Olfactory Function in Workers Exposed to Styrene in the Reinforced-Plastics Industry

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Background *Impairment of olfactory function in humans has been associated with occupational exposure to volatile chemicals. To investigate whether exposure to styrene was associated with olfactory impairment, olfactory function was examined in workers with a minimum of 4 years exposure to styrene in the reinforced-plastics industry (current mean exposure: 26 ppm, range: 10–60 ppm; historic mean dose: 156 ppm-years, range: 13.8–328 ppm-years) and in a group of age- and gender-matched, unexposed controls.*
Methods *Olfactory function was assessed using a standardized battery that included tests of threshold sensitivity for phenylethyl alcohol (PEA), odor identification ability, and retronasal odor perception. Odor detection thresholds for styrene were also obtained as a measure of specific adaptation to the work environment.*
Results *No differences were observed between exposed workers and controls on tests of olfactory function. Elevation of styrene odor detection thresholds among exposed workers indicated exposure-induced adaptation.*
Conclusions *The present study found no evidence among a cross-section of reinforced-plastics industry workers that current or historical exposure to styrene was associated with impairment of olfactory function. Taken together with anatomical differences between rodent and human airways and the lack of evidence for styrene metabolism in human nasal tissue, the results strongly suggest that at these concentrations, styrene is not an olfactory toxicant in humans.* Am. J. Ind. Med. 44:1–11, 2003. *© 2003 Wiley-Liss, Inc.*

KEY WORDS: olfactory function; styrene; occupational exposure; nasal effects; adaptation

INTRODUCTION

One of the potential consequences of occupational exposure to respirable chemicals, the impairment of olfactory function, has been noted in the medical literature for more than 100 years [Mackenzie, 1984]. Because the function of the olfactory system is to detect ambient chemicals, the receptors must constantly interact with chemical stimuli, some of which are potentially toxic. Olfactory receptors are located on specialized neurons that extend into the nasal cavity and are therefore uniquely and consistently exposed to the external environment. Numerous animal studies have shown that olfactory neurons are the only CNS neurons that are continuously replaced throughout the adult vertebrate lifespan, even in healthy animals housed in clean environments [e.g., Loo et al., 1996] suggesting that normal olfactory function is inherently damaging to the neurons. Exposure to higher levels of pollutants, such as can be found in some industrial environments, might therefore be hypothesized to surpass the regenerative capacity of the olfactory system and lead to dysfunction.

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While not, by itself, life-threatening, olfactory dysfunction can have serious consequences for the detection of many olfactory warning signals (e.g., smoke, spoiled food and gas leaks) [Cowart et al., 1997] and can have significant impact on nutritional status, eating satisfaction, and many other issues related to quality of life [Breslin et al., 1997]. In addition, a worker whose olfactory abilities are impaired may be at greater risk from exposure-related injuries due to the loss of an early warning system for chemical exposure.

Two lines of evidence suggest that occupational chemical exposure may impair olfactory function. First, animal toxicological studies have demonstrated selective and dose-dependent histopathologic alterations in the nasal cavity from experimental exposures to a diverse range of chemical substances, (e.g., methyl bromide, chlorine, isobutyraldehyde, formaldehyde) [Jiang et al., 1983; Hurtt et al., 1988; Monticello et al., 1991; Abdo et al., 1998]. The precise nature and distribution of these chemically induced nasal lesions can vary considerably as a function of regional deposition of the inhaled substance and the local susceptibility of the nasal tissue. However, inhalation exposure studies suggest that the olfactory epithelium is particularly vulnerable to damage by inhaled compounds [Jiang et al., 1983; Genter et al., 1998] with a variety of non-neoplastic lesions of the olfactory neuroepithelium and damage to central olfactory structures, such as the olfactory bulb, associated with chronic exposure to a variety of chemicals [Ekblom et al., 1984; Odkvist et al., 1985; Feron et al., 1986; Rose et al., 1992].

Second, a number of studies of individuals and/or populations occupationally exposed to various chemicals have reported problems with their sense of smell or apparent decrements on certain measures of olfactory function. For example, an analysis of work history and olfactory ability among the 712,000 (20–79 year old) US and Canadian respondents to the National Geographic Smell Survey revealed that factory workers reported poorer senses of smell and demonstrated objective evidence of poorer odor detection ability, although this effect was pronounced among elderly individuals [Corwin et al., 1995]. Notably, among this sample, factory workers reported the highest rates of olfactory decrements secondary to chemical exposure and head injury.

Amoore [1986] identified more than 100 airborne substances that were reported to disrupt olfactory function following either acute or chronic exposures, including organic solvents, metals, inorganic non-metallic compounds, and dusts. Much of this evidence is based on single case studies or anecdotal observations in occupational environments [Emmett, 1976; Amoore, 1986; Prudhomme et al., 1998], and relies on subjective reports of olfactory function or limited olfactory testing. Although several investigators have examined olfactory function in larger groups of chemically exposed workers and reported associations between exposure and olfactory function, the relationship is far from straightforward. For example, decrements in performance on the University of Pennsylvania Smell Identification Test (UPSIT), a 40-item test of olfactory identification ability, have been observed among some, but by no means all, workers exposed to organic solvents [Sandmark et al., 1989; Schwartz et al., 1990, 1991]; among workers exposed to acrylate and methacrylate vapors, small decrements in function were only observed for non-smokers [Schwartz et al., 1989]. In brief, although many chemicals have been implicated as causative agents in olfactory dysfunction [Amoore, 1986], considerable variation has been observed both in the type and degree of impairment associated with occupational exposures. Moreover, in the case of the few published epidemiological studies, well-matched control groups and/or assessments of current and historical chemical exposures are largely lacking.

Recent inhalation studies in animals have found that exposure to as little as 20–50 ppm styrene results in non-neoplastic, histopathological changes in the olfactory epithelium of rodents [Cruzan et al., 1997, 1998]. However, because of structural differences in the nasal passages between rodents and humans, and differences in the biochemistry of the nasal/olfactory tissue, and because the most common laboratory animals models are obligate nasal breathers, these changes might not occur in humans in comparable environments. Moreover, because animal toxicological studies have rarely incorporated functional measures, very little is known about the relationship between the type and severity of olfactory epithelial damage and the extent to which olfactory function is altered. Thus, the question of how to relate the observed structural damage in rodent olfactory epithelium to humans, and the functional consequences of such damage for either species, remains unknown. The goal of this study was to examine the effect of exposure to styrene under conditions that permitted a careful assessment of the olfactory function of both occupationally exposed workers and of a matched group of unexposed controls.

Styrene is a clear colorless liquid with a characteristic pungent odor. The primary use of styrene is in the production of polymers and copolymers, including polystyrene, styrene-butadiene-rubber, styrene-butadiene-latex, and a variety of different resins. Styrene monomer is combined with polyester resins and serves as a cross-linking agent in the manufacture of numerous fiberglass-reinforced products (bathtub and shower enclosures, boats, tanks, panels, etc.). The most significant occupational exposure to styrene vapor occurs in the reinforced-plastics industry, where exposure can occur both by inhalation and direct skin contact [Lemasters et al., 1985]. However, inhalation of styrene vapor is the major route of occupational exposure to styrene [Brooks et al., 1980] with the nasal epithelium as a point of entry. Thus, there is potential for adverse effects of occupational styrene exposure on nasal histopathology and olfactory function.

Our study was designed to evaluate whether repetitive exposure to styrene vapor at the upper range of concentrations likely to be encountered in the workplace [American Conference of Government Industrial Hygienists, 1998; Morgan, 1997] is associated with clinically significant olfactory dysfunction. To evaluate this, we measured olfactory function among workers who were occupationally exposed to styrene in the reinforced plastics industry, using a comprehensive battery of objective tests of olfactory function, developed and validated at the Monell-Jefferson Chemosensory Clinical Research Center. In addition to the potential for causing generalized olfactory dysfunction, continued or repetitive exposure to any odorous chemical will lead to olfactory adaptation, a compound-specific reduction in olfactory sensitivity to that chemical. As a measure of specific adaptation to the ambient chemical environment, we tested olfactory sensitivity to styrene vapor. All olfactory assessments were coupled with current and retrospective determinations of airborne styrene exposure for the workers [for details see Lees et al., 2003 (this issue)] and were compared with olfactory assessments performed on age- and gender-matched, non-exposed controls at each site, and with normative data obtained from healthy individuals.

MATERIALS AND METHODS

Overview of Olfactory Testing

We measured olfactory detection thresholds to two chemicals (styrene and phenylethyl alcohol) and odor identification ability using twenty chemicals in order to provide a comprehensive evaluation of olfactory function, similar to the clinical assessment used at the Monell-Jefferson Chemosensory Clinical Research Center. Each type of test can reveal a different component of olfactory function: detection thresholds can provide assessments of peripheral olfactory function, whereas tests of odor identification are assumed to tap more central components of olfaction as well. Because performance on both types of tests of olfactory ability can be influenced by a variety of demographic factors, (e.g., age, gender, and education) and dysfunction can arise from a variety of non-toxic etiologies [e.g., nasal-sinus disease or head injury; Cowart et al., 1993], it is essential to compare the performance of the exposed cohort with the performance of a suitably matched control or referent group.

There are compelling reasons to include comprehensive olfactory evaluations and appropriately matched control groups into occupational evaluations. In studies that measured both peripheral (odor detection) and central (odor identification) olfactory function within the same exposed individuals, occupational exposure to cadmium was shown to impair both the detection and the identification component of olfactory function [Rydzewski et al., 1998]. It is instructive to note that when olfactory function in a similar group of cadmium-exposed workers was compared with a group of age- and gender-matched controls, only detection sensitivity differed between the two groups [Rose et al., 1992]. The present study, therefore, attempted to address previous shortcomings in the characterization of chemical-exposure effects on olfaction by coupling a thorough evaluation of past and present styrene exposures with a battery of clinical tests that included an assessment of both peripheral and central components of general olfactory function (odor detection thresholds and odor identification tests, respectively) and a specific test of exposure-induced adaptation (detection thresholds for styrene).

If either historic or current exposure to styrene produced generalized olfactory damage, we would expect to find that the prevalence of olfactory dysfunction would be greater among workers with exposure to styrene than in the general population, as measured by our normative clinical database. However, due to the potential confounding effects of a variety of geographic, local environmental, and lifestyle factors, we also recruited and examined olfactory function in a group of referents who were similar to the workers with respect to socio-economic status, geographic location, and matched for age and gender. Comparisons between these groups were expected to reveal whether occupational exposure to styrene produces any olfactory loss or dysfunction above and beyond age- or health-associated effects found among workers of this socio-economic status, generally.

Site Selection

Workers in the reinforced-plastics industry were selected as the study group because the highest occupational exposures to styrene are reported to occur in this industry [Lemasters et al., 1985]. Solicitation letters were sent to approximately 30 candidate companies that manufactured reinforced-plastics and composite products, inviting them to participate in the study. Of those indicating interest in participating, we determined eligibility according to criteria described by Lees et al. [2002]. Based on these criteria, four facilities were selected to participate in the study: two factories engaged in the manufacture of fiberglass-reinforced shower and tub enclosures using an open-mold process, one factory that manufactured reinforced-fiberglass paneling, and one factory that produced a variety of reinforced-plastic products, including truck parts, sinks, and fan blades, using a closed-mold process.

Subjects

At each site, potentially eligible workers and controls were individually identified and recruited following a review of: (1) industrial hygiene surveys that indicated ambient

styrene concentrations for each job title and (2) personnel records identifying individual work histories. Through the company's personnel office, pre-screened workers were notified of their potential eligibility for the study and invited to fill out a medical and occupational history questionnaire, the answers to which were used to determine their suitability for the olfactory study.

To adjust for the potentially confounding effects of a wide range of social, economic, and environmental factors, we also tested a comparison worker group, comprised of workers from the same geographical region and having similar socio-economic status but no chemical exposure.

All medical/occupational questionnaire responses were masked with respect to cohort/control group membership and reviewed by the director of the chemosensory disorders clinic and the occupational physician to screen for medical conditions and prior exposure to environmental agents or chemicals, *other than styrene*, that were known or suspected to affect olfactory function. Respondents were sorted into three categories: (1) eligible, (2) eligible but requiring additional clarification on certain aspects of their medical/exposure history, and (3) ineligible. Workers in the first two categories were then invited to participate in the study, with 94% of the eligible workers agreeing to participate.

All workers and control subjects provided informed consent for their participation in the study using the form that was approved by the Committee for Studies Involving Human Beings of the Institutional Review Board at the University of Pennsylvania. Volunteers were advised that they were free to withdraw at any time. Workers continued to receive full wages during the 1–2 hr of data collection. The control subjects who participated on their own time were compensated for their participation.

Sixty-two exposed workers and sixty-seven controls were tested in the study. On the basis of follow-up questions at the time of test, eight of the exposed workers and three control subjects were excluded because of pre-existing medical conditions or exposure to other chemicals potentially causative of olfactory dysfunction. Two more workers were excluded based on age and failure to understand/comply with test instructions. To match the number, gender, and age of subjects in the worker-group, fifteen control subjects were dropped from the analysis, resulting in fifty-two subjects in each group. The selection procedure for dropping control subjects was blind with respect to olfactory performance; at each site, subjects were included in the order of testing until the control group was matched to the workers on age (within one decade) and gender.

Odor Stimulus Preparation

Styrene monomer (Sigma-Aldrich) and phenylethyl alcohol (Sigma-Aldrich) were used as stimuli in the odor detection tests. Styrene was diluted into odorless, light, white mineral oil in an 18-step binary dilution series, beginning with a concentration of 20% v/v. Prior to use, the mineral oil was filtered through a column of silica gel to remove odorous contaminants. Phenylethyl alcohol was diluted into glycerol (Sigma) in a 19-step semi-log dilution series, starting with a concentration of 100% v/v. The dilutions were placed into clean, 280 ml glass bottles, fitted with a flip-top cap into which a Teflon nosepiece was inserted. Each bottle contained 10 ml of the stimulus. For each subject, a fresh set of bottles, containing 10 ml of diluent only, served as blanks. When volunteers inhaled from the sniffing port, they sampled from the headspace inside two bottles (one sniffing port per nostril). In a typical sniff by adult males at rest, 500 ml of air is drawn through the nostrils. Thus, drawing the headspace from the two glass bottles (total volume 540 ml) should have provided an adequate volume of stimulus, undiluted by incoming air.

Banana, butterscotch, coffee, lemon, and peppermint, alcohol-free extracts (Frontier) were used as stimuli in the retronasal olfactory assessment. One-half milliliter of each extract was pipetted onto a cleaned, black, polyethylene snap cap (Wheaton) and covered with parafilm until ready for testing. One set of odorants was prepared for each participant and discarded after use.

The stimuli for the odor identification test consisted of twenty odorants that were presented in cleaned, boiled, 250 ml polypropylene squeeze bottles with flip caps (Wheaton). Each bottle contained a total of 5 ml of odorant or odorant and diluent combined. Table I lists the odorants used and presents the correct odor label for each stimulus.

New threshold series and odor identification stimuli were prepared for each week of testing and analyzed via gas chromatography immediately after the series was prepared to ensure reliability across and within series.

TABLE I. Stimuli Used in Odor Identification Test

Identification	Chemical/ odorant	Identification	Chemical/odorant
Menthol	L-Menthol	Cloves	Eugenol
Rye bread	D-Carvone	Licorice	Anethol
Turpentine	Terpinolene	Spearmint	L-Carvone
Banana	Amyl acetate	Smoke	Guaiaicol
Almond	Benzaldehyde	Wintergreen	Methyl salicylate
Strong cheese	Butyric acid	Cinnamon	Cinnamaldehyde
Fish	Triethylamine	Coconut	Octalactone
Peanut butter	Ethylpyrazine	Root beer	Safrol
Vinegar	Acetic acid	Rose	Phenylethyl alcohol
Vanilla	Vanillin	Strawberry	C-16 Aldehyde

Testing Procedure

All olfactory tests were administered in a styrene-free environment (as established by area samples using active-sampling technology to be below the analytical limit of detection, <1 ppm styrene). All exposed workers were tested during their regular 8- or 10-hr work-shift (day, evening, or night). Workers assumed their regular duties for a minimum of 1 hr and a maximum of 6 hr prior to the test. Except for workers at one site, all had worked full shifts on at least the two days immediately prior to their olfactory test. Scheduling constraints at one site meant that some workers had only worked one consecutive day prior to their test. Testing of controls and workers was alternated as much as possible during each day of testing.

All volunteers were tested individually in a single session that lasted approximately 1½ hr. Subjects were asked to refrain from smoking, eating, chewing candy, or drinking a beverage in the hour prior to the test. The session consisted of multiple assessments. First, odor detection thresholds were obtained for two compounds, phenylethyl alcohol—a measure of general olfactory function, and styrene—a measure of specific adaptation to the work environment. Next, a retronasal odor identification test was administered followed by an odor identification assessment.

Medical History and Screening Questionnaire

Prior to the olfactory evaluation, each volunteer's responses to the medical history questionnaire were again reviewed with the tester; when necessary, follow-up questions elicited additional information about specific nasal, allergy or sinus problems, head or facial injuries and surgeries, exposures to environmental agents, and medications used that may contribute to or cause olfactory dysfunction.

Olfactory Detection Thresholds

Olfactory detection thresholds were obtained by using an objective, two alternative, forced choice, modified staircase method, a procedure that is the method of choice for most clinical and experimental applications, since it provides a reliable threshold measure with a relatively small number of trials [Wetherill and Levitt, 1965; Dalton and Wysocki, 1996]. Thresholds for styrene and PEA were obtained sequentially, with the order of test counter-balanced across subjects. On each trial, the subject was presented with two pairs of bottles. One pair consisted of two blanks and the other pair consisted of one blank and the appropriate dilution step of styrene or PEA. As determined from a pilot study, the starting dilution step for styrene was step 8 and for PEA was step 11.

On receiving the set of bottles, volunteers inserted the nosepieces into each nostril and took a normal sniff from the air in the headspace. After sniffing from both pairs, volunteers were asked to identify which pair contained the odorants. Presentation of increasing or decreasing concentrations continued until the individual had achieved five reversals (a reversal is defined as two correct identifications followed by one incorrect one or one incorrect identification followed by two correct ones at a given concentration step).

Retronasal Odor Identification

Each of the five stimuli (peppermint, lemon, coffee, banana, and butterscotch) was presented twice retronasally and twice orthonasally in a pre-determined, semi-random order, as described by [Pierce and Halpern, 1996]. On each trial, the subject chose one of the five stimulus names from a given list.

Odor Identification Test

Each odorant was presented twice resulting in a forty-item test in which each presentation was associated with a choice of four labels. Each odorant name appeared twice as a correct response, twice as a near-miss, and four times as a far-miss. For example, amyl acetate (banana odor) was presented with the following labels: banana (correct), cloves (incorrect), strawberry (near-miss), smoke (incorrect). The volunteer was required to sniff from the bottle and choose from among the four options, the label that best fit the odor. Due to the inherent difficulty in the task, subjects were permitted to take more than one sniff prior to choosing the answer.

Criteria for Clinical Abnormality

The classification of olfactory dysfunction or loss among the workers tested was based upon previously established clinical criteria for the diagnosis of olfactory disorders [Cowart et al., 1997]. Each of the odor threshold measures and the identification tests were examined separately and in combination. Among the exposed workers, elevation of the styrene threshold (relative to the controls) when the PEA threshold and odor identification tests fell within normal limits was regarded as a measure of occupational adaptation to styrene, but not a clinical abnormality per se. If subjects reported odor quality distortions on the questionnaire, a primary diagnosis of dysosmia (distortions in odor quality) was assigned when (1) the PEA threshold fell within normal limits and (2) the odor identification score fell below criterion performance. A primary diagnosis of hyposmia was assigned when PEA threshold fell above the concentration step of 0.01% v/v (step 7) and an odor identification score below 31/40 for males and 34/40 for females (reflecting a gender

difference in the normative sample). These test scores were used as the basis for a diagnosis of hyposmia or anosmia. Generally, the upper (or lower) 2.5th percentile is regarded as the cutoff point for chemosensory abnormality [Feinstein, 1985]. Due to age-related declines in olfactory sensitivity observed among normative subjects beginning in the sixth or seventh decade, these criteria are based on scores obtained by subjects under 50 years of age; to avoid the necessity of age-adjusting scores in this study, the majority of our study group was comprised of individuals between 21–50 with only 5 individuals in each group who were between 50 and 60 years of age.

The retronasal odor identification test provided another unique measure of olfactory dysfunction, likely to be functionally evident in the diminished perception of everyday food flavor. Based on the limited normative sample, an identification score less than 8 out of 10 on either orthonasal or retronasal presentation was indicative of clinical abnormality.

Assessment of Current and Past Occupational Exposure to Styrene

Establishment of a relationship between occupational exposure to styrene and any observed olfactory dysfunction rests critically on the estimates and assessments of current and historical exposure to styrene among the exposed workers. Thus, personal exposure profiles were created for each of the styrene-exposed workers who participated in this study based on individual or surrogate sampling data from the facility, where available [for details about exposure reconstruction see Lees et al., 2003 (this issue)]. Current exposures to styrene were determined by full-shift, personal air sampling, and biological monitoring of each enrolled worker.

Since any effect of styrene on the human olfactory system might result either from the direct local effect of the air concentration acting on the nasal epithelium or from the effect of absorbed styrene, carried by the blood stream, we also evaluated current styrene exposures through urinalysis for metabolites of styrene (mandelic acid and phenylglyoxylic acid) [Gotell et al., 1972; Mizunuma et al., 1993]. For each worker, pre- and post-shift urine samples were collected on the day prior to the one in which they were tested for olfactory function. For each control subject, a single urine sample was obtained at the time of their olfactory test.

Data Analysis

Thresholds for PEA and styrene were calculated by taking the mean of the dilution steps representing the last four reversals in each test. If the subject was able to smell the stimulus at the weakest concentration available, they were assigned the next dilution step (e.g., step 19 for styrene and step 20 for PEA). Scores on the retronasal trials testing olfactory function were given one point for each correct answer out of a total possible of ten. The odor identification test was scored by assigning one full point for every correct answer and one-half point for every near-miss response.

To evaluate differences among the styrene-exposed workers and the control group on the clinical tests of olfactory function, separate, one-way Analysis of Variance (ANOVAs) were performed on the PEA thresholds, the retronasal scores and the odor identification scores. Potentially confounding variables were identified on the basis of prior research and included age, years of education, and smoking status (pack years); these were then used as covariates in the analysis. To evaluate differences among the workers and controls on styrene sensitivity, a one-way ANOVA was performed on the individual styrene thresholds. Multiple linear regression was used to evaluate significant differences, if any, among groups on any of the olfactory measures.

RESULTS

The demographic characteristics of each group are presented in Table II.

Current Workplace Environmental Exposures

The results of the personal air sampling during the two workshifts prior to olfactory testing are presented in Table III, for each study site. Workers were currently exposed to a range of styrene concentrations, with many exposure measurements considerably less than 30 ppm and a few exposure measurements in excess of 50 ppm. The highest air concentration measurements were confined to three individuals wearing respiratory protection, but the use of respirators ensured that their actual styrene exposures were considerably less than those measured.

In order to adjust the air exposure data, a respiratory protection factor (RPF) was determined for a group of workers at one site who were currently using respirators [Lees et al., 2002] and an RPF of 5 was subsequently used.

With the exception of Site 4, the measurements of current exposures were generally consistent with historic exposure measurements which had been made over the last ten or more years. During air sampling at Site 4, production (and, therefore, ambient concentrations) were significantly reduced from historic norms, which accounted for this difference.

TABLE II. Demographic Characteristics of Participants

	Total no.	Females	Age mean & range	Smokers	Styrene exp. duration (mean years & range)
Workers	52	6	37.6 (21–60)	35	12.5 (4–41)
Controls	52	6	36.7 (21–57)	27	0

TABLE III. Mean and Standard Deviations of Airborne Styrene Concentrations by Study Site

	Site 1	Site 2	Site 3	Site 4	Average
Day1 (ppm)	58.8 (34.6)	18.2 (23.3)	[a]	12.6 (9.1)	20.6
Day 2 (ppm)	65.5 (44.7)	15.8 (15.8)	14.1 (9.2)	11.3 (6.9)	24.5

[a]Air not sampled on day 1 at this site.

TABLE V. Means and Ranges of Historic Exposures to Airborne Styrene for Individual Workers (n = 52) (in ppm)

	Mean exposure	Cumulative mean exposure	Peak year exposure
Workers (all sites)	13.25 (3.5–31)	156 (13.8–328)	26 (5.2–76.5)

Urine samples were analyzed for the presence of the styrene metabolites, mandelic acid and phenylglyoxylic acid, corrected by measured creatinine levels. Creatinine determination was performed by colorimetric spectroscopy. Table IV shows the pre- and post-shift means and ranges of both metabolites for the workers tested. As expected, the levels of styrene metabolites for all control subjects were below the limit of detection (0.02 g/L).

Historic Workplace Environmental Exposures

Individual historic styrene exposure profiles were developed for every study participant using job title-based estimates of annual average styrene exposure for each year of employment. After accounting for respirator use, a cumulative ''effective'' estimate of styrene dose (calculated as the sum of annual average exposures and expressed in terms of ppm-years) was also established. Annual average exposures for workers at the individual sites basically mirrored styrene in air concentrations reported in the previous section except that the calculation of effective exposures (to account for the historic use of respirators by a small number of individuals), dramatically reduced the upper end of the exposure distribution (see Table V).

While both current and historic exposure measurements focused on styrene, a subset of workers in some of these facilities were exposed to a variety of other chemicals. In addition to a wide range of catalysts and other additives to the styrene resins (e.g., brominated flame retardants) used over the last two decades, laminators (gunners and rollers) historically used large quantities of acetone to clean rollers and chopper guns. Although the level of exposure to acetone is unknown, its use has been discontinued within the last 5 years at all sites studied except Site 3. In addition, Site 3 uses a small amount of methyl methacrylate in the production of panels. Air concentrations of methyl methacrylate ranged from 0 to 28 ppm (mean = 6.7 ppm) when measured in the late 1980s. More recent measurements are not available.

TABLE IV. Means and Ranges of Urinary Metabolites of Styrene

	MA Pre-shift	MA Post-shift	PGA Pre-shift	PGA Post-shift
Workers	0.10 (0.0–0.51)	0.62 (0.0–6.98)	0.07 (0.0–0.47)	0.17 (0.0–2.25)

All values for mandelic acid (MA) and phenylglyoxylic acid (PGA) are g/g creatinine corrected.

Evaluation of Olfactory Function: Exposed Workers Vs. Controls

The group means, standard deviations and ranges of scores for the three clinical tests of olfactory function are presented in Table VI. Separate, one-way ANOVAs performed on the phenylethyl alcohol thresholds, the retronasal test and the odor identification scores showed no significant differences between the performance of the styrene-exposed workers and the matched controls on any of the tests of olfactory function.

In marked contrast to the performance on the clinical olfactory assessments, odor detection thresholds for styrene were significantly different among the exposed and unexposed groups, $F\ (1,100) = 16.69$, $P = 0.00001$. On an average, the styrene threshold for exposed workers was almost four dilution steps (i.e., 32-fold) higher than for the unexposed control subjects (10.6 vs. 14.3, workers and controls, respectively). Stratifying the groups by age (in decades) revealed that this difference was not uniform across all workers in our sample. As shown in Figure 1, the youngest workers and controls showed small, but significant differences in their sensitivity to styrene during their 3rd (n = 26) and 4th (n = 36) decades. However, the greatest reduction in sensitivity to styrene was shown by the workers who were in their 5th (n = 28) and 6th (n = 14) decades.

Although there is a small, but significant correlation between the age of the workers and the duration of exposure to styrene ($r = 0.31$), the marked decline in sensitivity to styrene in older workers cannot be accounted for by the duration of their past exposure to styrene. When styrene thresholds for the exposed-worker population were regressed upon the variables of age, exposure duration, and other exposure factors (ppm-years, peak-year), age was the only significant predictor of styrene thresholds, and was still significant, even after adjusting for the effects of exposure duration ($P < 0.03$). Importantly, as shown in the inset graph depicting PEA thresholds as a function of age for both groups, there were no age-associated changes in sensitivity to PEA among workers or controls.

TABLE VI. Performance on the clinical tests of olfactory function

	Workers		Controls	
Test	**Mean (s.d.)**	**Range**	**Mean (s.d.)**	**Range**
PEA threshold (dilution step 0–20)	16.67 (4.5)	5.5–20.0	16.66 (4.8)	1.5–20.0
Retronasal ID (number correct of 10)	8.11 (1.8)	2.0–10.0	8.05 (2.0)	1.0–10.0
Odor ID (number correct of 40)	34.80 (2.6)	27.0–39.5	35.75 (3.0)	23.0–40.0

Evaluation of Olfactory Function: Incidence of Clinical Abnormality

To evaluate the incidence of clinical dysfunction (hyposmia, dysosmia, or anosmia) among either exposed-workers or controls, we compared each subject's threshold for PEA and their score on the odor identification test with the cut-off scores for olfactory function as determined by the normative sample obtained from the Monell-Jefferson CCRC database. No significant differences in test performance were found between the exposed workers and the controls.

Although a few more workers than controls reported disturbances of some sort on olfactory function, the only diagnosed abnormality of olfactory function among the entire sample was hyposmia, of varying severity, and the frequency of observed abnormality did not differ between groups. Moreover, a test of proportions revealed that the prevalence of olfactory dysfunction (as measured by the PEA threshold and odor identification test) was not higher among either group than would be expected to occur in the general population (2.5%; $z = 1.31$, $P = 0.08$).

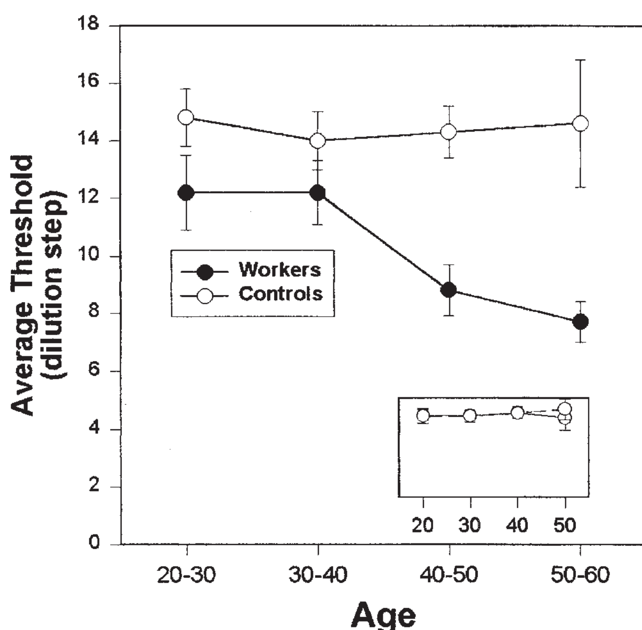

FIGURE 1. Styrene thresholds and pea thresholds (inset graph) as a function of occupational exposure and age (in decades).

Although scores on the retronasal olfactory evaluation were lower than expected in both workers and controls when compared to our normative population data, this outcome may have arisen from non-exposure related factors, such as dietary experience or cultural practices: odor identification tests are known to be influenced by previous exposure to odorants [Doty et al., 1996]. Our normative sample was drawn predominantly from an urban environment whereas participants in this study were drawn from relatively rural environments.

DISCUSSION AND CONCLUSIONS

Exposure to styrene vapor, in a cross-sectional analysis of workers in the reinforced-plastics industry, did not appear to be associated with any adverse effect on olfactory function. Performance on the tests of peripheral (PEA threshold) and central (odor identification test) olfactory function revealed no significant differences between the workers exposed to styrene and the comparison group of unexposed controls. Given the widespread use and validation of these or similar tests to diagnose and identify olfactory dysfunction in many clinical settings [e.g., Cain et al., 1988], we are confident that these evaluations have been able to reveal any patterns of generalized deficits in olfactory ability among the workers in this sample. Moreover, the lack of any observed effect on objective olfactory function in the present study correlates well with the workers' self-reported olfactory performance. Only a few workers reported problems with their sense of smell and of these complainants, only one individual showed evidence of any abnormality on objective olfactory evaluation.

Occupational exposure to styrene affected sensitivity to styrene, but this effect is expected and consistent with specific, exposure-induced effect of adaptation—which can be observed in a variety of field settings and also in the laboratory following even brief exposures to volatile chemicals [Åhlstrom et al., 1986; Dalton and Wysocki, 1996; Wysocki et al., 1997]. The present study revealed, however, that this exposure-related decrement was more pronounced among older workers than younger ones. Given that (1) all workers had been working in their regular position (and exposed to styrene vapor) for a minimum of one hour prior to olfactory testing and that (2) neither age nor exposure

duration were significant predictors of variation in any of the other olfactory measures, a plausible explanation for the age-related effect on styrene thresholds among the workers is that occupational olfactory adaptation (in this case to styrene) is both more profound and more persistent among older workers than younger ones. Age-related differences in the kinetics of olfactory adaptation have been noted previously when Stevens et al. found that the rate and degree of adaptation accelerated while recovery decelerated with age [Stevens et al., 1989]. Although exposure-induced adaptation effects appear attenuated or reversible following cessation of exposure [Åhlstrom et al., 1986; Gagnon et al., 1994; Mergler and Beauvais, 1992], the time course of recovery after long-term exposure is unknown. The more pronounced decrement in styrene sensitivity shown by the older workers may need to be taken into account in any situations where early detection of the ambient chemical is critical (i.e., detection of respirator failure).

Although there is recent evidence of histopathologic lesions in olfactory epithelium of rodents following exposure to styrene at levels similar to those experienced by many of the workers in this study, we found no evidence that such exposures in humans produced impairments in olfactory function. Since we did not examine the nasal or olfactory epithelium of the worker participants, we cannot determine from the present study whether exposure to styrene in humans results in changes in the nasal epithelium that are similar to those observed in animal toxicological studies. However, if such changes are present, they do not appear to have a functional impact on any aspect of olfactory ability as measured in this study.

How can we reconcile the failure to observe a relationship between long-term styrene exposure and adverse effects on olfactory function as demonstrated by the current study with the presence of significant olfactory lesions in experimental animals exposed at similar concentrations? Comparative studies of nasal uptake and metabolism in rodents and humans have identified several factors to account for discrepancies, and suggest that estimates of olfactory risks based on exposures to rodents may not be relevant to humans. The first factor is the presence of significant anatomical differences between the nasal passages of rats, mice, and humans that result in alterations in the volume and patterns of airflow. Thus, one explanation for the discrepancy between animal studies and the current functional assessment is that the inhaled styrene vapor may deposit in the nasal passages of humans, but unlike in rodents, high levels of deposition do not occur in areas that subserve olfaction. Evidence for this possibility comes from experimental data [Morgan and Monticello, 1990] and transport models of airflow that have been used to predict and model the deposition of inhaled chemicals in the nasal cavities of rodents [Kimbell et al., 1997] and humans [Keyhani et al., 1997]. Based on anatomical casts of the upper airways in both species, computer models reveal strikingly different patterns of airflow through the nasal compartment that result in substantial differences in chemical deposition and concentration in the olfactory regions [Morgan and Monticello, 1990]. Taken together with the fact that rodents are obligate nose breathers, these differences in structure and airflow would mean that exposure to the same airborne concentration of styrene would result in different doses and patterns of nasal deposition for humans and rodents.

Alternatively (or additionally), biochemical differences in the nasal tissue of rodents and humans could account for the disparity between the olfactory damage seen in styrene-exposed rodents and the lack of functional effects on humans exposed to styrene vapor. In the case of styrene, the primary metabolic pathway is the oxidation by cytochromes *P*-450 to two enantiomeric forms of styrene oxide [Bond, 1989]. When this metabolic process is prevented by pre-exposure to the cytochrome *P*-450 enzyme inhibitor (5-phenyl-1-pentyne), the development of olfactory lesions in rodents following exposure to styrene does not occur [Green et al., 2001]. This strongly suggests that the lesions found in rodent olfactory tissue are induced by the primary metabolite of styrene, styrene oxide, and not by exposure to styrene per se.

Evidence that this metabolite may not be present in human nasal epithelium exposed to styrene comes from a recent investigation that compared metabolic activity of styrene in vitro in rat, mouse, and human nasal respiratory tissue and found important differences in the metabolic activity of styrene across these species [Green et al., 2001]. Specifically, rat and mouse nasal respiratory fractions were found to contain high concentrations of the two cytochrome *P*-450 isoforms necessary for the conversion of styrene into styrene oxide, whereas human nasal fractions did not. Species differences in the nasal metabolism of other chemicals that produce rat nasal tumors have been recently reported as well [Green et al., 2000], suggesting that the relevance of nasal tumorigenesis studies in rodents for human risk prediction may need to be evaluated on a chemical-by-chemical basis. Both anatomical and metabolic differences could explain differences in the toxic effects of styrene on the olfactory epithelium in man and rodents.

One limitation of the present study is the potential self-selection bias of the workers that were tested. Classically, it is held that workers with perceived exposure-related health effects select out of high-exposure jobs and are thus unavailable for study. Evaluation of the remaining participants in a cross-sectional study may lead to serious underestimation of the effects under study. In the present study, however, it is equally possible that olfactory dysfunction status had an effect on exposure status, but in an opposite direction. That is, in an inversion of the 'healthy worker effect', workers with poorer olfactory ability may be more likely to remain in high-exposure job positions than workers with good olfactory ability. If this were the case, although worker complaints

about chemical odor/irritation have been reduced, we would have also expected to find a higher prevalence of olfactory dysfunction/lowered sensitivity among the worker population than the unexposed controls, which we did not observe.

Another limitation of a cross-sectional study is the inability to measure changes in olfaction from a pre-exposure or baseline level. We cannot, for example, rule out the possibility that the styrene-exposed workers, we tested, had better-than-average olfactory ability prior to styrene exposure. If this were the case, however, the comparison of the performance of the styrene-exposed workers with a carefully matched control group and the normative sample from our database strongly suggests that (1) both the exposed workers and the control group had roughly equivalent olfactory ability prior to workplace exposure, and the exposed workers gradually lost sensitivity to styrene alone and (2) exposure to styrene does not have effects on general olfactory function. Nevertheless, there may be subtle changes that occur in any chemically exposed population that could only be observed in undertaking a longitudinal study where workers who exhibit normal olfactory ability were enrolled prior to any exposure and, serving as their own controls, are assessed periodically across the course of their occupational exposure.

Much of the prior evidence for olfactory dysfunction subsequent to chemical exposure has been derived from anecdotal reports and case studies [Emmett, 1976; Prudhomme et al., 1998]. In the present study, each worker's personal exposure history was developed and documented through careful examination of work records, industrial hygiene surveys, plant records, and personal medical/occupational history interviews. Moreover, the performance of styrene-exposed workers was compared with the performance of an appropriately matched referent group and with the normative measures of performance from our clinical database. Equally important, the olfactory assessments used in the current study were logical, comprehensive, and known to be capable of detecting even subtle forms of olfactory dysfunction.

In summary, despite observations in animal studies that exposure to styrene at or below currently acceptable workplace limits produced lesions in the olfactory epithelium of rodents, the present study found no evidence among a cross-section of reinforced-plastics workers that current or historical exposure to styrene was associated with impairment of general olfactory function. Taken together with animal and human anatomical and metabolic data, the current results strongly suggest that styrene at these exposure levels is not an olfactory toxicant in humans.

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