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Background *Health risks of using styrene to manufacture windblades for the green energy sector are unknown.*

Methods *Using data collected from 355 (73%) current windblade workers and regression analysis, we investigated associations between health outcomes and styrene exposure estimates derived from urinary styrene metabolites.*

Results *The median current styrene exposure was 53.6 mg/g creatinine (interquartile range: 19.5–94.4). Color blindness in men and women (standardized morbidity ratios 2.3 and 16.6, respectively) was not associated with exposure estimates, but was the type previously reported with styrene. Visual contrast sensitivity decreased and chest tightness increased (odds ratio 2.9) with increasing current exposure. Decreases in spirometric parameters and FeNO, and increases in the odds of wheeze and asthma-like symptoms (odds ratios 1.3 and 1.2, respectively) occurred with increasing cumulative exposure.*

Conclusions *Despite styrene exposures below the recommended 400 mg/g creatinine, visual and respiratory effects indicate the need for additional preventative measures in this industry.* Am. J. Ind. Med. 58:1150–1159, 2015.

KEY WORDS: styrene; occupational exposure; color vision defects; contrast sensitivity; spirometry

INTRODUCTION

The manufacture of power-generating wind turbines, complex machines that include composite material windblades, is a relatively new industry that employed 30,000 workers in the United States in 2011 and is expanding rapidly with the increased focus on renewable energy sources [Platzer, 2012]. A previous investigation at the windblade manufacturing facility that is the subject of this study documented high inhalational exposures to styrene [Hammond et al., 2011]. Styrene, an aromatic hydrocarbon derived from benzene, is a colorless, volatile liquid with a sweet smell. It is used widely in the synthesis and manufacture of polystyrene, hundreds of different copolymers, and other industrial resins [NTP, 2014]. The highest potential exposures to styrene occur in the production of reinforced plastics [ATSDR, 2010], in which fibers of glass or other materials are used to enhance the strength and resistance of plastics. Fiberglass-reinforced plastics (often called simply "fiberglass" materials) are made with styrene as a cross-linking agent in polyester resins used in gel-coating and laminating operations; the resins generally contain between 30% and 50% styrene by weight [NTP, 2014]. Fiberglass products traditionally have included boats, bathtubs, shower

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stalls, tanks, and drums [NTP, 2014] and, more recently, some windblades [Platzer, 2012].

Styrene is associated with neurotoxic effects and respiratory effects [Chmielewski and Renke, 1975; Brigham and Landrigan, 1985; Moscato et al., 1987; Kolstad et al., 1995]. Notably, there are recent case reports of obliterative bronchiolitis in England and Taiwan in the fiberglass boat and water tank industries, which share similar exposures with fiberglass windblade manufacture [Chen et al., 2013; Cullinan et al., 2013].

A recent effort to decrease styrene exposures at this windblade manufacturing facility had been prompted by the documentation of high peak exposures (>100 part per million [ppm]) during task-based sampling [Hammond et al., 2011]. The facility had eliminated the highest exposure tasks, increased exhaust ventilation, increased respiratory protection, and changed from a gelcoat with 36–42% styrene [Hammond et al., 2011] to one with a lower percentage of styrene. In follow-up, the company requested that the National Institute for Occupational Safety and Health (NIOSH) conduct a health hazard evaluation of current workplace exposures and health effects. Prior to the medical survey, we evaluated airborne styrene concentrations at the plant. On 3 consecutive days, we collected 74 full-shift air samples for styrene in the breathing zone of production workers. The styrene concentrations ranged from <1 ppm to 51 ppm, with a median of 7 ppm. Only one of five departments that used styrene had concentrations above the NIOSH recommended exposure limit of 50 ppm for up to a 10-hr time weighted average, or the American Conference of Governmental Industrial Hygienists (ACGIH®) Threshold Limit Value® of 20 ppm averaged over an 8-hr workday, during a 40-hr workweek [ACGIH, 2011]. In this department, employees manually reinforced and patched the blades, both interior and exterior, using sheets of styrene resin-containing fiberglass.

Styrene is rapidly absorbed and eliminated, appearing in the blood immediately after the start of exposure and declining precipitously after the end of exposure [ACGIH, 2011]. The vast majority (90–97%) of absorbed styrene is eliminated as urinary metabolites, primarily mandelic acid and phenylglyoxylic acid [ACGIH, 2011]. Urinary elimination of these metabolites is biphasic, with half-times up to 10 and 40 hr [ACGIH, 2011]. Thus, the biologic exposure estimate for assessing urinary metabolites of styrene reflects acute, rather than chronic, exposure. Biological monitoring has advantages over air monitoring in understanding dose when multiple routes of exposure exist and respiratory and skin protection are used. To protect styrene-exposed workers from neurotoxic effects and ocular and respiratory irritation (not respiratory disease), the ACGIH has recommended an end-of-shift biologic exposure index (BEI®) maximum of 400 mg of styrene metabolites (mandelic acid + phenylglyoxylic acid) per gram (mg/g) of creatinine in the urine [ACGIH, 2011]. ACGIH estimates this BEI corresponds to an inhalation exposure at the TLV for styrene of 20 ppm averaged over an 8 hr workday, during a 40-hr workweek [ACGIH, 2011].

At the time of our study, this windblade manufacturing plant employed about 500 people who worked in shifts ranging from 8 hr to 12 hr. The number of people employed varied in recent years from 300 to 900, depending on market fluctuations. Many employees had been laid off and rehired, leading to gaps in their employment history.

Employees sprayed styrene-containing gelcoat into a clamshell-like mold, and then laid down balsa wood and fiberglass to form the blade structure. Employees bound the layers together with a styrene-based resin, infused in a closed process. The two halves of the blade were then closed together with styrene-containing glue and transported to another department for further refinements and repairs. There employees sanded the blade and placed pieces of fiberglass over errors and weak areas, both inside and outside the blade, and coated the repairs with styrene-based resin. Finally, each blade was painted with styrene-based paint, balanced, and finished. Workers with the same job title often performed different tasks, or changed primary tasks on a regular basis, leading to varying exposures from day-to-day or week-to-week.

To explore the visual and respiratory effects of styrene, and understand the current risk to workers at this facility, we conducted a cross-sectional study. We aimed to understand exposures using styrene biomarkers and compare these to health outcomes including color and contrast vision, respiratory symptoms, and lung function.

MATERIALS AND METHODS

Data Collection

The study was conducted in accordance with the NIOSH Institutional Review Board's policies for health hazard evaluations. Every worker currently employed was invited to participate in testing during the week of the study in July 2013. After providing written informed consent, each participant was interviewed with a computer-based questionnaire addressing respiratory symptoms, medical diagnoses, smoking history, work history, personal protective equipment (PPE) use, and demographic information. The respiratory health questions were adapted from validated survey instruments [Ferris, 1978; DHHS, 1996; Grassi et al., 2003].

We assessed visual acuity using a Rosenbaum pocket vision screener. The Lanthony desaturated D-15 (Gulden Ophthamalics, Elkins Park, PA), a color arrangement test recommended for detecting acquired vision loss [Fox and Boyes, 2001], was administered binocularly with daylight

filtering glasses. The caps were randomly clustered on the test surface; workers were asked to arrange the caps in color order by finding the cap closest in color to the reference cap and placing it nearby. They then continued for all 15 caps. Visual contrast sensitivity was tested monocularly with the functional acuity contrast test (FACT; Stereo Optical Company, Inc.; Chicago, IL), under conditions specified in the manual [Ginsburg, 1993].

Fraction of exhaled nitric oxide (FeNO) and lung function were assessed following American Thoracic Society (ATS) guidelines [Miller et al., 2005; Dweik et al., 2011] using the NIOX Mino© device for exhaled nitric oxide (Aerocrine; Solna, Sweden), and a dry rolling-seal spirometer. Repeat spirometry after administration of a bronchodilator was offered to any participant with an abnormal test. FeNO is associated with eosinophilic airway inflammation as often occurs with asthma and FeNO levels rise with exposure to allergens or other asthma triggers [Dweik et al., 2011]. Before FeNO was measured, participants were asked about potential confounders, including food and drink intake, smoking, recent illness, exercise, and steroid use. They were allowed six tries, and the first acceptable trial was used.

End-of-shift urine samples were assayed for mandelic acid (MA), phenylglyoxylic acid (PGA), and a third styrene metabolite, *N*-acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine + *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine (PHEMA) using ultra high performance liquid chromatography (Waters, Inc., Milford, MA) coupled with an electrospray ionization triple quadrupole mass spectrometry (Sciex API 5500 Triple Quad, Applied Biosystems, Foster City, CA) [Alwis et al., 2012]. Together, MA, PGA, and PHEMA represent the major products of styrene metabolism in humans. The limits of detection were 12 ng/ml for MA, 12 ng/ml for PGA, and 0.7 ng/ml for PHEMA. Urine creatinine was measured using the enzyme colorimetric method with the automated Roche/Hitachi Modular Analytics system, allowing us to normalize results to individual kidney function. More detailed information about the analyses of urine samples is available in the Online Supplement.

Statistical Methods

Statistical analyses were conducted using SAS software version 9.3 and JMP software version 10.0.1 (SAS Institute, Inc., Cary, NC). We chose a *P*-value of ≤ 0.05 for statistical significance, and $P \leq 0.1$ for borderline statistical significance.

For comparison to the BEI, we calculated a biologic exposure measurement for current styrene exposure for each individual by summing milligrams of MA and PGA, and dividing by grams of creatinine. To reduce the effect of outlier variables, we log-transformed (using the natural log) the current styrene exposure to compare it to our health outcomes using linear and logistic regression models. We compared the odds of symptoms and health outcomes in workers with a current styrene exposure above or equal to the median to those with exposure below the median.

To estimate workers' styrene exposure over the course of their work history at this plant, an average current styrene exposure was assigned to each department/job title combination based on those workers who participated in the study. For those job titles without an average current styrene exposure, we assigned one from another job in the same or a nearby department. These average current styrene exposures were then multiplied by the number of months a worker had spent in that job and summed to give a relative indicator of cumulative styrene exposure.

Asthma-like symptoms were defined as any of the following: current use of asthma medicine, wheezing or whistling in the chest in the past 12 months, awakening with a feeling of chest tightness in the past 12 months, or attack of asthma in the past 12 months [Grassi et al., 2003]. For all symptoms (including those mentioned above, eye irritation, nasal irritation, shortness of breath, usual cough, and usual phlegm), we calculated standardized morbidity ratios (SMRs) and 95% confidence intervals (CI) through comparisons with data obtained from the US population from the Third National Health and Nutrition Examination Survey (NHANES III) [DHHS, 1996], using indirect standardization for race (white or black), sex, age (17–39 or $\geq$40 years) and cigarette smoking (ever/never). We compared symptoms to styrene exposure (the natural log of our current and cumulative exposure markers) using logistic regression to calculate odds ratios (OR) and corresponding 95%CI, and controlled for smoking (current, former, never), gender, race, and age.

For color vision, using the published guidelines each participant was assigned a color confusion index (CCI) and a color angle [Geller, 2001; Toruk, 2014]. A CCI >1.65 was considered abnormal color vision, and the color angle then determined the type of color vision deficit (protan [red–green], deutan [red–green], tritan [blue–yellow], or other [unknown type]). We calculated SMRs comparing the prevalence of color vision abnormalities with those expected in a western population [Kalloniatis and Luu, 2007]. We used linear and logistic regression models to assess associations between exposure variables and both CCI and type of color blindness. We also evaluated grouped color vision categories (protan/deutan, tritan/unknown, and normal color vision), given that protan and deutan color vision deficits are typically congenital, tritan is more commonly acquired, and unknown could represent the combination of a congenital and an acquired deficit or more than one acquired deficit. Some literature suggests that occupational color blindness may be progressive, beginning with blue–yellow, and developing through combined blue–yellow and red–green color blindness to complete color vision loss [Geller and Hudnell, 1997; Fox and Boyes, 2001].

For visual contrast sensitivity, we created a score at each frequency (1.5, 3, 6, 12, and 18 cycles/degree [CPD]) for each participant by averaging the left and right eye scores [Ginsburg, 1993]. Using linear regression models, we looked for an association between visual contrast sensitivity and the exposure variables. All vision models were controlled for visual acuity, diabetes, glaucoma, macular degeneration, cataracts, age, alcohol consumption in the last 24 hr (as a surrogate for overall alcohol consumption), and smoking; for consistency with prior literature, we report the fully adjusted results. The B-estimate is a slope relating the contrast score and log of exposure (current styrene exposure or cumulative styrene exposure) given by the linear regression model; it was converted to an effect for the un-logged variables by multiplying the B-estimate by ln (101/100), giving our effect estimate for a 1% increase in the current styrene exposure or cumulative styrene exposure. To give a better sense of the impact of such a change, we calculated the absolute change in visual contrast with an increase in exposure from the 2.5th to 97.5th percentile [Cornell Statistical Consulting Unit, 2012].

FeNO results above 50 parts per billion (ppb) were considered abnormal and those between 25 ppb and 50 ppb were considered intermediate, according to the ATS guidelines [Dweik et al., 2011]. FeNO values below the limit of detection (LOD) were estimated using the formula LOD/($\sqrt{2}$). We used the FeNO value as a continuous outcome variable in linear models to assess the relationship with exposure variables. We adjusted the models for age, smoking status, respiratory illness in the last 7 days, and ingestion of nitrate-rich foods. As described above, we also calculated an effect estimate and an absolute change in FeNO with a change in exposure from 2.5th to 97.5th percentile.

Spirometry was interpreted based on prediction equations to define normal values for forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and the ratio of the two (FEV1/FVC) [Hankinson et al., 1999]. We defined obstruction as a value of FEV1/FVC below the lower limit of normal, restriction as FVC below the lower limit of normal, and the combination of both low values to define a mixed pattern of obstruction and restriction [Pellegrino et al., 2005]. The prevalence of workers with obstruction, restriction, or a mixed pattern on spirometry was compared to population values from NHANES III with SMRs. Associations between the biologic exposure measures and spirometry outcomes were investigated using linear regression models adjusted for smoking, body mass index (BMI), and in the case of FEV1/FVC, age. As with the visual contrast scores and FeNO outcomes, we converted the B-estimate to an effect estimate, and then calculated the absolute change in outcome with a change in exposure from the 2.5th to 97.5th percentile.

In this paper, we present results of analyses using current and cumulative styrene exposure metrics that were based on the biological exposure measurement (sum of MA and PGA). We also developed models using current and cumulative styrene exposure metrics that were based on the individual urinary metabolites (MA, PGA, and PHEMA alone). These single-metabolite models generally had similar estimates to those of the biological exposure measurement, so are not reported.

RESULTS

Overall, 355 (73%) of 486 eligible workers participated in at least one component of the study. Most participants completed most of the testing offered, including the questionnaire (n = 354; >99%) vision testing (n = 352; 99%), FeNO measurement (n = 341; 96%), spirometry (n = 343; 97%), and urine styrene metabolite analysis (n = 322; 91%). Bronchodilator was administered to 24 participants with obstruction on spirometry testing. The workforce was largely young, white and male, with short tenures, and a high proportion of current and former smokers (Table I).

Symptom frequencies are shown in Table II. Eye symptoms, shortness of breath, usual cough, and usual phlegm were less common than expected from adjusted comparisons with the US adult population, while other

TABLE I. Demographic Characteristics and Styrene Exposure of Windblade Manufacturing Plant Workers

Demographic Characteristic (n = 354)[a]	Value
Age, years, mean (range)	37.5 (19–65)
Male, n (%)	269 (75.9)
Race, n (%)	
White	263 (74.3)
Black	46 (13.0)
American Indian or Alaska Native	20 (5.7)
Other	4 (1.1)
More than one race	14 (4.0)
Missing	7 (2.0)
Smoking status, n (%)	142 (40.1)
Current	78 (22.0)
Former	134 (37.9)
Never	4.8, 5.5 (<1–22.7)
Tenure, years, mean, median (range)[b]	
Styrene Exposure (n = 322)[a]	Mean, median (range)
Current styrene exposure,[c] mg/g creatinine	69.5, 53.6 (0.7–941.0)
Cumulative styrene exposure,[d] mg-months/g creatinine	3945, 2426 (10.7–69800)

[a]354 workers completed the questionnaire, providing demographic information; 322 workers provided a urine sample.

[b]Tenure was calculated by summing all time employed at the plant. Gaps in employment (such as due to layoffs and rehiring) were not included in the tenure calculation.

[c]Urinary mandelic acid + phenylglyoxylic acid.

[d]Calculated for 354 participants using average current styrene exposure for a job multiplied by an individual's job tenure in months and summing across the jobs held by an individual, if applicable.

TABLE II. Symptoms and Lung Function Test Results of 355 Windblade Manufacturing Plant Workers, Overall and by Median Current and Cumulative Styrene Exposure*

		By median styrene exposure			
		Current (mg/g creat)		Cumulative (mg-months/g creat)	
	Overall	<53.6	≥53.6	<2,426.4	≥2,426.4
Symptom, n (%)[a]					
Shortness of breath	44 (12.4)	19 (11.8)	22 (13.7)	17 (9.6)	27 (15.3)
Usual cough	43 (12.1)	20 (12.4)	20 (12.4)	21 (11.9)	22 (12.4)
Usual phlegm	39 (11)	17 (10.6)	19 (11.8)	20 (11.3)	19 (10.7)
Wheeze	79 (22.3)	35 (21.7)	39 (24.2)	**31 (17.5)**	**48 (27.1)**
Chest tightness	31 (8.9)	**8 (5.0)**	**21 (13.0)**	16 (9.0)	15 (8.5)
Asthma-like symptoms	98 (27.7)	41 (25.5)	52 (32.3)	*41 (23.2)*	*57 (32.2)*
Nasal symptoms	182 (51.4)	89 (55.3)	77 (47.8)	**79 (44.6)**	**103 (58.2)**
Eye symptoms	108 (30.5)	49 (30.4)	52 (32.3)	**42 (23.7)**	**66 (37.3)**
Spirometry, n (%) or mean[b]					
Obstruction	28 (8.2)	15 (9.3)	9 (5.6)	**9 (5.1)**	**19 (10.7)**
Restriction	16 (4.7)	10 (6.5)	6 (3.8)	10 (5.9)	5 (3.5)
Mixed	3 (0.9)	—	—	—	—
Any abnormality	47 (13.7)	26 (16.2)	17 (10.6)	19 (10.7)	28 (15.8)
%predicted FEV1	99.9	99.7	100.7	100.7	98.9
% predicted FVC	102.5	102.4	103.0	102.5	102.3
% predicted MMEF	96.4	95.8	98.1	98.7	94.1
FEV1/FVC %	79.2	78.6	79.8	**80.3**	**78.1**
FeNO, n (%) or mean					
FeNO result, ppb	13.5	14.5	12.9	14.3	12.8
FeNO >25 ppb	30 (8.8)	15 (9.4)	13 (8.6)	17 (10.0)	13 (7.6)

creat, creatinine; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; FeNO, fraction of exhaled nitric oxide; MMEF, maximal mid-expiratory flow; ppb, parts per billion.

Statistically significant differences ($P \leq 0.05$) are in bold. Borderline statistically significant differences ($0.05 < P \leq 0.1$) are in italics.

*A total of 355 workers participated in at least one component of the study. For the results presented, the denominator ranged from 310 to 354, depending on participation in the health assessment component and the urine styrene metabolite analyses.

[a]Shortness of breath was defined as when hurrying on level ground or walking up a slight hill. Wheeze, chest tightness, nasal symptoms (stuffy, itchy, or runny nose), and eye symptoms (watery, itchy eyes) were reported for the last 12 months. Asthma-like symptoms were defined as any of the following: current use of asthma medicine, wheezing, or whistling in the chest in the past 12 months, awakening with a feeling of chest tightness in the past 12 months, or attack of asthma in the past 12 months.

[b]Obstruction defined as FEV1/FVC below the lower limit of normal, restriction defined as FVC below the lower limit of normal, mixed defined as both FEV1/FVC and FVC below the lower limit of normal. Mixed defects were not stratified by median styrene exposure due to the small number of participants with this physiology; the corresponding cells are marked with —.

symptoms and self-reported lifetime asthma diagnosis (n = 26; 7.3%) were about as common as expected (data not shown). The average percent of predicted FEV1 and FVC were within the normal range (Table II), and the prevalences of spirometric abnormalities were not elevated compared to the reference population (data not shown). Among participants with obstruction, 13 (46%) were current smokers and 7 (25%) were former smokers, with mean age of 42 years (range 29–63) and mean pack-years of 18.5 (range <1–47). Twenty-four workers with obstruction were given a bronchodilator. Five (21%) of these had reversible obstruction; 14 (74%) of those with fixed obstruction were current or former smokers. Just three participants had a FeNO result which was classified as abnormal (>50 ppb), while over half (n = 184; 52%) had abnormal visual contrast sensitivity; 8% (n = 7) of female workers and 18% (n = 49) of male workers had abnormal color vision. Fifteen men reported a history of doctor-diagnosed color blindness; 11 (73%) of these had abnormal color vision on our testing. Thus, most of those identified as having abnormal color vision did not report a history of doctor-diagnosed color blindness.

Styrene exposure (assessed through urine biomarkers) varied widely throughout the plant. The mean current styrene exposure was 69.5 mg/g creatinine, with a range of 0.7–941.0 mg/g creatinine (Table I). The median was 53.6 mg/g creatinine, and the interquartile range was

19.5–94.4 mg/g creatinine. Only one participant exceeded the ACGIH recommended BEI of 400 mg/g creatinine. The mean cumulative styrene exposure was 3,945 mg-month/g creatinine (Table I).

The prevalence of some symptoms differed by styrene exposure (Table II). Participants with a current styrene exposure greater than or equal to the median had higher odds of chest tightness than participants with a current styrene exposure below the median (OR 2.9, CI 1.2–6.7). Cumulative styrene exposure was associated with greater odds of wheeze (OR 1.3, CI 1.1–1.5), nasal symptoms (OR 1.2, CI 1.1–1.4), eye symptoms (OR 1.2, CI 1.1–1.4), and asthma-like symptoms (OR 1.2, CI 1.0–1.4). Since the models were run using the natural log of the cumulative exposure estimate, these ORs represent the change in odds of each symptom with a 2.7-fold change in the untransformed cumulative exposure.

The prevalences of both deutan and protan color deficiencies (red–green color blindness) in the workforce were similar to expected levels (Table III). Tritan color blindness (blue–yellow) is rare, and the prevalence of tritan color blindness in this workforce was elevated compared to the prevalence in the reference population (Table III). Because there is no information on expected prevalence of unknown abnormalities, SMRs could not be calculated for this outcome. The prevalence of color blindness overall was significantly elevated in both men and women at the plant (Table III). None of the color vision outcomes was associated with the markers of styrene exposure (not shown).

Visual contrast outcomes at 1.5, 3, 6, and 12 CPD were significantly related to the natural log of current styrene exposure in adjusted models (Table IV). This effect was strongest at 6 cycles/degree (Table IV). Visual contrast scores fell an estimated 8.8–22.1 points from the 2.5th to 97.5th percentile of untransformed current styrene exposure, that is, from 2.8 mg/g to 242.0 mg/g creatinine (Table IV).

The prevalence of obstruction on spirometry was significantly higher and the FEV1/FVC ratio significantly lower in participants with cumulative styrene exposure greater than or equal to the median (Table II). In regression models, cumulative styrene exposure was inversely associated with spirometric parameters (Table V). The reductions in both percent of predicted maximal mid-expiratory flow (MMEF) and FEV1/FVC were significant (Table V), while the reduction in percent of predicted FEV1 was borderline significant. The percent of predicted FVC was not associated with cumulative styrene exposure (Table V).

Cumulative styrene exposure was also inversely associated with FeNO in an adjusted model (Table V). We repeated the analysis looking at non-smokers only and found a similar trend (data not shown).

Certain health outcomes were correlated with each other, including decreased FEV1/FVC and the presence of asthma-like symptoms ($P < 0.0001$), and CCI and visual contrast sensitivity at 6 CPD ($P = 0.02$). The CCI was not associated with FEV1/FVC, the percent of predicted MMEF, or FeNO, and tritan color blindness was not associated with spirometry interpretation, or percent of predicted FEV1 or MMEF. Visual contrast sensitivity at 6 CPD (the frequency affected

TABLE III. Color Vision Abnormalities in Workers at a Windblade Manufacturing Plant (n = 352)

	n	Observed, n (%)	Expected[a], n (%)	SMR	95%CI
Protan					
Male	268	8 (3.0)	5.4 (2.0)	1.5	0.6–2.9
Female	84	0	0.02 (0.02)	α	α
Deutan					
Male	268	10 (3.7)	17.5 (6.5)	0.6	0.3–1.1
Female	84	0	0.3 (0.4)	α	α
Tritan					
Male	268	7 (2.7)	0.03 (0.01)	**270**	**105–536**
Female	84	3 (3.6)	0.01 (0.01)	**360**	**73–1031**
Unknown abnormality					
Male	268	24 (9.0)	n/a	β	β
Female	84	4 (4.8	n/a	β	β
Any abnormality[b]					
Male	268	49 (18.3)	21.5 (8.0)	**2.3**	**1.7–3.0**
Female	84	7 (8.3)	0.4 (0.5)	**16.6**	**6.6–33.9**

SMR, standardized morbidity ratio (based on percentages); CI, confidence interval; n/a, not available; α, unable to calculate because number observed was zero; ß, unable to calculate because population values do not exist.

[a]Expected percentages from literature [Geller and Hudnell, 1997].

[b]Any abnormality was defined as having a protan, deutan, tritan, or unknown color vision abnormality.

TABLE IV. Adjusted Models* of the Relationship Between Visual Contrast Sensitivity and Log-Transformed Current Styrene Exposure Estimates Among Windblade Manufacturing Workers (n = 351)

Frequency	Effect estimate*,[a]	*P*-value[b]	Change in outcome with current exposure change from 2.5th to 97.5th percentile[b]
1.5 cpd	−**0.020**	**0.04**	−**8.8**
3 cpd	−**0.034**	**0.03**	−**15.6**
6 cpd	−**0.049**	<**0.01**	−**22.1**
12 cpd	−**0.030**	**0.04**	−**13.2**
18 cpd	−0.015	0.06	−6.7

cpd, cycles per degree.

*Models were adjusted for age, smoking status, glaucoma, cataracts, macular degeneration, alcoholic consumption during last 24 hr, and visual acuity (of which only age and acuity were significant).

[a]Effect change per 1% change in current styrene exposure.

[b]Bolded *P*-values and estimates are statistically significant.

TABLE V. Lung Function Parameters and the Natural Log of Cumulative Styrene Exposure Estimates in Workers at a Windblade Manufacturing Plant

Lung function measure	Effect estimate[a,b]	*P*-value[b]	Change in outcome with cumulative exposure change from 2.5th to 97.5th percentile[b]
FEV1/FVC[c]	**−0.006**	**0.015**	**−3.5**
Percent of predicted FEV1[d]	−0.008	0.079	−5.0
Percent of predicted MMEF[d]	**−0.026**	**0.011**	**−15.6**
Percent of predicted FVC[d]	−0.002	0.7	−0.93
FeNO[e]	**−0.009**	**0.0013**	**−5.54**

FEV1, forced expiratory volume in one second; FVC, forced vital capacity; MMEF, maximal mid-expiratory flow; FeNO, fraction of exhaled nitric oxide; BMI, body mass index.
[a]Effect change per 1% change in cumulative styrene exposure.
[b]Bolded results are statistically significant.
[c]Model adjusted for age, smoking status, and BMI.
[d]Model adjusted for smoking status and BMI.
[e]Model adjusted for smoking status, respiratory illness, consumption of nitrate-rich foods, gender, and age.

most by styrene) was not associated with FeNO, FEV1/FVC, percent of predicted MMEF, or spirometry interpretation.

DISCUSSION

In a windblade manufacturing workforce without excess symptoms or spirometric abnormalities overall, we found associations between estimates of current and cumulative styrene exposure based on urine styrene metabolites and adverse vision and respiratory outcomes. We also found an excess of color vision abnormalities in the workforce that was not associated with exposure estimates.

The excess of color vision abnormalities in this workforce is striking and is likely related to occupational exposure, despite the lack of association with exposure variables in this study. Animal and human studies have linked the pathophysiology of acquired color blindness to styrene exposure, reporting that solvents such as styrene lead to blue–yellow (or tritan) color blindness, with red–green color blindness additionally occurring after further exposure [Geller and Hudnell, 1997; Vettori et al., 2000; Fox and Boyes, 2001; Kishi et al., 2001; Iregen et al., 2004]. The impact on blue–yellow color vision comes from outer retinal changes caused by the solvents, while red–green is secondary to optic nerve and central pathway damage [Fox and Boyes, 2001; ACGIH, 2011]. The prevalence of all types of color blindness in the plant was more than twice as high as expected, and the excess was mostly found in the tritan group and the unknown group (which may represent a combination of two or more abnormalities, as might be seen in progressive occupational color blindness). The majority of prior studies found blue–yellow color abnormalities in workers with exposures higher than those in our study, some of whom did not wear any respiratory protection [Eguchi et al., 1992; Campagna et al., 1995; Castillo et al., 2001; Kishi et al., 2001; Iregen et al., 2004]. If the color vision abnormalities reflect current styrene exposures, our study suggests that the effect threshold may be lower than previously thought and that current respirator use in this plant may not protect against color vision loss; furthermore, the high prevalence of unknown abnormalities may reflect complex color vision loss that deserves further study.

There are several reasons why our study may not have shown an association between color blindness and exposure estimates. The correlation of color blindness and visual contrast suggests that the same mechanism may be impacting both, but that there is something complicating the relationship between color vision and styrene exposure. Past studies have indicated that there may be some reversibility in color blindness when styrene exposure is decreased [Castillo et al., 2001; Triebig et al., 2001; Seeber et al., 2009]. Because of the changes at the plant and gaps in employees' work histories, such reversibility may have impacted our results. Furthermore, most exposures were clustered in a tight group well below the BEI of 400 mg/g creatinine; despite log-transformation of exposure variables, this clustering may have obscured our ability to see a dose–dependent relationship. Few workers at this plant were unexposed to styrene; if the effect threshold is lower than initially thought, it might be that we did not have a large enough sample of workers with minimal exposures to see a relationship between exposure and color vision. The large number of recent hires in high exposure areas may have impacted our results because such workers may not have been working long enough to show color vision changes that were classified as abnormal. Lastly, our use of binocular testing may have misclassified people with monocular acquired deficits as normal and obscured associations with exposure.

Current styrene exposure was related to visual contrast sensitivity at intermediate and low frequencies. The effect was strongest at the intermediate frequency of 6 cycles/degree, which supports prior literature reporting contrast sensitivity loss at intermediate and high frequencies due to neurologic injury from solvent exposure [Castillo et al., 2001; Waksman and Brody, 2007]. Furthermore, contrary to some studies, our findings support an association between contrast vision loss and current, rather than cumulative, exposure [Castillo et al., 2001].

Chest tightness in the past 12 months was nearly three times more common in workers with a current styrene exposure greater than or equal to the median than in those

below. For workers with higher cumulative exposure to styrene, symptom relationship to exposure was also noted, although effects were not as large; asthma-like symptoms reached statistical significance, as did wheeze, nasal irritation, and eye irritation. These findings support prior literature which indicates styrene may be a respiratory irritant and a cause of occupational asthma [Moscato et al., 1987; Helal and Elshay, 2012], as such symptoms are consistent with both asthma and other obstructive lung diseases such as obliterative bronchiolitis. Furthermore, workers with asthma-like symptoms had decreased FEV1/FVC, which indicates that workers with subjective symptoms also may have objective findings on examination. Such exposure-related symptoms were seen despite the absence of excess symptoms in the workforce overall (possibly secondary to the healthy worker effect), indicating increased focus on protection from exposure may be warranted.

The possibility of styrene-associated lung disease was further reinforced by the association between cumulative styrene exposure and spirometric parameters. FEV1/FVC below the lower limit of normal indicates an obstructive lung problem; a decreased ratio that nevertheless remains within the range of normal may represent an early change in the development of obstruction. In this workforce with other evidence of the healthy-worker effect, the lack of excess abnormal tests does not negate the importance of decreasing FEV1/FVC associated with increasing cumulative styrene exposure. Furthermore, the significant impact on percent of predicted MMEF suggests that the small airways are involved [Burgel, 2011]. Almost 80% of workers with obstruction who were given a bronchodilator had no significant change in their test parameters, which is consistent with fixed obstructive lung disease, including obliterative bronchiolitis. In a cross-sectional study of adults ages 40–79, 64% of those with obstruction did not respond to a bronchodilator [Doney et al., 2014], suggesting that fixed obstruction in our younger study population was higher than expected. The majority of participants with fixed obstruction were current or former smokers. Nonetheless, the relatively young ages and limited smoking histories make it difficult to attribute these abnormalities solely to smoking.

A link between styrene and obliterative bronchiolitis was suggested recently by two case series which described eight workers employed in the reinforced plastics industry (primarily boatbuilding) who had obliterative bronchiolitis, a rare irreversible lung disease [Chen et al., 2013; Cullinan et al., 2013]. Additionally, a large epidemiologic study described excess mortality from obstructive lung disease in highly exposed, short-term reinforced-plastics workers, which would be consistent with obliterative bronchiolitis [Collins et al., 2013; Cummings et al., 2014]. Furthermore, an occupational study in an Egyptian reinforced plastics factory found that styrene exposure correlated with decreased pulmonary function tests, including FEV1/FVC and percent of predicted FEV1, FVC, and MMEF, indicating that obstruction, especially mid-flow obstruction, might be a consequence of styrene exposure [Helal and Elshafy, 2012]. Animal studies support such respiratory findings; they have demonstrated that mice exposed to 40–160 ppm of styrene for 2 years had pathologic changes, including respiratory metaplasia of olfactory epithelium, decreased olfactory fibers, decreased eosinophilic staining of Clara cells in terminal bronchioles, and bronchiolar epithelial hyperplasia in alveolar ducts [Cruzan et al., 2001].

FeNO is an indirect marker of eosinophilic airway inflammation that may be increased in asthma [Pala et al., 2011; Quirce et al., 2012]. In patients with refractory asthma, low FeNO levels have been found to be associated with the presence of neutrophils in the sputum [Tseliou et al., 2010]. In our study, FeNO correlated inversely with cumulative styrene exposure, indicating that higher cumulative exposure (estimated through urine biomarkers and job history) was associated with less eosinophilic inflammation. Past studies have suggested that styrene could be associated with airway inflammation and asthma [Chmielewski and Renke, 1975; Moscato et al., 1987; Helal and Elshay, 2012] but to our knowledge, none have examined the type of inflammation. Thus, our finding of lower FeNO with increasing cumulative styrene exposure may reflect the development of neutrophilic (rather than eosinophilic) inflammation in the airways with styrene exposure, which could be associated with asthma or other airways disease. It is notable that in a study of microwave popcorns workers at risk for diacetyl-related obliterative bronchiolitis, participants with higher flavoring exposures had lower FeNO levels [Akpinar-Elci et al., 2006]. Thus, it is difficult to say whether there was a styrene-associated increase in asthma in this workforce, though the predominance of fixed obstruction may indicate that asthma is not a primary respiratory consequence of styrene exposure.

Limitations of this study include a potential under- or overestimation of current styrene exposure. Though the current recommendation is to collect urine at the end of shifts, as this correlates well with levels of styrene in the air [ACGIH, 2011], to our knowledge this has not been explicitly studied in workplaces with periodic exposures, such as are seen in this workplace. In addition, due to the changing nature of workers' tasks, the measurements on the day of the test may not have represented current exposure beyond the date of the test. Finally, the BEI was established under the assumption of 8-hr shifts and a 40-hr workweek [ACGIH, 2011]. Extended duration shifts and overtime hours may have occurred for some participants during our study, but were not formally quantified, and their impact on styrene exposure estimates is not known.

Some other important considerations include the significant changes in exposure over the past several years at the plant, which may have led to an underestimation of cumulative styrene exposure. Though we treated this

variable as an indication of relative exposure during work at the plant, this may have been misleading, as reductions were probably not uniform across the plant. Furthermore, the common occurrence of layoffs and rehires could have easily impacted some of our outcomes (such as color blindness), as such gaps may leave time for employees to recover from exposures. For a small number of workers who were English language learners, the language barrier may have been a problem. All participants spoke English well enough to follow directions in the workplace and answer simple questions, but some may have struggled with certain instructions. Additionally, workers who were rehired might have been healthier or less susceptible to the effects of styrene than workers who previously worked at the plant but were not rehired, reflecting survivor bias.

Strengths of this study include the large workforce with styrene exposure, the participation rate of 73%, and the use of biological markers to measure exposure. With this workforce's wide and varied use of PPE, air styrene measurements, while useful to target high-risk areas and determine the need for PPE, are not as useful in trying to link health effects to exposures. Biologic exposure markers may be more efficient and accurate than air monitoring estimates of exposure doses.

Our finding of associations between styrene biomarkers and health outcomes suggest that styrene may have health effects below previously identified thresholds. Our review of chemicals in use at the plant did not identify other potential causative exposures. Yet it is possible that styrene may be a marker for another chemical source of health effects, or that some of our effects are due to co-exposures to styrene and other workplace chemicals or dust. However, when added to the evolving evidence of sentinel cases, mortality studies, animal experiments, and cross-sectional workforce studies, this study certainly supports the presence of health hazards in the reinforced plastics industry and a role for styrene as an exposure of concern.

In summary, the association of health outcomes including decreased visual contrast, decreased lung function, and increased respiratory symptoms with styrene exposure below the ACGIH recommended BEI indicates that the threshold of 400 mg/g creatinine may not protect workers from the consequences of styrene exposure. Furthermore, the excess of acquired color-blindness, though not associated with styrene biomarkers, suggests that health effects may be a consequence of relatively low styrene exposures. The association of cumulative exposure with certain outcomes indicates that there are effects, particularly on respiratory health, that are not attributable to current styrene exposure only. Further studies may help to determine an effect threshold for these health outcomes and provide guidance toward targets for exposure reduction. Additionally, such studies could further elucidate the relationship between airways disease, inflammation, and styrene.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health or the Centers for Disease Control and Prevention.

Supplement Material

Methods

Analysis of styrene metabolites:

Spot urine samples collected from workers were stored at -70 °C until the assay. An aliquot of 50 µl of each sample was assayed for 29 volatile organic metabolites using an ultra high performance liquid chromatography system (Waters Inc., Milford, MA) coupled with electro spray tandem mass spectrometry (Sciex API 5500 Triple Quad, Applied Biosystems, Foster City, CA) method (UPLC/ESI-MSMS) [Alwis et al. 2004]. Urine samples were assayed at 1:10 dilution (50 µL urine + 25 µL working mixed internal standard + 425 µL 15 mM ammonium acetate). A mixture of 28 internal standards labeled with stable isotopes was used to normalize the results. Chromatographic separation was achieved using an Acquity UPLC® HSS T3 1.8 µm x 2.1 mm x 150 mm (Waters Inc, Milford, MA) column with 15 mM ammonium acetate pH 6.8 (Solvent A) and acetonitrile (Solvent B) as mobile phases. The eluent from the column was ionized using an electrospray interface to generate and transmit negative ions into the mass spectrometer. Comparison of relative response factors (ratio of native analyte to stable isotope labeled internal standard) with known standard concentrations yielded individual analyte concentrations for unknowns. The Analyst software (version 1.5.1, Applied Biosystems, Foster City, CA) was used to operate both the UPLC and the API5500 triple quadrupole. The mass spectrometer was operated under scheduled multiple reaction monitoring (SMRM) mode for negative ions, the ion source temperature was kept at 650°C, and the electro spray ion voltage at -4000 V. The mass parameters were optimized for each analyte. For PHEMA, m/z 282/153 and m/z 282/123, for MA m/z 151/107 and m/z 152/108, and for PGA m/z 149/77 and m/z

149/105 were monitored as quantitation and confirmation ion transitions respectively. We monitored m/z 288/159 for PHEMA_13C6, m/z 156/112 for MA_D5 and m/z 154/82 for PGA_D5. The limit of detection (LOD) for PHEMA 0.7 ng/mL and for MA and PGA was 12 ng/mL.

Analysis of urine creatinine:

The urine creatinine was assayed by an enzyme colorimetric method using the automated Roche/Hitachi Modular Analytics system. The color intensity produced was measured by a multiple wavelength spectrophotometer with a 12 volt tungsten halogen lamp as the light source. The wavelengths read for this assay were 546 and 700 nm. The color intensity produced is directly proportional to the concentration of creatinine in the sample. This method is standardized against isotope dilution mass spectrometry method (ID-MS). The lower detection limit of this colorimetric method is 0.6 mg/dL.