EFFECTS OF EXPOSURE TO ORGANIC SOLVENTS AND OCCUPATIONAL NOISE ON HEARING LOSS AND TINNITUS IN US ADULTS FROM 1999 TO 2004

by

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EFFECTS OF EXPOSURE TO ORGANIC SOLVENTS AND OCCUPATIONAL NOISE ON HEARING LOSS AND TINNITUS IN US ADULTS FROM 1999 TO 2004

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There is evidence that organic solvents are ototoxic, and studies suggest there is an interaction between organic solvents and noise on ototoxicity. The purpose of this study was 1) to explore the association between organic solvent exposure and hearing loss or tinnitus and 2) to determine if interaction exists between occupational noise and organic solvent exposure on hearing loss or tinnitus. This study used data from the National Health and Nutrition Examination Survey (NHANES) from 1999-2004 to analyze data on hearing outcomes and organic solvent exposure. The following organic solvents were studied: 1) 1,4-dichlorobenzene, 2) benzene, 3) ethylbenzene, 4) styrene, 5) toluene, 6) o-xylene, and 7) m-/p-xylene. Study participants were excluded if they had bilaterally unsymmetrical hearing loss, missing data on covariates, or detectable blood measurements that exceed the calibrated range of assay. The number of study participants included in this study was 2,513, but the sample size varied by analysis as the available data for each organic solvent and outcome

varied. Data from the Occupational Information Network (O*NET) was used to approximate occupational noise exposure in study participants based on their job. Logistic regression was used to determine associations between organic solvent exposure and hearing outcomes (i.e., self-reported hearing loss, audiometrically-assessed hearing loss, self-reported tinnitus, high-frequency hearing loss, and low-frequency hearing loss). The age of study participants ranged from 20-59 years. A majority of study participants had an income equal to or over $20,000 (80.5%) and were non-Hispanic white (49.7%), while a minority of study participants had recently used ototoxic medication (4.3%), were smokers (22.4%), were diabetic (5.0%), or were classified as exposed to non-occupational noise (30.0%). After adjusting for covariates, there was no evidence of an association between hearing loss or tinnitus and organic solvent exposure except for three solvents: higher levels of benzene, ethylbenzene and toluene were associated with high-frequency hearing loss (benzene adjusted odds ratio (OR)=1.43, 95% confidence interval (CI) 1.15- 1.78; ethylbenzene adjusted OR=1.24, 95% CI 1.02-1.50; and toluene adjusted OR=1.27, 95% CI 1.06-1.52). Moreover, the effect estimates for high-and low-frequency hearing loss were higher as compared to audiometrically-assessed hearing loss. Additionally, no evidence of interactions between organic solvent exposure and occupational noise on high-frequency hearing loss was observed. In conclusion, this dissertation found, in a large, diverse population with blood measurements of organic solvents, there was no indication of association between organic solvent exposure and self-reported and audiometrically-assessed hearing loss or self-reported tinnitus, but there was evidence of an association between organic solvents and high-frequency hearing loss.

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Literature Review

In the United States (US), some degree of hearing loss affects approximately 48 million adults [1], while tinnitus affects approximately 50 million US adults [2]. Hearing loss is the partial or complete inability to hear, and the threshold for debilitating hearing loss is 25 to 30 decibels (dB) [3]. A histological examination of a patient suffering from hearing loss may show damage or loss of hair and nerve cells [4]. There are two different types of hearing loss: sensorineural and conductive. Sensorineural hearing loss is initiated in the fibers of the auditory nerve or cochlea, while conductive hearing loss is caused by interference with the transmission of sound in the conducting apparatus (outer or middle ear) (Figure 1) [5]. Patients may have a mixture of each type of hearing loss (i.e., sensorineural and conductive). In some cases, an important early sign of sensorineural hearing loss is tinnitus (i.e., the sense of hearing ringing or other noise, when there is no other observable cause); although tinnitus can occur in patients without hearing loss [5].

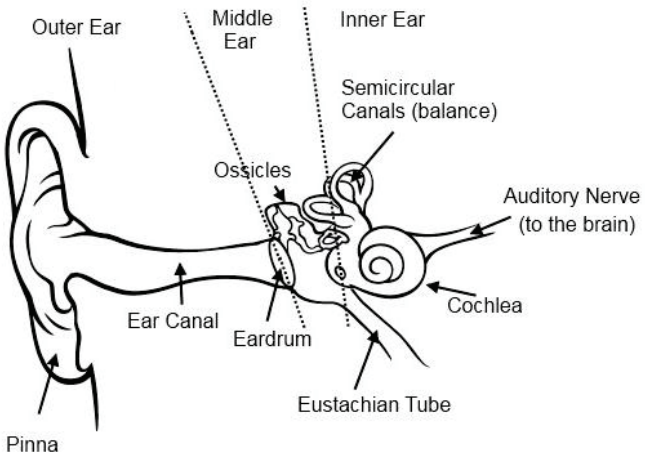


Figure 1. Diagram of the human ear
Source: See reference [6]

Comorbidities

Research has demonstrated the substantial effects hearing loss and tinnitus can have on health and well-being. Hearing loss and tinnitus have similar comorbidities that include: social interference (e.g., social rejection, avoidance of social situations, lifestyle change, loneliness), emotional difficulties (e.g., irritability, insecurity, annoyance, fatigue, anger, tension, depression, stress, negativity), and decreased overall health [7-10]. Additionally, studies have shown an association between tinnitus and difficulty sleeping [8, 10]. Other effects on hearing loss have been linked to decreased work-related capability (i.e., decreased performance, reduced earning power), impaired memory, weakened capacity to learn new tasks, decreased alertness, increased risk to personal safety, and reduced psychological health [7].

Causes of Tinnitus and Hearing Loss

Known causes of tinnitus and hearing loss include noise, presbycusis (i.e, age-related hearing loss), obstruction by physical agent, and otosclerosis (i.e., deafness caused by growth of bone in the inner ear) [4, 11, 12]. Other causes of hearing loss include: health conditions (e.g., disease, infection, medication use, and tumors), genetics (e.g., malformation of the inner ear, Ménière's Disease), or external contact (e.g., head trauma) [4, 12]. Exposure to certain industrial chemicals, such as organic solvents (i.e., carbon-based substances capable of dissolving or dispersing other substances) has also been suggested as a causal agent in the development of hearing loss and tinnitus [13-15]. Specific types of hearing loss, which include hearing loss that is bilaterally symmetrical, irreversible, high frequency,

sensorineural, and main damage occurs at cochlear hair cells, can be induced by exposure to organic solvents [16].

Exposure Sources

According to the Occupational Health and Safety Administration (OSHA), millions of workers are exposed to solvents every day during the production of agricultural products, dyes, pharmaceuticals, plastics, polymers, printing inks, and textiles [17]. Additionally, organic solvents are found in adhesives, degreasing/cleaning agents, glues, lacquers, paints, fuels, varnishes, and other products [13, 18]. Organic solvents that were of particular interest in the current proposed study were 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, trichloroethylene, and xylene. Organic solvents are used in a variety of different industries including construction, manufacturing, medical facilities, and the petrochemical industry (see Table 1 for additional details) [14, 15, 19-24]. Levels of exposure to organic solvents vary by occupation. The National Institute of Occupational Health and Safety (NIOSH) provides information on the recommended exposure limits (REL) for organic solvents, which are given as time-weighted averages for up to a 10 hour work day during a 40 hour work week [25]. Benzene, 1,4-dichlorobenzene, and trichloroethylene are considered by NIOSH to be potential occupational carcinogens [25]. Therefore, the REL for these solvents are equivalent to “no detectable exposure levels for proven carcinogenic substances” or a level that can be feasibility achieved using controls [25].

Table 1. Uses, potentially exposed Occupations, and National Institute of Occupational Safety and Health Recommended Exposure Limits (REL)* for selected organic solvents.

Chemical	Uses	Potentially exposed industries or occupations	NIOSH REL
1,4-Dichlorobenzene**	Mothball production, fumigants, lacquers, paints, insecticides, seed disinfection products, and deodorant blocks [26]	Chlorobenzene production [27]	As low as feasible
Benzene**	Gasoline additive and in the synthesis of numerous chemicals [28]	Benzene manufacturers, medical and surgical hospitals, petrochemical plants/ refineries, coke/coal chemicals, tire manufacturers, bulk terminals/plants, transportation, gasoline station workers, firefighters, dry cleaners [29]	0.1 ppm
Ethylbenzene	As a raw material, predominant use in the manufacturing of styrene [30]	Gasoline stations attendants, drivers, refinery personnel, varnish workers, auto paint shops, screen printing plants, spray-painting and gluing operations [31]	100 ppm
Styrene	Intermediate in the production of plastics, coatings, resins, and paints [30]	Reinforced-plastics industries, styrene polymerization, rubber manufacturing, styrene-polyester resin facilities, photocopy centers, vanish workers [32]	50 ppm
Toluene	Gasoline additive, solvent carrier in paints, thinners, inks, and adhesives [30]	Gasoline stations, printing industry, paint stripping operations, coke plant operations, commercial painting,	100 ppm

		printing workers [33]	
Trichloroethylene**	Degreaser, paint stripper, paint and varnish ingredient, adhesive solvent, and use in the manufacturing of organic chemicals [30]	Degreasing operations, fiberglass manufacturing, dry cleaning, insecticide production [34]	2 or 25 ppm
Xylene	Fuel, found in solvents in leather, paint, printing, and rubber industries [30]	Petroleum chemical, paint and plastics plants, service station attendants, street vendors, machinery (except electrical) workers, special trade contractors, fabricated metal products workers, and health services industries [35]	100 ppm

* RELs from the NIOSH Pocket Guide to Chemical Hazards [25]
** Potential occupational carcinogen

Not only can people be exposed to organic solvents at work, but they may also be exposed to environmental sources, both indoors and outdoors. Sources of environmental exposures typically found indoors include cigarette smoke, cleaning products, moth repellents, and water chlorination by-products [36, 37]. Sources of outdoor environmental exposures typically include residential proximity to refineries or chemical plants, exhaust fumes, and refueling [36, 37]. Both occupational and environmental exposures have the same potential mechanism for causing hearing loss. Su *et al.* reports higher exposures to organic solvents may be due to occupational exposure, while lower exposure levels may occur due to environmental exposure [38]. Biomarkers of solvent exposure reflect an individual's exposure from all source pathways. Typically the half-lives of solvents are relatively short, minutes to hours, and, therefore, measurements may represent only the most recent exposure.

Potential Mechanism

Research has shown noise can cause mechanical or metabolic injury (Figure 2) [39-41]. Mechanical injury is caused by overstimulation of stereocilia of hair cells, which leads to cell death [39, 42-44]. Additionally, cell death leads to metabolic activity, which may initiate the formation of free radicals (e.g., reactive oxygen species (ROS)). The production of free radicals is associated with cellular injury and thought to be part of the underlying mechanism for noise-induced hearing loss [45-48]. As compared to noise, the mechanism for solvent induced hearing loss is less well known. Research has shown organic solvents affect both the cochlea and the central auditory pathway. In the cochlea, exposure to organic solvents has produced cochlear lesions leading to hearing loss [49-52]. In the central auditory pathway, research shows organic solvents can: 1) inhibit the auditory efferent system [53, 54]; and 2) block the protective middle ear reflex [55], which leads to hearing loss. Research has shown noise may cause tinnitus by causing damage to the auditory hair cells [56], but the potential mechanism for organic solvent induced tinnitus is unclear.

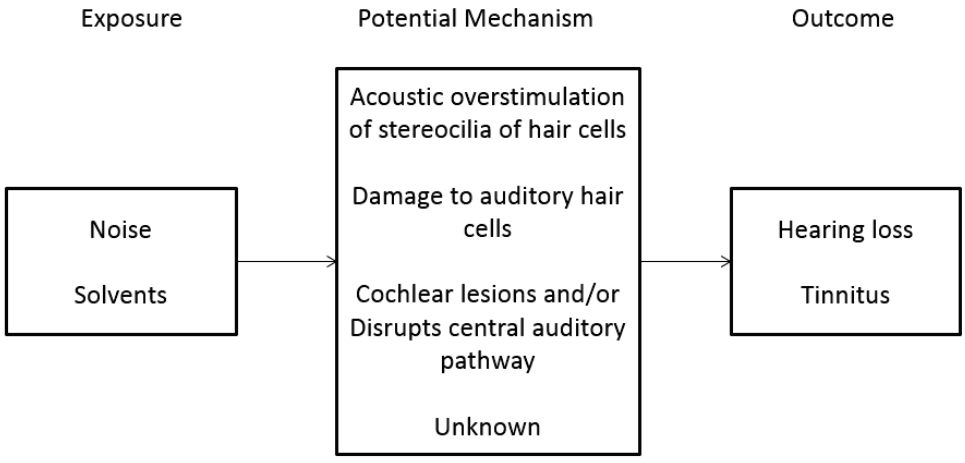


Figure 2. Potential mechanism of hearing loss and tinnitus due to organic solvent and noise exposure

Source: see reference [39-56]

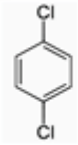
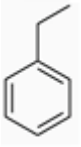
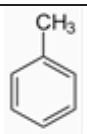
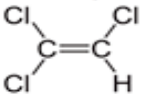
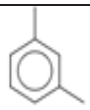
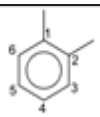
Previous Research

A summary of the epidemiologic findings on the ototoxicity of selected organic solvents can be viewed in Table 2. Based on the data collected by the Risk Assessment Information System, which is a program designed to provide information about risk assessment to the public and is sponsored by the U.S. Department of Energy Office of Environmental Management, there have been no studies on the ototoxicity of 1,4-dichlorobenzene, but this solvent has shown neurotoxic effects in human and animal studies and nephrotoxic effects in animal studies [26]. Nephrotoxicity and neurotoxicity are important characteristics because most chemicals that affect the auditory system also have potential neurotoxic and/or nephrotoxic effects [45]. Additionally, benzene has demonstrated neurotoxic effects and the ability to produce free radicals, which hints at a mechanism for ototoxicity [57].

Several animal studies have documented the ototoxicity of ethylbenzene [58-63], styrene [49, 64-66], toluene [52, 67-69], trichloroethylene [70-73], mixed xylenes [74] and p-xylene [60, 75-78]. Studies have reported a dose-dependent effect on ototoxicity associated with styrene [65] and toluene [79, 80]. In a study by Muijser *et al.*, rats exposed to trichloroethylene had hearing loss, as measured by reflex modification audiometry based on acoustic startle reflexes, at the low and mid-frequencies [70]. Other animal studies showed exposure to trichloroethylene affected the mid-frequency region of the cochlea as measured by reflex modification in rats [71-73]. The threshold shift in rats due to exposure from p-xylene was documented at high frequencies (≥8 kHz) using audiometry [76, 77]. Conversely, Fechter *et al.* found no ototoxic effects among guinea pigs exposed to styrene, but the authors suggested guinea pigs may not be suitable models for studying styrene induced hearing loss

as the rat's auditory system is more sensitive to ototoxic effects as compared to the guinea pig [81].

Table 2. Chemical structure and ototoxic potential of selected organic solvents

Chemical (CAS Number)	Symbol	Ototoxic findings		
		Human	Animal	Other*
1,4-Dichlorobenzene (106-467)				X
Benzene (71-43-2)				X
Ethylbenzene (100-41-4)			X	
Styrene (100-42-5)		X	X	
Toluene (108-88-3)		X	X	
Trichloroethylene (79-01-6)			X	
meta (m-) Xylene (108-38-3)				
ortho (o-) Xylene (95-47-6)				
para (p-) Xylene (106-42-3)		X	X	

* Other ototoxic findings include neurotoxicity, nephrotoxicity, and free radical production

Many studies have reported exposure to a mixture of organic solvents is associated with hearing loss. Among male steel workers exposed to acetone, styrene, resins, cobalt and other solvents, Botelho *et al.* found a statistically significant difference in audiometrically-assessed occupational hearing loss in men exposed to a solvent mixture and noise (18.3%) verses those only exposed to noise (6.0%) [82]. In paint and varnish factory workers exposed to xylene, toluene, ethyltoluene, styrene, n-propylbenzene, ethylbenzene, and trimethylbenzene isomers, Sulkowski *et al.* found 42.0% of the exposed group had high-frequency sensorineural hearing loss as compared to 5.0% of controls. Among various workers categorized for exposure by job title, there was a statistically significant association between audiometrically-assessed hearing loss in workers exposed to a mixture of solvents (i.e., benzene, ethanol, hexane, heptane, xylene, toluene, methyl ethyl ketone, methyl isobutyl ketone, or mineral spirits) and carbon monoxide versus those not exposed to solvents [14, 18, 22, 83]. On the contrary, Smedje *et al.* found aircraft maintenance personnel who self-reported exposure to solvents or chemicals did not have a statistically significant difference in audiometrically-assessed hearing loss as compared to those who were not exposed, but found these exposed workers were 2.6 times (95% CI 1.2-5.7) more likely to have self-reported hearing loss as compared to workers who were not exposed [84]. Among various other workers, other studies have shown a statistically significant association between self-reported hearing problems and mixed solvent exposure estimated by job title or self-report as compared to those not exposed [15, 85]. Among manufacturing plant workers exposed to toluene, methyl ethyl ketone, trichloroethylene, acetone, n-methyl, pyrrolidone, dimethlformamide, chlorobenzene, or isopropyl alcohol as determined by a job exposure matrix, Fuente *et al.* found a statistically significant difference in audiometrically-assessed

hearing loss greater than 30 dB as compared to those not exposed [21]. In a case study among an artist exposed to toluene, xylene, benzene, methyl ethyl ketone, toluene diisocyanate, acetone, and paint thinner, hearing loss was detected [86]. Although there is a large amount of literature on solvent mixtures, there are fewer studies on individual organic solvents.

Human studies have reported styrene [24, 87, 88], toluene [89], and p-xylene [90] are ototoxic. Morata *et al.* reported the odds of audiometrically-assessed hearing loss of 25 dB or greater increased 2.4 (95% CI 1.0-5.9) times for every one millimole increase in mandelic acid (a biological marker for styrene) per gram of creatinine in urine [87]. Likewise, Triebig *et al.* reported the odds of audiometrically-assessed hearing loss of 25 dB or greater were 7.5 (95% CI 1.1-51.4) times higher in workers employed at a boat building plant exposed to high styrene levels (greater than 501 mg mandelic acid + phenylglyoxylic acid [biological marker for styrene] per gram creatinine) for at least 10 years as compared to workers exposed to low styrene levels (less than 100 mg mandelic acid + phenylglyoxylic acid per gram creatinine) for a short time (mean=6.4 years, standard deviation (SD) = 3.4 years, range = 2-16 years) [88]. In a study among printing workers, there was a 1.8 (95% CI 1.0-3.0) times increase in the odds of audiometrically-assessed hearing loss of 25 dB or greater for every one gram increase in hippuric acid (a marker for toluene in urine) per gram of creatinine [89]. In a small study conducted in Chile, Fuente *et al.* found lab workers exposed to a p-xylene mixture had worse audiometrically-assessed hearing thresholds than non-exposed participants (Student's t test p-value < 0.05) [90]. Four case-studies documented self-reported exposure to toluene or xylene and self-reported tinnitus in the study subject, although the study subjects also self-reported exposure to noise [91].

Conversely, some studies did not report an ototoxic effect associated with organic solvents. In a few studies that found a null association between organic solvents and audiometrically-assessed hearing loss of 25 dB or greater, the exposure levels were relatively low as compared to the levels of organic solvents in studies that reported a statistically significant association [92, 93]. Therefore, the authors posited there may be a threshold effect as these studies may have found an association if greater concentration levels were studied. In the study by Hoffman *et al.*, there was no statistically significant difference in the mean values of audiometrically-assessed hearing thresholds between styrene-exposed workers, who were categorized for styrene exposure by job title, and controls. The findings may be explained by the small sample size used in the study or that the controls had measureable concentrations of mandelic acid and phenylglyoxylic acid [94]. Muijser *et al.* reported no difference in audiometrically-assessed hearing thresholds among factory workers who were exposed to styrene (hearing threshold shift= 34.1 ± 10.2 dB sound pressure level (SPL)), where exposure was estimated by job title, as compared to factory workers who were not exposed (hearing threshold= 35.3 ± 8.0 dB SPL) [95].

Noise-induced hearing loss is caused by months or years of high level noise exposure [5]. Indications of noise-induced hearing loss include: sensorineural hearing loss, permanent hearing loss, and equal degree of hearing loss in both ears [5]. The NIOSH REL is 85 dB for an 8 hour time-weighted average [96]. Thus, any exposure averaging greater than or equal to this level is considered hazardous to hearing. In the US, 9% of workers had noise-induced hearing loss that was attributable to occupational exposure [97]. Major industrial groups affected by daily noise levels over 85 dB include: agriculture, mining, construction, manufacturing and utilities, transportation, and the military [96]. Hearing loss caused by

exposure to non-occupational noise, such as recreational or environmental noise (e.g., guns, loud music, and power tools), affect the ear the same as occupational noise. Furthermore, combined exposure to noise and organic solvents appear to have synergistic effects on hearing loss.

Animal studies have shown organic solvents and noise interact to increase hearing loss. Using morphological analysis, researchers showed a positive interaction between styrene and noise [98-100] and between ethylbenzene and noise on increased outer hair cell loss in rats [58]. A positive interaction between toluene and noise on hearing loss, which was measured by brainstem audiometry, has been reported in animals [101-103]. A positive interaction between noise at 95 dB SPL and trichloroethylene at 3000 ppm was shown for threshold shifts in rats using reflex-modification testing at 4, 8, 16, and 20 kHz [70].

Furthermore, epidemiologic studies on workers have also found joint exposure to noise and a mixture of organic solvents affects hearing loss [20, 82, 104-110]. Among workers employed in various occupations, who were assessed for exposure to solvent mixture (i.e., xylene, toluene, and paint thinner) and noise by job title, abnormal hearing, which was assessed audiometrically, was found in 62.9% of the workers exposed to noise, in 63.4% of workers exposed to noise and solvents, and in 16.9% of workers who were not exposed to noise or solvents [104]. Among workers exposed to benzene, toluene, xylene, and tetrachloroethylene as categorized by mean concentration of solvent in ambient air at job location and noise as categorized by a sound level meter at an automobile plant, the odds ratio (OR) of audiometrically-assessed hearing loss of 25 dB or more was 1.8 (95% CI 1.1-3.0) times higher in workers exposed to noise and solvents at levels lower than permitted (which was defined according to the American Conference of Governmental Industrial

Hygienists Threshold Limit Values (ACGIH-TLV) for each equivalent exposure to the solvent mixture) and 4.1 (95% CI 2.6-6.6) times higher in workers exposed to noise and mixed solvents at levels higher than permitted as compared to workers only exposed to noise [20]. In paint and lacquer manufacturing workers exposed to xylene isomers, ethyl acetate, white spirit, toluene, butyl acetate, and ethyl benzene as measured by dosimetry and noise as measured by sound pressure level meter, the relative risk (RR) of audiometrically-assessed hearing loss was 2.8 (95% CI 1.6-4.9) times higher in those exposed to only solvents and 2.8 (95% CI 1.6-4.9) times higher in those exposed to both solvents and noise greater or equal to 85 dB as compared to those who were not exposed to solvents or noise [110]. The same study reported 61.5% of the workers exposed to solvents and noise, 57.5% of workers exposed to solvents only, and 36.0% of workers not exposed to solvents or noise had hearing loss [110].

Additional studies have found joint exposure to noise and individual solvents affects hearing loss. Among petrochemical and power station workers in China categorized for noise and ethylbenzene exposure by job location, the odds of audiometrically-assessed hearing loss of 25 dB or more among workers exposed to ethylbenzene and noise (OR=124.0; 95% CI 11.7-651.0) was higher compared to workers exposed to a noise and not ethylbenzene (OR=15.3; 95% CI 5.7-52.9), although the wide confidence intervals preclude making strong statements about the observed differences [111]. Studies have also reported statistically significant interaction between exposure to styrene and noise on hearing loss, but given the differences in definitions of exposure and outcome, there are difficulties in directly comparing the effect estimates [24, 87, 106]. Toluene may also exacerbate hearing loss in noisy environments [22, 112]. Among manufacturing plant employees categorized for exposure to toluene and noise by job division, Chang *et al.* found the prevalence of

audiometrically-assessed hearing loss of 25 dB or more was 86% in the workers exposed to toluene and noise, 45% in the workers exposed to noise and not toluene dB, and 5% in workers not exposed to toluene or noise [112]. After categorizing workers for noise and toluene exposure based on their job division, Morata *et al.* found audiometrically-assessed hearing loss of 30dB or more was 4.1 (95% CI 1.4-12.2) times higher in those exposed to noise and not toluene in the range of 88-97 dB versus those unexposed, 10.9 (95% CI 4.1-28.9) times higher in those exposed to toluene and noise in the range of 88-97 dB versus those unexposed, and 5.0 (95% CI 1.5-17.5) times higher in those exposed to a solvent mixture including toluene and no noise versus those unexposed [22].

Also, Sliwinska-Kowalska *et al.* (2004) and Sliwinska-Kowalska *et al.* (2005) reported audiometrically-assessed hearing loss of 25 dB or greater was 4.9 and 6.7 times greater, respectively, in workers exposed to a solvent mixture comprising of mostly xylene as measured by individual dosimetry and noise as measured by sound pressure level [106, 107]. On the contrary, other research reported different findings for the interaction between noise and solvent exposure on hearing loss [85, 113]. In the study by Hughes *et al.*, no statistically significant interaction was shown between noise ≥ 85 dB reported by industrial hygiene noise exposure monitoring data and solvent exposure as estimated by job task documentation (left ear RR= 1.2; 95% CI 0.9-1.5; right ear RR=1.0; 95% CI 0.8-1.4); the authors posited low levels of solvent exposure, as the levels were below occupational limits, may have affected the study results [113]. In the study by Jacobsen *et al.*, the RR of hearing loss was 1.9 (95% CI 1.7-2.1) times higher in study participants exposed to noise and not solvents, while no additional effect on hearing loss was shown in study participants exposed to both noise and

solvents (RR= 1.8; 95% CI 1.6-2.1); information bias may have affected the results as study participants self-reported noise, hearing impairment, and solvent exposure [85].

Addressing Gaps

Quantitative audiometric tests for hearing loss include: 1) air-conduction threshold test, which measures sound conducted to the eardrum through air to establish hearing thresholds and 2) bone-conduction threshold test, which determines if hearing loss is conductive or sensorineural [5]. Another way to assess hearing loss related outcomes is through self-reports. This method is also used for tinnitus because there are no quantitative measurements to diagnose tinnitus. Hearing loss was analyzed by both audiometric measurement and self-report to showcase the differences in these reporting mechanisms in relation to solvent and noise exposure. Thus, in this his dissertation, three outcomes were analyzed: 1) audiometrically-assessed hearing loss, 2) self-reported hearing loss, and 3) self-reported tinnitus in relation to solvent and noise exposure.

As described previously, there is evidence that organic solvents are ototoxic [58-63, 70-73, 91]. Additionally, studies suggest there is an interaction between organic solvents and noise on ototoxicity [22, 24, 70, 87, 106, 111, 112, 114]. Limitations of previous studies include a scarcity of information on the association of individual solvents with hearing loss in human studies and limited research available on the association of tinnitus and solvent exposure. Furthermore, a comparison of previous studies also shows mixed results in the association of some individual solvents with hearing loss [24, 87-90, 92-94, 115]. Lastly, there is difficulty in comparing the effect of solvent and noise exposure on hearing loss and tinnitus as definitions often vary from study to study. This study compared the effect of a

multitude of individual solvents and noise exposure on hearing loss and tinnitus using similar definitions for noise, hearing loss and tinnitus.

Public Health Significance

In the US, millions of workers are exposed to organic solvents during the production of agricultural products, dyes, pharmaceuticals, plastics, polymers, printing inks, and textiles [13], but little is known about the association of hearing loss and tinnitus with exposure to organic solvents. This cross-sectional study assessed the ototoxicity of 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, trichloroethylene, o-xylene, and m-/p-xylene in a US population and determined the need for researching the association in other study designs. The assessment of interaction between potentially ototoxic organic solvents (1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, trichloroethylene, o-xylene, and m-/p-xylene) and noise on hearing loss and tinnitus will provide evidence for policies that provide additional protection for workers exposed to both ototoxic organic solvents and noise. Overall, the study results will lead to improvements in the understanding of the relationship of ototoxic organic solvents with hearing loss and tinnitus, especially in the workforce. Ideally, policymakers will use information generated in this study in conjunction with results of other studies to develop exposure standards that will reduce the incidence of hearing loss and tinnitus caused by ototoxic organic solvents. The long term goal of this research was to improve the workplace environment.

Specific Aims

The long-term goal of this study was to inform potential strategies to improve the health of workers by reducing hearing loss and tinnitus due to exposure to ototoxic organic

solvents. The aims of this study can help address the long-term goal by providing additional information on the ototoxicity of 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, o-xylene, and m-/p-xylene and by identifying the interaction of organic solvents and noise with hearing loss and tinnitus.

Aim 1: To examine the association of 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, o-xylene, and m-/p-xylene with hearing loss and tinnitus.

Hypothesis 1: Based on the findings of previous studies [18, 24, 86-90, 93, 115]*, I predicted exposure to 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, and m-/p-xylene will be positively associated with audiometrically-assessed hearing loss, self-reported hearing loss, and self-reported tinnitus.*

Using available data from the National Health and Nutrition Examination Survey (NHANES), 21 logistic regression models were built to answer Aim 1. Separate, individual logistic regression models were built for each of the seven organic solvents and each the following outcomes, separately: audiometrically-assessed hearing loss, self-reported hearing loss, and self-reported tinnitus.

Aim 2: To assess if hearing loss and tinnitus vary by 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, o-xylene, and m-/p-xylene exposure and occupational noise level.

Hypothesis 2: Based on the findings of previous studies [22, 24, 87, 106, 107, 111, 112, 114]*, I predicted occupational noise and organic solvents will interact to influence the*

effect on audiometrically-assessed hearing loss, self-reported hearing loss, and self-reported tinnitus.

The ototoxic effects of noise and organic solvents may interact. Using available data from NHANES and O*NET (Occupational Information Network), the interaction between the organic solvents and occupational noise exposure in association with hearing loss and tinnitus were tested.

METHODS

NHANES – The National Health and Nutrition Examination Survey

NHANES, a major program of the National Center for Health Statistics of the United States Centers for Disease Control and Prevention (CDC), is a continuous cross-sectional survey with a complex, multistage sampling design. This survey was designed to select a representative sample of US non-institutionalized, civilian residents currently living in the 50 US states or the District of Columbia. The four stage sampling design includes: 1) selection of primary sampling units, 2) selection of segments within primary sampling units, 3) selection of households within those segments, and 4) selection of individuals within those households [116]. To accurately represent the US population, NHANES oversampled Mexican-American persons, black persons, and, beginning in 2000, white and other persons at or below 130% of federal poverty [116]. The purpose of NHANES is to collect data on health risk factors of the US population. Not all NHANES study participants are included in every component of data collection. For all NHANES participants, data on health behaviors, personal environment and lifestyle are collected during household interviews. Additionally, physical examinations, including measurement of audiometry, were conducted on a random subsample of ½ of the NHANES participants, lab testing, including measurement of blood concentrations of many environmental contaminants, on a random subsample of ¼, ⅓, or ½ of the NHANES participants and medical interviews, including assessment of hearing/audiometry and demographic covariates, on the full sample of NHANES participants [117]. In 1999, the survey became a continuous program that collects data every year and releases data in waves, representing two years. For the present analysis, the NHANES study

waves 1999-2000, 2001-2002, and 2003-2004 were collapsed into a single dataset from 1999-2004. The present study was also restricted to individuals from NHANES 1999-2004 who had laboratory testing and available data on blood concentrations of the following solvents: 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, trichloroethylene, o-xylene, and m-/p-xylene. Although NHANES tests the same number of blood samples for organic solvents, the final sample size of organic solvent blood measurements in NHANES varies due to laboratory quality control measures (see Appendix A) [118, 119].

Hearing loss and tinnitus assessment

Audiometrically-assessed hearing loss was assessed in NHANES through a pure-tone air conduction threshold test at seven frequencies (0.5, 1, 2, 3, 4, 6, and 8 kHz) over an intensity range of -10 dB to 120 dB [120]. The examinations were conducted in a sound-isolated room by health technicians, who were trained by a NIOSH certified audiologist. Test instrumentation included audiometer (model AD226; Interacoustics, Assens, Denmark), headphones (model TDH-39), and insert earphones (Etymotic EarTone 3A) [120]. As part of a quality control measure, study participants were tested twice in each ear at the 1 kHz frequency and audiograms with a difference greater than or equal to 10 dB were classified as unreliable [120]. In the present study, I computed the pure-tone average by averaging the air-conduction thresholds at 0.5, 1, 2, and 4 kHz. Hearing loss was defined as a pure-tone average threshold change greater than or equal to 25 dB in either ear, which was consistent with the definition used by the World Health Organization [121].

Self-reported hearing loss was assessed in NHANES using questionnaires. Study participants were asked “which statement best describes your hearing (without a hearing

aid)?" Participants responded: good, a little trouble, a lot of trouble, or deaf. In the present study, study participants were categorized as having self-reported hearing loss if they responded to the question with "a little trouble, a lot of trouble, or deaf". Study participants were categorized as not having self-reported hearing loss if they responded to the question with "good". This classification was consistent with previous studies on self-reported hearing loss [122, 123].

Self-reported tinnitus was assessed in NHANES using questionnaires. Study participants were asked "in the past 12 months, have you ever had ringing, roaring, or buzzing in your ears?" in the present study, participants who responded yes were classified as having self-reported tinnitus, and those who answered no were classified as not having self-reported tinnitus. Details on the sample size for each outcome are provided in the "Exclusion Criteria" section.

Organic solvents assessment

The results of previous studies were used to inform the ototoxic potential of organic solvents included in NHANES from 1999-2004. Based on this information the following eight organic solvents were selected for examination in this proposed study: 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, trichloroethylene, o-xylene, and m-/p-xylene.

As part of NHANES data collection, the concentration of 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, trichloroethylene, o-xylene, and m-/p-xylene in human blood was analyzed using solid-phase microextraction with gas chromatography and bench top quadrupole mass spectrometer. The concentration of the organic solvent in blood was

reported as a continuous value. If a result was below the limit of detection (LOD), which is indicated in Table 3, NHANES reported the value as the detection limit divided by the square root of two [124]. Since a large percentage of trichloroethylene values (96-99%) were below the limit of detection, this solvent was not included in this study analysis. The distribution of organic solvent concentrations was skewed with a long tail to the right. Several transformations of these variables were explored, including a natural log transformation, but for these data the cubic root transformation provided the best normalization.

Table 3. Limit of detection (LoD), and percent below LoD for selected organic solvents in US adults from the National Health and Nutrition Examination Survey, 1999-2004

			1999-2000		2001-2002		2003-2004	
Solvent	**Half-life** (in hours)	**LoD** (ng/mL)	**N**	**% <LoD**	**N**	**% <LoD**	**N**	**% <LoD**
1,4-Dichlorobenzene	7-8 [125]	0.120	287	12	802	49	1320	46
Benzene	0.5 [126]	0.024	300	0	837	47	1345	42
Ethylbenzene	27.5 [31]	0.024	261	10	879	39	1299	33
m-/p- xylene	4 [127]	0.034	294	0	962	4	1346	2
o-xylene	4 [127]	0.049	308	42	981	60	1365	63
Styrene	3.9 [128]	0.030	283	5	948	46	1245	59
Toluene	4.5 [129]	0.025	298	1	953	5	1336	5
Trichloroethylene	10-12 [130]	0.012	285	96	922	97	1228	99

Source: see reference [131, 132]

Occupational noise exposure assessment

In the present dissertation, to attribute occupational noise, I linked information from the O*NET occupational noise exposures estimates with NHANES data on each participant's longest job. In NHANES, the longest job was ascertained by the following question: "thinking of all the paid jobs or businesses you ever had, what kind of work were you doing the longest?" Participants could report 40 different occupations (see Table 4). Data on noise exposure used in this study was collected by O*NET from March 2002 to July 2015.

Table 4. National Health and Nutrition Examination Survey occupational groups, 1999-2004

NHANES occupational groups
Executive, administrators, and managers
Management related occupations
Engineers, architects and scientists
Health diagnosing, assessing and treating occupations
Teachers
Writers, artists, entertainers, and athletes
Other professional specialty occupations
Technicians and related support occupations
Supervisors and proprietors, sales occupations
Sales representatives, finance, business, & commodities ex. Retail
Sales workers, retail and personal services
Secretaries, stenographers, and typists
Information clerks
Records processing occupations
Material recording, scheduling, and distributing clerks
Miscellaneous administrative support occupations
Private household occupations
Protective service occupations
Waiters and waitresses
Cooks
Miscellaneous food preparation and services occupations
Health service occupations
Cleaning and building service occupations
Personal service occupations
Farm operators, managers, and supervisors
Farm and nursery workers
Related agricultural, forestry, and fishing occupations
Vehicle and mobile equipment mechanics and repairers
Other mechanics and repairers
Construction trades
Extractive and precision production occupations
Textile, apparel, and furnishings machine operators
Machine operators, assorted materials
Fabricators, assemblers, inspectors, and samplers
Motor vehicle operators
Other transportation and material moving occupations
Construction laborers
Laborers, except construction
Freight, stock, and material movers, hand
Other helpers, equipment cleaners, hand packagers and laborers

O*NET is a national source of occupational information, including detailed information on job tasks and hazards, developed through a grant to the North Carolina Department of

Commerce from the US Department of Labor/Employment and Training Administration (USDOL/ETA). Data collection by O*NET uses a two-stage design: 1) identify a random sample of businesses expected to employ workers within needed occupational groups, and 2) randomly sample workers within those identified businesses. O*NET is continuously updated by surveying workers in a variety of occupations [133].

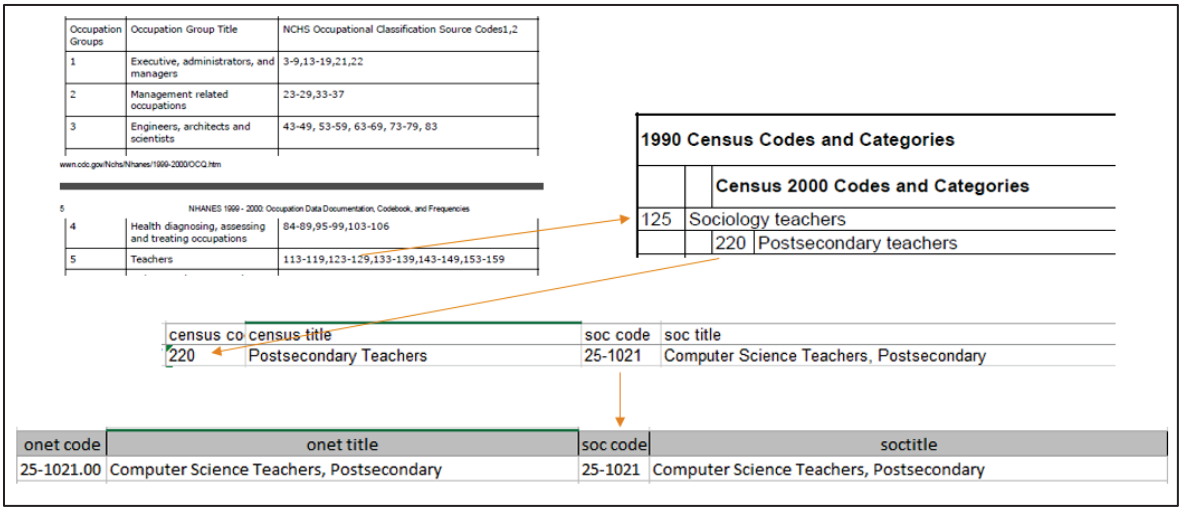


Figure 3. Example of National Health and Nutrition Examination Survey/Occupational Information Network linking process
Source: See reference [134-136]

There is not a direct crosswalk between NHANES and O*NET occupational coding. Therefore, for NHANES data, occupational codes were first linked with the 1990 census codes [134]. Coding changed from the 1990 census to the 2000 census, so an occupational crosswalk for comparing data from the 1990 Census codes to the 2000 Census codes was used [135]. Another crosswalk was used to link the 2000 Census codes to the Standard Occupational Classification (SOC) system [136]. Finally, the SOC codes were linked to the O*NET-SOC codes [136]. See figure 3 for an example of the linking process that was used. Additional details on these methods can be found elsewhere [137]. NHANES occupational

coding linked to multiple O*NET-SOC occupational categories. Based on the distribution of noise scores, the mean was used to represent the cumulative measure of the O*NET-SOC noise scores for each NHANES occupational category.

Noise exposure was assessed in O*NET by the following question, "how often does this job require working exposed to sounds and noise levels that are distracting or uncomfortable?" with the following answer choices, never (1), once a year or more but not every month (2), once a month or more but not every week (3), once a week or more but not every day (4), and every day (5). For the occupational noise score, the mean number of respondents per occupation was 29 workers. The median value of the occupational noise score (1-5) was assigned to each occupation in O*NET. In the present study, after linking NHANES study participants with their O*NET occupational noise score, study participants were divided into high and low occupational noise exposure groups based on a median cut point (median score=2.84 (interquartile range (IQR)= 2.59-3.67) [138].

Covariates

The following variables were explored as covariates in the present analysis: age (years), gender (male, female), non-occupational noise exposure (yes, no), diabetes (yes, no), race/ethnicity (Hispanic, non-Hispanic black, non-Hispanic white, other), smoking (yes, no), total household income (<$20,000, ≥$20,000), and use of ototoxic medication (yes, no). These variables were chosen based on prior literature suggesting that they may influence the degree and occurrence of hearing loss [16, 30, 139, 140]. In NHANES, the use of prescription medication was recorded using the following question, "in the past month have you used or taken medication for which a prescription is needed?" If the study participant

responded “yes,” then the NHANES data collector recorded the generic drug name/s. In the present study, the use of ototoxic medications was defined as self-reported use of any aminoglycosides, antineoplastic drugs, nonsteroidal anti-inflammatory drugs, or loop diuretics during the past month as recorded in the NHANES dataset. Data on smoking status was collected in NHANES using the following questions, “have you ever tried cigarette smoking, even 1 or 2 puffs?” and “does anyone who lives here smoke cigarettes, cigars, or pipes anywhere inside this home?” In the present study, study participants were classified as smokers if they responded positively to either of these questions. Data on diabetes was collected in NHANES using the following question, “other than during pregnancy, have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?” In the present study, diabetes was classified using NHANES data on self-reported physician diagnosis. Self-reported non-occupational noise data are available in NHANES. Participants were classified as exposed to non-occupational noise if they reported they have: 1) “outside of work, ever been exposed to firearms noise for an average of at least once a month for a year”, or 2) “ever been exposed to other types of loud noise, such as noise from power tools or loud music, for an average of at least once a month for a year” where loud noise means noise so loud that they had to speak in a raised voice to be heard.

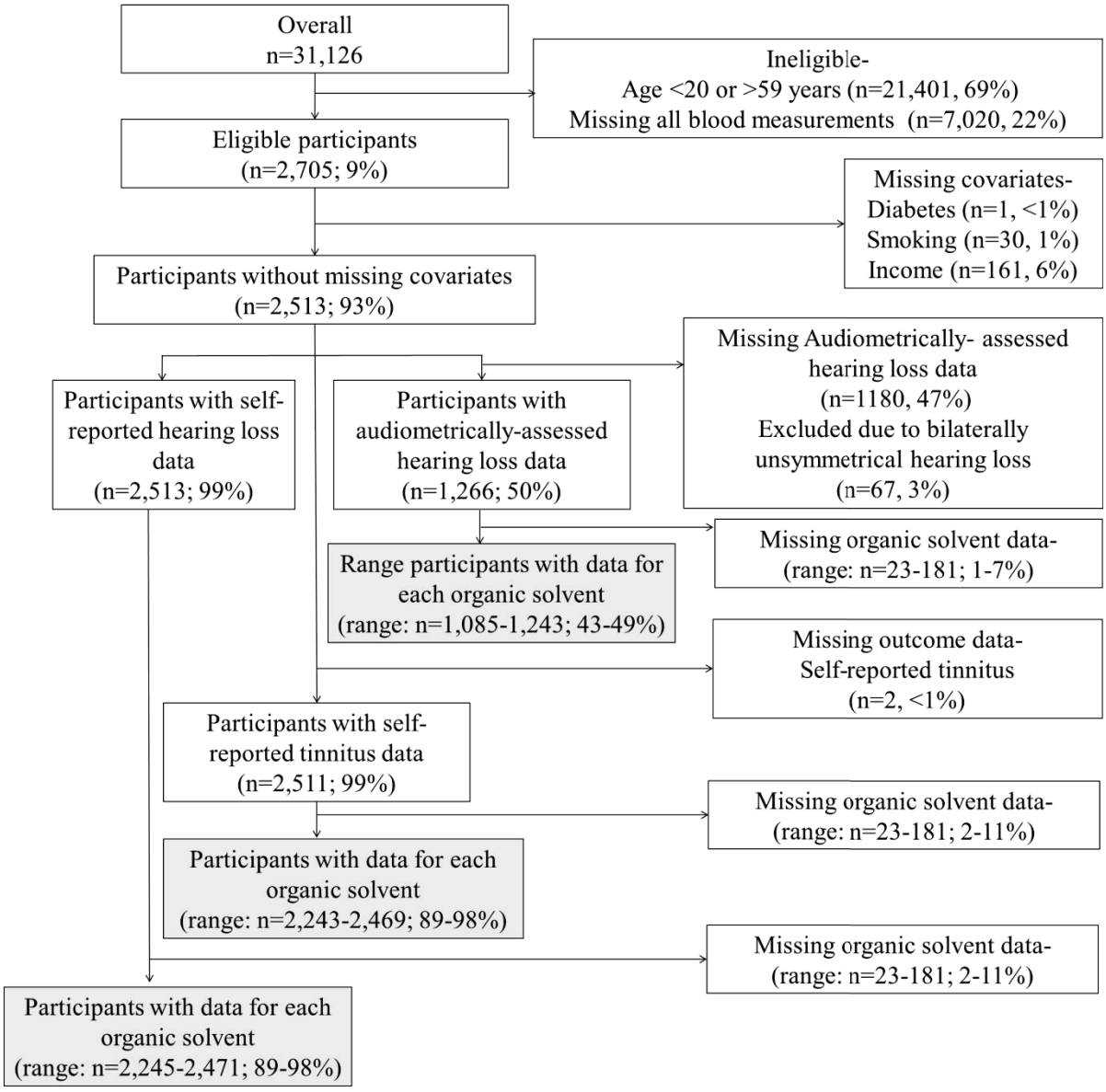


Figure 4. Sample size of NHANES study participants by inclusion/exclusion criteria, 1999-2004

Sample size for the analyses of each combination of solvent and outcome data varies. See text for details.

Inclusion/Exclusion criteria

In 1999-2004, NHANES collected data on a total of 31,126 individuals. Eligibility for the present analysis was based on age (20-59 years) and having a valid blood measurement for at least one of the organic solvents of interest (i.e., 1,4-dichlorobenzene, benzene, ethylbenzene,

styrene, toluene, o-xylene, and m-/p-xylene). This age range was chosen because, for these years, NHANES only measured blood concentrations of volatile organic compounds among this age group. Of the individuals in NHANES, 9,725 participants were 20-59 years and among these, 2,705 participants had a valid blood measurement for at least one of the organic solvents of interest and were therefore eligible for the present analysis. All of these individuals also had data on at least one outcome (i.e., self-reported hearing loss, audiometrically-assessed hearing loss, or self-reported tinnitus) although not all subjects had data on all three outcomes. Study subjects with missing information on covariates were excluded: 30 (1%) were missing smoking data, 1 (<1%) was missing diabetes data, and 161 (6%) were missing income data. Thus, data on 2,513 study participants were available for the present analysis (see Figure 4).

Of the 2,513 participants without missing covariates 2 (<1%) were excluded from the self-reported tinnitus analysis and 1,180 (47%) were excluded from the audiometrically-assessed hearing loss analysis due to missing outcome data. In addition, 67 (2%) were excluded from the audiometrically-assessed hearing loss analysis because they had bilaterally unsymmetrical hearing loss greater than 10 dB, which is not characteristic of organic solvent exposure and may complicate the interpretation of study results.

The sample sizes for the analyses of each outcome varied due to differences in the availability of data reported by NHANES for individual organic solvents. Although blood concentrations which exceeded the calibrated range of the assay were reported by NHANES, I excluded individual solvent measurements for 39 participants as these measured concentrations may be imprecise [141]. Note, although the solvent measurements for 39 subjects exceeded the calibrated range of the assay for a single solvent, this did not result in

the exclusion of the study participant from all analyses, rather, they were only excluded from the individual solvent analysis for which their blood measurement was deemed imprecise. Further, while NHANES tests each blood sample for all of the organic solvents, they may not release individual organic solvent measurements for some samples due to laboratory quality control measures related to potential cross-contamination (e.g., results from chromatograms of laboratory air, quality control samples for analytes) [118, 119]. Therefore, final sample sizes were as follows: between 2,245 and 2,471 participants for the analysis of self-reported hearing loss, between 2,243 and 2,469 participants for the analysis of self-reported tinnitus, and between 1,085 and 1,243 participants for the analysis of audiometrically-assessed hearing loss. The detailed sample sizes and number of cases for each outcome is shown in Appendix A.

The final sample size for Aim 2 was further reduced because these analyses were limited to study participants with NHANES data regarding longest job (i.e., non-workers were excluded) while Aim 1 was not limited to the working population and differed from aim 2 (range of total number of study participants for each organic solvent was between 1,097 and 1,215 for self-reported hearing loss; between 522 and 607 for audiometrically-assessed hearing loss; and between 1,096 and 1,214 for self-reported tinnitus).

Statistical analysis

All analyses were completed using Stata (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). To aid interpretation, odds ratios (ORs) presented represent the odds of hearing loss per increase in the IQR of solvent concentration. ORs were considered statistically significant if the 95% confidence interval did not include

1.0 or the p-value was less than 0.05. The specific analytic methods are described for each aim below.

Aim 1: To examine to association of 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, o-xylene, and m-/p-xylene with hearing loss and tinnitus.

Twenty one logistic regression models were built to address Aim 1. Three separate, individual logistic regression models were built for each of the seven included organic solvents: 1) 1,4-dichlorobenzene, 2) benzene, 3) ethylbenzene, 4) styrene, 5) toluene, 6) o-xylene, and 7) m-/p-xylene. In these models, the organic solvent was the independent variable, while the dependent variable was either: 1) self-reported hearing loss, 2) audiometrically-assessed hearing loss, or 3) self-reported tinnitus.

Using audiometrically-assessed hearing loss as the dependent variable and toluene as the independent variable I ran models to identify potential confounders for the final models. I started by running a full model that included all potential confounders (i.e., age, gender, non-occupational noise exposure, diabetes, race/ethnicity, smoking, income, and use of ototoxic medications) and selecting those which were statistically significantly ($p<0.05$) associated with the outcome. In this step, age, gender, non-occupational noise exposure, diabetes, and race/ethnicity were identified as confounders and a reduced model containing only these variables was constructed.

Next, every possible combination of the variables found to be statistically non-significant in the full model (i.e., smoking, income, and use of ototoxic medications) was added to the reduced model. In this step, the addition of smoking and income produced the model with the lowest Akaike information criterion (AIC) value, which measures the relative quality of statistical models for a given set of data, and was deemed the 'best' model. A

combination of statistical significance testing and AIC scores was used for model selection because a model only retaining statistically significant predictors may not have the lowest AIC [142]. Therefore, the final model consisted of adjustment for: age, gender, non-occupational noise exposure, diabetes, race/ethnicity, smoking, and income. This set of covariates was also tested among other solvents, and this model (see below) was the consistently the 'best'.

$$\text{Logit}(p_{\text{hearing loss}}) = B_0 + B_1(\text{organic solvent}) + B_2(\text{age}) + B_3(\text{gender}) + B_4(\text{non-occupational noise}) + B_5(\text{diabetes}) + B_6(\text{race/ethnicity}) + B_7(\text{smoking}) + B_8(\text{income}),$$

where $p_{\text{hearing loss}}$ equals the probability of having hearing loss/tinnitus. The intercept is denoted by B_0, and (B_1, B_2, …, B_8) are the beta coefficients of all linear factors.

Aim 2: To assess if hearing loss and tinnitus vary by 1,4-dichlorobenzene, benzene, ethylbenzene, styrene, toluene, o-xylene, and m-/p-xylene exposure and occupational noise level.

The solvents found to be statistically significantly associated with hearing loss using the final adjusted models in Aim 1 were tested for interaction with occupational noise exposure. Covariates included those identified as confounders in Aim 1. Please see the following model:

$$\text{Logit}(p_{\text{hearing loss}}) = B_0 + B_1(\text{organic solvent}) + B_2(\text{occupational noise}) + B_3(\text{occupational noise} \times \text{organic solvent}) + B_4(\text{age}) + B_5(\text{gender}) + B_6(\text{non-occupational noise}) + B_7(\text{diabetes}) + B_8(\text{race/ethnicity}) + B_9(\text{smoking}) + B_{10}(\text{income}),$$

where $p_{\text{hearing loss}}$ equals the probability of having hearing loss/tinnitus. The intercept is denoted by B_0, and (B_1, B_2, …, B_{10}) are the beta coefficients of all linear factors, including the interaction term between occupational noise and organic solvent.

Sensitivity analyses

Two different sensitivity analyses were run to determine the robustness of the study findings:

1) To determine the impact blood concentrations below the limit of detection had on study results, I examined the differences in the strength of association between solvent exposure and hearing loss by excluding the values below the LOD and compared the results with effect estimates including values below the limit of detection. I expected the results of the sensitivity analysis to be similar to the effect estimates including blood concentrations below the limit of detection. Three separate logistic regression models were built for each of the seven included organic solvents: 1) 1,4-dichlorobenzene, 2) benzene, 3) ethylbenzene, 4) styrene, 5) toluene, 6) o-xylene, and 7) m-/p-xylene. In these models, the organic solvent was the independent variable, while the dependent variable was either: 1) self-reported hearing loss, 2) audiometrically-assessed hearing loss, or 3) self-reported tinnitus. These models were adjusted for: age, gender, non-occupational noise exposure, diabetes, race/ethnicity, smoking, and income.
2) As studies in the literature use different definitions of hearing loss, a sensitivity analysis using a different definition of audiometrically measured hearing loss was used to explore the robustness of study findings. Results will be compared between high-frequency hearing loss, low-frequency hearing loss, and audiometrically-assessed hearing loss as defined by the World Health Organization [121]. If differences exist in these results, it will be concluded that studies with different definitions of hearing loss are not comparable. In this sensitivity analysis, high-frequency hearing loss was defined as average air conduction pure-tone thresholds at

3, 4, 6, 8 kHz greater than 15 dB in either ear, while low-frequency hearing loss was defined as average air conduction pure-tone thresholds at 0.5, 1, 2 kHz greater than 15 dB in either ear [143]. Two separate logistic regression models were built for each of the seven included organic solvents: 1) 1,4-dichlorobenzene, 2) benzene, 3) ethylbenzene, 4) styrene, 5) toluene, 6) o-xylene, and 7) m-/p-xylene. In these models, the organic solvent was the independent variable, while the dependent variable was either: 1) high-frequency hearing loss or 2) low-frequency hearing loss. These models were adjusted for: age, gender, non-occupational noise exposure, diabetes, race/ethnicity, smoking, and income.

3) There is limited evidence of alcohol use being ototoxic [144-148]. Thus, as a sensitivity analysis, I wanted to identify whether inclusion of alcohol use impacted my main findings. Data on alcohol use was collected in NHANES using the following questions, “in your entire life, have you had at least 12 drinks of any type of alcoholic beverage?”. If a study participant responded “no,” the study participant was recorded as a non-drinker (i.e, 0 drinks). If the study participant responded “yes,” the study participant was asked “in the past 12 months, on those days that you drank alcoholic beverages, on the average, how many drinks did you have?”. Alcohol use was a continuous variable based on a) the average number of drinks the study participant reported on a day the study participant drank in the past 12 months or b) if the study participants reported not having at least 12 drinks in their entire life, alcohol use was recorded as zero drinks. There were a large number of missing data (n=521) on alcohol use in NHANES. Of the 2,513 participants available for this study, after including alcohol use as a covariate, there were between 1,793 and 1,963 participants

available for the analysis of self-reported hearing loss, between 1,791 and 1,961 participants available for the analysis of self-reported tinnitus, and between 893 and 1,013 participants available for the analysis of audiometrically-assessed hearing loss. To determine the impact of controlling for alcohol use on the analysis, the analysis was repeated including alcohol use as a predictor (Appendix B and C).

4) Another sensitivity analysis was conducted to determine the impact of including non-workers in the analysis of aim 1. The potential differences in solvent exposure level of these populations (i.e. workers and non-workers) are relevant because some workers may have occupational exposure to organic solvents at a higher level than non-workers. If so, the effect of exposure to organic solvent could be diluted if non-worker exposure estimates are included in the analyses. In addition, as previously indicated, the sample size largely decreased due to the number of non-workers who participated in NHANES which may be in detriment of the main analyses of aim 1. For these reasons, I have considered the effect estimates on the population excluding study participants without measurements on longest job reported in NHANES as an additional sensitive analysis. These results are presented in appendices D and E.

Human subjects protection

NHANES is a publically available dataset. Protocols to recruit and collect data on study participants were approved by the NHANES Institutional Review Board , which changed its name to the NCHS Research Ethics Review Board in 2003 [149]. Additionally, this proposal was approved by The University of Texas Health Science Center at Houston (UTHealth) Committee for the Protection of Human Subjects (HSC-SPH-16-0064).

RESULTS

Demographics

Table 5 summarizes other characteristics of the study participants. The mean age of study participants was 38.2 years (SD=11.1, range = 20−59). About half of study participants were non-Hispanic white (49.7%). A majority of study participants had an income equal to or over $20,000 (80.5%), while a minority of study participants had recently used ototoxic medication (4.3%), were smokers (22.4%), were diabetic (5.0%), or were classified as exposed to non-occupational noise (30.0%). The median blood concentration of styrene (0.28 ng/mL, IQR= 0.28, 0.39) was the lowest, while the concentrations of 1,4- Dichlorobenzene (0.52 ng/mL, IQR= 0.44, 0.75) and m-/p-xylene (0.52 ng/mL, IQR= 0.45, 0.60) were the highest (see Table 6).

Association between Exposure to Organic Solvents and Hearing-related Outcomes

The relation between blood concentrations of organic solvents and odds of hearing loss and tinnitus is shown in Table 7. The odds of each outcome shown were per IQR increase in transformed organic solvent blood concentration. The associations between audiometrically-assessed hearing loss and benzene (OR=1.50; 95% CI 1.15-1.94), ethylbenzene (OR1.31; 95% CI 1.04-1.67), and toluene (OR=1.29; 95% CI 1.04-1.60) were statistically significant. Once adjusted for covariates, the odds ratio between audiometrically-assessed hearing loss and benzene (OR=1.20; 95% CI 0.85-1.68), ethylbenzene (OR=1.02; 95% CI 0.73- 1.41) and toluene (OR=1.00; 95% CI 0.77-1.30) were reduced. In addition, the associations between self-reported hearing loss and benzene (OR=1.30; 95% CI 1.15-1.47), ethylbenzene (OR=1.20; 95% CI 1.06-1.36), and toluene (OR=1.17; 95% CI 1.04-1.31) were

statistically significant. Once adjusted for covariates, the odds ratios between self-reported hearing loss and benzene (OR=1.11; 95% CI 0.96-1.29), ethylbenzene (OR=0.99; 95% CI 0.86-1.15), and toluene (OR=0.96; 95% CI 0.84-1.10) were reduced. The association between self-reported tinnitus and benzene (OR=1.16; 95% CI 1.03-1.31) and ethylbenzene (OR=1.14; 95% CI 1.01-1.28) were statistically significant, but were reduced once adjusted for covariates (benzene adjusted OR=0.98; 95% CI 0.85-1.13; ethylbenzene adjusted OR=0.98; 95% CI 0.85-1.12). None of the adjusted ORs in the analysis between organic solvents and hearing loss (audiometrically-assessed and self-reported) and self-reported tinnitus were statistically significant ($p \geq 0.05$). Hence, no analysis was conducted on the interaction of occupational noise with organic solvent exposure and self-reported hearing loss, audiometrically-assessed hearing loss or self-reported tinnitus.

Sensitivity Analysis 1- excluding <LOD

As part of the sensitivity analysis, study participants with organic solvent concentrations lower than the limit of detection were excluded from analysis (Table 8). For this analysis, 44-94% of study participants were included in the analysis of self-reported hearing loss, 21-47% of study participants were included in the analysis of audiometrically-assessed hearing loss, and 40-94% of study participants were included in the analysis of self-reported tinnitus. See sample size and number of cases for this analysis in Appendix F.

After excluding subjects with solvent concentrations below the limit of detection, the pattern of point estimates was similar among many estimates (Table 8 versus Table 7). Exclusions consist of the adjusted ORs between o-xylene and benzene with audiometrically-assessed hearing loss. Among those exposed to o-xylene as compared to those not exposed,

the adjusted odds of audiometrically-assessed hearing loss was lower among the study population that excluded study participants with measurements lower than the limit of detection (OR=0.79; 95% CI 0.57-1.12) as compared to the study population that included study participants with measurements lower than the limit of detection (OR=0.98; 95% CI 0.82-1.17). Among those exposed to benzene as compared to those not exposed, the adjusted odds of audiometrically-assessed hearing loss was lower among the study population that excluded study participants with measurements lower than the limit of detection (OR=1.02; 95% CI 0.71-1.47) as compared to the study population that included study participants with measurements lower than the limit of detection (OR=1.20; 95% CI 0.85-1.68). Other differences between estimates excluding versus including values below the limit of detection were found among crude estimates of audiometrically-assessed hearing loss and benzene (OR=1.20; 95% CI 0.90-1.59 versus OR=1.50; 95% CI 1.15-1.94), respectively.

Sensitivity Analysis 2- different hearing loss definition

To examine whether organic solvent exposure may differentially affect the occurrence of different types of hearing loss, I ran models testing the association of each of the organic solvents with high-frequency and low-frequency hearing loss, separately (Table 9). Of the final sample of 2,513 study participants, 43-49% of the study participants had audiometric data available at 0.5, 1, 2, 3, 4, 6, and 8 kHz with a range of 22-25% being cases with high-frequency hearing loss and 10-12% being cases with low-frequency hearing loss. See sample size and number of cases for this analysis in Appendix G. As an overall comparison of hearing loss, the odds of hearing loss were higher among the association between organic solvent exposure and high-/low-frequency hearing loss as compared to

audiometrically-assessed hearing loss, which defined as a pure-tone average threshold change greater than or equal to 25 dB in either ear. After controlling for covariates, benzene, ethylbenzene, and toluene concentrations remained statistically significantly associated with increased odds of high-frequency hearing loss (benzene adjusted OR=1.43, 95% CI 1.15-1.78; ethylbenzene adjusted OR=1.24, 95% CI 1.02-1.50; and toluene adjusted OR=1.27, 95% CI 1.06-1.52).

Sensitivity Analysis 3- alcohol as a covariate

As previously mentioned, a sensitivity analysis was conducted to determine the impact of using alcohol as a covariate on the study results. The results that controlled for alcohol use followed the pattern of the results from the main analysis (see Appendices B and C). All the effect estimates that adjusted for alcohol use were in the same direction and of similar magnitude than the effect estimates without adjustment for alcohol. A comparison of the results with and without alcohol use as a covariate showed the many effect estimates being within hundredths of each other.

Sensitivity Analysis 4- exclusion of non-workers

As mentioned in the methods section, the final sample size for aim 2 differed from aim 1. Repeating the analyses for Aim 1 with the same sample as in Aim 2 (i.e., restricting the analysis to 'workers') did not change the overall pattern of results (appendix D and E). As shown in Appendix D, with the exception of the ORs for ethylbenzene and self-reported hearing loss (Aim 1 OR= 0.99 95% CI 0.86-1.15 versus sensitivity analysis OR= 1.04 95% CI 0.82-1.32) and audiometrically-assessed hearing loss (Aim 1 OR= 1.02 95% CI 0.73-1.41 versus sensitivity analysis OR= 0.91 95% CI 0.51-1.65), all the effect estimates were in the

same direction and of similar magnitude as compared to Aim 1. As shown in Appendix E, the majority of the effect estimates were in the same direction and of similar magnitude as compared to Aim 1.

Interaction between organic solvents and low-and high-frequency hearing loss

To address Aim 2, whether or not the association of organic solvents and hearing loss varied by occupational noise level, I first estimated the association of occupational noise with high-frequency hearing loss. The odds of high-frequency hearing loss were statistically significantly higher among those classified as exposed to occupational noise (OR= 1.63; 95% CI 1.19-2.24) as compared to those not classified as exposed to occupational noise. After including the interaction term for occupational noise and organic solvent exposure and controlling for covariates, there was not a statistically significant interaction (benzene × occupational noise adjusted OR= 1.21, 95% CI 0.08-18.03; ethylbenzene × occupational noise adjusted OR=0.03, 95% CI 0.00, 3.42; toluene × occupational noise adjusted OR=0.75; 95% CI 0.11-5.3) between occupational noise and organic solvent exposure on high-frequency hearing loss (see Table 10). For these interaction terms, the odds of high-frequency hearing loss were for every one unit increase in benzene exposure in those study participants exposed to occupational noise. Given the small concentrations of organic solvent exposure, a one unit increase in this measurement explains why the confidence interval for the interaction term is so large.

Table 5. Number and percentage of study participants with organic solvents (ng/mL) measurements collected by the National Health and Nutrition Survey, 1999-2004

Characteristics	**N**	**%**
Gender		
Male	1195	47.6
Female	1318	52.5
Race/ethnicity		
Hispanic	649	25.8
Non-Hispanic White	1250	49.7
Non-Hispanic Black	507	20.2
Other	107	4.3
Income		
<$20,000	489	19.5
≥$20,000	2024	80.5
Ototoxic medication		
Yes	107	4.3
No	2406	95.7
Smoker		
Yes	564	22.4
No	1949	77.6
Diabetes		
Yes	125	5.0
No	2388	95.0
Occupational noise exposure		
Low	617	51.0
High	594	49.1
Non-occupational noise exposure		
Yes	753	30.0
No	1760	70.0
Age (mean, SD)	38.2	11.1

Table 6. Median and interquartile range (IQR) of organic solvents (ng/mL) in US adults from the National Health and Nutrition Survey, 1999-2004

Organic solvent (ng/mL)	Median	IQR
1,4- Dichlorobenzene	0.52	0.44, 0.75
Benzene	0.32	0.26, 0.45
Ethylbenzene	0.31	0.26, 0.38
m-/p-xylene	0.52	0.45, 0.60
o-xylene	0.33	0.33, 0.38
Styrene	0.28	0.28, 0.39
Toluene	0.49	0.39, 0.65

Table 7. Odds ratios (OR, per interquartile range increase) and corresponding 95% confidence intervals (CI) for the association between transformed organic solvent exposure (ng/mL) and hearing loss and tinnitus in US adults from the National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	Hearing loss				Self-reported tinnitus	
	Self-reported		Audiometrically-assessed			
	Crude OR (95% CI)	Adjusted* OR (95% CI)	Crude OR (95% CI)	Adjusted* OR (95% CI)	Crude OR (95% CI)	Adjusted* OR (95% CI)
1,4-Dichlorobenzene	0.93 (0.86, 1.00)	1.00 (0.93, 1.08)	0.96 (0.82, 1.13)	1.04 (0.87, 1.24)	0.96 (0.90, 1.03)	1.01 (0.94, 1.08)
Benzene	**1.30 (1.15, 1.47)**	1.11 (0.96, 1.29)	**1.50 (1.15, 1.94)**	1.20 (0.85, 1.68)	**1.16 (1.03, 1.31)**	0.98 (0.85, 1.13)
Ethylbenzene	**1.20 (1.06, 1.36)**	0.99 (0.86, 1.15)	**1.31 (1.04, 1.67)**	1.02 (0.73, 1.41)	**1.14 (1.01, 1.28)**	0.98 (0.85, 1.12)
m-/p- xylene	1.10 (0.99, 1.22)	0.95 (0.84, 1.07)	1.20 (0.97, 1.48)	0.95 (0.73, 1.23)	1.07 (0.97, 1.18)	0.98 (0.88, 1.10)
o-xylene	1.06 (1.00, 1.14)	1.00 (0.93, 1.08)	1.09 (0.96, 1.24)	0.98 (0.82, 1.17)	1.01 (0.95, 1.08)	0.97 (0.90, 1.04)
Styrene	1.05 (0.96, 1.15)	0.96 (0.85, 1.09)	1.06 (0.93, 1.20)	0.94 (0.72, 1.25)	1.03 (0.95, 1.12)	0.95 (0.84, 1.06)
Toluene	**1.17 (1.04, 1.31)**	0.96 (0.84, 1.10)	**1.29 (1.04, 1.60)**	1.00 (0.77, 1.30)	1.09 (0.98, 1.22)	0.93 (0.82, 1.06)

* Models adjusted for age, gender, race/ethnicity, diabetes, non-occupational noise exposure, smoking, and income.

Table 8. Odds ratios (OR, per interquartile range increase) and corresponding 95% confidence intervals (CI) for the association between transformed organic solvent exposure (ng/mL) and hearing loss and tinnitus excluding subjects with solvent concentration less than the limit of detection, in US adults from the National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	Hearing loss				Self-reported tinnitus	
	Self-reported		Audiometrically-assessed			
	Crude OR (95% CI)	Adjusted* OR (95% CI)	Crude OR (95% CI)	Adjusted* OR (95% CI)	Crude OR (95% CI)	Adjusted* OR (95% CI)
1,4-Dichlorobenzene	0.94 (0.83, 1.07)	1.03 (0.91, 1.17)	0.98 (0.77, 1.25)	1.07 (0.83, 1.40)	0.97 (0.86, 1.08)	1.06 (0.94, 1.18)
Benzene	**1.30 (1.12, 1.52)**	1.12 (0.93, 1.34)	1.20 (0.90, 1.59)	1.02 (0.71, 1.47)	1.07 (0.92, 1.25)	0.90 (0.75, 1.07)
Ethylbenzene	**1.16 (1.03, 1.31)**	1.01 (0.87, 1.17)	1.18 (0.95, 1.46)	0.99 (0.73, 1.33)	1.05 (0.93, 1.18)	0.94 (0.81, 1.08)
m-/p- xylene	1.10 (0.99, 1.23)	0.96 (0.85, 1.08)	**1.25 (1.02, 1.55)**	1.00 (0.77, 1.29)	1.06 (0.96, 1.17)	0.97 (0.87, 1.09)
o-xylene	1.06 (0.94, 1.20)	1.02 (0.89, 1.17)	0.99 (0.78, 1.26)	0.79 (0.57, 1.12)	0.96 (0.85, 1.09)	0.94 (0.82, 1.08)
Styrene	1.02 (0.92, 1.14)	0.97 (0.83, 1.13)	0.98 (0.78, 1.22)	0.81 (0.55, 1.19)	0.99 (0.89, 1.11)	0.92 (0.79, 1.08)
Toluene	**1.19 (1.06, 1.33)**	0.98 (0.85, 1.12)	**1.30 (1.04, 1.62)**	1.03 (0.79, 1.35)	1.10 (0.98, 1.23)	0.94 (0.82, 1.07)

* Models adjusted for age, gender, race/ethnicity, diabetes, non-occupational noise exposure, smoking, and income.

Table 9. Odds ratios (OR, per interquartile range increase) and corresponding 95% confidence intervals (CI) for the association between transformed organic solvent exposure (ng/mL) and high and low frequency hearing loss in US adults from the National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	**High-frequency hearing loss**		**Low-frequency hearing loss**	
	Crude OR (95% CI)	**Adjusted* OR (95% CI)**	**Crude OR (95% CI)**	**Adjusted* OR (95% CI)**
1,4-Dichlorobenzene	0.94 (0.87, 1.02)	0.99 (0.90, 1.09)	0.91 (0.82, 1.01)	0.95 (0.85, 1.07)
Benzene	**1.59 (1.34, 1.89)**	**1.43 (1.15, 1.78)**	**1.43 (1.20, 1.72)**	1.12 (0.89, 1.39)
Ethylbenzene	**1.54 (1.30, 1.82)**	**1.24 (1.02, 1.50)**	**1.33 (1.13, 1.57)**	1.08 (0.89, 1.31)
m-/p- xylene	**1.29 (1.14, 1.46)**	1.08 (0.93, 1.24)	**1.17 (1.02, 1.33)**	1.00 (0.86, 1.16)
o-xylene	**1.14 (1.04, 1.25)**	1.03 (0.93, 1.13)	1.08 (0.99, 1.18)	1.00 (0.90, 1.11)
Styrene	**1.19 (1.04, 1.36)**	1.04 (0.94, 1.17)	**1.15 (1.01, 1.31)**	1.05 (0.94, 1.17)
Toluene	**1.58 (1.36, 1.82)**	**1.27 (1.06, 1.52)**	**1.32 (1.14, 1.53)**	1.03 (0.87, 1.23)

*Models adjusted for age, gender, race/ethnicity, diabetes, non-occupational noise exposure, smoking, and income.

Table 10. Odds ratios (OR, per interquartile range increase) and corresponding 95% confidence intervals (CI) for the interaction between occupational noise and transformed organic solvent exposure (ng/mL) on high frequency hearing loss in US adults from the National Health and Nutrition Examination Survey, 1999-2004

Predictors	**Adjusted* OR (95% CI)**		
	Benzene	**Ethylbenzene**	**Toluene**
Occupational noise	1.02 (0.32, 3.24)	3.67 (0.66, 20.24)	1.37 (0.42, 4.44)
Solvent	1.40 (0.93, 2.12)	**1.86 (1.19, 2.92)**	**1.61 (1.10, 2.35)**
Solvent × Occupational noise	1.21 (0.08, 18.03)	0.03 (0.00, 3.42)	0.75 (0.11, 5.30)

*Models adjusted for age, gender, race/ethnicity, diabetes, non-occupational noise exposure, smoking, and income.

DISCUSSION

The purpose of this study was 1) to explore the association between organic solvent exposure and hearing loss or tinnitus and 2) to determine if interaction exists between occupational noise and organic solvent exposure on hearing loss or tinnitus. After adjusting for covariates, there was no evidence of an association between hearing loss or tinnitus and organic solvent exposure except in three cases: higher exposure to benzene, ethylbenzene, and toluene was associated with greater likelihood of high-frequency hearing loss. The main findings in this study were that for every IQR unit increase in benzene, ethylbenzene, and toluene blood concentration, the odds of high-frequency hearing loss statistically significantly increased in study participants. No statistically significant interaction between exposure to organic solvents and occupational noise on high-frequency hearing loss was identified. Therefore, this study found no evidence of interaction between organic solvent exposure and occupational noise on high-frequency hearing loss.

There is limited epidemiologic evidence on the association between hearing loss and benzene exposure. Research has shown benzene has neurotoxic effects and can produce free radicals, which suggest a mechanism for ototoxicity [57]. Additionally, occupational studies have shown exposure to a mixture of solvents which included benzene, where exposure was categorized by job title, was positively associated with hearing loss [14, 18, 22, 83]. Animal studies have shown ethylbenzene exposure [58-63] has ototoxic effects, while toluene [52, 67-69, 79, 80, 89] exposure has demonstrated ototoxic reactions in animal and human studies. Similar to the Triebig *et al.* study, my study used blood concentrations of organic solvents and found a statistically significant association between high-frequency hearing loss

and 1) benzene 2) ethylbenzene and 3) toluene exposure. However, I did not identify a statistically significant association between other organic solvents and high -frequency or between organic solvents and self-reported/ audiometrically-assessed/ low-frequency hearing loss.

The odds of hearing loss were higher for the associations between organic solvent exposure and high-/low-frequency hearing loss as compared to audiometrically-assessed hearing loss. My results show the frequency of hearing loss varies by organic solvent exposure. In animal studies, Cappaert reported hearing loss, which varied by frequency, in rats and guinea pigs after exposure to ethyl benzene [59, 61, 62]. Research has shown exposure to organic solvents can produce cochlear lesions in the inner ear leading to hair cell loss, which is important as hair cells control the frequencies the ear can hear [49-52]. Given the potential mechanism for hearing loss at specific frequencies, Morata *et al.* suggests analyzing high-frequency hearing loss (hearing loss at frequencies of 3, 4, 6 and 8 kHz) when looking at the association between hearing loss and organic solvent exposure, but many researchers use the World Health Organization definition of hearing loss, which is a pure tone average greater than or equal to 25 dB in either ear at the frequencies of 0.5, 1, 2, and 4 kHz [16, 121]. As the frequency of hearing loss can vary by exposure to organic solvents, researchers should consider analyzing the association of organic solvent exposure by the individual frequency of hearing loss as opposed to a general definition of hearing loss.

In addition to the differences found in hearing loss based on frequency, there could be important differences in hearing loss assessed by audiometry versus a questionnaire. In the present study, self-reported hearing loss and audiometrically-assessed hearing loss followed

similar patterns in there effect estimates for each organic solvent studied. These results are in agreement with previous studies [85, 150-155]. For example, in a study conducted by Sindusake in 2001, pure-tone audiometry data was compared to hearing loss assessed by the following question, "do you feel you have a hearing loss?" For hearing loss greater than 25 dB, sensitivity was 78% and specificity was 67%, and, for hearing loss greater than 40 dB, sensitivity was 93% and specificity was 56% [151]. Therefore, self-reported data on hearing loss can be used to access hearing loss, but audiometry data should be used whenever it is available.

Previous research demonstrates mixed conclusions on the ototoxicity of organic solvents. Using biomarkers in urine samples, Schaper *et al.* found a null association between toluene exposure and audiometrically-assessed hearing loss of 25 dB or greater [93]. Fechter *et al.* reported a null association between styrene exposure and cochlear function in guinea pigs [81], while human studies also reported a null association between styrene exposure (as measured by job categorization and personal air sampling) in workers and audiometrically-assessed hearing thresholds [92, 94, 95], which supports my results. Contrary to my results, other studies have identified a statistically significant association between organic solvent exposure and hearing loss. Animal studies have shown trichloroethylene [70-73] exposure to have ototoxic effects. Likewise, p-xylene [60, 75-78, 90] exposure has demonstrated ototoxic reactions in animal studies as well as in certain human studies. There is also evidence of an association between hearing loss and styrene. In a laboratory setting, animals given a controlled dose of styrene exposure have shown hearing loss [49, 64-66], while human studies, where styrene exposure was measured by urine and air samples, provide evidence for

the association between hearing loss and styrene exposure [24, 87]. Additionally, Triebig *et al.* used blood measurements of styrene and found a statistically significant relationship with styrene exposure and hearing loss [88].

A possible reason for the difference between my findings and those of previous studies is the measurement used to quantify exposure among study participants. Exposure was quantified based on blood measurements in the current study, while urine concentrations, personal air sampling, and job categorization were used for exposure measurements in previous studies that found a statistically significant result [89, 90, 92, 94, 95]. Blood concentrations of organic solvents mainly characterize recent exposure due to the short half-life, and, therefore, may not be a good biomarker for predicting the long-term effect of organic solvent exposure on hearing loss or tinnitus. For instance, the blood concentrations of organic solvents (i.e., 1,4-dichlorobenzene, benzene, ethylbenzene, m-/p- xylene, o-xylene, styrene, and toluene) found in this study were all below the NIOSH REL [25]. Ideally, this dissertation would incorporate a measure of long-term exposure to organic solvents, but those data were not available in NHANES.

To my knowledge, this dissertation is the first epidemiologic study to evaluate the association between tinnitus and organic solvent exposure. A previous case-study documented self-reported exposure to toluene or xylene and self-reported tinnitus in the study subject [91]. My results were contrary to those previous findings and extend limited evidence of the null association between tinnitus and organic solvent exposure.

With regards to the differences in populations of Aim 1 and Aim 2, the study population of Aim 1 included workers and non-workers; while Aim 2 only included study

participants who reported their longest job (i.e. Aim 2 excluded non-workers). To determine the impact of the study population differences, I ran a sensitivity analysis reanalyzing the effect estimates of Aim 1 excluding study participants without measurements on longest job reported in NHANES. The results showed there was minimal difference in the effect estimates of Aim 1 when including or excluding study participants without measurements on longest job reported in NHANES.

For the second aim of this study, I determined there was no interaction between occupational noise and benzene, ethylbenzene, and toluene exposures with high-frequency hearing loss. To the best of my knowledge, there are no previous studies on the interaction of noise and exposure to benzene as an individual solvent on hearing loss. Researchers have reported a null association for the interaction between noise and solvent exposure on hearing loss [85, 113]. On the contrary, a positive interaction on hearing loss between individual organic solvents (including ethylbenzene and toluene but not including benzene) and noise has been demonstrated in animal studies [58, 70, 98-103] along with human studies [22, 24, 87, 106, 107, 111, 112]. Further, epidemiologic studies on workers have also found joint exposure to noise and a mixture of organic solvents, including benzene and styrene, affects hearing loss [20, 82, 104-110].Occupational noise exposure estimation was indirect, based on linking longest held job reported in NHANES to estimates of occupational noise in O*NET. There is a degree of uncertainty in using multiple crosswalks to link two databases. For example, certain census and SOC codes may be incorrectly matched causing study participants to be misclassified. The occupational noise score estimation used in this study was based on the median value of the occupational noise from a representative sample of US

workers with the same jobs. Therefore, this estimate does not account for inter-individual variability and could explain the lack of association in reported in this study.

Previous research has demonstrated an association between exposure to occupational and non-occupational noise and hearing loss and tinnitus [22, 85, 104, 112]. Additionally, previous studies assessing the association between organic solvents and hearing loss or tinnitus have demonstrated the potential confounding effects of non-occupational noise [14, 16, 89, 156]. Therefore, if this factor had not been accounted for in this study, there would be a potential for residual confounding, which could have resulted in a spurious relationship between organic solvent exposure and hearing loss or tinnitus.

Previous studies have suggested that alcohol use may confound the association between solvent exposure and hearing loss [14, 16, 88, 89, 111, 112, 156, 157]. For this study, I was limited by self-reported use of alcohol as collected by NHANES. Here, alcohol use was defined as the self-reported average number of drinks consumed per day in the past 12 months. However, there is limited evidence of the direct ototoxicity of alcohol use and thus, this variable was not included as a covariate in the main analysis. I did conduct a sensitivity analysis including alcohol use as a covariate and found little difference between the effect estimates controlling for alcohol use and the effect estimates that did not control for alcohol use. Therefore, in these data, there was little evidence of confounding of the association between solvent exposure and hearing loss by alcohol use. However, there are potential limitations of the alcohol use data collected by NHANES including not having beverage–specific questions, having potentially embarrassing open–ended responses, and measurement of alcohol use in standard drink sizes [158].

A comparison of the analysis that included measurements below the limit of detection and the analysis that excluded those measurements showed the pattern of point estimates was similar for the majority of organic solvents- although some differences were present. For the adjusted analysis, the results from this sensitivity analysis differed in magnitude for the odds ratios of o-xylene and benzene with audiometrically-assessed hearing loss. For the crude analysis, the results from this sensitivity analysis differed in magnitude for the odds ratios of benzene with audiometrically-assessed hearing loss. Overall, the sensitivity analyses demonstrated there were minimal differences in the effect estimates based on the exclusion of blood concentrations below the limit of detection. These results can be useful in the methodology for future studies as my results show little difference in results based on inclusion of measurements below the limit of detection.

Research shows exposure to organic solvents is decreasing in the general population [37, 38]. In a study by Su *et al.*, the median levels of benzene, toluene, ethylbenzene, m-/p-xylene, o-xylene, and styrene in 1999-2000 decreased (2.1-18.2%) as compared to the levels in 2003-2004 [38]. However, Su *et al.* concluded it was possible these changes could occur due to unreported anomalies in the study dataset that affected the 1999-2000 cohort [38]. Batterman *et al.* states emission controls and process changes may be responsible for reduced organic solvent exposure [37]. Even though exposure levels are decreasing, it is still important to identify if these levels of organic solvent exposure can affect hearing loss.

Unfortunately, although NHANES collected data on trichloroethylene, this organic solvent was not analyzed as part of this study because over 95% of the measurements on trichloroethylene blood concentrations in the study population were below the limit of

detection. Therefore, many of the measurements for this organic solvent would be the same, making the analysis of no value. Trichloroethylene, which is a potential occupational carcinogen, is used as a degreaser, paint stripper, paint and varnish ingredient, adhesive solvent, and in the manufacturing of organic chemicals [30]. Occupational groups that are potentially exposed to trichloroethylene are degreasing operators, fiberglass manufacturers, dry cleaners, and insecticide producers [34]. A possible reason for the high number of measurements below the limit of detection may be that the occupational groups, which would be potentially exposed to trichloroethylene, were not well represented by the data. Future research should analyze if there are statistically significant differences between occupational groups and organic solvent blood concentrations as measured in NHANES.

As mentioned in the methods section, the data was transformed using a cubic root transformation. A log-normal transformation was tested, but the cubic root transformation best normalized the data. Transformation of the data makes the interpretation of the results less intuitive, but, from a statistical standpoint, the transformation was necessary to ensure appropriate data analysis. When interpreting these dissertation results, the reader must keep in mind that the odds of hearing loss or tinnitus were for every IQR increase in transformed concentrations of organic solvent exposure.

The important strengths of this study include the use of NHANES data, which supports adjustment for potentially important confounding variables, uses strict quality control measures, and includes a large, diverse sample of the US population. Additionally, the O*NET database, an occupational exposure assessment tool, was used to control for occupational noise exposures. O*NET is constantly updated and offers a national source of

data on job tasks and hazards. To the best of my knowledge, this study was one of the first to combine the NHANES and O*NET databases, which could potentially be a beneficial and an innovative data source for the field of occupational health research.

Several limitations of this study must also be considered. NHANES has a cross-sectional design, which limits the validity of causal interpretations of the association between exposure to organic solvents and hearing loss or tinnitus. There is a potential for selection bias as not all randomly selected participants may have participated in the survey or study participants may have only completed the survey in part. Residual confounding may be present in study results as there may be additional confounding factors that were not considered or there may be errors in the classification of study participants with regards to confounding variables. Lastly, I ran many regressions, and multiple comparisons increase the possibility of detecting a statistically significant association by chance. In this case, detection of statistically significant associations (e.g., among the high-/low-frequency outcomes) by chance seems unlikely because the effect estimates were consistently larger for the high-/low-frequency hearing loss outcomes for each exposure variable as compared to hearing loss assessed by the World Health Organization definition.

Additionally, there is potential for information bias in the measurement of tinnitus, noise and other covariates. Recall bias may be present in the measurement of tinnitus, noise variables and covariates as study participants may incorrectly remember events from the past.

Lastly, not all NHANES study participants were included in every component (i.e., laboratory or examination components) of data collection. The subsample of study participants that were selected for additional examination and laboratory testing were chosen

at random. Subsample weights should be used to reflect the representative proportions of these groups in the population. Unfortunately, the subsamples only partially overlap, which makes the NHANES weighting inappropriate [116]. Given that the aim of this study was to look at associations and not to represent proportions or be representative of worker populations, the un-weighted data is likely to be unbiased regarding the accuracy of my study results. Although, oversampling of certain study populations may create potential for selection bias.

In conclusion, this dissertation found, in a large, diverse population with blood measurements of organic solvents, there was no indication of association between organic solvent exposure and hearing loss (self-reported and audiometrically-assessed) or self-reported tinnitus but there was evidence of an association between organic solvents and high-frequency hearing loss. Moreover, the effect estimates for high-/low-frequency hearing loss were higher as compared to audiometrically-assessed hearing loss defined according to the World Health Organization's definition of hearing loss. Future research should identify which definition of hearing loss should be used in studies on organic solvent-induced hearing loss. In addition, this study was able to link NHANES with O*NET, which is potentially very useful for occupational health studies. Future research should also assess the accuracy of linking NHANES with O*NET. These steps in combination with the work of other researchers can help improve the knowledge base on the association between organic solvent exposure and hearing loss or tinnitus.

APPENDICES

Appendix A: Sample size for the association between organic solvent exposure (ng/mL) and hearing loss and tinnitus in US adults from the National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	Hearing loss				Self-reported Tinnitus	
	Self-reported		Audiometrically-Assessed			
	N	Cases	N	Cases	N	Cases
1,4-Dichlorobenzene	2245	439	1085	82	2243	520
Benzene	2315	453	1130	90	2313	537
Ethylbenzene	2285	443	1125	86	2283	533
m-/p- xylene	2416	461	1208	92	2414	564
o-xylene	2471	472	1243	99	2469	578
Styrene	2295	433	1153	87	2293	540
Toluene	2405	463	1205	99	2403	561

Appendix B: Sample size, odds ratios (OR, per interquartile range increase) and corresponding 95% confidence intervals (CI) for the association between organic solvent exposure (ng/mL) and hearing loss and tinnitus in US adults from the National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	Hearing loss				Self-reported tinnitus	
	Self-reported		Audiometrically-assessed			
	N	Adjusted* OR (95% CI)	N	Adjusted* OR (95% CI)	N	Adjusted* OR (95% CI)
1,4- Dichlorobenzene	1793	1.00 (0.91, 1.09)	893	1.06 (0.87, 1.29)	1791	1.02 (0.95, 1.10)
Benzene	1844	1.07 (0.90, 1.27)	923	1.19 (0.82, 1.75)	1842	0.94 (0.79, 1.10)
Ethylbenzene	1815	0.98 (0.82, 1.15)	921	1.00 (0.69, 1.45)	1813	0.97 (0.83, 1.13)
m-/p- xylene	1924	0.94 (0.82, 1.08)	988	0.97 (0.73, 1.29)	1922	0.96 (0.84, 1.09)
o-xylene	1963	0.98 (0.89, 1.07)	1013	0.97 (0.79, 1.19)	1961	0.96 (0.88, 1.05)
Styrene	1812	0.92 (0.79, 1.07)	938	0.90 (0.65, 1.26)	1810	0.93 (0.82, 1.06)
Toluene	1914	0.93 (0.80, 1.08)	984	1.03 (0.77, 1.38)	1912	0.89 (0.77, 1.03)

*Models adjusted for age, gender, race/ethnicity, diabetes, non-occupational noise exposure, smoking, income, and alcohol.

Appendix C: Sample size, odds ratios (OR, per interquartile range increase) and corresponding 95% confidence intervals (CI) for the association between organic solvent exposure (ng/mL) and high and low frequency hearing loss in US adults from the National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	**High-frequency hearing loss**		**Low-frequency hearing loss**	
	N	**Adjusted* OR (95% CI)**	**N**	**Adjusted* OR (95% CI)**
1,4-Dichlorobenzene	943	0.98 (0.88, 1.09)	941	0.96 (0.84, 1.09)
Benzene	975	**1.49 (1.16, 1.91)**	973	1.13 (0.88, 1.45)
Ethylbenzene	969	**1.24 (1.01, 1.52)**	967	1.09 (0.88, 1.34)
m-/p- xylene	1040	1.07 (0.91, 1.27)	1038	1.02 (0.86, 1.22)
o-xylene	1064	1.05 (0.94, 1.16)	1062	1.02 (0.91, 1.14)
Styrene	987	1.03 (0.92, 1.16)	985	1.05 (0.93, 1.18)
Toluene	1034	**1.26 (1.04, 1.54)**	1032	1.09 (0.91, 1.31)

*Models adjusted for age, gender, race/ethnicity, diabetes, non-occupational noise exposure, smoking, income, and alcohol.

Appendix D: Sample size, odds ratios (OR, per interquartile range increase) and corresponding 95% confidence intervals (CI) for the association between organic solvent exposure (ng/mL) and hearing loss and tinnitus in US adults who reported longest job from the National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	Hearing loss				Self-reported tinnitus	
	Self-reported		Audiometrically-assessed			
	N	Adjusted* OR (95% CI)	N	Adjusted* OR (95% CI)	N	Adjusted* OR (95% CI)
1,4- Dichlorobenzene	1097	1.02 (0.92, 1.13)	522	1.08 (0.84, 1.39)	1096	1.02 (0.93, 1.11)
Benzene	1122	1.17 (0.93, 1.48)	538	1.09 (0.58, 2.04)	1121	0.87 (0.70, 1.09)
Ethylbenzene	1123	1.04 (0.82, 1.32)	545	0.91 (0.51, 1.65)	1122	0.97 (0.78, 1.21)
m-/p- xylene	1186	0.93 (0.78, 1.11)	586	0.84 (0.55, 1.30)	1185	0.96 (0.82, 1.13)
o-xylene	1215	0.98 (0.87, 1.10)	607	0.96 (0.71, 1.28)	1214	0.96 (0.86, 1.07)
Styrene	1135	0.86 (0.69, 1.07)	567	0.93 (0.55, 1.57)	1134	0.90 (0.74, 1.08)
Toluene	1180	0.91 (0.73, 1.13)	589	0.94 (0.57, 1.56)	1179	0.92 (0.75, 1.11)

*Models adjusted for age, gender, race/ethnicity, diabetes, non-occupational noise exposure, smoking, and income.

Appendix E: Sample size, odds ratios (OR, per interquartile range increase) and corresponding 95% confidence intervals (CI) for the association between organic solvent exposure (ng/mL) and high and low frequency hearing loss in US adults who reported longest job from the National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	High-frequency hearing loss		Low-frequency hearing loss	
	N	Adjusted* OR (95% CI)	N	Adjusted* OR (95% CI)
1,4-Dichlorobenzene	554	1.05 (0.92, 1.20)	551	1.02 (0.87, 1.18)
Benzene	572	**1.47 (1.04, 2.07)**	569	1.03 (0.71, 1.48)
Ethylbenzene	576	**1.55 (1.11, 2.18)**	573	1.16 (0.85, 1.59)
m-/p- xylene	620	**1.28 (1.01, 1.61)**	617	0.98 (0.77, 1.24)
o-xylene	640	1.09 (0.94, 1.26)	637	1.00 (0.85, 1.17)
Styrene	600	1.31 (0.98, 1.76)	597	0.99 (0.73, 1.34)
Toluene	622	**1.57 (1.16, 2.11)**	619	0.99 (0.73, 1.33)

*Models adjusted for age, gender, race/ethnicity, diabetes, non-occupational noise exposure, smoking, and income.

Appendix F: Sample size for the association between organic solvent exposure (ng/mL) and hearing loss and tinnitus excluding subjects with solvent concentration less than the limit of detection in US adults from the National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	Hearing loss				Self-reported Tinnitus	
	Self-reported		Audiometrically-Assessed			
	N	Cases	N	Cases	N	Cases
1,4-Dichlorobenzene	1280	284	634	228	1278	45
Benzene	1417	355	707	299	1415	70
Ethylbenzene	1541	383	784	314	1540	67
m-/p- xylene	2355	553	1179	449	2353	88
o-xylene	994	248	534	206	993	53
Styrene	1190	298	635	238	1189	58
Toluene	2296	536	1152	441	2294	95

Appendix G: Sample size for the association between organic solvent exposure (ng/mL) and high and low frequency hearing loss in US adults, National Health and Nutrition Examination Survey, 1999-2004

Organic solvent (ng/mL)	Hearing loss			
	High-frequency		Low-frequency	
	N	Cases	N	Cases
1,4-Dichlorobenzene	1149	544	1146	261
Benzene	1197	577	1194	280
Ethylbenzene	1188	571	1185	267
m-/p- xylene	1275	614	1272	292
o-xylene	1309	632	1306	302
Styrene	1217	585	1214	282
Toluene	1270	617	1267	297

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