and exposure assessment. WH and SIB organised study logistics for the ANA analysis. AAF-A and KDD oversaw the ANA laboratory analysis. JR contributed to the data analysis. All authors critically revised and approved the final version of this manuscript. QL, NR and KDD contributed equally to this paper.

**Funding** This project was supported by Intramural Research Program funding from the National Cancer Institute (Bethesda, Maryland, USA) and funding from the Department of Science and Technology of Guangdong Province, China (2007A050100004 to XT).

**Competing interests** None declared.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and was approved by ethical committees/IRBs at the US National Cancer Institute (Special Studies Review Board) and Guangdong Poison Control Center (FWA00009945). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available upon reasonable request.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

## ORCID iDs

Mark Purdue <http://orcid.org/0000-0003-1177-3108>

Luoping Zhang <http://orcid.org/0000-0001-7866-8391>

Roel Vermeulen <http://orcid.org/0000-0003-4082-8163>

## REFERENCES

- Cooper GS, Makris SL, Nietert PJ, et al. Evidence of autoimmune-related effects of trichloroethylene exposure from studies in mice and humans. *Environ Health Perspect* 2009;117:696–702.
- Nietert PJ, Sutherland SE, Silver RM, et al. Is occupational organic solvent exposure a risk factor for scleroderma? *Arthritis Rheum* 1998;41:1111–8.

- 3 Diot E, Lesire V, Guilmot JL, *et al.* Systemic sclerosis and occupational risk factors: a case-control study. *Occup Environ Med* 2002;59:545–9.
- 4 Garabrant DH, Lacey JV, Laing TJ, *et al.* Scleroderma and solvent exposure among women. *Am J Epidemiol* 2003;157:493–500.
- 5 Lan Q, Zhang L, Tang X, *et al.* Occupational exposure to trichloroethylene is associated with a decline in lymphocyte subsets and soluble CD27 and CD30 markers. *Carcinogenesis* 2010;31:1592–6.
- 6 National Toxicology Program. *Report on carcinogens*. 15th ed. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, 2021. <https://ntp.niehs.nih.gov/go/roc15>
- 7 Arbuckle MR, McClain MT, Rubertone MV, *et al.* Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med Overseas Ed* 2003;349:1526–33.
- 8 Slight-Webb S, Lu R, Ritterhouse LL, *et al.* Autoantibody-Positive healthy individuals display unique immune profiles that may regulate autoimmunity. *Arthritis Rheumatol* 2016;68:2492–502.
- 9 Satoh M, Chan EKL, Ho LA, *et al.* Prevalence and sociodemographic correlates of antinuclear antibodies in the United States. *Arthritis Rheum* 2012;64:2319–27.
- 10 Guo Y-P, Wang C-G, Liu X, *et al.* The prevalence of antinuclear antibodies in the general population of China: a cross-sectional study. *Curr Ther Res Clin Exp* 2014;76:116–9.
- 11 Dinse GE, Parks CG, Weinberg CR, *et al.* Increasing prevalence of antinuclear antibodies in the United States. *Arthritis Rheumatol* 2020;72:1026–35.
- 12 Cooper GS, Stroehla BC. The epidemiology of autoimmune diseases. *Autoimmun Rev* 2003;2:119–25.
- 13 Aringer M, Costenbader K, Daikh D. European League against Rheumatism/American College of rheumatology classification criteria for systemic lupus erythematosus. *Arthritis Rheumatol* 2019;2019:1400–12.
- 14 Satoh M, Vázquez-Del Mercado M, Chan EKL. Clinical interpretation of antinuclear antibody tests in systemic rheumatic diseases. *Mod Rheumatol* 2009;19:219–28.
- 15 Menor Almagro R, Rodríguez Gutiérrez JF, Martín-Martínez MA, *et al.* Association between antinuclear antibody titers and connective tissue diseases in a rheumatology department. *Reumatol Clin* 2017;13:150–5.