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NeuroToxicology 26 (2005) 241–255

NeuroToxicology

Amalgam Exposure And Neurological Function

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Received 1 September 2004; accepted 24 September 2004

Abstract

Concerns regarding the safety of silver–mercury amalgam fillings continue to be raised in the absence of any direct evidence of harm. The widespread population exposure to amalgam mandated that a thorough investigation be conducted of its potential effects on the nervous system. The National Institute of Dental and Craniofacial Research and U.S. Air Force investigators collaborated in the ongoing Air Force Health Study (AFHS) of Vietnam era veterans. The primary study question involved adverse health effects associated with exposure to herbicides or dioxin. An assessment of exposure to dental amalgam fillings was added to the 1997–1998 health examination to investigate possible associations between amalgam exposure and neurological abnormalities. Our study population consisted of 1663 dentate AFHS participants, comprised of 986 AFHS controls and 677 Ranch Hand veterans who were exposed to dioxin in Vietnam. Two hundred and fifty-two of the participants had confirmed diabetes mellitus. Study outcomes included clinical neurological signs, vibrotactile thresholds, and summary variables for different levels of peripheral neuropathy. A limitation of our study is that our database did not include more sensitive continuous measures such as nerve conduction studies. No significant associations were found between amalgam exposure and clinical neurological signs of abnormal tremor, coordination, station or gait, strength, sensation, or muscle stretch reflexes or for any level of peripheral neuropathy among our study participants. A statistically significant association was detected between amalgam exposure and the continuous vibrotactile sensation response for the combined non-diabetic participants and separately for non-diabetic AFHS controls. No significant association in this measure was detectable for non-diabetic Ranch Hand veterans or among the combined diabetic participants. The association is a sub-clinical finding that was not associated with symptoms, clinically evident signs of neuropathy, or any functional impairment. Overall, we found no association between amalgam exposure and neurological signs or clinically evident peripheral neuropathy. Our findings do not support the hypothesis that exposure to amalgam produces adverse, clinically evident neurological effects.

Keywords: Amalgam exposure; Mercury; Neurological signs; Peripheral neuropathy; Epidemiology

INTRODUCTION

An estimated 190 million persons living in the U.S. have amalgam restorations and 70 million restorations are placed annually (ADA, 2002). Concerns have been raised regarding the safety of dental amalgams, due to the release of small amounts of mercury vapor. Eighty percent of inhaled mercury vapor enters the blood through the lungs. Most of this mercury is excreted (Cherian et al., 1978; Clarkson, 1989). However, some mercury is deposited in the brain (Nylander et al., 1987; Eggleston and Nylander, 1987) and other body tissues, most notably the kidney (Clarkson, 1992; Clarkson, 2002). Given the widespread exposure,

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doi:10.1016/j.neuro.2004.09.008

any possible health effects caused by dental amalgam are of great public health importance and mandate thorough study.

Estimates of total daily intake of mercury vapor from amalgams are highly variable, ranging from 5 to 9 μg/day (Mackert, 1997). Intake is dependent on both the number of amalgam fillings present and the total surface area of exposed amalgam (Clarkson, 2002). Mastication of certain foods and nicotine chewing gum also contribute to total intake of mercury vapor (Sallsten et al., 1996).

In general, no adverse clinical health effects have been associated with dental amalgams other than localized mucosal reactions in those relatively rare individuals who are allergic (Munksgaard, 1992; Ekstrand et al., 1998; Camisa et al., 1999). In the past decade, several studies showed no adverse health effects associated with amalgam fillings in general populations (Pedersen et al., 1991; Saxe et al., 1995; Bjorkman et al., 1996; Ahlqwist et al., 1999; Factor-Litvak et al., 2003). However, some studies of occupationally exposed dentists reported preclinical effects on symptoms, mood, and motor function for a group whose urinary mercury concentrations averaged as low as 36 μg mercury/l (Ngim et al., 1992; Echeverria et al., 1995; Echeverria et al., 1998; Langworth, 1997). In a review article on the toxicity of mercury and its associated clinical manifestations, it was concluded that currently there is no substantial evidence of adverse effects associated with amalgam fillings (Clarkson et al., 2003).

There are no current standards for safe levels of mercury exposure from dental amalgams. However, standards for occupational exposure to inorganic mercury are currently 50 $\mu g/m^3$ in air and 50 μg/g creatinine in urine (WHO, 1991). Occupational exposure to elemental mercury has been associated with a higher prevalence of accentuated postural tremor, impaired coordination, positive Romberg sign, and reduced distal sensation suggestive of a peripheral neuropathy among exposed workers (Neal and Jones, 1938; Andersen et al., 1993; Albers et al., 1982; Albers et al., 1988; Letz et al., 2000). Although these signs may persist after removal from exposure, the abnormalities are typically mild and in most cases cannot be classified as disease (Andersen et al., 1993).

The exposure profile investigated in this study is a low-dose, long-term exposure to mercury vapor from dental amalgams. The goal of this study was to investigate possible associations between amalgam exposure and the prevalence of neurological signs including impaired coordination, accentuated postural tremor, reflex and sensory signs, and quantitative vibration thresholds suggestive of clinical and sub-clinical peripheral neuropathy. We selected these outcome variables because they are known to be sensitive in identifying clinically relevant neurological dysfunction of the type associated with occupational exposure to elemental mercury (Albers et al., 1982; Albers et al., 1988). We also investigated possible associations between amalgam exposure and quantitative vibration thresholds in order to detect evidence of sub-clinical neuropathy.

MATERIALS AND METHODS

Study Design

The Air Force Health Study (AFHS) was designed to determine whether Vietnam veterans of Operation Ranch Hand demonstrated adverse health effects that could be attributed to exposure to herbicides or dioxin (Wolfe et al., 1990). In 1992, the National Institute of Dental and Craniofacial Research (NIDCR) and the Air Force added an oral health examination to the AFHS medical examination. This collaboration was motivated because peripheral neuropathy is one of the most important adverse neurological effects associated with elemental mercury exposure, and these variables were available in the AFHS database. The protocol and consent forms were approved by the institutional review boards of Scripps Clinic and Loma Linda Dental School. Over 95% of eligible 1997–1998 AFHS participants enrolled in the oral health study. They were compensated for their participation. The goal of the 1997–1998 NIDCR Amalgam Study was to determine whether any adverse neurological health effects were associated with amalgam exposure. Two groups of subjects were studied: Ranch Hand veterans who were exposed to Agent Orange during the Vietnam conflict and AFHS controls who served in southeast Asia in the same historic period but who had no exposure to Agent Orange.

MEDICAL HISTORIES AND PARTICIPANT SELECTION

Lifetime medical histories were obtained from each participant using a standardized questionnaire. Medical records were reviewed to detect medical conditions that potentially produce nervous system abnormalities (e.g., diabetes mellitus or alcohol-related disorder) or

precluded a complete evaluation (e.g., quadriplegia, paraplegia, limb injuries or amputations). A participant was defined as having diabetes based on a physician's diagnosis or a 2-h post-100 g oral glucose test result at any AFHS examination exceeding 200 mg/dl. Alcohol ingestion was derived from the AHFS interview and computed as the number of drink-years of alcohol consumption, where one drink-year was defined as the equivalent of 1.5 Oz of whisky (or equivalent), 5 Oz of wine, or 12 Oz of beer per day for 1 year. Dioxin exposure was based on documented serum dioxin levels (Wolfe et al., 1988).

NEUROLOGICAL EXAMINATION

All neurological examinations were performed by a board-certified neurologist to identify clinically evident signs using conventional and standardized techniques (DeJong, 1979; Mayo Clinic and Mayo Foundation, 1991). These techniques have known reliability and sensitivity for specific neurological conditions (Chimowitz et al., 1990; Syndulko et al., 1996; Siderowf et al., 2002), including peripheral neuropathy (American Diabetes Association, 1988; Dyck et al., 1991; Dyck et al., 1996). The evaluation of postural tremor and the clinical signs used to identify neuropathy also have been used to identify adverse effects of elemental mercury exposure, and the clinical thresholds for identifying neuropathy and abnormal postural tremor were found to be comparable to quantitative measures used to identify impaired function (Albers et al., 1988).

The neurological examination included evaluation of selected cranial nerve function (olfaction, visual fields, pupillary light response, extraocular movements, facial sensation, masseter and facial strength, and gag reflex); coordination (finger-to-nose, heel-to-shin); alternate motion rate (hand); presence of abnormal movement (postural and resting tremor); station (Romberg); gait; distal strength (wrist extension, ankle and toe dorsiflexion), proximal strength (arm abduction, hip flexion); sensation (light touch, pin-prick and joint position at the great toe, vibration at the ankle); muscle stretch reflexes (biceps brachii, triceps, knee, and ankle); and documentation of abnormal pyramidal tract signs (increased resistance to passive limb movement or abnormal Babinski response).

For many measures (e.g., reflexes), separate determinations were made for right and left sides and used to define unilateral and bilateral abnormalities. An abnormal unilateral response required deficits in either the right or left measure, while an abnormal bilateral measure required deficits in both right and left sides. Composite variables (such as peripheral neuropathy, described below) included bilateral responses that were combined to form a single response. A composite response was classified as "normal" if all component determinations were normal and "abnormal" otherwise.

QUANTITATIVE VIBROTACTILE THRESHOLD MEASUREMENTS

The Vibratron II® device was used to measure vibrotactile threshold on both the left and right great toes using the methods-of-limits protocol (Michalek et al., 2001). The Vibratron ® provides a non-invasive means of measuring the sensitivity to vibration, which reflects the integrity of sensory transduction, as well as neural conduction from periphery to cortex. Threshold values were first recorded in vibration units (VU) on a 0–23 VU scale, and then transformed to displacement in microns using the formula:

$$\text{displacement } (\mu\text{m}) = 0.55 \times \text{VU}^{2.02217}$$

derived by Letz and Grubbs (written personal communication). Displacement measurements were converted to natural logarithms. A value of 23 VU (i.e., highest value accurately measured by the Vibratron II®) was entered for those participants that could not detect vibration at any level tested. The left and right toes were analyzed separately. For each great toe, the average (in log microns) of four of seven trials was determined, after elimination of the first trial and the high and low reading of the other six trials. Abnormal bilateral responses to quantitative vibrotactile measures required both right and left toe values to exceed 4.02, the 90th percentile of the distribution of individual minimum vibrotactile responses within the AFHS control group.

OUTCOME MEASURES FOR PERIPHERAL NEUROPATHY

Participants with clinical evidence of *any peripheral abnormality* potentially consistent with a symmetrical stocking or stocking glove distribution sensory or sensorimotor peripheral neuropathy were identified. This variable was considered the most sensitive but least specific indicator of neuropathy. A more specific indicator of clinically evident sensory or sensorimotor peripheral neuropathy was defined using evidence of symmetric stocking distribution sensory loss among

tests of vibration sensation at the ankle, pinprick sensation at the great toe, and absent ankle reflexes. A diagnosis of *possible peripheral neuropathy* required a single bilateral abnormality among vibration sensation, pinprick sensation, or ankle reflexes. A diagnosis of *probable peripheral neuropathy* required a bilateral abnormality of two or three of the aforementioned three measures. A diagnosis of confirmed peripheral neuropathy required a diagnosis of probable peripheral neuropathy and a bilaterally abnormal quantitative vibrotactile threshold measurement. These measures are well accepted and their validity has been established in previous investigations of peripheral neuropathy (Diabetes Control and Complications Trials Research Group, 1993; Albers et al., 1999; Michalek et al., 2001). We investigated the sensitivity of the dichotomous neurological measures to detect peripheral neuropathy associated with diabetes by comparing diabetic and non-diabetic AFHS control participants.

AMALGAM EXPOSURE ASSESSMENT

Experienced dentists performed the oral health examinations. The examination consisted of a soft-tissue assessment, an evaluation of dental caries using the number of decayed, missing and filled surfaces index (Radike, 1972), and a complete enumeration of all restorative materials present, including 3rd molars. Restorative materials were grouped into five categories: amalgam, resins, porcelain/cement/temporary (P/C/T); gold, and other metals. A question on recent dental visits and one on the use of chewing gum were asked of each participant. Current use of chewing gum was scored as: never, occasionally, or regularly. Special training sessions were held to calibrate the dentists in scoring all dental restorative materials.

The primary measure of amalgam exposure used in this study was the total number of tooth surfaces with amalgam fillings (total number of amalgam surfaces; TNAS). A four-level variable based on the quartiles of the TNAS distribution was defined by 0–7, 8–14, 15–23, and 24–61 surfaces, respectively, and served as the primary exposure variable in our analytical models.

EXCLUSION CRITERIA

Participants who were edentulous, had physician diagnosed alcohol-related disorders (ICD 2912, 30300, 4255, 2918, 30301, 30390-30393, 30500-30503, 3575, 5710-5713), medical co-morbidity [paraplegia (ICD 3444, 3445), spinal cord injury (ICD 9072), amputation of the legs (ICD 8970, 8972, 8974)], or missing covariate information were excluded (Table 1).

GENERAL STATISTICAL ANALYSIS

The primary study population consisted of dentate AFHS controls and Ranch Hand veterans. We performed statistical analyses for each neurological measure and each summary variable using the TNAS and four-level amalgam exposure variable. Multivariate logistic regression models were fitted to each dichotomous neurological response as a function of amalgam exposure and relevant covariates. Covariates were initially incorporated as confounders and effect modifiers. All first-order interactions involving amalgam exposure were tested using $p < 0.10$. We performed separate parallel analyses for the sub-groups in which first-order interactions were detected.

Patterns among odds ratios (OR) based on the four-level amalgam exposure variable were investigated for a potential dose–response, and tests for trend in amalgam exposure were conducted for the TNAS exposure variable. Logistic regression models were also used to analyze the clinically defined vibrotactile sensation measures.

Multiple linear regression and analysis of variance models incorporating the same covariates were fitted to the continuous vibrotactile threshold measures using TNAS counts and the four-level amalgam exposure variables, respectively. These measures were also investigated for dose–response and linear trend. No adjustments for multiple tests were used for any of the statistical analyses.

COVARIATE SELECTION

All confounding variables available in the AFHS database that were known to be associated with neurological health responses and amalgam exposure, such as age, military occupation (used here as a surrogate for socio-economic status), exposure to dioxin, diabetic status, current smoking history, body mass index (BMI), current use of chewing gum, and history of alcoholic consumption were included in all models (Gerr et al., 1990; Letz et al., 2000). The number of permanent teeth of the participant was also included as a covariate in these models due to disparate frequencies across amalgam exposure quartiles. Important covari-

Table 1
Participant characteristics of dental study population

	Distribution of covariates by amalgam exposure level: amalgam exposure group[a]				
	0–7	8–14	15–23	24–61	Total
Initial dental cohort (*N*)	615	466	502	455	2038
Exclusions (*N*)					
Edentulous	127	0	0	0	127
Confirmed alcoholism	66	48	40	34	188
Medical exclusion[b]	1	3	2	5	11
Missing covariates	13	11	12	13	49
Study population (*N*)	408	404	448	403	1663
Following covariate distributions are for the study population (*N* = 1663)					
Dioxin (*N*)					
Ranch Hand veterans	149	164	191	173	677
AFHS controls	259	240	257	230	986
Diabetics (*N*)					
Ranch Hand veterans	24	33	32	16	105
AFHS controls	56	36	31	24	147
Age (y)					
Median	61	56	56	56	57
Interquartile range	52–65	51–64	51–63	52–62	51–64
Number of teeth (*N*)					
Median	24	26	27	27	26
Interquartile range	12–27	22–28	24–28	25–28	23–28
BMI (kg/m^2)					
Median	28.0	28.4	28.4	28.1	28.3
Interquartile range	25.7–30.7	25.7–31.5	25.4–30.8	25.7–30.7	25.6–30.9
Occupation (%)					
Officer	38.5	41.6	43.3	45.2	42.2
Enlisted flyer	17.1	13.6	14.1	15.6	15.1
Enlisted ground crew	44.4	44.8	42.6	39.2	42.8
Drink-years (%)					
Non-drinker	6.6	6.7	5.8	6.2	6.3
Low (1–40)	67.4	72.8	71.0	72.7	71.0
High (40+)	26.0	20.5	23.2	21.1	22.7
Current smoking pattern (cigarettes/day)					
90th percentile	20.0	20.0	15.0	15.0	20.0
Interquartile range	0–0	0–0	0–0	0–0	0–0
Gum chewing (%)					
None	66.9	61.6	60.3	60.0	62.2
Occasionally	26.7	31.0	32.3	31.3	30.3
Regularly	6.4	7.4	7.4	8.7	7.5

[a] Amalgam exposure is the total number of amalgam surfaces (TNAS).
[b] Quadriplegia, paraplegia, limb injuries, or amputations.

ates for specific dietary intake profiles such as foods requiring substantial masticatory activity were not available in the database.

All analyses modeled the neurological response as a function of amalgam exposure, adjusting for age, number of teeth, occupation (rank in service), dioxin exposure, current smoking history, body mass index, current use of chewing gum, and history of alcohol use as main effects separately for non-diabetic and diabetic participants.

MODEL SENSITIVITY

Given the sample size of the overall study population (n = 1663), the statistical power (sensitivity) ran-

ged between 65 and 85% to detect odds ratios of 2.5 to 3.0 for prevalence of abnormalities of 3% or more assuming a 1:1 ratio of high-to-low exposure groups. Several neurological response measures occurred too rarely to model. Statistical power for the continuous vibrotactile sensation measure was very high for the TNAS exposure variable. For example, using a type I error rate of alpha = 0.05, a residual variation estimate of 1.2 log microns, and the 11.1 standard deviation for the TNAS variable, a 0.010 slope parameter value would be detectable with 90% power among non-diabetics ($n = 1382$). However, a slope parameter of 0.040 would be needed to achieve 90% power in the diabetic sub-group ($n = 240$).

RESULTS

Participant Characteristics

The full NIDCR cohort consisted of 2038 participants of whom 1911 were dentate. One hundred and twenty-seven (6.2%) of the participants were edentulous. Among dentate participants, the distribution of TNAS ranged from 0 to 61 with a mean (s.d.) of 16.1 (11.2) and a median of 15 (inter-quartile range, 8-23). Ninety two (4.8%) dentate participants had no amalgam fillings. The number of decayed or filled surfaces (DFS scores) with dental caries for this cohort (ranged between 34.8 and 42.5) were higher than those reported for NHANES III (Winn et al., 1996), which ranged between 27.8 to 29.7 for the same male age cohort. However, the NHANES III survey did not include caries on 3rd molar teeth which were included in our study. Eighty four percent of the participants were non-smokers and 35% used chewing gum. The findings by amalgam quartiles are presented in Table 1.

One hundred and eighty-eight participants were excluded for alcohol related disorders, 11 for paraplegic and amputee status, and 49 for missing covariate values. The distribution of exclusions among the amalgam quartiles is presented in Table 1. The study population analyzed had 1663 participants comprised of 986 AFHS controls and 677 Ranch Hand veterans. There were 252 confirmed diabetic study participants. The non-diabetic and diabetic study participants were analyzed separately because significant first-order interactions were detected between dioxin and diabetic status and amalgam exposure for most neurological responses in models for the 1663 combined participants. The 1411 non-diabetic AFHS controls and Ranch Hand groups were analyzed together and separately to determine whether there were differences for these different risk subgroups.

Distributional properties of covariate distributions for the 1663 study participants by amalgam quartile exposure groups are presented in Table 1. The median age of study participants ranged from 56 to 61, the median number of teeth varied from 24 to 27, and the median BMI ranged from 28.0 to 28.4 across amalgam exposure quartiles. There were larger percentages of officers in the higher amalgam quartiles and larger percentages of high alcohol consumers in the lowest quartile. Thirty eight percent of the participants currently use chewing gum with roughly 8% reported as regular gum users. The differences in gum use across amalgam quartiles were very small.

Multivariate Models

The multivariate logistic models detected significant first-order interactions between amalgam exposure and dioxin or diabetic status for many neurological responses. Separate parallel analyses for dentate non-diabetic and diabetic participants resulted in models without first-order interactions. We present the findings for dentate non-diabetic and diabetic participants separately.

Prevalences of bilateral abnormalities for neurological signs (cranial nerves, motor, gait, station, sensory, muscle stretch reflexes) and outcome measures for peripheral neuropathy are presented in Tables 2 and 3 for non-diabetic and diabetic participants, respectively. The prevalence of sensory abnormalities, muscle stretch reflexes abnormalities and outcome measures for peripheral neuropathy responses were much larger in diabetic participants.

Adjusted odds ratios are presented in Figs. 1 and 2 for neurological signs and outcome measures among non-diabetic and diabetic participants, respectively, for responses having sufficient abnormalities to model. No significant associations were detected between neurological signs and any amalgam quartile exposure variables among non-diabetic or diabetic participants. Although the separate parallel multivariate models included covariates only as potential confounders, evidence of effect modification can be seen by comparing the exposure odds ratios for a specific response in non-diabetic and diabetic participants. For example, although the odds ratios were non-significant, the degree and directionality of odds ratios for several abnormal signs (knee and ankle reflexes) and outcome measures of peripheral neuropathy differed between non-diabetic and diabetic participants.

Table 2
Frequency of neurological signs and outcome measures for peripheral neuropathy by amalgam exposure quartiles[a]

	Study population: non-diabetics					
	n	Percent abnormal	Quartile			
			0–7	8–14	15–23	24–61
Signs						
Cranial nerves						
Any abnormality	1400	2.1	10	7	5	8
Motor (bilateral abnormality)						
Coordination						
Finger–nose–finger	1410	0.2	1	0	0	2
Heel–knee–shin	1411	0.4	3	1	0	1
Alternate motion rate	1411	0.4	2	0	2	1
Involuntary movement						
Postural tremor	1411	5.5	21	14	24	19
Resting tremor	1411	0.2	0	0	1	2
Proximal strength						
Arm abduction	1411	0.0	0	0	0	0
Hip flexion	1411	0.0	0	0	0	0
Distal strength						
Wrist extension	1410	0.1	0	1	0	0
Ankle/toe dorsiflexion	1411	0.2	1	1	0	1
Gait (tandem)	1411	3.8	16	10	14	13
Station (Romberg)	1411	0.4	2	0	2	2
Sensory (bilateral abnormality)						
Any sensory abnormality	1411	2.5	12	9	11	3
Light touch (toe)	1411	1.1	3	7	5	1
Pin-prick (toe)	1411	1.6	7	4	10	2
Vibration (ankle)	1411	1.4	7	3	6	3
Joint position (toe)	1411	0.3	1	1	2	0
Muscle stretch reflexes (absent bilaterally)						
Biceps brachii	1410	0.4	1	1	3	1
Triceps	1410	0.5	1	1	3	2
Knee	1411	1.5	6	3	4	8
Ankle	1410	9.8	48	28	32	30
Outcomes measures for peripheral neuropathy[b]						
Any symmetric abnormality	1411	11.2	50	34	39	35
Possible neuropathy	1411	10.8	50	33	37	33
Probable neuropathy	1411	1.5	10	2	7	2
Abnormal vibrotactile threshold	1395	8.5	33	33	25	28
Confirmed neuropathy	1411	0.7	3	2	3	2

[a] Amalgam exposure is the total number of amalgam surfaces (TNAS).
[b] Symmetrical stocking or stocking glove distribution.

No significant trends between any neurological signs and the TNAS exposure variable were detected, except for a positive trend in abnormal reflexes at the knees among non-diabetic participants. Abnormalities among tests of resting tremor, coordination, alternate motion rate, proximal and distal strength and station were too rare to model. No association was evident for symmetrical peripheral abnormalities or other aspects of peripheral neuropathy and amalgam exposure. Even though there were sufficient numbers of abnormalities among measures of gait, vibration (ankle), knee reflexes and the outcome measure *probable neuropathy* among the non-diabetic and diabetic sub-groups, many odds ratio estimates had very wide confidence intervals indicative of non-stable estimates, illustrated in Fig. 1 as truncated confidence intervals. No clinical effect of vibrotactile threshold deficit was evident by amalgam exposure, and no evidence of peripheral neuropathy

Table 3
Frequency of neurological signs and outcome measures for peripheral neuropathy by amalgam exposure quartiles[a]

	Study population: diabetics					
	n	Percent abnormal	Quartile			
			0–7	8–14	15–23	24–61
Signs						
Cranial nerves						
Any abnormality	250	4.8	3	1	4	4
Motor (bilateral abnormality)						
Coordination						
Finger-nose-finger	252	1.2	0	1	2	0
Heel-knee-shin	252	0.8	0	0	2	0
Alternate motion rate	252	0.8	0	0	0	2
Involuntary movement						
Postural tremor	252	4.0	1	7	1	1
Resting tremor	252	0.0	0	0	0	0
Proximal strength						
Arm abduction	252	0.0	0	0	0	0
Hip flexion	252	0.4	0	1	0	0
Distal strength						
Wrist extension	252	0.4	1	0	0	0
Ankle/toe dorsiflexion	252	1.2	1	2	0	0
Gait (tandem)	252	7.1	4	7	4	3
Station (Romberg)	252	2.0	1	1	3	0
Sensory (bilateral abnormality)						
Any sensory abnormality	252	13.5	9	14	8	3
Light touch (toe)	252	8.3	7	7	5	2
Pin-prick (toe)	252	13.5	9	14	8	3
Vibration (ankle)	252	5.6	4	7	3	0
Joint position (toe)	252	2.8	2	3	1	1
Muscle stretch reflexes (absent bilaterally)						
Biceps brachii	252	2.0	0	3	1	1
Triceps	252	3.2	2	3	2	1
Knee	251	7.2	6	6	4	2
Ankle	252	28.2	25	23	13	10
Outcomes measures for peripheral neuropathy[b]						
Any symmetric abnormality	252	33.7	29	29	15	12
Possible neuropathy	252	32.9	28	28	15	12
Probable neuropathy	252	9.5	6	11	6	1
Abnormal vibrotactile threshold	241	13.3	17	10	9	8
Confirmed neuropathy	252	3.2	2	4	2	0

[a] Amalgam exposure is the total number of amalgam surfaces (TNAS).
[b] Symmetrical stocking or stocking glove distribution.

associated with amalgam exposure was detected. No individual responses had consistent, significantly elevated odds ratios across amalgam exposure quartiles.

In separate analyses for non-diabetic AFHS controls and Ranch Hand veterans (not presented here) we found 4 observed odds ratios in directional disagreement and 14 in agreement among the 6 clinical conditions that were testable in each subgroup. None of the four directional disagreement odds ratios was substantially different to produce a significant interaction in the corresponding model.

The median of the maximum adjusted generalized coefficient of determination (R^2) for the logistic models was 0.09 and ranged from 0.04 to 0.29. These R^2 values attempt to mimic the R^2 values obtained in multiple linear regression models.

The findings for the continuous version of the vibrotactile measurements are presented in Table 4

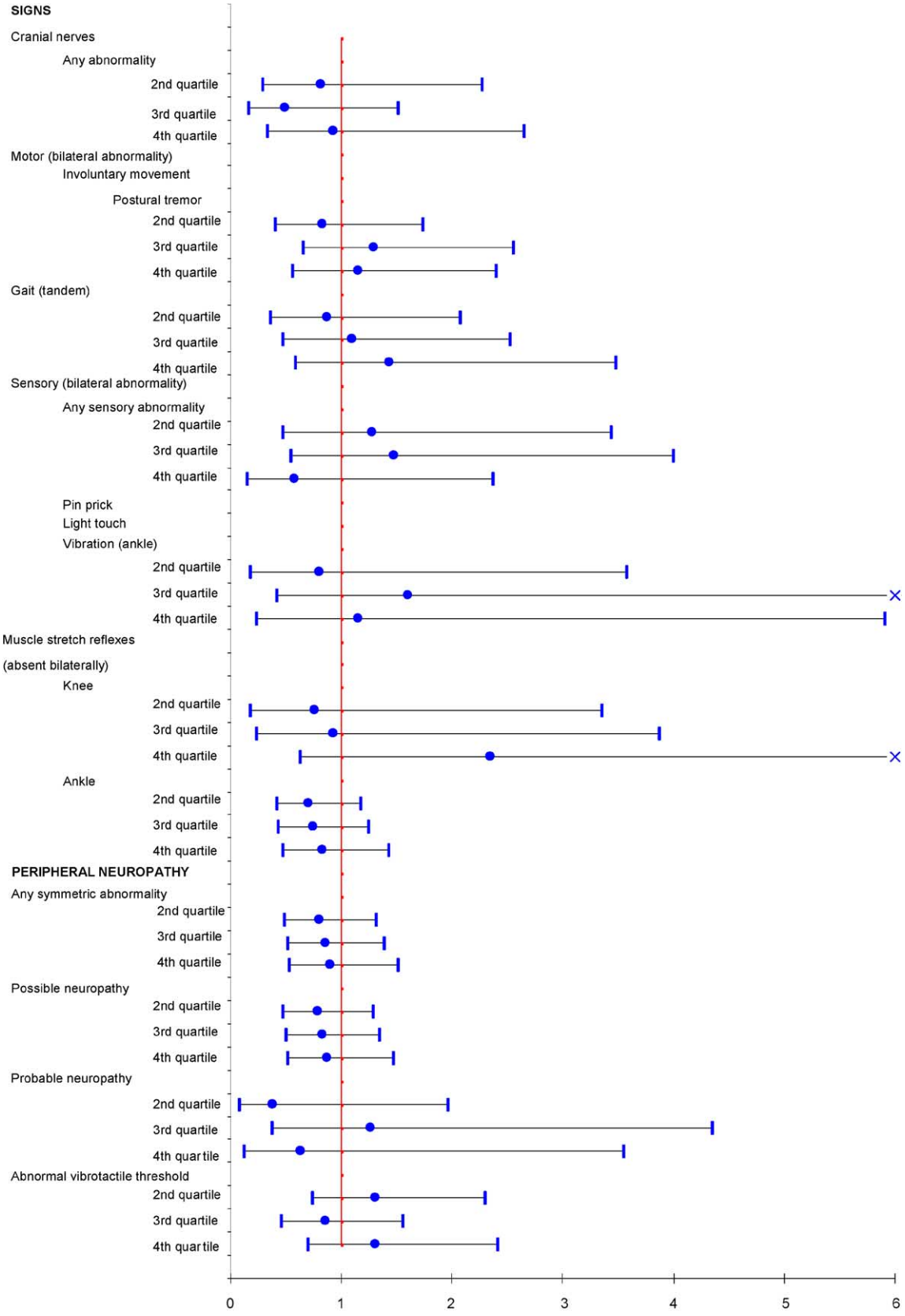

Fig. 1. Adjusted* odds ratios and associated 95% confidence intervals for neurological signs and outcome measures of peripheral neuropathy associated with amalgam exposure quartiles relative to 1st quartile among non-diabetic participants. (|-------|) (95% CIs for adjusted odds ratios*). (●) Adjusted odds ratio. (*) Adjusted for age, number of teeth, current smoking, occupation, body mass index, use of chewing gum and history of alcohol use. (×) Indicates a truncated upper limit to the confidence interval.

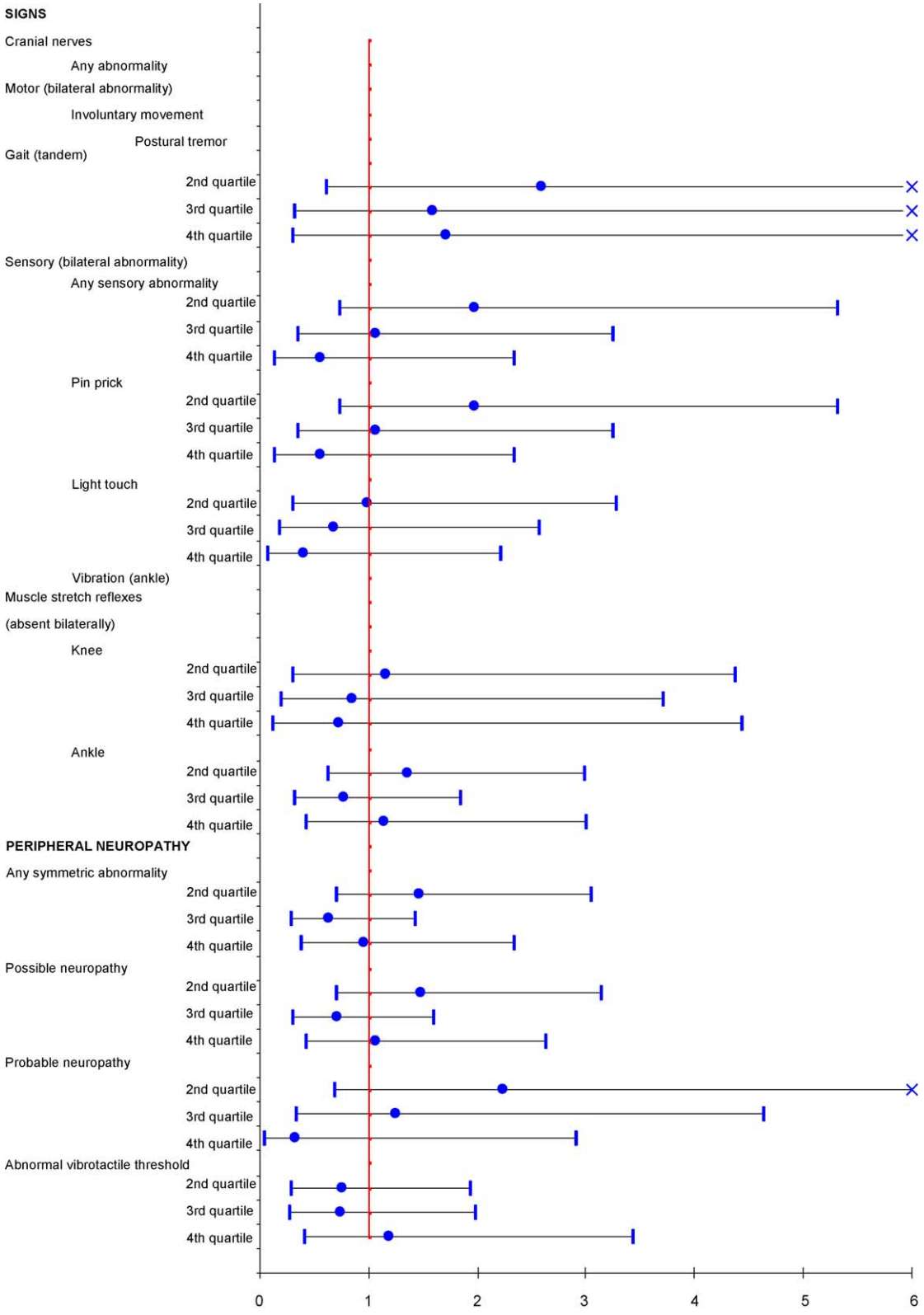

Fig. 2. Adjusted* odds ratios and associated 95% confidence intervals for neurological signs and outcome measures of peripheral neuropathy associated with amalgam exposure quartiles relative to 1st quartile among diabetic participants. (|-------|) (95% CIs for adjusted odds ratios*). (●) Adjusted odds ratios. (*) Adjusted for age, number of teeth, current smoking, occupation, body mass index, use of chewing gum and history of alcohol use. (×) Indicates a truncated upper limit to the confidence interval.

Table 4
Continuous vibrotactile thresholds by amalgam exposure quartiles[a]

TNAS (exposure quartile)	Study population ($N = 1622$)				Linear regression test for trend	
	N	Adjusted mean	95% CI	p-value	Beta	p-value
Non-diabetic participants ($N = 1382$)						
0–7	316	2.06	–		0.009[b]	0.005
8–14	332	2.25	[−0.01, 0.37][c]	0.053		
15–23	379	2.10	[−0.03, 0.35]	0.094		
24–61	355	2.31[d]	[0.05, 0.44]	0.012		
Non-diabetic controls ($N = 828$)						
0–7	198	1.96	–		0.011[e]	0.004
8–14	203	2.25[d]	[0.07, 0.53][c]	0.011		
15–23	225	2.13	[−0.05, 0.41]	0.129		
24–61	202	2.38[d]	[0.18, 0.66]	0.001		
Non-diabetic Ranch Hand veterans ($N = 554$)						
0–7	118	2.23	–		0.005[e]	0.353
8–14	129	2.23	[−0.32, 0.33][c]	0.985		
15–23	154	2.35	[−0.20, 0.43]	0.482		
24–61	153	2.22	[−0.31, 0.35]	0.916		
Diabetics ($N = 240$)						
0–7	76	2.38	–		0.016[f]	0.088
8–14	63	2.55	[−0.31, 0.63][c]	0.503		
15–23	62	2.84	[−0.03, 0.94]	0.068		
24–61	39	2.77	[−0.17, 0.94]	0.173		

Abnormal vibrotactile measure >4.02.

[a] Amalgam exposure = total number of amalgam surfaces (TNAS).

[b] Beta coefficient for TNAS exposure in model including age, number of teeth, dioxin, current smoking, occupation, BMI, gum chewing, and history of alcohol use.

[c] Confidence interval for the increases (decreases) in average score compared to the reference quartile mean.

[d] Significantly different than reference quartile mean.

[e] Beta coefficient for TNAS exposure in model including age, number of teeth, current smoking, occupation, BMI, gum chewing, history of alcohol use.

[f] Beta coefficient for TNAS exposure in model including age, number of teeth, dioxin, current smoking, occupation, BMI, gum chewing, history of alcohol use.

for the non-diabetic and diabetic study population. Findings for non-diabetic AFHS controls and Ranch Hand veterans are also given in Table 4 separately. A statistically significant association (slope parameter beta = 0.009, $p = 0.005$) was found between the continuous vibrotactile measures and the TNAS exposure variable in the multiple linear regression model for non-diabetic study participants, after adjusting for covariates. The multiple regression model explained 15% of the variation in vibrotactile sensation measures. An overall average vibrotactile score of 2.21 (s.d. = 1.17) was obtained by an analysis of variance model with adjusted vibrotactile sensation group means ranging between 2.06 and 2.31 across amalgam exposure quartiles. The 0.25 log μm difference was statistically significant (CI = 0.05, 0.44, $p = 0.012$). No significant linear trend among odds ratios for diabetic participants could be detected (slope parameter beta = 0.016, $p = 0.088$). The overall average vibrotactile sensation scores in the ANOVA model were 2.61 (s.d. = 1.32) for diabetic participants. The adjusted vibrotactile sensation means ranged between 2.38 and 2.84 ($p = 0.068$) for diabetic participants across the amalgam exposure quartiles.

The results for non-diabetic AFHS controls and Ranch Hand veterans also are presented in Table 4. The test for linear trend among odds ratios for non-diabetic AFHS controls was significant (slope parameter beta = 0.011, $p = 0.004$), but no linear trend among corresponding odds ratios could be detected among non-diabetic Ranch Hand veterans (slope parameter beta = 0.005, $p = 0.353$). The overall average vibrotactile sensation scores in the ANOVA model were 2.18 (s.d. = 1.11) for AFHS control participants and 2.26 (s.d. = 1.25) for the Ranch Hand veterans. The adjusted vibrotactile sensation means ranged between 1.96 and 2.38 ($p = 0.001$) for the control participants and 2.22 and 2.35 ($p = 0.482$) for the Ranch Hand veterans across the amalgam exposure quartiles. The associated confidence intervals for these differences are given in Table 4.

Comparison of participants with and without diabetes mellitus among all AFHS controls ($n = 986$) for the dichotomous neuropathy measures showed significant evidence of impairment among diabetic participants for measures of gait (OR = 2.6, CI = 1.2, 5.6), light touch (toe) (OR = 5.4, CI = 1.8, 16.4), pinprick (toe) (OR = 9.8. CI = 4.1, 23.5), triceps reflexes (OR = 8.5, CI = 2.3, 31.0), knee reflexes (OR = 5.2, CI = 2.2, 12.7), ankle reflexes (OR = 2.4, CI = 1.5, 3.8), and all peripheral neuropathy outcome measures; possible neuropathy (OR = 3.0, CI = 1.8, 4.3), probable neuropathy (OR = 6.0, CI = 2.1, 16.9) and abnormal vibrotactile sensation (OR = 2.1, CI = 1.3, 3.6).

DISCUSSION

Overall we found no detectable associations among non-diabetic or diabetic study populations between amalgam exposure and either clinically evident signs of neuropathy using conventional clinical measures or quantitative vibrotactile threshold testing in separate parallel analyses. Analyses for the combined diabetic and non-diabetic participants produced several first-order interactions. For this reason, we presented our findings for the non-diabetic and diabetic sub-groups separately, including responses in which significant interaction was not detected. Partial evidence for these interactions can be seen by a comparison of corresponding odds ratios presented in Figs. 1 and 2. There were 10 out of 24 odds ratios common to diabetic participants and non-diabetic participants in directional disagreement as well as several large differences in degree for those odds ratios that were in directional agreement. The significant positive trend in odds ratios for abnormal knee reflexes among non-diabetic participants was accompanied by a non-significant negative trend among diabetics (Figs. 1 and 2). The clinical significance of this trend is unclear, as a bilateral abnormality of the ankle reflexes is the more sensitive and specific indicator of neuropathy, and a similar trend was not observed for this clinical sign.

We also detected no significant associations within either non-diabetic AFHS controls or Ranch Hand participants when analyzed separately. For most of the neurological signs, the odds ratios and patterns among odds ratios for the non-diabetic AFHS controls were similar to those presented in Fig. 1 for the effects we were able to model. In 4 of 18 instances, the odds ratios were in directional disagreement, but differences were too small to be detected as a first-order interaction between dioxin and amalgam exposure.

Our negative findings complement those reported in other studies (Pedersen et al., 1991; Saxe et al., 1995; Bjorkman et al., 1996; Ahlqwist et al., 1999; Factor-Litvak et al., 2003). A few studies among dentists occupationally exposed to amalgam have suggested sub-clinical neurobehavioral effects (Ngim et al., 1992; Echeverria et al., 1995; Echeverria et al., 1998). Comparable sub-clinical measures were not available in this study.

The statistically significant trends among the non-diabetic study population and non-diabetic AFHS controls for the continuous vibrotactile measure of sensation and amalgam exposure is a sub-clinical finding that was not associated with symptoms, clinically evident signs of neuropathy, or any functional impairment. The trend in the continuous vibrotactile sensation measure among amalgam exposure quartiles was not statistically significant for diabetic participants or for the non-diabetic Ranch Hand veteran subgroup. The lack of a consistent association complicates the interpretation of these findings. The estimated magnitude of the increase in log micron displacement per surface exposure increase among diabetic participants was larger than that for non-diabetic participants, but the lack of significance is likely due to the small number of diabetic participants. However, we feel the parameter estimate obtained for the AFHS control subgroup is the most accurate estimate for the continuous vibrotactile sensation measure because it is based on the least potentially confounded subset of participants.

Our study had several strengths and limitations. We had access to a large study population of 1663 middle-aged males who had a high level of dental care, substantial amalgam fillings and neurological evaluations performed which included measures of postural tremor, measures of peripheral neuropathy and vibration detection threshold. We speculate that our study population represents a relatively high amalgam exposure group because caries scores observed in this study population were somewhat higher than those for the similar aged U.S. male population from NHANES III (Winn et al., 1996). Thus we speculate that the number of amalgam fillings, if anything, is slightly higher than the corresponding national average for males.

The dichotomous measures of neuropathy used in our study were sensitive enough to detect neuropathy among diabetic participants compared with non-diabetic participants. They also were sufficiently sensitive to document neuropathy associated with dioxin exposure for this study population (Michalek et al., 2001). Thus, we speculate that any effect of exposure to

amalgam, if present, must be smaller than those shown for either diabetes status or dioxin exposure.

The TNAS exposure variable we used represents a compromise between the total number of teeth having an amalgam filling and the total tooth surface area covered by an amalgam filling. The best surrogate exposure variable is believed to be represented by either the total volume of amalgam placed or the surface area covered with amalgam. There is no consensus as to which of the two measures is the more relevant for mercury vapor from amalgam fillings. We believe the TNAS score is as accurate as a surrogate measure of amalgam exposure can be that is available from a cross-sectional epidemiological study without dental radiographs. Dentists performing these evaluations were well-trained and adequately calibrated in scoring amalgam exposure at the surface level.

We stress that the results reported here are based on cross-sectional analyses. However, a significant association between urinary mercury concentrations and the number of amalgam fillings was demonstrated in a subset of study participants who had urinary mercury assays performed (Kingman et al., 1998) suggesting that measurable absorption of mercury vapor from amalgams occurs in adults.

Our initial intent was to use the TNAS-years as a longitudinally based amalgam exposure variable for each participant. However, we were unable to calculate this exposure variable due to a lack of information, even after extensive efforts to obtain archived military and civilian dental records. Undoubtedly some exposure misclassification exists due to currently missing teeth that previously had amalgam fillings. Although the extent of this misclassification remains unknown, it undoubtedly produced a shift to the left in the amalgam exposure distribution, which most likely would attenuate the results towards the null. However, the magnitude and degree of misclassification will be a function of the pattern of shift. Another aspect of missing teeth patterns relates to when they were lost or extracted. Teeth lost several years previously would not have as strong an impact on the participant's amalgam exposure as those lost more recently. The net effect of amalgam exposure misclassification due to missing teeth will likely inflate the Type II error, which effectively results in a loss of statistical power.

In spite of our large study population, we were unable to successfully model rare neurological conditions. Additionally, less than 5% of the study population was African American and no females were included. Thus no information for females and African Americans is available from this study.

It would have been beneficial to have access to inherently more sensitive continuous measures of neuropathy such as nerve conduction studies to confirm or refute the internal validity of the findings for the vibrotactile sensation measure, but such measures were not available in the AFHS database. We feel that further investigations are needed, preferably prospective in nature. Two long-term clinical trials are currently underway to assess the effect of amalgam exposure on neurological function.

ACKNOWLEDGEMENTS

The authors would like to thank Drs. Michael Duggan, Lynn Martin and Matty Abbate; Loma Linda University School of Dentistry, and Dr. John Reinhardt, University of Nebraska Dental School for their expertise and participation in the oral health component of this study.

DISCLOSURES

Dr. Albers has at times been retained as a consultant or served as an expert witness in litigation for firms or companies, concerned with the manufacture or use of amalgam or elemental mercury. Support of these activities has included both personal and institutional remuneration.

Dr. Garabrant has at times served as an expert witness in litigation for firms concerned with industrial use of elemental mercury and its compounds.

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