Chem. Senses 32: 739–747, 2007
doi:10.1093/chemse/bjm041
Advance Access publication June 29, 2007

Evaluation of Long-Term Occupational Exposure to Styrene Vapor on Olfactory Function

Pamela Dalton[1], Peter S.J. Lees[2], Michele Gould[1], Daniel Dilks[1], Aleksandr Stefaniak[2], Michael Bader[3], Andreas Ihrig[3] and Gerhard Triebig[3]

[1]Monell Chemical Senses Center, Philadelphia, PA, USA, [2]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA and [3]Institute and Policlinic of Occupational and Social Medicine, University Hospital Heidelberg, Heidelberg, Germany

Correspondence to be sent to: Pamela Dalton, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104-3308, USA. e-mail: pdalton@pobox.upenn.edu

Downloaded from https://academic.oup.com/chemse/article-abstract/32/8/739/582959 by Centers For Disease Control user on 04 May 2020

Abstract

The primary sensory neurons of the olfactory system are chronically exposed to the ambient environment and may therefore be susceptible to damage from occupational exposure to many volatile chemicals. To investigate whether occupational exposure to styrene was associated with olfactory impairment, we examined olfactory function in 2 groups: workers in a German reinforced-plastics boat-manufacturing facility having a minimum of 2 years of styrene exposure (15–25 ppm as calculated from urinary metabolite concentrations, with historical exposures up to 85 ppm) and a group of age-matched workers from the same facility with lower styrene exposures. The results were also compared with normative data previously collected from healthy, unexposed individuals. Multiple measures of olfactory function were evaluated using a standardized battery of clinical assessments from the Monell–Jefferson Chemosensory Clinical Research Center that included tests of threshold sensitivity for phenylethyl alcohol (PEA) and odor identification ability. Thresholds for styrene were also obtained as a measure of occupational olfactory adaptation. Styrene exposure history was calculated through the use of past biological monitoring results for urinary metabolites of styrene (mandelic acid [MA], phenylglyoxylic acid [PGA]); current exposure was determined for each individual using passive air sampling for styrene and biological monitoring for styrene urinary metabolites. Current mean effective styrene exposure during the day of olfactory testing for the group of workers who worked directly with styrene resins was 18 ppm styrene (standard deviation [SD] = 14), 371 g/g creatinine MA + PGA (SD = 289) and that of the group of workers with lower exposures was 4.8 ppm (SD = 5.2), 93 g/g creatinine MA+PGA (SD = 100). Historic annual average exposures for all workers were greater by a factor of up to 6×. No differences unequivocally attributable to exposure status were observed between the Exposed and Comparison groups or between performance of either group and normative population values on thresholds for PEA or odor identification. Although odor identification performance was lower among workers with higher ongoing exposures, performance on this test is not a pure measure of olfactory ability and is influenced by familiarity with the stimuli and their sources. Consistent with exposure-induced sensory adaptation, however, elevated styrene thresholds were significantly associated with higher occupational exposures to styrene. In summary, the present study found no evidence among a cross-section of reinforced-plastics workers that current or historical exposure to styrene was associated with a general impairment of olfactory function. When taken together with prior studies of styrene-exposed workers, these results suggest that styrene is not a significant olfactory toxicant in humans at current exposure levels.

Key words: adaptation, biomonitoring, occupational exposure, olfactory function, styrene

Introduction

The potential for olfactory impairment from occupational exposure to volatile chemicals results from the chronic exposure of the olfactory receptor neurons to the external ambient environment. Although the olfactory system exhibits remarkable regenerative capacity, some types of exposures may exceed this capacity and lead to sensitivity loss or other dysfunction. Although not directly life threatening, olfactory dysfunction may reduce the impact of many warning signals (e.g., smoke, spoiled food, and gas leaks) (Cowart et al. 1997; Doty and Hastings 2001; Santos et al. 2004; Dalton and Opiekun 2006) and may affect nutritional status, eating satisfaction, and many other issues related to quality of life (Hummel and Nordin 2005). Among individuals occupationally exposed to chemicals, however, loss of olfaction

© The Author 2007. Published by Oxford University Press. All rights reserved.
For permissions, please e-mail: journals.permissions@oxfordjournals.org

may lead to greater risk from exposure-related injuries due to the loss of an early warning system for chemical exposure (Bramerson et al. 2007).

The goal of this study was to evaluate the impact of occupational exposure to styrene on olfactory function. Styrene is a clear colorless liquid with a characteristic pungent odor that is primarily used 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 many fiberglass-reinforced plastic products including boats, as was the case in this study. Because inhalation of styrene vapor is the major route of occupational exposure (Brooks et al. 1980) with the nasal epithelium as the point of entry, there is significant potential for damage to nasal mucosa and olfactory function from occupational styrene exposure.

Animal studies have found that exposure to airborne concentrations of styrene at 20–50 ppm produced nonneoplastic, histopathological changes in the olfactory epithelium of rodents (Cruzan et al. 1997, 1998). Although a recent study evaluating olfactory function in workers exposed to styrene in the reinforced-plastics industry did not find an association between occupational exposure to styrene and olfactory dysfunction (Dalton et al. 2003), the majority of workers tested in that cohort were exposed to styrene at concentrations falling in the lower end of the range that elicited olfactory lesions in rodents (e.g., ~20–30 ppm). Hence, we sought to replicate this investigation in a cohort of workers with historical exposure to higher concentrations of styrene vapor. Workers exposed to styrene vapor in the process of laminating the decks and hulls of reinforced-plastic (fiberglass) boats have been documented to have historic styrene exposures at the higher end of the range of interest (Triebig et al. 1989). An additional advantage of evaluating this cohort was the presence of a long and well-documented medical and occupational exposure history for individual workers, based on biological monitoring (Triebig et al.1989), thereby allowing us to develop individual effective exposure histories, corrected for respirator use, for each worker whose olfactory function was evaluated. Thus, the goal of the present study was to use biomonitoring data to evaluate whether workers employed at a boat-manufacturing plant with current and historic occupational exposure to airborne styrene vapor exhibited any functional impairment in olfaction, relative to unexposed workers or normative controls.

Materials and methods

Subjects

Workers employed in a boat-manufacturing facility, and whose occupational exposures had been documented for a number of years, were recruited to participate in this study. Styrene-exposed workers were individually identified and recruited following a review of 1) historic air and biological monitoring surveys that established the relevant styrene concentrations for each individual/job title, 2) personnel records identifying individual work histories, and 3) medical records confirming eligibility for the study. All workers provided informed consent for their participation in the study using a form that had been approved by the Institutional Review Board at the University of Pennsylvania. They were advised and they stated that they understood they were free to withdraw from the study at any time. All participants continued to receive full wages during the 1–2 h of data collection.

Individual workers were divided into 2 groups. Fifteen workers whose jobs directly exposed them to the highest concentrations of styrene vapor in this facility were assigned to the ''Exposed'' group, whereas a matched group of 16 workers whose jobs exposed them to lower concentrations (or no) styrene were assigned to the ''Comparison'' group. The majority of workers in the Exposed group were directly involved in laminating the decks or hulls of boats with fiberglass resin, whereas workers in the Comparison group were employed in the warehouse or as finishers, electricians, or metalworkers and thus had indirect and substantially lower exposure to styrene. Workers in the Exposed group had a mean age of 39.6 years (range 29–51) and an average of 8.7 years (standard deviation [SD] = 4.1) of employment in the facility. Workers in the Comparison group had a mean age of 41 years (range 30–53) and an average of 3.7 years (SD = 4.6) of employment in the facility. The data from one comparison subject was excluded because of an inability to follow instructions and perform the required tasks, thus leaving 15 subjects in each group.

Procedures

Olfactory testing took place in a styrene-free environment (<0.1 ppm) on the premises and was conducted for each subject in a single session that lasted approximately 1–2 h. The odor evaluation consisted of 2 parts, based on the protocol for evaluating olfactory dysfunction established by the Monell–Jefferson Chemosensory Clinical Research Center (MJCCRC): 1) detection thresholds for 2 compounds, styrene and phenylethyl alcohol (PEA) and 2) an 18-item odor identification test. To ensure uniformity in directions, all instructions for each part of the study were recorded on audiotape and then played for each of the subjects immediately prior to each phase of the experimental session.

Odor detection thresholds

Styrene monomer and PEA were used as the stimuli. Styrene (Sigma-Aldrich, St Louis, MO) was diluted in filtered mineral oil (Sigma-Aldrich) and placed into glass bottles in an 18-step binary dilution series, starting with a concentration of 20% v/v liquid (headspace concentration = ~11 000 ppm). PEA (Sigma-Aldrich) was diluted into glycerol (Sigma-Aldrich) and placed into glass bottles in a 19-step

Downloaded from https://academic.oup.com/chemse/article-abstract/32/8/739/582959 by Centers For Disease Control user on 04 May 2020

semilog dilution series, starting with a concentration of 100% v/v liquid. Each bottle contained 10 ml of a stimulus (or the diluent alone). When volunteers inhaled from the sniffing ports, they sampled from the ~270 ml headspace inside each of the 2 bottles (one sniffing port per nostril). Vapor concentrations were verified by analyzing headspace above each stimulus concentration with a gas chromatograph (Agilent Technologies 6890, Santa Clara, CA). Ten-point calibration curves for styrene and for PEA were generated from injection of known amounts and their associated chromatographic readings and were used to convert the readings from the dilution steps into concentration units (ppm by volume). The coefficient of determination (r^2) for each calibration curve was greater than 0.99.

Olfactory detection thresholds were obtained using an objective, 2-alternative, forced-choice, up–down, staircase method with a 5-reversal criterion (Wetherill and Levitt 1965).

Odor identification

The odor identification assessment consisted of 18 odorants that were presented in cleaned, boiled, 250-ml polypropylene squeeze bottles with flip caps (Wheaton Plastics, Millville, NJ). The test was modified from the original version administered in the MJCCRC in 2 ways: 1) by eliminating 2 odorants (root beer and wintergreen) that were infrequently encountered, and thus unfamiliar, among this population and 2) by providing both the name and a picture of the test item to facilitate identification of each odorant. Each bottle contained a total of 5 ml of odorant and diluent combined (for dilutions and concentrations see [Dalton et al. 2003]). Table 1 identifies the chemical and the correct label for the odor.

Each odor presentation was associated with a choice of 4 word identification labels, and each word label was coupled with a picture of the odor source on a 20×30 cm answer card. Following the presentation of each odor, the subject was shown the appropriate answer card and asked to identify which of the 4 choices on the card best corresponded to the presented odor. For example, the odor of amyl acetate (banana odor) would be presented with the following word labels and illustrations: banana (correct), cloves (far miss), strawberry (near miss), and smoke (far miss).

Due to the inherent difficulty in the identification task, subjects were permitted to take more than one sniff prior to choosing an answer. Each odor stimulus was presented in 2 separate trials, resulting in a 36-six item test in which each odorant label/picture appeared twice as a correct response, twice as a near miss and 4 times as a far miss.

Table 1 Odorants and labels used in odor identification test

Identification	Chemical/odorant	Identification	Chemical/odorant
Menthol	Menthol	Vanilla	Vanillin
Rye Bread	D-Carvone	Cloves	Eugenol
Turpentine	Terpinolene	Licorice	Anethol
Banana	Amyl acetate	Spearmint	L-Carvone
Almond	Benzaldehyde	Smoke	Guaiaicol
Strong cheese	Butyric acid	Cinnamon	Cinnamaldehyde
Fish	Trimethylamine	Coconut	Octalactone
Peanut butter	Ethylpyrazine	Rose	PEA
Vinegar	Acetic acid	Strawberry	C-16 aldehyde

Assessment of current and historic occupational exposure to styrene

Each worker's current styrene exposure was determined through the use of air sampling and biological monitoring of styrene metabolites in urine. Historic exposure profiles were constructed for each of the styrene-exposed workers who participated in this study based on individual air and biological sampling data collected over the course of their employment at the facility.

Because the historic record of exposures as measured by urinary metabolites was far more extensive than the air sampling data, urinary markers were used as the primary measure of current and historical exposure. These urinary biological indicators of exposure to styrene (mandelic acid [MA] and phenylglyoxylic acid [PGA]) have been shown to correlate well with styrene concentrations measured in air, blood, and breath (Gotell et al. 1972); a quantitative relationship between air styrene concentration and urinary metabolite concentrations in similar reinforced-plastic fabrication operations has been developed previously by the authors (Lees et al. 2003). The use of biological monitoring has the advantage over traditional air sampling in this circumstance as it allows direct comparison of biological uptake, that is, "effective" exposures for those workers who wore respirators with those who did not wear respirators. The ability to link internal styrene dose with olfactory performance measures is also an important feature of this study because styrene may impact the olfactory epithelium either through direct airborne exposure or through blood-borne circulation in the olfactory cleft.

Samples to assess current airborne exposures were collected using passive organic vapor monitors (Model 3500, 3M Corp., St Paul, MN) and analyzed in conformance with the National Institute for Occupational Safety and Health (1994) Method 1501. After collection, samples were sealed and stored in a cool environment prior to shipping. The samples were shipped and analyzed as a single batch using gas chromatography with a flame ionization detector by Clayton Environmental Consultants (Novi, MI). This commercial laboratory is fully accredited under the American Industrial Hygiene Association's Industrial Hygiene Laboratory Accreditation Program for the gas chromatographic method used for analysis of these styrene samples.

Current styrene exposures were also evaluated on the day prior to each participant's olfactory assessment through

Downloaded from https://academic.oup.com/chemse/article-abstract/32/8/739/582959 by Centers For Disease Control user on 04 May 2020

end-of-shift collection of urine samples and analysis of urinary MA and PGA concentrations. These metabolites were also measured in urine samples collected at the time of the olfactory exam. Because olfactory exams were scattered throughout the workday, workers were exposed from approximately 3–7 h prior to the collection of their urine sample. Once collected, all samples were immediately frozen and at the end of data collection shipped on dry ice to the analytical laboratory (National Medical Services, Willow Grove, PA) where they were maintained at −10 °C until analyzed. Urinary MA and PGA were analyzed by a method employing anion exchange chromatography with suppressed conductivity detection derived from Murer et al. (1994); creatinine was analyzed by a standard test kit based on a colorimetric spectroscopic method (IL Test creatinine; Instrumentation Laboratory Company, Lexington, MA). Measured MA and PGA concentrations were normalized to measured creatinine levels to compensate for variations in hydration, that is, urinary dilution. Following the recommendation of the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (Deutsche Forschungsgemeinschaft Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area 2004), the sum of creatinine-corrected urinary MA and PGA concentrations (MA + PGA) was used as the surrogate of styrene exposure.

Exposure histories were reconstructed for each worker participating in the study from the extensive historical record of urinary metabolite measurements at this facility. Exposure reconstruction was done on an individual basis, that is, for each worker, only his own historic urinary metabolite values were used to reconstruct exposure history. As with current exposures, exposure was calculated as the sum of MA and PGA. When there were multiple urinary metabolite measurements in a calendar year, the annual average (MA + PGA) value was used. If a measurement was missing for a given year, a value was imputed using a linear interpolation between the values for the years preceding and following the missing year.

For both current and historic exposures, the results of the biological monitoring were entered into a previously developed regression model (Lees et al. 2003) to calculate the corresponding airborne (respiratory) exposure:

$$\log(\text{urinary MA} + \text{PGA, mg/g creatinine}) = 1.0513 \log(\text{air styrene, ppm}) + 1.2490.$$

This calculated airborne styrene concentration does not correspond to measured airborne concentrations as it takes the protective capacities of respirator use into account. In effect, it represents the airborne styrene concentration inside of the respirator and is a measure of the actual biological exposure. A comparison of measured airborne styrene concentrations and metabolite-derived effective exposures suggests that the powered air-purifying respirators used by the laminators and sprayers provided a protection factor of approximately 3–6×. Given the known capabilities of this type of respirator and the observed usage patterns by this population, this is a reasonable value.

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). Threshold and odor identification test scores were used as the basis for a diagnosis of hyposmia (a decreased sensitivity to odors), anosmia (a loss of ability to detect odors), or dysosmia (a distortion in odor quality). Although Feinstein (Feinstein 1985) has identified the upper (or lower) 2.5th percentile as the cutoff point for chemosensory abnormality, most chemosensory clinical research centers (Doty et al. 1984; Cowart et al. 1997; Kobal et al. 2000) regard the 10th percentile as the cutoff for hyposmia, which we also do here. 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 from 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 and 50 years old with only 3 individuals who were between 50 and 53 years of age.

Each of the odor threshold measures and the identification tests were examined separately and in combination. A primary diagnosis of dysosmia is typically assigned 1) when the subject complains of odor quality distortions, 2) the PEA threshold falls within normal limits (<0.01% v/v), and 3) the odor identification score falls below criterion performance (<77.5% correct for males). A primary diagnosis of hyposmia is assigned when PEA threshold falls above the concentration step of 0.01% v/v (Step 7) and the odor identification score falls below 77.5%. Elevation of the styrene threshold following exposure when the PEA threshold and odor identification tests fall within normal limits is regarded as a measure of occupational olfactory adaptation to styrene, but not a clinical abnormality.

Data analysis

Thresholds for PEA and styrene were determined by calculating the mean of the dilution steps representing the last 4 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). 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 between the Exposed and Comparison groups on the threshold scores, separate, nonparametric Mann-Whitney *U* tests were performed (as is typical for sensory thresholds, scores are not normally distributed).

Downloaded from https://academic.oup.com/chemse/article-abstract/32/8/739/582959 by Centers For Disease Control user on 04 May 2020

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 between the groups on odor identification performance, a *t*-test was performed. Multiple regression analysis was used to explore differences, if any, between groups on any of the olfactory measures as a function of current and historical (lifetime) exposure to styrene. Urinary metabolite values were used as measures of exposure in all analyses.

Results

Current workplace environmental exposures

The results of the biological monitoring and the calculated equivalent concentration of styrene in air during the work shift on the day of the olfactory function testing are presented in Table 2.

These results indicate that the directly exposed laminator and sprayer populations are currently exposed to effective styrene concentrations in the range of 10–25 ppm. Measured airborne styrene concentrations (not shown) for this group were in the range of 70–80 ppm. The wider range of equivalent airborne exposures is thought to be a function of the wide range of respirator-use practices between individuals. Workers in the other job categories did not work directly with styrene resins but worked in the vicinity of spraying and laminating operations or conducted their work in or on the recently completed boat shell. As expected, levels of urinary metabolites in this group indicated minimal to very moderate levels of exposure to styrene during the day of the olfactory assessment. The mean values presented in Table 2 for these indirectly exposed workers are skewed by single unexpectedly high metabolite values in each of the electrician, finisher, and warehouseman job categories. The reason for these high values was not readily apparent. Overall, the exposure data do not indicate an "exposed" and an "unexposed" population, but rather a range of exposures varying by a factor of almost 50×.

Table 2 Summary of current styrene exposures, mean metabolite concentrations, and calculated effective mean concentration of styrene in air by job title

Job title	*N*	Respirator use	Mean ± SD (MA + PGA, mg/g creatinine)	Mean ± SD equivalent airborne styrene concentration (ppm)
Hull laminator	6	Yes	277 ± 194	14 ± 10
Deck laminator	7	Yes	463 ± 363	22 ± 18
Sprayer	2	Intermittent	220 ± 14	11 ± 1
Deck mounter	1	No	55	2.9
Woodworker	3	No	73 ± 6	3.8 ± 0.3
Metalworker	1	No	10	0.6
Finisher	3	No	80 ± 56	4.2 ± 3.0
Electrician	4	No	141 ± 182	7.1 ± 9.2
Warehouseman	3	No	78 ± 98	4.1 ± 5.1

Historic workplace environmental exposures

Individual historic exposure profiles were developed for every study participant using annual average (MA + PGA) concentrations for each year of employment. An estimate of cumulative lifetime styrene dose was derived for each worker from the historic exposure profiles. Both mean annual exposure and cumulative dose metrics were then used to analyze current olfactory acuity as a function of historical styrene exposure.

A summary of annual mean exposure levels as measured by urinary biomarkers and the derived effective airborne concentrations is presented in Table 3. Mean exposures are grouped by job title for 5-year intervals. Examination of the data for the most recent period (1995–1999) shows that the directly exposed population of laminators and sprayers are exposed at a level approximately 2× that of the current measured exposure (collected at the end of 1999). It further appears that the average level of effective exposure for the laminator and sprayer population ranged from approximately 30–50 ppm over the decade preceding this study. Annual average effective exposures for this group were considerably higher, in the range of 50–85 ppm during the earliest years of their work history. Although the historic exposure data for the indirectly exposed population are considerably sparser, the same general pattern of exposures is exhibited relative to the current measures. Overall, the historic exposure data do not indicate an exposed and an unexposed population, but rather a range of exposures varying by a factor of almost 100×.

Evaluation of olfactory sensitivity: Exposed versus Comparison groups

The group means, SDs, and ranges of scores for the clinical tests of olfactory function are presented in Table 4. There was no significant difference in odor detection threshold concentrations for PEA between the 2 groups of workers, $U = 92$, $P = 0.57$. In marked contrast, however, odor detection thresholds for styrene were significantly different between the Exposed and Comparison groups, $U = 56$, $P = 0.01$. On average, the styrene odor detection threshold for the Exposed group was almost 10 times higher than that of the Comparison group (56 vs. 6 ppm, respectively). Interestingly, regression analysis on both groups combined revealed that styrene thresholds were only predicted by styrene exposure concentration on the day of the olfactory test ($r^2 = 0.45$, $P < 0.01$). For the group with the highest historical exposures, neither the exposures measured on the previous day ($r^2 = 0.12$, $P > 0.10$; $r^2 = 0.33$, $P > 0.10$) nor their lifetime

Downloaded from https://academic.oup.com/chemse/article-abstract/32/8/739/582959 by Centers For Disease Control user on 04 May 2020

Table 3 Summary of historic styrene exposures, mean metabolite concentrations, and calculated effective mean concentration of styrene in air by job title and year

Job title	1984–1989				1990–1994				1995–1999			
	n	person-years	MA + PGA (mg/g creatinine)	Air equivalent (ppm)	*n*	person-years	MA + PGA (mg/g creatinine)	Air equivalent (ppm)	*n*	person-years	MA + PGA (mg/g creatinine)	Air equivalent (ppm)
Hull laminator	3	13	1876	84	6	24	720	34	6	30	729	34
Deck laminator	1	5	1240	57	5	13	732	34	7	35	694	33
Sprayer	0	0	NA	NA	0	0	NA	NA	2	8	1015	47
Deck mounter	0	0	NA	NA	0	0	NA	NA	1	3	110	6
Woodworker	1	6	326	16	2	6	328	16	3	11	174	9
Metalworker	0	0	NA	NA	1	5	299	15	1	5	315	15
Finisher	0	0	NA	NA	1	3	27	1	3	7	113	6
Electrician	0	0	NA	NA	0	0	NA	NA	4	4	71	4
Warehouseman	0	0	NA	NA	1	5	11	0.6	3	11	12	0.7

NA, not applicable.

Table 4 Group threshold means and SDs (in parentheses) for PEA (in ppb air) and for styrene (in ppm air); odor identification task (no. correct out of 36 possible)

Group	PEA (ppb)	Styrene (ppm)	Odor identification (no. correct)
Exposed	4.1 (6.4)	56.8 (10.4)	28.2 (4.2)
Comparison	2.4 (8.8)	6.2 (9.5)	31.1 (2.7)

exposures ($r^2 = 0.17$, $P > 0.10$; $r^2 = 0.67$, $P > 0.10$) were predictive of olfactory sensitivity to either PEA or styrene, respectively. In light of 1) our failure to observe an association between lifetime exposure and styrene sensitivity among the group with the highest exposures and 2) no significant differences in PEA sensitivity between the 2 worker groups, the most likely explanation for the decreased sensitivity to styrene among workers with higher exposure is olfactory adaptation. Olfactory adaptation is a well-documented and typically reversible decrease in sensitivity to a stimulus resulting from recent prior exposure to that stimulus (Ähls trom et al. 1986; Dalton et al. 1997; Wysocki et al. 1997).

When the PEA thresholds for the 2 groups in this study were compared with those of styrene-exposed workers and matched unexposed controls in the United States (Dalton et al. 2003), the results were highly consistent (2.75 ppb and 3.1 ppb for exposed workers and controls in the United States vs. 2.45 ppb and 4.1 ppb for the 2 groups in this study). Despite differences in both the concentration and length of exposure to styrene in the 2 studies, elevations of styrene thresholds among workers with regular exposure were also quite comparable.

In contrast with this earlier study, however, a *t*-test performed on the odor identification test scores revealed significant differences in response between the 2 groups of workers ($t(28) = 4.80$, $P = 0.03$). As a group, those workers with higher exposures to styrene had lower scores on the test of olfactory identification than did workers in the other group. However, among the workers with lower scores on odor identification, multiple regression analysis showed no relationship between current styrene exposure levels (i.e., on the day of olfactory testing or on the preceding day) or historical exposures and performance on the odor identification test ($P = 0.90$ and $P = 0.78$, respectively). Although not definitive, this outcome suggest that the difference in performance observed may arise from factors other than current or cumulative styrene exposure.

Evaluation of olfactory function: incidence of clinical abnormality

To evaluate the incidence of clinical dysfunction (hyposmia, dysosmia, or anosmia), we compared each subject's threshold for PEA and their score on the odor identification test with the cutoff scores for olfactory function as determined by the normative sample obtained from the MJCCRC database.

One individual among the group with higher styrene exposures was diagnosed as hyposmic, as indicated by a relatively high threshold for PEA and a below-average score on the odor identification test. Six workers with higher styrene exposures (40%) and 3 workers with lower exposures (20%) had scores on the odor identification test at or just below the criteria cutoff for males (<77.5%) as defined by the normative database established at the MJCCRC. This represents a higher proportion of abnormal responders in both groups than would be expected to occur in the general population (10%) (Doty et al. 1984; Cowart et al. 1997; Kobal et al. 2000). When coupled with a normal PEA threshold and clinical complaints of odor quality distortions, a suboptimal score on odor identification performance is generally

Downloaded from https://academic.oup.com/chemse/article-abstract/32/8/739/582959 by Centers For Disease Control user on 04 May 2020

considered to reflect a diagnosis of olfactory dysosmia. However, results from the screening questionnaire indicated no complaints of quality distortions reported in either group. It should also be noted that, unlike detection thresholds, performance on the odor identification test is not a pure measure of olfactory ability but instead can be influenced by nonsensory factors such as education level and cultural familiarity with the odorant stimuli used (Doty et al. 1985). Notably, the odor identification test was modified to exclude 2 odorants that are not familiar among Germans, but many of the eligible participants, especially those with higher exposures, were recent immigrants from different cultural backgrounds. Thus, it is possible that the relatively marginal performance on this test by individuals in both groups is attributable to differences in familiarity with the test stimuli and their sources or labels, rather than deficits in olfactory acuity. Evidence supporting this hypothesis comes from 1) a lack of association between performance on this test and current or historical styrene exposures and 2) a slightly higher prevalence of individuals who reported familiarity with the test stimuli among the group with lower styrene exposures. In this facility, however, both the airborne styrene concentrations and metabolite levels were higher among some of the workers than in a previous study where no differences in olfactory performance between exposed and unexposed workers were observed (Dalton et al. 2003). Although the average duration of employment in this facility was less than the average duration in the prior study (7.5 years vs. 10. 8 years), the possibility exists that peak exposures, rather than chronic exposure, to styrene at the concentrations experienced in this boatbuilding facility may result in aberrations in the quality of odor sensation. The occurrence of dysosmia, leading to poorer performance on an odor identification task, has been associated with acute, high exposures to solvents or other olfactory toxicants (Doty and Hastings 2001; Dalton and Opiekun 2006). Given that neither the current nor historic exposure monitoring in this cohort would be capable of capturing such an occurrence, the possibility that some workers may have had previous or other chemical exposure-induced olfactory impairment cannot be ruled out. Nonetheless, the comparable performance on PEA sensitivity between these workers, the prior workers tested, and the normative database coupled with the lack of association between current and historic exposure and odor identification ability suggest that at current exposure levels in this facility, exposure to styrene may not be associated with olfactory toxicity.

Discussion

In this cross-sectional analysis of workers in the reinforced-plastics industry, historic effective exposures to styrene vapors at concentrations in the range of <1–85 ppm did not appear to be associated with adverse effects on general olfactory function (e.g., PEA sensitivity and odor identification). Performance on threshold tests of olfactory function (PEA threshold) revealed no significant differences between the 2 groups. Performance on the odor identification test, which is a measure of both olfactory and cognitive (naming, identifying) ability, showed a significant difference between the 2 groups, with individuals who were exposed to higher concentrations of styrene having lower scores on odor identification than those exposed to lower concentrations. However, the possibility of alternative interpretations of this marginal finding, such as cultural and educational differences between the 2 groups, raise significant questions about whether this test can be viewed as a measure of olfactory deficits in a population for which we lack extensive normative data on the test stimuli. Only one individual showed clear evidence of hyposmia on objective olfactory evaluation, and although this individual was in the group with higher exposures, the frequency of this finding is not greater than would be expected by chance alone.

A study by Chang et al. (2004) compared the olfactory performance of a group of styrene-exposed, injection-molding workers with a unexposed reference group using thresholds for *n*-butanol and odor identification tests both prior to and following a work shift. They found no difference from pre- to postshift among the groups on the test of odor identification, but they did observe a small, but significant, postshift elevation in butanol thresholds among the group with styrene exposure. However, the similarity among the groups in their preshift threshold for butanol suggests that any effect of styrene exposure on butanol sensitivity may be quite transient. Unfortunately, because neither air nor biological measures of worker styrene exposure were obtained for the exposed cohort in the Chang study, it is difficult to compare the results from that study with those reported here.

Although the number of individuals evaluated in this study was relatively few, the results of this study are consistent with an earlier study of styrene-exposed workers and matched controls conducted in facilities in the United States (Dalton et al. 2003) where 56 workers were evaluated and compared with matched controls and normative values. Although, on average, participants in the current study had higher historical exposures to styrene than in the US cohort, no significant differences were observed on measures of threshold sensitivity to PEA among any of the exposed workers and the nonexposed controls, whereas similar elevations in threshold for styrene were found among the directly exposed workers in both studies. In the absence of any alterations on PEA sensitivity, the most plausible explanation for the elevation in styrene threshold is olfactory adaptation, a specific, exposure-induced effect of repetitive exposure on olfactory sensitivity (Ählstrom et al. 1986; Dalton and Wysocki 1996; Wysocki et al. 1997). Exposure-induced adaptation effects appear to be attenuated or reversible following cessation of exposure (Ählstrom et al. 1986; Mergler and Beauvais 1992; Gagnon et al. 1994). Nevertheless, the pronounced decrement in styrene sensitivity found among workers in the Exposed group may need to be taken into account in

Downloaded from https://academic.oup.com/chemse/article-abstract/32/8/739/582959 by Centers For Disease Control user on 04 May 2020

any situations where early detection of the ambient chemical is critical (i.e., detection of respirator failure).

Although the presence of histopathologic lesions in the olfactory epithelium of rodents exposed to styrene at concentrations similar to those experienced by workers in this study raises the possibility of risks to human nasal function (Cruzan et al. 1997, 1998), there was no clear evidence from this study that exposure to styrene is associated with impairments in olfactory function. We did not biopsy or otherwise examine the nasal or olfactory epithelium of the worker participants and thus 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 did not affect sensitivity to PEA and may not influence odor identification if cultural factors can account for the differences observed. This is especially likely given the lack of association between odor identification performance and measures of current or historic uptake of styrene vapor.

Comparative studies of nasal uptake and metabolism in rodents and humans have identified several factors that 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 histological evaluations and the current functional assessment in humans 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 containing olfactory neuroepithelium. 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 the site of chemical absorption 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 suggest that exposure to the same airborne concentration of styrene could result in different doses and patterns of nasal deposition for humans and rodents.

Differences in the biochemistry of the nasal tissue of rodents and humans could also account for disparities 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 2 enantiomeric forms of styrene oxide (Bond 1989). When this metabolic process is prevented by preexposure 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, Toghill, and Foster 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 styrene.

Evidence that this metabolite may not be present in human nasal epithelium exposed to styrene comes from a study that compared metabolic activity of styrene in rat, mouse, and human nasal respiratory tissue and found important differences in the metabolic activity of styrene across these species (Green, Lee et al. 2001). Specifically, rat and mouse nasal respiratory fractions were found to contain high concentrations of the 2 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 reported as well (Green et al. 2000), suggesting caution in interpreting the relevance of nasal tumorigenesis studies in rodents for human risk prediction. Both anatomical and metabolic differences could explain differences in the toxic effects of styrene on the olfactory epithelium in man and rodents.

Animal studies have shown that exposure to styrene, at or below concentrations experienced by our study group, produces lesions in the olfactory epithelium of rodents. The present study, however, found no robust associations between duration or concentration of exposure to styrene vapor and general olfactory ability (e.g., PEA sensitivity and odor identification) in a cross-section of workers employed in a boat-manufacturing facility. The evaluation of olfactory function in the workplace is an important tool to protect the health and safety of chemically exposed workers but is of greatest value when coupled with ongoing, preferably biological, exposure monitoring to correlate any observed effects with personal exposure history. Utilizing these methods, results from the current study, taken together with a prior study showing no impairment of olfactory function in styrene-exposed workers (Dalton et al. 2003), suggest that at the concentrations studied here, styrene may not be associated with olfactory toxicity in humans.

Funding

The European Chemical Industry Council; the National Institutes of Health to P.D.

Acknowledgements

The performance site was the Institute and Out-patient Clinic of Occupational and Social Medicine of the University Hospital Heidelberg, Heidelberg, Germany. The authors wish to thank Eva Hungerland and Nadine Doolittle for their assistance in the conduct of this study.

References

Ählstrom R, Berglund B, Berglund U, Lindvall T, Wennberg A. 1986. Impaired odor perception in tank cleaners. Scand J Work Environ Health. 12:574–581.

Downloaded from https://academic.oup.com/chemse/article-abstract/32/8/739/582959 by Centers For Disease Control user on 04 May 2020

Bond JA. 1989. Review of the toxicology of styrene. CRC Crit Rev Toxicol.19:227–249.

Bramerson A, Nordin S, Bende M. 2007. Clinical experience with patients with olfactory complaints, and their quality of life. Acta Otolaryngol. 127:167–174.

Brooks SM, Anderson L, Emmett E, Carson A, Tsay J, Elia V, Buncher R, Karbowsky R. 1980. The effects of protective equipment on styrene exposure in workers in the reinforced plastics industry. Arch Environ Health. 35:287–294.

Cheng SF, Chen ML, Hung PC, Chen CJ, Mao IF. 2004. Olfactory loss in poly (acrylonitrile-butadiene-styrene) plastic injection-moulding workers. Occup Med (Lond). 54:469–474.

Cowart BJ, Young IM, Feldman RS, Lowry LD. 1997. Clinical disorders of smell and taste. Occupational medicine: state of the art reviews. Philadelphia (PA): Hanley & Belfus, Inc. p. 465–483.

Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Hardy CJ, Coombs DW, Mullins PA, Brown WR. 1998. Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. Toxicol Sci. 46:266–281.

Cruzan G, Cushman JR, Andrews LS, Granville GC, Miller RR, Hardy CJ, Coombs DW, Mullins PA. 1997. Subchronic inhalation studies of styrene in CD rats and CD-1 mice. Fundam Appl Toxicol. 35:152–165.

Dalton P, Lees PSJ, Cowart BJ, Emmett EA, Dilks DD, Stefaniak A, Gould M. 2003. Olfactory function in workers exposed to styrene in the reinforced-plastics industry. Am J Ind Med. 44:1–11.

Dalton P, Opiekun RE. 2006. Effects of inhaled toxicants on the nose and nasal function. In: Salem H, Katz S, editors. Inhalation Toxicology. Boca Raton (FL): CRC Press. p. 373–404.

Dalton P, Wysocki CJ. 1996. The nature and duration of adaptation following long-term exposure to odors. Percep Psychophys. 58:781–792.

Dalton P, Wysocki CJ, Brody MJ, Lawley HJ. 1997. Perceived odor, irritation and health symptoms following short-term exposure to acetone. Am J Ind Med. 31:558–569.

Deutsche Forschungsgemeinschaft Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. 2004. List of MAK and BAT values: maximum concentrations and biological tolerance values at the workplace. Weinheim (Germany): Wiley-VCH.

Doty RL, Applebaum S, Zusho H, Settle RG. 1985. Sex differences in odor identification ability: a cross-cultural analysis. Neuropsychologia. 23:667–672.

Doty RL, Hastings L. 2001. Neurotoxic exposure and olfactory impairment. Neurotoxicology. 1:547–573.

Doty RL, Shaman P, Dann M. 1984. Development of the University of Pennsylvania mell identification test: a standardized microencapsulated test of olfactory function. Physiol Behav. 32:489–502.

Feinstein AR. 1985. Clinical epidemiology: the architecture of clinical research. Philadelphia (PA): WB Saunders Co.

Gagnon P, Mergler D, Lapare S. 1994. Olfactory adaptation, threshold shift and recovery at low levels of exposure to methyl isobutyl ketone (MIBK). Neurotoxicology. 15:637–642.

Gotell P, Axelson O, Lindelof B. 1972. Field studies on human styrene exposure. Work Environ Health. 9:76–83.

Green T, Lee R, Moore RB, Ashby J, Willis GA, Lund VJ, Clapp MJ. 2000. Acetochlor-induced rat nasal tumors: further studies on the mode of action and relevance to humans. Regul Toxicol Pharmacol. 32: 127–133.

Green T, Lee R, Toghill A, Meadowcroft S, Lund V, Foster J. 2001. The toxicity of styrene to the nasal epithelium of mice and rats: studies on the mode of action and relevance to humans. Chem Biol Interact. 137: 185–202.

Green T, Toghill A, Foster JR. 2001. The role of cytochromes P-450 in styrene induced pulmonary toxicity and carcinogenicity. Toxicology. 169:107–117.

Hummel T, Nordin S. 2005. Olfactory disorders and their consequences for quality of life. Acta Otolaryngol. 125:116–121.

Keyhani K, Scherer PW, Mozell MM. 1997. A numerical model of nasal odorant transport for the analysis of human olfaction. J Theor Biol. 186: 279–301.

Kimbell JS, Godo MN, Gross EA, Joyner DR, Richardson RB, Morgan KT. 1997. Computer simulation of inspiratory airflow in all regions of the F344 rat nasal passages. Toxicol Appl Pharmacol. 145:388–398.

Kobal G, Klimek L, Wolfensberger M, Gudziol H, Temmel A, Owen CM, Seeber H, Pauli E, Hummel T. 2000. Multicenter investigation of 1,036 subjects using a standardized method for the assessment of olfactory function combining tests of odor identification, odor discrimination, and olfactory thresholds. Eur Arch Otorhinolaryngol. 257:205–211.

Lees PSJ, Stefaniak A, Emmett EA, Dalton P. 2003. Exposure assessment for study of olfactory function in workers exposed to styrene in the reinforced-plastic industry. Am J Ind Med. 44:12–23.

Mergler D, Beauvais B. 1992. Olfactory threshold shift following controlled 7-hour exposure to toluene and/or xylene. Neurotoxicology. 13: 211–216.

Morgan KT, Monticello TM. 1990. Airflow, gas deposition, and lesion distribution in the nasal passages. Environ Health Perspect. 88:209–218.

Murer AJL, Christensen JM, Midtgard T. 1994. Determination of the urinary metabolites of styrene: estimation of the method evaluation function and evaluation of reference values in Danish subjects. Int Arch Occup Environ Health. 65:313–318.

National Institute of Occupational Health and Safety. 1994. NIOSH Manual of Analytical Methods, P & CAM #125. Vol. 1. Washington (DC): National Institute of Occupational Health and Safety.

Santos DV, Reiter ER, DiNardo LJ, Costanzo RM. 2004. Hazardous events associated with impaired olfactory function. Arch Otolaryngol Head Neck Surg. 130:317–319.

Triebig G, Lehrl S, Weltle D, Schaller KH, Valentin H. 1989. Clinical and neurobehavioural study of the acute and chronic neurotoxicity of styrene. Br J Ind Med. 46:799–804.

Wetherill GB, and Levitt H. 1965. Sequential estimation of points on a psychometric function. Br J Math Stat Psychol. 18:1–10.

Wysocki CJ, Dalton P, Brody MJ, Lawley HJ. 1997. Acetone odor and irritation thresholds obtained from acetone-exposed factory workers and from control (occupationally non-exposed) subjects. Am Ind Hyg Assoc J. 58:704–712.

Accepted May 8, 2007

Downloaded from https://academic.oup.com/chemse/article-abstract/32/8/739/582959 by Centers For Disease Control user on 04 May 2020