Title
Ambient geothermal hydrogen sulfide exposure and peripheral neuropathy

Authors
Pope, Karl
So, Yuen T
Crane, Julian
et al.

Publication Date
2017-05-01

DOI
10.1016/j.neuro.2017.02.006

Peer reviewed

HHS Public Access

Author manuscript

Neurotoxicology. Author manuscript; available in PMC 2018 May 01.

Published in final edited form as:

Neurotoxicology. 2017 May ; 60: 10–15. doi:10.1016/j.neuro.2017.02.006.

Ambient geothermal hydrogen sulfide exposure and peripheral neuropathy

Karl Pope[a], **Yuen T. So**[b], **Julian Crane**[c], and **Michael N. Bates**[a,*]

[a]Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA, USA [b]Department of Neurology, Stanford University, Palo Alto, CA 94305, USA [c]Department of Medicine, School of Medicine and Health Sciences, University of Otago, Wellington, New Zealand

Abstract

The mechanism of toxicity of hydrogen sulfide (H_2S) gas is thought mainly to operate through effects on the nervous system. The gas has high acute toxicity, but whether chronic exposure causes effects, including peripheral neuropathy, is yet unclear. The city of Rotorua, New Zealand, sits on an active geothermal field and the population has some of the highest measured ambient H_2S exposures. A previous study in Rotorua provided evidence that H_2S is associated with peripheral neuropathy. Using clinical methods, the present study sought to investigate and possibly confirm this association in the Rotorua population.

The study population comprised 1,635 adult residents of Rotorua, aged 18–65. Collected data relevant to the peripheral neuropathy investigation included symptoms, ankle stretch reflex, vibration sensitivity, as measured by the timed-tuning fork test and a Bio-Thesiometer (Bio-Medical Instrument Co., Ohio), and light touch sensitivity measured by monofilaments. An exposure metric, estimating time-weighted H_2S exposure across the last 30 years was used. Principal components analysis was used to combine data across the various indicators of possible peripheral neuropathy. The main data analysis used linear regression to examine associations between the peripheral nerve function indicators and H_2S exposure. None of the peripheral nerve function indicators were associated with H_2S exposure, providing no evidence that H_2S exposure at levels found in Rotorua is a cause of peripheral neuropathy. The earlier association between H_2S exposure and peripheral neuropathy diagnoses may be attributable to the ecological study design used. The possibility that H_2S exposure misclassification could account for the lack of association found cannot be entirely excluded.

*Corresponding author: School of Public Health, 50 University Hall, 2199 Addison St., University of California, Berkeley, CA 94720-7367, USA. Tel.:+1 510 504 5424. m_bates@berkeley.edu (M.N. Bates).

The authors declare they have no actual or potential competing financial interests.

Keywords

Cross-sectional Study; Geothermal; Hydrogen Sulfide; New Zealand; Peripheral Neuropathy

1. Introduction

Hydrogen sulfide (H_2S) is a toxic gas responsible for the second highest number of occupational gas-related deaths, after carbon monoxide(Bronstein et al., 2007). This gas is emitted from a number of natural and industrial sources, including geothermal areas, oil and gas fields and refineries, sewage treatment plants, and confined animal feeding operations (CAFOs or “factory farms”) (Lewis and Copley, 2015). It is also endogenously produced in humans and animals by gut bacteria and by the cells of some organs, where it has important physiological functions(Guidotti, 2015). H_2S has a “rotten egg” smell, with a detection threshold of 10 ppb or lower. As the acute exposure concentration increases, the gas becomes an irritant to the eyes and lungs. At concentrations of 150–200 ppm, it can paralyze the olfactory nerves so that it is no longer detectable by smell. Death from respiratory paralysis can occur at around 1,000 ppm(U.S. Environmental Protection Agency (EPA), 1978).

The mechanism of acute toxicity of H_2S appears to involve effects on the nervous system, raising the question of whether long-term low-level H_2S exposures may cause chronic neurological damage. Only a few studies have produced data that address this possibility. Of most relevance to the present study was an ecological epidemiology study in New Zealand that found a positive association between the estimated H_2S exposures where people lived and hospital discharge diagnoses for disorders of the peripheral nervous system (ICD-9 codes 350–359) (Bates et al., 2002). For persons categorized as living in “high”, “medium” and “low” H_2S exposure areas, the standardized incidence ratios were 2.59 (95% confidence interval: 1.91, 3.44), 1.94 (1.36, 2.67) and 1.76 (1.48, 2.09), respectively, relative to the rest of New Zealand. A study of chronic environmental H_2S exposure compared H_2S-exposed populations in Odessa, Texas, and Puna, Hawaii with a reference population drawn from unexposed communities in the two states. This study produced an overall odds ratio of 12.7 (7.59–22.00 95% CI) for self-reported central nervous system symptoms (Legator et al., 2001). However, interpretation of the results is limited by questions about the comparability of the reference communities, co-exposure to other emissions and litigation (Odessa) and an ongoing political controversy (Puna).

There is also some support from animal studies for the possibility of nervous system effects caused by H_2S. Chao et al. (2012) found evidence that H_2S interacted with Na^+ channels in mouse brains, possibly causing neuronal injury(Chao et al., 2012). A month-long exposure of rats to emissions from a gas field with a high content of H_2S was associated with demyelination of central nervous system axons(Solnyshkova, 2003). However, the natural gas emissions also contained hydrocarbons and mercaptans, which are highly associated with central and peripheral neurotoxicity(LoPachin and Gavin, 2015), casting doubt on any causal relationship with H_2S. In a review article, Lewis and Copley (2015), found that most studies that examined the effects of H_2S on the central nervous system involved self-reported

H_2S exposure and that few were likely to involve exposure to H_2S alone, making interpretation difficult(Lewis and Copley, 2015)

In this study, we examined indicators of peripheral neuropathy in a sample of the population of the city of Rotorua, New Zealand, continually exposed to ambient H_2S from vents in the geothermal field on which it is situated. There is considerable H_2S exposure variation across the city and some of the highest exposures occur in the central business district. From an epidemiologic investigation perspective, geothermal sources have the advantage over other ambient H_2S-producing entities of not being known to produce other gases with the potential to confound results. Other geothermal gases are mainly carbon dioxide and water vapor, with small amounts of hydrogen, nitrogen, methane and carbon monoxide (Horwell et al., 2004). Other possible air pollutants in Rotorua, such as vehicle emissions, are not known to cause peripheral neuropathy. Therefore they would not be likely to confound any relationship of peripheral neuropathy with geothermal emissions.

Study results for respiratory, cognitive and ocular outcomes have previously been published(Bates et al., 2013, 2015, 2016; Reed et al., 2014). The purpose of the study component covered by this publication was to investigate whether there is evidence of an association between long term exposure to H_2S and indicators of peripheral neuropathy, after controlling for potential confounding factors.

2. Methods

2.1 Ethics Statement

Prior Institutional Review Board approvals were obtained from the University of California, Berkeley, and from the Northern Ethics Committee in New Zealand. All subjects provided written informed consents before their participation.

2.2 Participant recruitment

This has previously been described in some detail (Bates et al., 2013, 2015; Reed et al., 2014). Briefly, a total of 1637 residents of Rotorua, aged 18–65, participated in the study during the period April, 2008, to December, 2010. An estimated 98% of the city's population is included in a centralized patient registry, which was used as the basis for selecting potential participants. The city was initially stratified into three H_2S-exposure level areas (high, medium, low), based on the Rotorua investigation of Horwell et al. (2004) (Horwell et al., 2004), and as previously used Bates et al. (2002)(Bates et al., 2002). Approximately equal numbers of participants were sought from residents in each of these 3 exposure level areas. This initial stratification was to ensure a good distribution of H_2S exposures, particularly to avoid a preponderance of low-exposure participants. However, since more accurate exposure data were collected during the study, the initial exposure stratification was not used in the data analysis. Participants were invited to the study clinic where a questionnaire was administered and various clinical tests were performed.

Ineligible for participation were persons not resident in Rotorua for at least the last 3 years, persons unable to speak and write English, anyone unable to visit the study clinic due to a

disability, and anyone who was blind. Women who reported they were pregnant were also excluded because of the use of mydriatics in another part of the study.

2.3 H_2S exposure estimation

H_2S exposure assessment has been described in detail elsewhere(Bates et al., 2015, 2013; Reed et al., 2014). Briefly, residential, workplace and school location histories were obtained by questionnaire and the locations geocoded. Subjects were also asked the number of hours spent at each location, and lengths of time spent away from Rotorua (vacations or temporary reassignments). Separately, three networks of passive H_2S passive samplers (Radiello, Sigma-Aldrich Co. LLC), were placed in various sites spaced across Rotorua for 2 week periods in 2010 and 2011. Most sampling sites were the same across the collection periods. Concentration surfaces were created for the Rotorua urban area using kriging. From the maps, average ambient H_2S concentrations were assigned to each participant-reported location. An average time-weighted H_2S exposure over the last 30 years was calculated for each participant by applying their time data to estimated concentrations at the geocoded locations. Study participants were categorized into quartiles of this exposure metric.

2.4 Neuropathy measures

Although nerve conduction study is often considered the “gold standard” for diagnosis of neuropathy, we did not utilize this procedure, because it was not feasible to obtain a trained technician to carry out the testing. The test requires technical expertise that was beyond the scope of our field study. Moreover, the discomfort associated with the procedure would likely have discouraged study participation. There is general consensus that an accurate clinical diagnosis of neuropathy can be made with relatively simple screening examinations(England et al., 2005). We chose 5 measures that have been validated as indicators of the possible presence of neuropathy. A single specially trained examiner carried out neuropathy screening for all subjects, to ensure consistency of the data collection. 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 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 to 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 of at night?
    - **iv.** Do you have areas of constant numbness in your lower legs or feet, occurring at rest or at night?
2. Ankle-stretch reflex was tested on both sides using a Queen Square hammer. 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 2.83 (0.07g), 3.61 (0.4g), 4.31 (2.0g), 4.56 (4.0g), 5.07 (10g), and 6.65 (300g). The lightest monofilament that the participant reported feeling was recorded; lower values equate to better sensation. For the analysis, the average lightest monofilament weight detected by the two feet was used.

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. For the analysis, the average time of detection by the two feet was used.

5. Finally, a Bio-Thesiometer (Bio-Medical Instrument Co., Ohio) was also used to test vibration sensitivity following the procedure recommended by the manufacturer. The intensity of vibration at the detection threshold was recorded on a 0 to 50 scale; lower values indicate higher sensitivity to vibration. For the analysis, the average lowest perceived vibration (on a voltage scale) from the two feet was used.

2.5 Statistical analysis

Age, ethnicity, education, and income were all analyzed as categorical variables. Age was categorized into 5 brackets (18–29, 30–39, 40–49, 50–59, and 60+ years). Ethnicity had 3 categories based on subjects' self-identified 1st choice: European, Maori, and "other". Education status was categorized as no secondary qualification, secondary qualification, trade qualification or certification, bachelor's degree, and postgraduate degree attainment. Income had 6 categories in 20,000 NZ Dollar steps and included a category of don't know or refused. Smoking status had 3 categories: never smoked, ex-smoker, and current smoker. Being diagnosed with diabetes and alcohol consumption, those who ever drank at least once a week, were binary variables.

As well as examining the relationships between exposure and results of each of the tests separately, we used principal components analysis (PCA) to combine test results into a single composite variable. The first principal component was selected for use in the data analysis, because the tests were designed to explain the presence of a single outcome, peripheral neuropathy. We refer to this as the neuropathy composite index score (NCIS). The mean value of a principal component is 0 and for the NCIS, a positive value indicates a combined positive outcome from all the tests in a direction consistent with peripheral neuropathy. The NCIS was correlated with both age and diabetes, both of which have well-established associations with neuropathy. Linear regression analysis was used to examine the association between H_2S exposure and results of the physical tests, as well as the NCIS. The statistical analysis was performed using Stata 14 (StataCorp 2015 College Station, TX).

3. Results

The participation rates have been previously described and discussed(Bates et al., 2015, 2013). In brief, a total of 3,522 eligible people were contacted and 1,927 (54.7%) agreed to participate, but only 1,639 (46.5%) actually participated due to field work duration constraints. An additional four were excluded from this analysis because of incomplete data collection.

The range of 30-year averaged exposure to H_2S was between 0 and 58 ppb, the median and average were 11 and 13 ppb respectively. The average age of the participants was 46.7 years, with a range of 18.5 to 65.8 years. Table 1 shows unadjusted test results across categories of study demographic variables and covariates. Providing some confirmation of data validity, the table shows poorer performance with increasing age, in the expected direction, for all 5 indicators of possible peripheral neuropathy. The other measures, being unadjusted by age, are less interpretable.

Pearson's correlation coefficients were used to examine the association 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-Thesiometer 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.

Separate bivariate analyses of individual variables with the outcome and the exposure showed that age, race, diabetes, and tobacco use were potential confounders of the relationship between H_2S exposure and peripheral neuropathy indicators. That analysis is partially reflected by the *p*-values. Gender was included in a full regression analysis with the other covariates as an exploratory variable.

4. Discussion

Our results provide no evidence of an association of any of the indicators of peripheral neuropathy with exposure to ambient H_2S over a period of up to 30 years. Possibly the strongest epidemiologic indication of an association between H_2S and peripheral neuropathy came from a previous ecological study in the same city—Rotorua–using New Zealand hospital discharge data, in which evidence for an exposure-related association was found (Bates et al., 2002).

It is necessary to consider whether uncontrolled confounding or selection or information biases could have been responsible for the apparent absence of effect. To negatively confound results, a causal factor for peripheral neuropathy would need to be inversely associated with H_2S exposure. It is difficult to imagine what this factor would be. The obvious possibility is other toxic exposures, such as the solvent n-hexane. For such an exposure to negatively confound a neurodegenerative effect of H_2S, the two exposures would need to be negatively correlated. It is possible to conceive of scenarios that might tend towards this. For example, people working in industrial settings with prolonged exposures to n-hexane might work predominantly in areas with low H_2S exposure levels. However, in the

absence of evidence to the contrary, it seems unlikely that any such other exposure, which would need to be common in the Rotorua population, exists.

The possibility of selection bias in the study population has been addressed in previous publications from this study(Bates et al., 2015, 2013; Reed et al., 2014). The participation rate was approximately 55%. It is hypothetically possible that those who did and those who did not participate were in some way systematically different and their participation was in a way that it could account for the lack of association with H_2S in this study. However, we previously found no indication of a differential participation with respect to H_2S exposure, suggesting that selection bias related to willingness to participate is unlikely to account for the lack of association in this study.

Information bias through misclassification of the exposure or outcome is a third major area of consideration. Ideally, we would have used nerve conduction velocity testing, as the ‘gold standard’ of peripheral neuropathy diagnosis, but this was not practicable for our study. Nonetheless, we found the expected associations between study outcomes and both increased age and having diabetes. This supports the validity of the study outcome measures as indicators of possible peripheral neuropathy and does not support a hypothesis that outcome misclassification is likely to account for the lack of H_2S effect.

We can be sure that the H_2S exposure measures used were imperfect and would have introduced some exposure misclassification. We calculated our H_2S exposure estimates on the basis of where and when participants lived, worked and went to school. It was not possible to account for all the complexities of individual movement patterns and our estimates were based on an assumption that H_2S emission sources stayed reasonably constant over the last 30 years. Errors and uncertainties in such classification would likely have been non-differential across the study population and would have tended to obscure any true association of H_2S exposure with peripheral neuropathy. However, previously we found evidence of plausible associations with respiratory measures,(Bates et al., 2015, 2013) suggesting that our exposure metrics are reflecting real exposures. So, while imperfect, they are much more detailed than the simple, essentially ecological measures of H_2S exposure used in the previous Rotorua study, based on hospital discharge data, that suggested an association with peripheral neuropathy(Bates et al., 2002). In that study, subjects were classified solely on the basis of living at the time of data collection in suburbs with “high”, “medium” or “low” ambient H_2S exposure levels. No account was taken of time spent at work, school, or living in other areas.

In conclusion, this study has found no evidence that ambient H_2S, at levels found in the city of Rotorua, are associated with peripheral neuropathy. The previously found association was likely a consequence of the limitations of the ecological study method employed(Bates et al., 2002). We cannot, however, entirely rule out the possibility that the lack of effect found in this study was the result of exposure misclassification or possibly some unknown negative confounding factor. Despite those caveats, the results are generally reassuring and provide no basis for concern.

Table 2 presents the linear regression analysis results for the 30-year average H_2S exposure quartiles on results for each of the assessment instruments and the neuropathy composite index score (NCIS). The adjusted mean values and confidence intervals around the mean outcome associated with the lowest exposure level (Q1) are shown. The differences in the means from the Q1 exposure level and their 95% confidence intervals are shown for exposure levels Q2–Q4 for all of the instruments and the NCIS. None of the 95% confidence intervals for quartiles Q2 to Q4 excluded the baseline, Q1, value.

Acknowledgments

This work was supported by the National Institute of Environmental Health Sciences of the National Institutes of Health (grant number R01ES014038). Support for the study was also provided by the New Zealand Regional Councils Environment Bay of Plenty and Environment Waikato. The authors thank Shane Iremonger (Environment Bay of Plenty) and Nick Kim (Environment Waikato), Dr. Phil Shoemack (Medical Officer of Health, Bay of Plenty District Health Board), and Dr. Nick Garrett (AUT, Auckland) for assistance. And thanks to Melinda Sando, Dr. Sandra Linenberger, Fiona Paignton, Kataraina George, Nerene Lynskey, John McKeogh and Dr. Peter Turner for data collection and management. Assistance with participant recruitment and data management was provided by the Rotorua Area Primary Health Service (RAPHS). Thanks to Kirsten Stone, David Bradford, Chris Walmsley, Jennifer Anastasi and Miranda Whitwell.

References

Bates MN, Bailey IL, Dimartino RB, Pope K, Crane J, Garrett N. Lens opacity and hydrogen sulfide in a New Zealand geothermal area. Optom Vis Sci. 2016

Bates MN, Crane J, Balmes JR, Garrett N. Investigation of hydrogen sulfide exposure and lung function, asthma and chronic obstructive pulmonary disease in a geothermal area of new zealand. PLoS One. 2015; 10:e0122062.doi: 10.1371/journal.pone.0122062 [PubMed: 25822819]

Bates MN, Garrett N, Crane J, Balmes JR. Associations of ambient hydrogen sulfide exposure with self-reported asthma and asthma symptoms. Environ Res. 2013; 122:81–87. DOI: 10.1016/j.envres.2013.02.002 [PubMed: 23453847]

Bates MN, Garrett N, Shoemack P. Investigation of Health Effects of Hydrogen Sulfide from a Geothermal Source. Arch Environ Health. 2002; 57:405–411. [PubMed: 12641180]

Bronstein AC, Spyker DA, Cantilena LR, Green J, Rumack BH, Heard SE. 2006 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS). Clin Toxicol (Phila). 2007; 45:815–917. DOI: 10.1080/15563650701754763 [PubMed: 18163234]

Chao D, He X, Yang Y, Balboni G, Salvadori S, Kim DH, Xia Y. Hydrogen sulfide induced disruption of Na+ homeostasis in the cortex. Toxicol Sci. 2012; 128:198–208. DOI: 10.1093/toxsci/kfs125 [PubMed: 22474073]

England JD, Gronseth GS, Franklin G, Miller RG, Asbury AK, Carter GT, Cohen JA, Fisher MA, Howard JF, Kinsella LJ, Latov N, Lewis RA, Low PA, Sumner AJ, American Academy of Neurology, American Association of Electrodiagnostic Medicine, American Academy of Physical Medicine and Rehabilitation. Distal symmetric polyneuropathy: a definition for clinical research: report of the American Academy of Neurology, the American Association of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation. Neurology. 2005; 64:199–207. DOI: 10.1212/01.WNL.0000149522.32823.EA [PubMed: 15668414]

Guidotti, TL. Handbook of Clinical Neurology. 1st. Elsevier BV; 2015. Hydrogen sulfide intoxication.

Horwell CJ, Allen AG, Mather TA, Patterson JE. Evaluation of a Novel Passive Sampling Technique for Monitoring Volcanogenic Hydrogen Sulfide. J Environ Monit. 2004; 6:630–635. [PubMed: 15237295]

Legator MS, Singleton CR, Morris DL, Philips DL. Health Effects from Chronic Low-Level Exposure to Hydrogen Sulfide. Arch Environ Health. 2001; 56:123–131. [PubMed: 11339675]

Lewis RJ, Copley GB. Chronic low-level hydrogen sulfide exposure and potential effects on human health: A review of the epidemiological evidence. Crit Rev Toxicol. 2015; 45:93–123. [PubMed: 25430508]

LoPachin RM, Gavin T. Toxic neuropathies: Mechanistic insights based on a chemical perspective. Neurosci Lett. 2015; 596:78–83. DOI: 10.1016/j.neulet.2014.08.054 [PubMed: 25218479]

Reed BR, Crane J, Garrett N, Woods DL, Bates MN. Chronic ambient hydrogen sulfide exposure and cognitive function. Neurotoxicol Teratol. 2014; 42:68–76. DOI: 10.1016/j.ntt.2014.02.002 [PubMed: 24548790]

Solnyshkova TG. Demyelination of Nerve Fibers in the Central Nervous System Caused by Chronic. 2003; 136:328–332.

U.S. Environmental Protection Agency (EPA). Hydrogen Sulfide. 1978

Highlights

- Largest epidemiology study of peripheral neuropathy and hydrogen sulfide exposure.
- The geothermal source has the advantage of no likely emitted confounding exposures.
- No association found between H_2S exposure and peripheral neuropathy.

Table 1

Means and standard deviations of test results by variable category.

Test (Range) Direction[a]	N (%)	Mean Score (SD)[†]					
		Symptoms (0 – 4) ↑	Ankle Reflex Test (0 – 2) ↑	Filament Test (1 – 6) ↑	Tuning Fork (0 – 35 sec) ↓	Bio-Thesiometer (0 – 50 volts) ↑	NCIS (−4.5 – 10.8) ↑
N	1,635	1,635	1,635	1628	1,635	1,635	1,627
Mean (SD)		0.13 (0.44)	0.18 (0.37)	2.24 (0.78)	16.08 (5.19)	13.09 (7.14)	0 (2.01)
H_2S exposure quartile							
Q1	411 (25%)	0.10 (0.39)	0.14 (0.33)	2.21 (0.77)	16.82 (5.21)	12.05 (6.65)	−0.32 (1.95)
Q2	406 (25%)	0.14 (0.50)	0.17 (0.36)	2.22 (0.79)	16.33 (5.01)	12.80 (7.14)	−0.10 (2.02)
Q3	408 (25%)	0.13 (0.41)	0.20 (0.38)	2.26 (0.71)	15.78 (4.89)	13.62 (6.64)	0.14 (1.83)
Q4	409 (25%)	0.13 (0.45)	0.22 (0.39)	2.29 (0.83)	15.37 (5.52)	13.89 (7.92)	0.27 (2.17)
p value[b]		*0.164*	*0.002*	*0.077*	*< 0.001*	*< 0.001*	*< 0.001*
Age							
18 to 29	168 (10%)	0.06 (0.34)	0.02 (0.14)	1.96 (0.74)	19.85 (3.60)	8.12 (4.33)	−1.54 (1.19)
30 to 39	305 (19%)	0.06 (0.30)	0.06 (0.23)	1.99 (0.63)	18.23 (4.03)	9.72 (3.87)	−1.06 (1.20)
40 to 49	452 (28%)	0.10 (0.37)	0.16 (0.35)	2.19 (0.70)	16.96 (4.68)	11.98 (5.62)	−0.32 (1.58)
50 to 59	470 (29%)	0.16 (0.50)	0.26 (0.41)	2.40 (0.84)	14.31 (4.91)	15.36 (7.54)	0.74 (2.07)
60+	239 (15%)	0.24 (0.60)	0.34 (0.46)	2.55 (0.80)	12.50 (5.40)	18.54 (8.43)	1.60 (2.18)
p value[b]		*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Race							
European	1304 (80%)	0.10 (0.39)	0.17 (0.36)	2.24 (0.75)	15.99 (5.28)	13.29 (7.25)	0.01 (2.01)
Maori	269 (16%)	0.23 (0.63)	0.26 (0.42)	2.28 (0.90)	16.21 (4.65)	12.27 (6.43)	0.00 (0.00)
Other	60 (4%)	0.10 (0.35)	0.11 (0.29)	2.11 (0.74)	17.48 (5.24)	12.46 (7.54)	0.00 (0.00)
p value[b]		*0.011*	*0.141*	*0.291*	*0.079*	*0.034*	*0.509*
Gender							
Female	979 (60%)	0.15 (0.48)	0.19 (0.37)	2.17 (0.70)	16.11 (4.88)	12.77 (6.55)	−0.07 (1.85)
Male	654 (40%)	0.09 (0.38)	0.18 (0.36)	2.35 (0.87)	16.03 (5.62)	13.58 (7.92)	0.11 (2.22)
p value[b]		*0.002*	*0.690*	*< 0.001*	*0.962*	*0.344*	*0.558*
Smoking Status							

Test (Range) Direction[a]	N (%)	Mean Score (SD)[†] Symptoms (0 – 4) ↑	Ankle Reflex Test (0 – 2) ↑	Filament Test (1 – 6) ↑	Tuning Fork (0 – 35 sec) ↓	Bio-Thesiometer (0 – 50 volts) ↑	NCIS (−4.5 – 10.8) ↑
Never-smoker	830 (51%)	0.11 (0.39)	0.17 (0.36)	2.19 (0.74)	16.31 (5.36)	13.15 (7.34)	−0.09 (2.00)
Ex-smoker	475 (29%)	0.12 (0.43)	0.20 (0.38)	2.30 (0.74)	15.34 (5.14)	13.68 (7.22)	0.23 (2.01)
Current smoker	329 (20%)	0.18 (0.56)	0.20 (0.39)	2.30 (0.91)	16.56 (4.69)	12.10 (6.37)	−0.09 (2.00)
p value[b]		*0.057*	*0.132*	*0.061*	*0.994*	*0.117*	*0.557*
Diabetes DX							
No	1563 (96%)	0.13 (0.47)	0.18 (0.37)	2.20 (0.79)	16.37 (5.12)	12.43 (6.94)	−0.08 (1.95)
Yes	71 (4%)	0.12 (0.43)	0.18 (0.37)	2.25 (0.76)	15.97 (5.21)	13.29 (7.12)	1.70 (2.53)
p value[b]		*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
Drink Alcohol							
No	420 (26%)	0.13 (0.47)	0.18 (0.37)	2.20 (0.79)	16.37 (5.12)	12.43 (6.94)	−0.14 (2.03)
Yes	1212 (74%)	0.12 (0.43)	0.18 (0.37)	2.25 (0.76)	15.97 (5.21)	13.29 (7.12)	0.04 (1.99)
p value[b]		*0.850*	*0.875*	*0.083*	*0.233*	*0.016*	*0.035*
Education							
No secondary qual.	213 (13%)	0.19 (0.58)	0.23 (0.41)	2.35 (0.88)	15.09 (4.82)	14.13 (7.79)	0.43 (2.17)
Secondary qual.	375 (23%)	0.13 (0.46)	0.18 (0.36)	2.25 (0.81)	16.41 (5.47)	12.68 (6.90)	−0.10 (2.02)
Trade qual. or cert.	623 (38%)	0.12 (0.40)	0.17 (0.36)	2.22 (0.75)	16.28 (5.03)	12.79 (6.52)	−0.10 (1.86)
Bachelor’s degree	275 (17%)	0.12 (0.45)	0.20 (0.38)	2.28 (0.75)	15.91 (5.36)	13.70 (8.26)	0.14 (2.25)
Postgrad degree	148 (9%)	0.07 (0.31)	0.16 (0.35)	2.07 (0.62)	16.13 (5.20)	12.81 (6.90)	−0.21 (1.76)
p value[b]		*0.018*	*0.134*	*0.038*	*0.131*	*0.392*	*0.040*
Income							
<=$20,000	357 (22%)	0.23 (0.65)	0.21 (0.39)	2.29 (0.89)	15.87 (5.53)	13.52 (8.10)	0.19 (2.35)
$20,001–40,000	472 (29%)	0.14 (0.44)	0.18 (0.37)	2.23 (0.78)	16.05 (5.14)	12.82 (6.60)	−0.04 (1.94)
$40,001–60,000	337 (21%)	0.07 (0.27)	0.15 (0.35)	2.24 (0.72)	16.28 (5.20)	13.08 (6.84)	−0.09 (1.81)
$60,001−80,000	227 (14%)	0.06 (0.31)	0.19 (0.37)	2.26 (0.71)	16.06 (4.95)	12.78 (7.06)	−0.04 (1.93)
>=$80,001	192 (12%)	0.08 (0.31)	0.19 (0.38)	2.18 (0.70)	16.01 (5.20)	13.52 (7.22)	0.01 (1.97)
DK/Refused	48 (3%)	0.13 (0.33)	0.15 (0.33)	2.20 (0.83)	16.78 (4.04)	12.44 (6.80)	−0.29 (1.72)
p value[b]		*0.018*	*0.365*	*0.596*	*0.321*	*0.840*	*0.360*

[a] Arrows indicate the direction of the tests’ outcomes for higher risk of neuropathy diagnosis

[b] *p*-values were calculated using a non-parametric trend test which is an extension of the Wilcoxon rank-sum test.

Table 2

Linear regression of 30-year average H_2S quartile exposures on each test and the neuropathy composite index score

	N[a]	Q1 0–5.6 ppb	Q2 5.6–10.6 ppb	Q3 10.6–18.4 ppb	Q4 18.4–57.9 ppb
Tests[b]		**Mean (95% CI)[c]**	**Difference from Q1[d] (95% Confidence Interval)**		
Symptoms	1,635	0.12	0.02	0.02	−0.01
(↑)		(0.08, 0.16)	(−0.04, 0.08)	(−0.04, 0.08)	(−0.07, 0.05)
Ankle Reflex	1,635	0.17	0.01	0.03	0.01
(↑)		(0.14, 0.21)	(−0.04, 0.05)	(−0.02, 0.07)	(−0.04, 0.06)
Filament Test	1,628	2.27	−0.03	−0.02	−0.05
(↑)		(2.20, 2.34)	(−0.14, 0.07)	(−0.12, 0.08)	(−0.15, 0.05)
Tuning Fork	1,635	16.20	−0.06	−0.27	−0.15
(↓)		(15.74, 16.65)	(−0.70, 0.58)	(−0.91, 0.38)	(−0.80, 0.50)
Bio-Thesiometer	1,635	12.92	0.16	0.46	0.06
(↑)		(12.30, 13.54)	(−0.71, 1.04)	(−0.41, 1.34)	(−0.83, 0.95)
NCIS	1,627	−0.03	0.02	0.12	0.00
(↑)		(−0.20, 0.13)	(−0.22, 0.25)	(−0.11, 0.36)	(−0.24, 0.24)

[a] N, number of participants in the model

[b] Arrows indicate the direction of the tests' outcomes for higher risk of neuropathy diagnosis

[c] Adjusted mean values (95% confidence interval)

[d] Table values are the mean (95% CI) of the difference between the estimated outcome mean for exposure quartile 1 and quartiles 2–4 in models adjusted for age categories, sex, ethnicity, diabetes status, and smoking status