



Full Length Article

Hexane exposure and persistent peripheral neuropathy in automotive technicians

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ABSTRACT

Automotive technicians are commonly exposed to organic and chlorinated solvents, particularly through use of cleaning products. Mainly during the period 1989–2002, n-hexane was a component of some of these products. In other occupational contexts, n-hexane has been shown to be a cause of peripheral neuropathy. The purpose of the present study was to investigate whether previous exposures to low concentrations of n-hexane were a cause of persistent peripheral neuropathy in automotive technicians.

Enrolled in the study were 830 San Francisco Bay Area automotive technicians. Each participant underwent a battery of tests to investigate peripheral nervous system impairment. Test results regressed against estimated hexane and total solvent exposures showed only limited evidence of association with solvent exposures. Exposures to both hexane and general solvents were well below their occupational exposure limits.

Generally, our results provide reassurance about persistent peripheral neuropathic effects in automotive technicians who previously used hexane-containing automotive cleaning products. This may reflect repair processes, since the exposures occurred some years previous to the study. However, we cannot exclude the possibility that the absence of observed effect in this study may be attributable to low exposures, exposure misclassification and/or the healthy worker survivor effect.

1. Introduction

Automotive technicians are commonly exposed to organic and chlorinated solvents, particularly through use of spray cans to clean brakes and engine parts, but also from solvent tanks into which engines and parts are cleaned by dipping.

This study was prompted by the 1998 finding of 3 San Francisco Bay Area automotive technicians from one car repair facility with evidence of mild sensory or motor nerve conduction abnormalities (MMWR, 2001). Since 1989, automotive spray cans containing up to 85% hexane, often with acetone, had been used in California and other states. The technical grade of mixed hexanes used in industry typically contained 20–80% of the n-hexane isomer. N-hexane is well-established as a peripheral neurotoxin, acting through the formation of the metabolite 2,5-hexanedione (LoPachin and Gavin, 2015). In animal models its neurotoxic effects are enhanced by concurrent exposure to acetone (Norberg and Arlien-Soborg, 2000). However, as far as we are aware the combined effects of n-hexane and acetone have not been studied in

humans. Generally, the levels of hexane exposure associated with automotive spray can use were shown to be well below the current occupational exposure limits for exposure to n-hexane (Wilson et al., 2007).

The peripheral neuropathy findings (and color vision deficits in the same persons) prompted the California Department of Health Services in June 2001 to issue a Health Hazard Advisory through Hazard Evaluation System and Information Service (HESIS). Because of the size of the Californian market, in the two years following the Advisory, manufacturers removed hexane from all automotive spray cleaning products used in the U.S. (Wilson, 2003).

The main purpose of this study was to investigate whether previous exposures to hexane, previously shown to be below the threshold limit value (TLV) for n-hexane (50 ppm or 176 mg/m³ as a time-weighted average) established by the American Conference of Governmental Industrial Hygienists (ACGIH) (Wilson et al., 2007) posed a persistent neurotoxic hazard. Although the study focus was hexane, data were obtained on all solvents used in the automotive repair facilities at which

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Table 1

Description of the study population, by quartiles (Q1 to Q4) of cumulative inhaled solvent exposures and trichotomized (T1 to T3) cumulative hexane exposures.

	N (col%)	All Solvent Exposure (mg/m ³)-years				Any Hexane Exposure (mg/m ³)-years			
		Q1 < 605	Q2 605 to < 1197	Q3 1197 to < 2069	Q4 ≥ 2069	T1 0	T2 > 0 to < 32	T3 ≥ 32	Unknown
Number of participants	830 (100%)	208	209	207	206	385	210	217	18
5 year age bands									
< 45	176 (21.2%)	33.7%	24.9%	16.4%	9.7%	19.5%	16.8%	30.4%	0%
45 to < 50	194 (23.4%)	20.2%	25.4%	26.1%	21.8%	20.5%	28.4%	24%	22.2%
50 to < 55	237 (28.6%)	24.5%	29.7%	29.5%	30.6%	28.8%	31.7%	24.4%	38.9%
55+	223 (26.9%)	21.6%	20.1%	28%	37.9%	31.2%	24%	21.2%	38.9%
Union Local									
Oakland-San Leandro	662 (79.8%)	80.3%	78.9%	80.2%	79.6%	77.7%	81.7%	83.9%	61.1%
Sacramento	60 (7.2%)	7.7%	5.7%	7.7%	7.8%	8.1%	7.2%	5.1%	16.7%
San Mateo	108 (13%)	12%	15.3%	12.1%	12.6%	14.3%	12%	11.1%	22.2%
Race									
White	647 (78%)	80.3%	75.6%	74.4%	81.6%	78.7%	83.7%	71.4%	83.3%
Black	25 (3%)	3.4%	3.3%	2.4%	2.9%	3.4%	2.9%	2.3%	5.6%
Asian	78 (9.4%)	10.6%	8.6%	11.6%	6.8%	8.8%	6.3%	13.8%	5.6%
Native, PI	24 (2.9%)	0.5%	4.8%	3.4%	2.9%	2.1%	2.4%	5.1%	0%
Multi-race	56 (6.7%)	5.3%	7.7%	8.2%	5.8%	7%	5.8%	7.4%	5.6%
Ethnicity									
Hispanic or Latino	132 (15.9%)	14.4%	15.8%	16.4%	17%	16.4%	15.4%	15.7%	16.7%
Not Hispanic or Latino	697 (84%)	85.1%	84.2%	83.6%	83%	83.4%	85.6%	84.3%	83.3%
Unknown	1 (0.1%)	0.5%	0%	0%	0%	0.3%	0%	0%	0%
Education									
High school only	267 (32.2%)	26%	30.6%	35.7%	36.4%	32.5%	34.6%	31.8%	5.6%
College, no degree	366 (44.1%)	49%	41.6%	41.5%	44.2%	44.7%	40.9%	44.7%	66.7%
College degree	197 (23.7%)	25%	27.8%	22.7%	19.4%	22.9%	25.5%	23.5%	27.8%
Household income									
< \$40,000	84 (10.1%)	12.5%	7.7%	7.7%	12.6%	10.9%	7.7%	11.1%	11.1%
\$40,001 - \$60,000	125 (15.1%)	16.8%	10%	17.9%	15.5%	13.2%	15.4%	18.4%	11.1%
\$60,001 - \$80,000	189 (22.8%)	16.8%	27.8%	21.3%	25.2%	20.3%	26.4%	24%	22.2%
\$80,001 - \$100,000	156 (18.8%)	17.3%	21.1%	18.8%	18%	19.2%	17.8%	18%	33.3%
\$100,001 or more	268 (32.3%)	34.6%	33%	33.3%	28.2%	35.1%	32.7%	28.1%	22.2%
Missing	8 (1%)	1.9%	0.5%	1%	0.5%	1.3%	1%	0.5%	0%
Alcohol consumption frequency									
Non-drinker	189 (22.8%)	27.4%	19.1%	20.3%	24.3%	23.9%	16.8%	28.1%	5.6%
≥ once/month	69 (8.3%)	8.7%	5.3%	9.7%	9.7%	7.8%	8.2%	9.7%	5.6%
≥ 4 times/month	142 (17.1%)	15.4%	20.1%	16.4%	16.5%	17.4%	18.8%	14.3%	27.8%
≥ 3 times/week	148 (17.8%)	17.3%	20.6%	18.4%	15%	15.8%	24.5%	15.2%	16.7%
≥ 4 times/week	282 (34%)	31.3%	34.9%	35.3%	34.5%	35.1%	32.7%	32.7%	44.4%
Binge Drinking									
Never	356 (42.9%)	46.6%	41.6%	39.6%	43.7%	44.9%	40.9%	43.8%	16.7%
Less than monthly	217 (26.1%)	23.1%	24.9%	30.4%	26.2%	24.4%	30.3%	24.9%	33.3%
Monthly	101 (12.2%)	14.4%	14.4%	9.2%	10.7%	9.6%	15.9%	13.4%	11.1%
Weekly	102 (12.3%)	10.6%	14.4%	12.1%	12.1%	13.2%	8.7%	12.9%	27.8%
Daily or almost daily	54 (6.5%)	5.3%	4.8%	8.7%	7.3%	7.8%	5.3%	5.1%	11.1%
Diabetic and/or A1C ≥ 6.5%									
No	690 (83.1%)	86.5%	87.1%	80.2%	78.6%	82.1%	85.6%	83.9%	77.8%
Yes	140 (16.9%)	13.5%	12.9%	19.8%	21.4%	17.9%	15.4%	16.1%	22.2%

Dermal exposure adds about 10–15% increase in exposure for these quartiles.

our participants worked. Our main hypothesis was that we would find hexane exposure below the TLV to be associated with persistent deficits in peripheral nerve function.

2. Methods

Institutional Review Board approvals for study procedures were obtained at the University of California, Berkeley, CA, and at Stanford University, Palo Alto, CA. Prior informed consent in writing was obtained from all participants. Each was paid \$75 to cover transport to and from the study clinic and as some compensation for their time.

2.1. Participants

Participants were recruited from members of 3 Northern California locals of District 190 of the International Association of Machinists and Aerospace Workers (IAMAW). Eligibility required that participants be males ≤ 60 years old at time of participation, have worked as an automotive technician for at least one year in the period 1990–2000

(when hexane was in use), and currently living in or near the San Francisco Bay Area. Women were not included as there were few female members of the IAMAW during the period of hexane use, and another part of the study investigated possible testicular effects of hexane, manifesting in infertility. Current work as an automotive technician was not a requirement.

Recruitment began with members of the Oakland-San Leandro local, moving to locals in San Mateo and Sacramento when further eligible and willing members of the first local were unavailable for recruitment. The first step in recruitment was obtaining the Oakland-San Mateo membership database, which contained records of former and current members. From this database we identified all members who fit the eligibility criteria, including their most up-to-date contact details. Current addresses were confirmed using other means, including Experian, California Department of Motor Vehicles, and California voter registration records. Recruitment was initiated by sending a letter to the current address, including a brochure describing the study and a stamped and addressed response envelope with a form to be returned indicating willingness to participate. If necessary, a further letter was

Table 2

Regression results for the peripheral neuropathy measures for hexane-related exposures after controlling for covariates. T1 is the mean value for the non-exposed group for either hexane alone or hexane with acetone, with its standard error. Results, T2-T3, the mean differences from T1 with their 95% confidence intervals (CI), are in bold type face when the CI excludes the null value.

	N	D	Any Hexane Exposure (mg/m ³ -years)			Hexane with Acetone Exposure (mg/m ³ -years)		
			T1 0	T2 ^a > 0 to < 32	T3 ^a ≥ 32	T1 0	T2 ^a > 0 to < 18	T3 ^a > = 18
Symptom sum (0-4)	804	↓	0.6 (0.05)	0.0 (-0.22, 0.14)	0.1 (-0.06, 0.30)	0.6 (0.05)	0.0 (-0.17, 0.20)	0.1 (-0.07, 0.31)
Monofilament (g)	786	↓	4.8 (1.68)	-1.8 (-7.40, 3.83)	5.0 (-0.63, 10.58)	4.9 (1.51)	-0.7 (-6.50, 5.08)	4.6 (-1.33, 10.58)
Bio-Thesimeter (volts)	724	↓	17.3 (0.48)	-0.8 (-2.35, 0.84)	-0.3 (-1.87, 1.28)	17.2 (0.43)	-1.1 (-2.70, 0.58)	0.1 (-1.59, 1.77)
Tuning fork (secs)	789	↑	15.2 (0.26)	0.2 (-0.68, 1.08)	0.3 (-0.53, 1.23)	15.4 (0.24)	-0.2 (-1.10, 0.72)	-0.1 (-1.04, 0.83)
Ankle jerks (Y/N)	784	↓	0.2 (0.02)	0.0 (-0.04, 0.08)	-0.1 (-0.11, 0.01)	0.2 (0.02)	0.0 (-0.03, 0.10)	0.0 (-0.09, 0.04)
MNCV (m/sec)	364	↑	44.9 (0.32)	-0.8 (-1.90, 0.21)	-0.5 (-1.54, 0.61)	44.8 (0.28)	-0.8 (-1.86, 0.34)	-0.2 (-1.41, 1.00)
SSPL (m/sec)	418	↑	4.0 (0.03)	0.1 (0.02, 0.21)	0.0 (-0.09, 0.10)	4.0 (0.03)	0.1 (-0.01, 0.19)	0.0 (-0.09, 0.12)
PCA1	705	↓	0.1 (0.09)	-0.1 (-0.40, 0.23)	-0.1 (-0.46, 0.16)	0.0 (0.08)	-0.1 (-0.37, 0.27)	0.0 (-0.32, 0.34)
PCA2	306	↓	0.0 (0.14)	0.1 (-0.38, 0.62)	-0.2 (-0.69, 0.31)	0.0 (0.13)	0.2 (-0.30, 0.73)	-0.2 (-0.77, 0.35)

N, Number of participants in the model.

D, Direction of better performance (e.g., ↓ means a lower number implies better performance).

T1 Adjusted mean and SE values for the unexposed group.

T1-T3 Exposure categories in mg/m³-years.

MNCV: Peroneal motor nerve conduction velocity; SSPL: Sural sensory peak latency.

^a Values are means of differences from T1, with 95% confidence intervals, adjusted for age, local union affiliation, race, diabetes, alcohol consumption, binge drinking, and education.

sent, followed by calls to the last recorded telephone number. Where necessary, efforts were made to find new or alternative telephone numbers. Recruitment efforts proceeded until we had received participation acceptance or refusal, the invitee was found to have moved out of the area, was found to be deceased, or we had made no contact after multiple (up to 30) attempts to do so.

2.2. Data collection

Participants from Oakland-San Leandro and San Mateo attended a dedicated clinic in San Leandro. A mobile clinical van was used for participants from Sacramento. At the clinic they responded to questions in a questionnaire and underwent a series of clinical tests of neuropathy.

2.2.1. Questionnaire

The questionnaire was programmed using Casic Builder™ (West Portal Software Corporation), for direct data entry. As they were entered, data were downloaded to a dedicated study server. In addition to collecting data on demographics, medical history, tobacco smoking, alcohol consumption, education and income, the questionnaire obtained detailed automotive technician work histories, including names of employers, the frequency that tasks using solvents were performed at each workplace, and names and amounts of solvent products used. A booklet containing color pictures of 121 spray-can products was provided as a memory aid. The booklet was incomplete, as pictures of 17, mostly older, products were unavailable.

The work history module of the questionnaire was developed using a focus group of 14 experienced auto-technicians to identify tasks with potential solvent exposures, work practices and historical changes in those that would have affected solvent exposure. A draft questionnaire was pilot-tested and revised; this procedure was repeated until no further change was necessary.

2.2.2. Hemoglobin A1c

HbA1c, a biomarker for possible diabetes, was measured with a point-of-care In2it A1c Analyzer (Bio-Rad Laboratories, Inc). Results are expressed as the percent of hemoglobin that is glycated.

2.2.3. Neuropathy measures

Nerve conduction testing comprising sensory nerve conduction study of the right sural nerve and motor nerve conduction study of the

right peroneal nerve was carried out in a subset of participants, using a XLTEK NeuroMax EMG machine. The parameters used for data analysis were peroneal motor nerve conduction velocity (MNCV) and sural sensory peak latency (SSPL).

Although nerve conduction study is the “gold standard” of peripheral neuropathy detection, there is general consensus that an accurate clinical diagnosis of neuropathy can also be made with relatively simple screening examinations (England et al., 2005). We chose 5 such measures that have been validated as indicators of the possible presence of neuropathy. The study neurologist provided the initial training as well as continual data quality monitoring throughout the study.

The 5 neuropathy measures were as follow:

- 1 A 4-symptom questionnaire that asked about balance and pain in the legs and feet, scoring 1 when a symptom was reported present and 0 when it was not. These scores were summed for analysis (0–4). Questions were:
 - i Do you feel unsteady when you walk?
 - ii Do you have constant pain or tenderness in your lower legs or feet?
 - iii Do you have constant prickling sensations in your lower legs and feet, occurring at rest or at night?
 - iv Do you have areas of constant numbness in your lower legs or feet, occurring at rest or at night?
- 2 Using a Queen Square hammer, ankle stretch reflexes were tested on both sides. The results were recorded as a binary measure (0 normal, 1 abnormal), based on the presence or absence of the reflex. Possible scores were 0 if both sides were normal, 1 if only one side was abnormal, and 2 if both sides were abnormal.
- 3 Light touch sensation was tested with a series of Semmes-Weinstein monofilaments calibrated at sizes 2.83 (0.07 g), 3.61 (0.4 g), 4.31 (2.0 g), 4.56 (4.0 g), 5.07 (10 g), and 6.65 (300 g). The lightest monofilament that the participant reported feeling was recorded; lower values equate to better sensation. The analysis used the average lightest monofilament weight detected by the two feet.
- 4 A tuning fork that vibrates at 128 Hz was struck and applied to the hallux nail fold. The time in seconds that the subject reported being able to feel the vibration was recorded. The analysis used the average time of detection by the two feet. Longer times indicate higher sensitivity.
- 5 A Bio-Thesimeter (Bio-Medical Instrument Co., Ohio) was also used to test vibration sensitivity at the hallux following the procedure

Table 3
Regression results for peripheral neuropathy measures for all solvent exposures, after controlling for covariates Q1 is the mean value for the non-exposed group for all solvents exposure by inhalation, with or without dermal exposure, and its standard error. Results, Q2-Q4, the mean differences from Q1 with their 95% confidence intervals (CI), are in bold type face when the CI excludes the null value.

	N	All solvents exposure–Inhalation and dermal (mg/m ³ -yrs)				All solvents inhalation exposure only (mg/m ³ -yrs)			
		Q1 < 676	Q2 ^a 676 to < 1381	Q3 ^a 1381 to < 2491	Q4 ^a ≥ 2491	Q1 < 605	Q2 ^a 605 to < 1197	Q3 ^a 1197 to < 2069	Q4 ^a ≥ 2069
Symptom sum (0-4)	822	0.5 (0.07)	0.0 (-0.22, 0.18)	0.2 (0.00, 0.40)	0.3 (0.11, 0.53)	0.5 (0.07)	0.0 (-0.23, 0.17)	0.2 (-0.05, 0.36)	0.4 (0.15, 0.56)
Monofilament (g)	803	4.0 (2.30)	-1.0 (-7.41, 5.37)	0.9 (-5.58, 7.28)	6.5 (-0.03, 12.97)	3.9 (2.32)	-1.0 (-7.34, 5.43)	-0.6 (-7.08, 5.95)	8.0 (1.50, 14.60)
Bio-Thesimeter (volts)	740	16.7 (0.66)	0.2 (-1.66, 1.98)	0.6 (-1.21, 2.49)	0.5 (-1.41, 2.33)	16.7 (0.67)	0.3 (-1.51, 2.15)	0.3 (-1.61, 2.15)	0.7 (-1.20, 2.59)
Tuning fork (secs)	807	15.5 (0.37)	0.0 (-0.99, 1.05)	-0.3 (-1.34, 0.70)	-0.4 (-1.46, 0.61)	15.5 (0.37)	0.2 (-0.78, 1.26)	-0.4 (-1.43, 0.65)	-0.5 (-1.59, 0.50)
Ankle jerks (Y/N)	802	0.2 (0.03)	0.0 (-0.12, 0.03)	0.0 (-0.09, 0.06)	0.0 (-0.08, 0.06)	0.2 (0.03)	0.0 (-0.08, 0.06)	0.0 (-0.09, 0.06)	0.0 (-0.07, 0.08)
MNCV (m/sec)	370	44.8 (0.44)	-0.3 (-1.50, 0.93)	-1.0 (-2.28, 0.20)	0.6 (-0.63, 1.89)	44.7 (0.44)	-0.3 (-1.57, 0.88)	-1.0 (-2.29, 0.22)	0.7 (-0.55, 1.99)
SSPL (m/sec)	425	4.0 (0.04)	0.0 (-0.09, 0.13)	0.0 (-0.07, 0.16)	-0.1 (-0.18, 0.06)	4.0 (0.04)	0.0 (-0.10, 0.12)	0.0 (-0.07, 0.15)	-0.1 (-0.19, 0.05)
PCAI	720	-0.1 (0.13)	0.0 (-0.40, 0.31)	0.1 (-0.27, 0.45)	0.2 (-0.19, 0.54)	-0.1 (0.13)	0.0 (-0.36, 0.35)	0.1 (-0.30, 0.43)	0.2 (-0.14, 0.60)
PCA2	312	0.0 (0.20)	0.0 (-0.55, 0.58)	0.2 (-0.42, 0.72)	-0.2 (-0.76, 0.40)	0.1 (0.20)	-0.2 (-0.75, 0.38)	0.1 (-0.46, 0.68)	-0.2 (-0.81, 0.36)

N, Number of participants in the model.
 D, Direction of better performance (e.g., ↑ means a higher number implies better performance).
 Q1 Adjusted mean and SE values for the unexposed group.
 Q1-Q3 Exposure categories in mg/m³-years.
 MNCV: Peroneal motor nerve conduction velocity; SSPL: Sural sensory peak latency.

^a Values are means of differences from Q1, with 95% confidence intervals, adjusted for age, local union affiliation, race, diabetes, alcohol consumption, binge drinking, and education.

recommended by the manufacturer. The intensity of vibration at the threshold of detection was recorded on a 0 to 50 scale; lower values indicate higher sensitivity to vibration. The analysis used the average lowest perceived vibration from the two feet.

2.3. Solvent exposure assessment

Exposure to total solvents was calculated by combining task-based air sampling data with estimated amounts of solvent used during each task, taking into account the solvent application method, duration of task performance and quantity of solvent products used with self-reported tasks performed by each participant. Dermal exposure was calculated and converted to its inhalation-equivalent, in mg/m³. The full-shift time-weighted average (TWA) concentration was calculated by combining exposures from all of a participant's daily tasks with estimates of workplace background levels. Exposures were expressed as 8-h time-weighted averages in mg/m³, estimated for each month. Cumulative exposure, in mg/m³-years, was calculated by summing the monthly 8 h TWA across the period of working as an auto-technician and dividing by twelve.

Hexane was present only in aerosol cans. Exposure to hexane was calculated by applying the percent of hexane in a product, obtained from calendar year-specific material safety data sheets (MSDS) for the commercial products used in each workplace, to the total estimated solvent exposure from that product. Over three hundred MSDS for solvent products, containing composition information, were collected from manufacturers and online sources. The proportions of hexane that were the n-isomer were not stated, so all calculations are based on total hexane. Acetone exposure was not quantified, but was classified as either present or absent in any hexane formulation.

2.4. Statistical analysis

As well as examining the relationships between solvent exposure and the neuropathy measures for each of the tests separately, we used principal components analysis (PCA) to combine test results into composite variables. PCA reduces correlated test variables into fewer components. Since each test is intended to investigate essentially the same outcome, their results should be highly correlated. Two separate PCAs were carried out, but only the first component of each PCA is presented because we believe there is only a single underlying construct (i.e., peripheral neuropathy) in these data. The first PCA (PCA1) included results of 5 neuropathy measures (symptom score, Bio-Thesimeter, tuning fork, monofilament, and ankle reflexes), but not nerve conduction testing, and the second (PCA2) included all the tests in PCA1 plus the nerve conduction test measurements. PCA2 was calculated separately because the two nerve conduction measures (MNCV and SSPL) were collected from less than 40% of the participants. The mean value of a principal component is 0 and a positive value indicates a combined positive outcome from all the tests in a direction consistent with peripheral neuropathy. Linear regression analysis was used to examine the association between solvent exposures and the results of each of the physical tests, as well as the two PCA variables. The statistical analysis was performed using Stata 14 (StataCorp 2015 College Station, TX).

For simplicity and comprehensibility, we used a common set of covariates (age, union local, race/ethnicity, education, income, alcohol, diabetes) for all analyses. Age was categorized as shown in Table 1. Race was categorized as White, Black, Asian, Native American/Pacific Islander, and multi-race. Education was a categorical variable with 3 levels: high school only, some college, college degree. Income was an ordinal variable with 5 levels. There were two variables to describe alcohol use: consumption frequency (5 levels) and how often 6 or more drinks were consumed on one occasion (5 levels). Diabetes was a binary variable, for which a positive result was recorded for any participant who reported as having been diagnosed by a doctor with diabetes or

Table 4
Pearson correlation coefficients for the four hexane and solvent exposure categories used in this analysis.

	Hexane only	Hexane & acetone	All solvent inhalation	All solvent inhalation and dermal
Hexane only	1			
Hexane & acetone	0.442	1		
All solvent inhalation	0.227	0.251	1	
All Solvent inhalation and dermal	0.153	0.176	0.959	1

who had an HbA1c value of 6.5 or more. Categories are shown in Table 1.

3. Results

Study participation took place during 2009–2012. Using telephone and address information from electronic union records, we attempted to contact 4,186 potentially eligible auto-technicians. Of the 2,848 subjects contacted, 1,765 were verified as eligible to participate. Of these, 908 (52%) refused to participate. Those who declined participation were asked for a reason. The largest number stated a lack of interest ($n = 262$), others considered that they lived too far away to travel to the study site ($n = 132$), and others were too busy ($n = 129$). A few respondents were too ill to travel to our clinic ($n = 15$), and several ($n = 13$) repeatedly failed to appear for appointments. In 78 cases, a respondent (usually a family member) informed the researcher that the subject was not interested in participation and an additional 68 subjects declined by returning an invitation postcard sent to them. Seven respondents stated that the amount of compensation offered for participation was inadequate for the time and effort required.

A total of 831 motor vehicle technicians participated and provided usable data for the study. Nerve conduction investigation was conducted only in a subset of these participants (see Table 3), as this component was intended to confirm results of the primary metrics.

We divided participants into quartiles for cumulative exposure to total solvents, including hexane, and we categorized hexane exposures as either not exposed or above or below the median among those with exposures (three categories), and also separately for when acetone was present with the hexane. Table 1 shows the distribution of demographic factors according to quartiles of estimated cumulative solvent exposure and hexane exposure (regardless of whether it was combined with acetone).

Not unexpectedly, older ages were more frequent in the higher solvent exposure quartiles, both because they have more years of workplace exposure and because the use of solvents by automotive technicians has been decreasing over the past two decades; however, this pattern was not so evident in the hexane exposure groups, probably because eligibility required working as a technician during the years hexane was in use in automotive cleaning products (1989–2002), which is much less correlated with age or years as a technician. No other major differences were evident across the solvent and hexane categories.

The estimated mean 8-hr TWA hexane (n-hexane plus other hexanes) exposure for those study participants exposed to any hexane was 14 mg/m^3 (SD: 21 mg/m^3 , range: $0.1\text{--}201 \text{ mg/m}^3$), with a median of 7.7 mg/m^3 . Figures S1 and S2 in the Online Supplement show distributions, respectively, of inhalation exposure for any hexane and for hexane when combined with acetone.

Pearson's correlation coefficients were used to examine the correlation between the left and right feet for the physical tests. The correlation coefficients (r) between the right foot and the left foot for the ankle reflex, the tuning fork vibration, and the Bio-Thesimeter were all 0.83. The correlation coefficient for the filament test was 0.68. These high correlations indicate that, in general, nerve damage was not likely to be trauma-related.

Table 2 shows the results of tests and PCA variables across the

exposure categories for hexane, both for any hexane and for hexane with acetone. For PCA1 the first principal component explained 38% of the variance while the second was only 17%; for PCA2 the first component explained 33%, and the second, 16% of the variance. As expected, the second components explain less of the variance than the first and are not considered further in this publication. More information on the PCA variables is presented in the Online Supplement (Tables S1 and S2).

There is no evidence of exposure-related associations for any of the outcome categories. Both PCA1 and PCA2 were correlated with age and diabetes, both of which have well-established associations with neuropathy.

We also compared those with any hexane exposure to those with no hexane exposure, but there was no difference between the two categories (results not shown).

Table 3 shows similar results for solvent quartiles, based both on estimated inhalation exposure only and on estimated dermal and inhalation exposure combined. With both symptom sum and the monofilament test there is evidence of associations with all-solvents for the highest exposure quartiles.

Figures S3 and S4 in the Online Supplement show distributions, respectively, of inhalation exposure for all solvents and for combined inhalation and dermal exposure for all solvents.

Table 4 shows correlation coefficients for the four hexane and solvent categories examined in this analysis.

4. Discussion

To the best of our knowledge, this is the first epidemiologic study of peripheral neuropathy in relation to hexane and general solvent exposure in automotive technicians. For exposure to hexane, with or without acetone, the results of our analyses are generally reassuring, but, for all solvents, the symptom reporting and monofilament results provide evidence of associations with higher exposures. These findings are not supported, however, by the other test results, including those of the nerve conduction testing. Since no corresponding associations with symptoms and monofilament testing were found for hexane exposure, interpretation of these results is uncertain. In any case, they do not exclude the possibility that peripheral neuropathy could have been more clearly apparent in this population closer to the period of exposure to hexane—1989–2002, as testing occurred at least 7 years after hexane automotive cleaning products were removed from the California market and there is evidence of the reversibility of peripheral neuropathy with time (Chang, 1990; Kutlu et al., 2009; Misirli et al., 2008; Wang et al., 2014).

Our primary focus was on hexane, as the n-hexane isomer is a well-established cause of peripheral neuropathy in occupational groups (Chang et al., 1993; Kutlu et al., 2009; Misirli et al., 2008; Neghab et al., 2012; Puri et al., 2007), although we did not have data specific to n-hexane itself. Although there has been a case series report involving several automotive technicians (MMWR, 2001), they had not yet been studied epidemiologically. We took the opportunity at the same time to investigate associations with all solvents. In terms of solvent types, this study is complicated by the fact that many different solvents were used in the various automotive cleaning products. Some were used in spray cans, but others were used in solvent tanks (although solvent tanks had

generally changed to aqueous formulations by the 1990s). The wide variety of solvents used and the fact that there were significant gaps in the data reported, meant that, for other than hexane, it was impractical for us to attempt to carry out an analysis based on individual solvent types.

There are occupational exposure limits for n-hexane exposure: 500 ppm for the 8-hr TWA for the OSHA permissible exposure limit (PEL) (equivalent to 1760 mg/m³), 50 ppm (equivalent to 176 mg/m³) for the ACGIH TLV and Cal/OSHA, and 180 mg/m³ for the German MAK (Maximale Arbeitsplatz Konzentration). The estimated mean 8-hr TWA hexane (all isomers) exposure in our study was 14 mg/m³ (range: 0.1–201 mg/m³), indicating that most participants were exposed to n-hexane at concentrations an order of magnitude below commonly applied occupational exposure limits. Only 10 participants had estimated annual average TWA exposures to hexane that exceeded half the TLV, and only one of these appeared to have exceeded it.

Other possible explanations for why we found no positive associations involve confounding, selection bias and information bias. Negative confounding might explain the lack of evidence for an effect in our study, if there were an exposure that was correlated with lower n-hexane exposures and also associated with higher risk of peripheral neuropathy. While theoretically possible, it is difficult to conceive of a plausible scenario.

Since we attempted to recruit actively working, as well as former or retired, automotive technicians, this would have reduced some of the potential for the healthy worker effect to impact our results. However, even if follow-up extends beyond termination, the healthy worker survivor effect may operate if workers with higher levels of peripheral nerve dysfunction were more likely to terminate work—thereby reducing exposure. The healthy worker survivor effect, a problem of time-varying confounding and selection bias, causes downward bias toward the null and beyond (Eisen et al., 2012). Moreover, the low participation rate introduces another potential source of selection bias. If people who were disabled were more likely to participate, then this would possibly lead to an exaggerated measure of effect, if disabilities were associated with exposure. However, since we found no evidence of effect, our data do not support this possibility. Conversely, if people disabled by hexane exposure were less likely to participate, since they needed to be able to travel to our study clinic, then this could have dampened detection of any effect of solvents. Again, although this is theoretically possible, few (n = 15) reported poor health as a reason for not participating; we have no record of any being reported by family members as having been deceased, but that number is likely to have been small.

We used a broad battery of tests and think misclassification of the outcome, although it almost certainly occurred to some extent, is a lesser likelihood than misclassification of exposure. Exposure misclassification is quite likely, since we were relying on participants' memories of product use going back two or more decades. The overall impact of this misclassification would probably have been a bias towards the null. Such assumptions would mostly have affected estimates of hexane exposure and have had less impact on estimates of overall solvent exposure.

If our results have been influenced by a bias, we think the most likely explanations are misclassification of hexane exposure and selection bias. This will almost certainly have caused a bias towards the null of any true hexane-related effect, but we cannot say with any certainty whether such an effect actually occurred in our study population.

5. Conclusions

In conclusion, our results provide some reassurance about persistent peripheral neuropathy in automotive technicians who previously used hexane-containing automotive cleaning products. Possibly the lack of a

consistent effect observed in this study is attributable to low exposures in this occupational group. However, the possibilities that it might be attributable to peripheral nerve repair processes since hexane exposure ceased or to exposure misclassification or the healthy worker survivor effect cannot be discounted. In particular, there are studies showing that n-hexane-induced neuropathy does reverse, partially or completely, over time (Chang, 1990; Kutlu et al., 2009; Misirli et al., 2008; Wang et al., 2014). Much effort was expended to create detailed longitudinal solvent exposure profiles for each participant and it seems unlikely that exposure misclassification would have obscured a strong effect on peripheral nerve function. Overcoming this exposure misclassification would probably necessitate a prospective study, although the likelihood of this ever taking place is now slight, since hexane has not been used in automotive spray products for over a decade.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.neuro.2019.08.008>.

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