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Behavioral Effects of Low-Level Exposure to Hg° Among Dentists

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ECHEVERRIA, D., N. J. HEYER, M. D. MARTIN, C. A. NALEWAY, J. S. WOODS AND A. BITTNER, JR. *Behavioral effects of low-level exposure to Hg° among dentists.* NEUROTOXICOL TERATOL 17(2) 161-168, 1995. — Exposure thresholds for health effects associated with elemental mercury (Hg°) exposure were examined by comparing behavioral test scores of 19 exposed (mean urinary Hg = 36 μg/l) with those of 20 unexposed dentists. Thirty-six μg Hg/l is 7 times greater than the 5 μg Hg/l mean level measured in a national sample of dentists. To improve the distinction between recent and cumulative effects, the study also evaluated porphyrin concentrations in urine, which are correlated with renal Hg content (a measure of cumulative body burden). Subjects provided an on-site spot urine sample, were administered a 1-h assessment consisting of a consent form, the Profile of Mood Scales, a symptom and medical questionnaire, and 6 behavioral tests: digit-span, symbol-digit substitution, simple reaction time, the ability to switch between tasks, vocabulary, and the One Hole Test. Multivariate regression techniques were used to evaluate dose-effects controlling for the effects of age, race, gender and alcohol consumption. A dose-effect was considered statistically significant below a p value of 0.05. Significant urinary Hg dose-effects were found for poor mental concentration, emotional lability, somatosensory irritation, and mood scores. Individual tests evaluating cognitive and motor function changed in the expected directions but were not significantly associated with urinary Hg. However, the pooled sum of rank scores for combinations of tests within domains were significantly associated with urinary Hg, providing evidence of subtle preclinical changes in behavior associated with Hg exposure. Coproporphyrin, one of three urinary porphyrins altered by mercury exposure, was significantly associated with deficits in digit span and simple reaction time. The prophyrin pooled sums of rank scores were as sensitive as the urinary Hg analyses within the cognitive and motor domains but were less sensitive for the overall battery of tests. The reported effects were detected among dentists with a mean urinary Hg level of 36 μg/l, which lies between the proposed biologic thresholds of 25 and 50 μg Hg/creatinine, suggesting the need for a more comprehensive study to determine the threshold of adverse biologic effects.

Elemental mercury   Dental exposure   Behavior   Central nervous system

THE OCCUPATIONAL effects of elemental mercury (Hg°) exposure on human behavior and mood have been recognized as early as the 1860s (22), as a clinical disorder among felters who made hats ("mad as a hatter"). Recently, the effects of mercury at much lower levels have been reevaluated. Information indicating that background mercury levels in the general population are associated with seafood intake, environmental exposure, and the number of dental fillings containing mercury (13) has given this work greater importance and made determination of mercury exposure thresholds for adverse behavior, mood, and symptoms a significant public health question. This question is best answered by evaluating performance among an occupationally exposed group whose exposures range from equivalent to significantly greater than that of the general population (20). In 1991, mean urinary levels of mercury among a national sample of dentists was 5.0 μgs/l, which is only 2 to 3 μgs/l greater than background levels attributable to dental amalgam exposure and the con-

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sumption of seafood. The current study is an evaluation among dentists of sensitive measures of effect (symptoms, mood, and behavior) and measures of exposure (Hg and porphyrins in urine), in an effort to identify if potential adverse effects are associated with urinary mercury levels below 50 μgs/l.

RESULTS AMONG CHLORALKALI AND FLUORESCENT LAMP WORKERS

Behavioral deficits have been well-characterized among industrial workers chronically exposed to mercury with urinary levels above 100 μgs/l (14,15,19,30,31). These studies present a pattern generally consistent with the signs and symptoms characteristic of classic cases of mercurialism. Typically, mild deficits were reported for measures of mood, short-term memory (15,30), tremor (19), and motor function such as manual dexterity (8).

More variable results have been reported for exposures related to urine mercury levels between 40–100 μgs/l conducted in China and Europe (25,32,39). In 1980, electromyographic disturbances were reported among workers with mean urinary mercury levels of 93.4 μgs/l (39). In 1984, deficits in short-term memory and deficits on the WAIS verbal concept "Similarities" subtest were reported among workers with a mean urinary mercury level of 58 μgs/l (25). In 1990, fluorescent lamp workers were reevaluated and deficits in short-term memory, mood, and symptoms were associated with urinary mercury levels averaging 27.7 μgs/l, over 9 years of exposure (32).

More recently, a 1993 Chinese occupational health study (16) evaluated behavior at a mean 24-h urinary mercury level of 24 μgs/l, corresponding to 0.033 mg/m^3 in air. In this study, performance among 88 fluorescent lamp workers was compared to performance among 70 controls recruited from an embroidery factory. Whereas adverse effects were found on mental arithmetic, 2-digit search, switching attention, visual choice reaction time (CRT), and finger tapping, the study results may be subject to considerable confounding by variation between the two exposure groups in education, alcohol consumption, and other factors that were not controlled for analytically.

RESULTS AMONG DENTISTS

Dentists provide an excellent population for the study of threshold effects of mercury, as they span the range of exposure to be evaluated and are more homogenous than most exposed populations with respect to manual dexterity, education, training, and test-taking ability. Thus, as a study population, dentists amplify our ability to detect potential subtle preclinical effects. Three previous dental studies have reported health effects associated with adverse mercury exposure (23,28,34). Unfortunately, each study employs diverse epidemiological methods with weaknesses in the recruitment of subjects, definition of acute versus chronic effects, or reliable dose-effect information. Thus, a threshold effect level has yet to be established, though efforts on the part of several researchers have led to proposed biological threshold limit values of 25 (32) or 50 (26,37) μgs/l in urine. As a point of comparison, creatinine-corrected Hg levels have approximately the same numeric value as uncorrected Hg concentrations.

The largest study, conducted in Singapore (23), evaluated 98 dentists and 54 controls who were not dentists. Exposures of 16.7 $\mu gs/m^3$ Hg° in air were associated with poorer performance in mood, finger tapping, trailmaking, symbol-digit, digit span, logical memory, and visual reproduction. The cognitive test battery was extensive, but sensory tests and hand tremor were omitted. The use of university staff as referents rather than unexposed dentists increased the risk of confounding due to factors such as the use of vibrating tools such as dental drills (which affect fine motor manual dexterity), socioeconomic status, and test-taking training. Equally important, the mercury exposure assessment relies on highly variable air levels which reflect very recent exposure and blood levels which are also influenced by dietary intake (5). Urinary Hg levels provide a more reliable estimate of recent exposure/dose.

Two other dental studies (28,34) relied on nonstandard X-ray fluorescence of Hg° in bones of the wrist and head in attempts to measure chronic accumulation. Thus, their results are difficult to interpret with respect to more standard measures of exposure. In addition, these studies used limited test batteries and only evaluated 23 and 13 exposed subjects, respectively. Nevertheless, visuographic memory deficits were noted in both studies on the Bender-Gestalt (1 of 4 tests) and Rey's recurrent figures tests (1 of 6 tests), which included the PASAT, Rey's AVL, finger tapping, and the grooved peg board. Deficits in electrophysiologic tests (1), indicating slower sural sensory ($\Delta = 3\ ms^{-1}$) and median motor ($\Delta = 2\ ms^{-1}$) conduction velocities, were less impressive because reported differences were within the control subjects' range.

The combined results of these earlier dental studies provides an impetus to better understand and define the lower threshold of central and peripheral health effects of mercury in a dose-effect manner and underscores the need for improved methodology. Our study uses dentists as controls, standard measures of urinary mercury, potential biomarkers of body burden, and a behavioral test battery designed in accordance with World Health Organization's (38) recommendations in an attempt to evaluate such methods as a useful tool for establishing the mercury threshold of adverse effects for the general population.

METHOD

Study Design

A stratified cross-sectional study was conducted as part of the American Dental Association's (ADA) Health Screening Program offered to members of the ADA who attend the annual professional meetings. A spot sample urinary mercury analysis, whose concentration in μgs/l is usually available within 1 h of collection, has been a recent component of the program (21). In 1991, to assist in the interpretation of the health significance of these mercury levels, a battery of behavioral tests was administered to a selected group of dentists participating in the screening program. Subject eligibility was based on their urinary mercury levels. Participants with a level of 19 μgs/l or greater were asked to participate, while an equal number were randomly selected from those with nondetectable urinary mercury levels. Participation was completely voluntary.

Urinary mercury has a biological half-life 40–80 days reflecting more recent exposure over the last 3 months (26,29). Thus, our primary hypothesis was that exposed subjects would demonstrate acute preclinical effects of mercury associated with recent exposures. Our secondary hypotheses explored possible associations between adverse effects and the chronic body burden of longer term mercury exposure. Chronic measures of exposure included porphyrin concentrations in urine (metabolic bioindicators of mercury exposure) and chronic indexes of exposure based on work histories. Many dentists may be exposed to chronic exposure which may continue up to or near the time of assessment. Such subjects would incur both acute and chronic exposure.

Porphyrin concentrations in the urine were measured as an indicator of cumulative mercury exposure (4,35,36). Porphyrins with 8-, 7-, 6-, 5-, and 4-carboxylated side chains are excreted in the urine of normal subjects in a well-established pattern (35). The pattern associated with mercury exposure is characterized by highly elevated levels of porphyrins with 4 or 5 carboxyl groups (penta- and copro- porphyrins) and by the appearance of an atypical porphyrin ("precopro-porphyrin"), which may be pathognomonic of Hg exposure. The etiology of these changes involves both Hg-directed impairment of specific heme biosynthetic pathway enzymes in the kidney, as well as Hg-facilitated oxidation of reduced porphyrins which accumulate in kidney cells because of impairment of porphyrin metabolism. These measures require validation as a potentially sensitive biomarkers of chronic Hg exposure in human subjects.

Work practices (17) and occupational histories were available to calculate chronic exposure indices. An industrial hygienist and dentist weighted each job title, assigning an exposure rating between 0 and 3 to each entry in our dictionary of job titles. These ratings were then weighted by either the calendar period of exposure or the calendar period of the individual's graduation from dental school. The indices of cumulative exposure were the sum of the products of the time spent on each job multiplied by the weighted rating for that job.

The assessment of adverse preclinical nervous system effects is difficult due to large individual variation in most functions and the number of individual factors that may affect these functions. The process of standardization across behavioral studies is still a research question, but considerable progress has been made both in the design of studies, use of control measures, and in the test measures themselves. This study was designed to be consistent with several guiding principles. First, the study population provides a high degree of uniformity, reducing background noise, and amplifying the possibility of detecting preclinical effects. Second, an exposure gradient is studied instead of utilizing the conventional comparisons with a control group that may differ from the exposed group in unknown or unmeasured ways. This approach combines greater uniformity with the ability to estimate the dose-effect relationship. Third, the study relies on well characterized measures of the central nervous system, based on clinical and previous epidemiologic study results.

Subjects

In 1991, 9,566 of the 143,560 ADA member dentists (7%) attended the annual convention in Seattle, WA. A total of 1,706 dentists (17%) participated in the ADA Health Screening Program, and 1,502 (88%) had their urinary mercury concentration measured. Among these, 29 dentists (2%) were classified as exposed because their mercury levels exceeded 19 μgs/l in urine, and 19 (66%) agreed to participate. Among the remaining 10 eligible dentists, 6 refused due to time constraints and 4 were not located. There were 150 dentists (10%) with nondetectable levels of mercury in their urine, of which 20 were randomly chosen to be behaviorally tested.

Procedure

As urinary mercury levels were quantified, identification numbers for eligible subjects were posted at the exit gates. Eligible subjects were invited to participate in the study but were not informed of their exposure status until completion of their participation. If subjects agreed to participate, they were directed to the test area to schedule a 1 h assessment. Each participant filled out a consent form, a computerized symptom checklist, a Profile on Mood Scale (POMS), and a brief medical and occupational questionnaire. Test administrators were blind with respect to exposure status.

Symptom and Medical Questionnaires

One component of the medical questionnaire was designed to evaluate conditions that may influence test performance. Variables of interest included age, race, gender, education, medical history of neurologic disorders, hypertension, diabetes, and pharmaceutical use. Questions regarding personal habits included a detailed history of alcohol, caffeine, and nicotine use, as well as exposures received from hobbies.

The symptom questionnaire included measures of physiologic, psychologic, and somatic symptoms which have been reported following acute and chronic exposures. The symptom questionnaire was based in part on the Swedish "Questionnaire 16" (11) and items used in previous studies of mercury (1,12) organic solvents (6,7), and lead (2). This information supplements the ADA questionnaire that covers sources of mercury exposure such as work practices, the number of amalgams each dentist has, and seafood consumption.

Mood and Vocabulary Scores

An evaluation of mood (Profile of Mood States) (18) was included as changes in affect have been consistently reported in workers with Hg concentrations between 30–100 μgs/l (8,25,26,32,39). The mood scales are based on 65 mood descriptors which are rated on a 5-point scale ranging from *none at all* to *extreme*. The items comprise six mood scales: tension, depression, anger, fatigue, confusion, and vigor. Time: 5 min.

Vocabulary scores have often been used as a control variable to account for differences in education, socioeconomic status, and test-taking ability. However, among a uniformly educated and high-economic-status group such as dentists, the variation in response may better reflect a variation in the dentists' ability to concentrate.

The Behavioral Test Battery

The study used a set of behavioral tests covering a broad range of functions previously found to be affected at urinary levels of Hg° above 50 μg/l, as well as tests recommended by the World Health Organization and by the Agency for Toxic Substances and Disease Registry (ATSDR) to quantify neurotoxic effects attributable to low-level chemical exposures. The following section provides a brief description of the tests.

Cognition: memory/attention: digit span (30). The computerized digit span test computes a more precise estimate of a subject's 50% threshold digit span for correct serial recall using probit analysis to provide a maximum likelihood estimate. The test first conducts a range-finding digit span in 20 trials. Forty trials are then administered using 5 set sizes of 8 trials each. The test is recommended by WHO and has previously been shown to be sensitive to urinary Hg° concentrations as low as 111 μg/l. Score: digit span. Time: 23 min.

Cognition: complex attention: the switching task (33). This computer-presented task requires subjects to press a "same" or "different" button when confronted with a pattern comparison, semantic letter comparison, or semantic graphical comparison, presented in apparent random order. The order actually follows a complete Latin square procedure, balanced for residual effects. This test modifies the traditional design by inserting extra trials to achieve greater stability of the estimates of switching between tasks and to avoid the ability of subjects to predict the next task. These additions provide a total of 8 repetitions for the 6 switching combinations and between 10 to 16 repetitions for the 3 control conditions. Score: switching time and the number correctly chosen. Time: 4 min.

Cognition: attention: NES simple reaction time (SRT) (3). This computerized task requires subjects to press a button every time a stimulus appears on the screen with the right and later left index finger. Score: reaction time. Time: 4 min.

Cognitive: coding: NES symbol-digit substitution (3). The test is a psychomotor task. It is a computerized modification of the Digit Symbol Test from the Wechsler Adult Intelligence Scale-R (WAIS-R) which is in the World Health Organization's core set of tests. In this task, 5 sets of 9 symbols matched with nine numbers at the top of the screen in one panel are presented sequentially. The subject is presented a scrambled symbol array without numbers at the bottom of the screen. The subject has to manually enter the correct number to be paired with the symbols at the bottom. The number of incorrect pairs and the latency for each pair are recorded. There is one practice session. The pairing of symbols with numbers is varied between sets to avoid learning effects. Score: Latency to complete task elements. Time: 4 min.

Verbal skill: NES vocabulary (3). The NES computerized test is a modification of the Armed Forces Qualifying Test (AFQT). Twenty-five words are presented by computer and the subject is asked to select, from a set of four words, the synonym for the word originally presented. This negative control test is not expected to vary with Hg exposure and is a stable measure of CNS function indicating a level of test-taking ability. Score: Number correct. Time: 4 min.

Motor: manual dexterity: the one hole test (27). This test can independently assess component tasks such as the time it takes to grasp, move, position, and reach while transferring small pins from a large target to a small target hole. The test has been found sensitive to other neurotoxicants such as toluene and ethanol (33) and by previously exposed mercury workers (1). Scores: the number of pins, and four response times to grasp, move, position, and reach accompanied by the number of fumbles is recorded. Time: 7 min.

Urinary Mercury Analysis

Spot urine samples were collected in 50 ml flasks from the subjects. Urine specimens were analyzed twice, initially at the convention site (21) and later using the Hatch and Ott cold vapor atomic absorption spectrophotometric method (10). All elevated specimens were reanalyzed on-site to confirm the analytic findings. On-site urinary mercury results tend to underestimate the total mercury content due to the lack of time needed for sample digestion, however, these two methods provide results which are highly correlated with a correlation coefficient of $\simeq 0.90$ (21).

Porphyrin Analysis

Porphyrin concentrations in spot urine samples were analyzed using high pressure liquid chromatography (HPLC) separation and quantification with a spectrofluorometric detection method described by Woods et al. (36). This procedure permits quantitation of urinary porphyrins with a detection sensitivity of 0.5 pmoles.

Data Analysis

Multivariate regression (SAS-PC, SAS Institute, 1989) techniques were used to evaluate possible associations between recent and chronic measures of mercury exposure and adverse changes in symptoms, mood, and behavioral function. All continuous variables used in the analysis were graphically reviewed (AXUM, TriMetrix, 1992) to evaluate their distributions. Variables that contained extreme values that might dominate the analysis or that were too divergent from normal distributions were log transformed. Recent exposure was evaluated using the concentration of mercury in urine. Chronic exposure was evaluated using porphyrin levels or calculated chronic indices just described.

Tests of multiple individual outcomes were performed for hypothesis generating purposes only, due to the small number of subjects and large number of outcomes being tested. For hypothesis generation, we considered a one-sided p value of < 0.05 as statistically significant if the association was in the hypothesized direction. Correction for multiple comparisons (e.g., Bonferroni) was inconsistent with the goal of hypothesis generation in a study population of this size ($n = 39$). Age in years, age-squared, gender, race, alcohol consumption (a 5-level scale based on frequency of drinking per week), alcohol use on the day of the testing (dichotomous), medical history of neurological importance, and nitrous oxide use were each evaluated as potential confounders that would be included in the final analyses if they had either a significant association with the outcome variable, or if their inclusion substantially altered the observed association between exposure and outcome.

Sums of ranked scores, a conservative and robust measure of overall trend for tests with widely divergent measures and underlying distributions (24), were calculated for various a priori groupings of test scores. The sums of ranked scores were calculated by first running a multivariate regression with all important confounders for each outcome variable and saving the residual scores for each subject. One may interpret each residual score as an adjusted score. The residual scores for each test in the summary score were then ranked in order of decreasing performance, and these ranks were summed for each subject across outcome variables. Finally, these sums were analyzed by regression with only the exposure variables in the equation. This method of calculating the sums of

TABLE 1

DESCRIPTIVES OF POTENTIAL CONFOUNDING VARIABLES FOR ANALYSES OF SYMPTOMS, MOOD, AND BEHAVIOR

	Unexposed ($n = 20$)	Exposed ($n = 19$)
Age	45 (SD = 13)	52 (SD = 15)
Vocabulary (% correct)	87 (SD = 8)	85 (SD = 13)
% Male	70	89
% Caucasian	85	95
% N_2O use	53	18
% Alcohol use	75	63
% Alcohol today	55	32
% Alcohol frequency		
< 1 × /month	35	55
< 1 × /week	25	26
1–2 days/week	15	16
3–5 days/week	25	0

TABLE 2

WORK PRACTICE CHARACTERISTICS OF THE STUDY POPULATION

Characteristic	Hg Unexposed (n = 20) x̄, SD	Hg Exposed (n = 19) x̄, SD
% Own practice	30	16
Years in practice	21 (12)	25 (13)
Years in current office*	12 (9)	19 (10)
Amalgams placed/week*	19.8 (15.7)	28.0 (12.7)
Amalgams removed/week	17.5 (15.4)	17.8 (10.1)
Save amalgam scrap	77.8%	84.6%
Use squeeze cloths*	9.8%	53.8%
Ever had Hg spill*	43.2%	73.7%
Number of Hg spills in last year	0.1 (0.4)	2.3 (3.8)

*$p < 0.05$.

ranked scores allows each outcome measure to have an independent association with the confounding variables. Three sums of ranked scores statistics were calculated including (a) all major test scores (percent total symptoms, overall mood score, digit span, averaged simple reaction time, averaged switching time, symbol digit, one-hole rate of number of pins/min); (b) all major "objective" test scores (excluding the self-reported symptoms and mood scores); and (c) the four major "cognitive" test scores (digit span, reaction time, switching and one-hole).

RESULTS

Study Population

The exposed and unexposed dentists differed primarily in their age distribution, frequency of alcohol consumption, and use of nitrous oxide (Table 1). Older dentists received different medical training than younger dentists, and this may explain the difference in the use of nitrous oxide between the two exposure groups. This factor, however, was found to have no significant effect on the performance outcomes. Frequency of alcohol use and use of alcohol on the day of testing were not importantly different in their effect on the outcomes, and alcohol frequency was selected in subsequent analyses. Age squared did not improve control for confounding over age alone, and medical history had no effect on the outcomes. Vocabulary, often used as a surrogate measure of education, economic status and test taking ability was significantly associated with Hg exposure but had no important impact on other observed associations. It was thus treated as an outcome rather than a control variable (see Discussion section). Age, race, gender, and alcohol consumption where selected as control variables in the final analyses.

Work Practice

Dental practice characteristics were examined for the larger pool of eligible dentists from which the current study subjects were taken (Table 2). Regression analysis indicated that among the measures reported in the table, the use of squeeze cloths accounted for the largest portion of the variance associated with the concentration of mercury in urine. In addition, the numbers of years in the current office, accidental mercury spills, and amalgams placed per week were significantly different between the exposed and unexposed dentists. No significant differences were found between the two groups for the overall number of years in practice, the number of amalgams removed per week, or in the manner in which scrap amalgam was saved or disposed.

Exposure Assessment

The mean on-site mercury concentration in urine was 36.4 μgs/l (SD = 20.0 μgs/l) among exposed dentists and was non-detectable among the control group (see Table 3). The concentrations of the three porphyrins that have been observed to be altered during mercury exposure in animal studies (i.e., pentacarboxyl-, precopro-, and copro- porphyrins) are also presented. The mean concentrations of all three porphyrins were significantly elevated among exposed dentists, exceeding the unexposed group values by three- to four-fold. There were only moderate correlations between the three porphyrin concentrations and urinary mercury levels. The Pearson correlation coefficients were 0.62, 0.58, and 0.19 (r^2 = 0.39., 0.34, and 0.04) between Hg and copro-, precopro-, and pentacarboxyl- porphyrins, respectively. The cumulative exposure index did not prove useful in this study due to its extremely high correlation with the important covariate of age. The Pearson correlation coefficient was 0.96 (r^2 = 0.92) making this variable virtually interchangeable with age.

Vocabulary, Symptom, Mood, and Behavior

Associations between measures of exposure and test outcomes are described in Table 4 and Table 5. For each test, the overall mean of the test for all subjects is given followed by the maximum score possible for that test. These are followed by the SD and the differences in scores adjusted for confounding factors. The final four columns provide adjusted p values for significant associations (one-sided $p < 0.05$) with the exposure measures of Hg in urine and the three porphyrins. Adjusted scores were obtained using multivariate regression to control for the effects of age, race, gender, and ethanol consumption.

Vocabulary was found to be associated with urine mercury levels as were symptoms associated with mental concentration and emotion (Table 4). Associations between symptom scores and the porphyrin exposure measures were less consistent. There was no statistically significant association between total symptoms and any exposure measure.

The total mood score was very significantly associated with urine Hg levels and was associated at borderline significance with all porphyrin measures (Table 4). This pattern was generally repeated for the specific mood categories of tension, fatigue, and confusion. The mood scales for vigor and depression were more marginally associated with several exposure measures.

Table 5 shows that there were considerably fewer significant associations between behavioral measures and the four

TABLE 3

URINARY MERCURY AND PORPHYRIN CONCENTRATIONS, x̄(SD) (μgs/l)

	Unexposed	(n = 20)	Exposed	(n = 19)
Mercury*	0.0	(0.0)	36.4	(20.0)
Copro*	23.5	(24.6)	72.4	(34.6)
Precopro*	2.3	(2.6)	7.2	(3.9)
Penta*	1.1	(0.9)	6.1	(9.8)

*$p < 0.05$ (based on t tests).

TABLE 4

VERBAL, SYMPTOMS, AND MOOD TESTS

Function	Test	Mean/Max	SD	Adjusted Difference Between Groups	*p* Values for Exposures			
					Hg	COPRO	PRECOPRO	PENTA
Verbal Skill	Vocabulary	21.5/25	2.7	1.3	.01			
Symptoms	Total symptoms	4.3/42	5.3	3.5				
	Emotional	1.5/11	1.9	1.1	.03			.04
	Concentration	0.1/2	0.4	0.2	.01		.01	
	Somatic	0.5/8	0.9	0.4				.03
	Head	0.8/4	1.1	0.3				
	Memory	0.8/4	1.2	0.3				
	Coordination	0.3/6	0.6	0.1				
	Motor	0.2/4	0.5	0.2				
	Chest	0.2/3	0.5	0.1				
Mood	Summary score	31.2	21.8	14.9	<.001	.04	.04	.05
	Tension	7.2	4.3	3.3	.002	.03	.03	.04
	Fatigue	6.2	4.9	3.5	.003	.04		.003
	Confusion	4.2	3.6	2.6	.004	.04	.05	.02
	Vigor	21.5	5.0	3.4	.02			.03
	Depression	4.0	4.9	2.1	.05			
	Anger	31.2	5.6	1.3				

Results of Multivariate Regression adjusted for age, race, gender, and ethanol consumption.

measures of mercury exposure. The copro-porphyrin exposure measure did have significant associations with the digit span and the nondominant hand simple reaction time measures. Among 4 measures, the mean time it took to "move" a pin in the one-hole test was significantly associated with urine Hg levels and precopro-porphyrin levels. We report this subscore only because it has proven sensitive in our other neurobehavioral research.

Sum of Ranked Scores Measures

The sum of ranked scores technique measures the overall performance across the various tests included in the score. The significant associations reported in Table 6 indicate lower performance with increasing Hg exposure. In fact, almost all scores were in this anticipated direction. Urinary mercury levels were associated with the overall test battery results at a high level of significance, primarily reflecting their association with the mood scores. However, even when the more subjective responses involving symptoms and mood scores are excluded, leaving just cognitive and motor tests, there was still a significant association with urinary mercury levels. This also held true for the summary score including only the four main cognitive measures. The copro-porphyrin measure was also significantly associated with all three sum of ranked scores measures. However, the precopro-porphyrin measure was only associated with the overall summary score, while the pentacarboxyl porphyrin was only associated with the cognitive summary score.

DISCUSSION

This hypothesis-generating study showed highly significant correlations between mood scores and most measures of low-level Hg exposure. The specific mood measures most associated with Hg exposure were tension; fatigue, and confusion.

TABLE 5

COGNITIVE AND MOTOR TESTS

Function	Test	Mean	SD	Adjusted Difference Between Groups	*p* Values for Exposures			
					Hg	COPRO	PRECOPRO	PENTA
Cognitive								
Memory	Digit span	6.4	1.1	0.2		.03		
Simple attention	Simple reaction time							
	Dominant	355	35	11.3				
	Nondominant	358	34	9.8		.03		
Complex attention	Switching (average)	1.6	0.4	0.1				
Coding	Symbol digit	22.8	5.8	1.4				
Motor								
Manual dexterity	One hole	35.3	5.9	1.4				

Results of Multivariate Regression adjusted for age, race, gender, and ethanol consumption.

TABLE 6

SUMMARY RESULTS BASED ON THE SUMS OF RANKED SCORE TESTS

Domain	p Values for Exposures			
	Hg	COPRO	PRECOPRO	PENTA
Cognitive, motor, mood, symptoms	.003	.02	.05	
Cognitive and motor	.03	.02		
Cognitive	.04	.04		.03

Results of Multivariate Regression adjusted for age, race, gender, and ethanol consumption.

The strength of these associations suggests that mood should be included for investigation in future hypothesis-testing studies. Self-reported symptoms, in contrast, were not as significantly associated with Hg exposure as mood. However, symptoms associated with problems of mental concentration were significant for both urinary Hg levels and precopro-porphyrin levels. It was interesting that memory symptoms were not associated with any exposure measure, as memory problems are frequently affected by Hg exposure (15,31).

A strong association between vocabulary scores and urinary Hg levels was also noted. Vocabulary is frequently used as a control variable because it is considered a measure of intelligence and education that is usually robust against CNS insults. However, in this population, which is already highly selective with respect to education and intellectual ability, we believe that the observed association may better reflect the level of "immediate mental concentration". This conclusion is supported by the observed associations between exposures and symptoms related to concentration and we believe that this association deserves further investigation.

Among the cognitive and motor function tests, only the digit span and simple reaction time (nondominant hand) scores were associated with any measure of exposure. Both were marginally associated with only the copro-porphyrin measure. It is difficult to make recommendations for future hypotheses based on these weak results.

Some of the most interesting results from this study are found in the summary scores. Urinary mercury levels were not significantly associated with any of the individual cognitive or motor function measures. However, the sum of ranked scores measures for these function groups were associated with urinary Hg levels. This indicates that, while the differences in individual scores were too small to reach significance, their general direction was consistent (i.e., reduced performance with increased exposure). This suggests that Hg exposure may be associated with a more generalized effect that will be most efficiently evaluated with a full battery of tests.

Overall, urinary Hg levels were more highly correlated to mood and symptom measures, while the copro-porphyrin levels were most highly correlated to cognitive function measures. It is possible that cognitive effects may be more closely associated with chronic mercury exposures. The consistency of the directionality of associations for urinary mercury and copro-porphyrins is noteworthy. In this study, urinary copro-porphyrin was clearly the most useful porphyrin biomarker. This may be due to the fact that copro-porphyrin appears in much greater concentrations than the other two porphyrins which are altered by mercury exposure (Table 3), and its variability may be easier to determine accurately. We had initially expected that precopro-porphyrin might be the best biomarker, as this porphyrin seems to be uniquely characteristic of mercury exposure. Correlations between urinary mercury levels and copro- and precopro- porphyrins showed that recent exposure accounted for 34%–39% of the variation in the two porphyrins. This is a reasonable degree of association between acute and chronic exposure markers. On the other hand, urinary mercury levels accounted for only 4% of the variation in the pentacarboxyl porphyrin, indicating that this porphyrin was not a clear bioindicator of either chronic or acute mercury exposure in the present study.

Finally, some interesting associations between work practices and exposure were of note. Nitrous oxide, a known neurotoxin at high concentrations, was more extensively used among the control group (53% vs. 18%). Despite analytic control, such a large difference in N_2O use may explain some of the dilution of differences in performance between exposure groups. However, if it were to be a major factor, performance among the controls would have been worse than the exposed group that was not evident in the data. The use of squeeze cloths and the number of years in the current office were highly associated with exposure while total number of years of practice was not. A recent change of office might be associated with the introduction of new technology.

Due to the relatively small number of subjects, we were unable to simultaneously control for all possible confounders, which would have made the results too unstable. The small size also allows a few individuals to have considerable impact on some results. However, major differences in education, socioeconomic status and test taking ability, as well as possible confounding due to occupational exposures, were generally controlled for by restricting the study to dentists. Other possible confounders, such as medical history, use of glasses and handedness have been individually tested and did not have major influence on the overall test results. Individual subjects who initially appeared to have potentially large impacts on the outcomes were compared to the total group, and they do not seem unreasonably different from other subjects.

Other limitations to this study include the use of spot urines (less reliable than 24-h urine mercury levels) and possible selection bias associated with attending the ADA meeting and participating in the screening program and behavioral study. However, the possibility of exposure bias is unlikely because both exposed and unexposed dentists chose to participate in the screening program for reasons unrelated to previous or current knowledge of their mercury level in urine.

Overall, and despite the small size of the study population, this investigation found some evidence of adverse preclinical effects at mercury doses averaging 36 μgs/l in urine. The mood and symptom results of this study agree with prior evaluations of both high (14,15,30,31) and low (9,32,39) urinary mercury doses. These preliminary survey findings support a critical evaluation of the adequacy of the 50 μg/g creatinine biologic threshold for mercury proposed by the World Health Organization (37). This is the first U.S. dental study to detect potential behavioral deficits at such a low level of exposure. A larger and more comprehensive study is required to accurately determine a biologic threshold of adverse central and peripheral nervous system effects for elemental mercury.

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