Neurobehavioral effects from exposure to dental amalgam Hg^o: new distinctions between recent exposure and Hg body burden

DIANA ECHEVERRIA,[*,†,1] H. VASKEN APOSHIAN,[‡] JAMES S. WOODS,[*,†] NICHOLAS J. HEYER,[†] MARY M. APOSHIAN,[‡] ALVAH C. BITTNER, JR.,[*,†] RODERICK K. MAHURIN, AND MARGARET CIANCIOLA[§]

*Battelle Centers for Public Health Research and Evaluation, Seattle, Washington 98105, USA; [†]Department of Environmental Health, University of Washington, Seattle, Washington 98195, USA; [‡]Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721, USA; [§]Department of Psychiatry, University of Washington, Seattle, Washington 98195, USA

ABSTRACT **Potential toxicity from exposure to mercury vapor (Hg^o) from dental amalgam fillings is the subject of current public health debate in many countries. We evaluated potential central nervous system (CNS) toxicity associated with handling Hg-containing amalgam materials among dental personnel with very low levels of Hg^o exposure (i.e., urinary Hg <4 μg/l), applying a neurobehavioral test battery to evaluate CNS functions in relation to both recent exposure and Hg body burden. New distinctions between subtle preclinical effects on symptoms, mood, motor function, and cognition were found associated with Hg body burden as compared with those associated with recent exposure. The pattern of results, comparable to findings previously reported among subjects with urinary Hg >50 μg/l, presents convincing new evidence of adverse behavioral effects associated with low Hg^o exposures within the range of that received by the general population.—Echeverria, D., Aposhian, H. V., Woods, J. S., Heyer, N. J., Aposhian, M. M., Bittner, A. C., Jr., Mahurin, R. K. Neurobehavioral effects from exposure to dental amalgam Hg^o: new distinctions between recent exposure and Hg body burden. *FASEB J.* 12, 971–980 (1998);**

Key Words: behavior · elemental mercury · dentists · DMPS

THE CRITICAL TARGET organ of elemental mercury vapor (Hg^o)[2] is the central nervous system (CNS) (1). Although there is little debate regarding the toxicity of exposure to Hg^o associated with urinary Hg concentrations above 50 μg/l, no consensus exists with respect to a safe lower Hg^o exposure level among either dental populations that handle Hg amalgam or the general population with amalgam restorations. Hg^o exposures in this study are relevant to both groups, since they were assessed in a dental population but extend over a continuum of urinary Hg levels from 0 to 4 μg/l, comparable to the low exposure levels observed in the general U.S. population. General population levels provided by Dr. P. Factor (personal communication) and Dr. A. Kingman (2) range respectively from 1.3 to 18 μg/l (mean=9 μg/l creatinine corrected) and from 0 to 34 (mean=3.1 μg/l). Thus, this study addresses public health concerns for Hg^o toxicity of dental amalgams.

Interpretation of health effects observed among people with Hg^o exposures resulting in urinary Hg levels of less than 50 μg/l has previously been hampered by the inability to distinguish behavioral effects associated with recent exposure vs. those associated with chronic body burden. This study adopted a novel approach to distinguish between these effects by examining differences in behavior in relation to urinary Hg concentrations measured both before (prechelation) and after (postchelation) treatment of subjects with the Hg mobilizing agent, sodium 2,3-dimercapto-propane-1-sulfonate (DMPS). Urinary mercury levels (HgU) subsequent to DMPS challenge have been reviewed extensively (3, 4) and shown to constitute a better approximation of Hg body burden (5).

A central question is the validity of using prechelation HgU as a proxy for CNS dose. This indirect measure has been commonly accepted, because the lipophilic property and low vapor pressure of Hg^o (0.005 mm Hg^o at 37°C) permit 76–80% of the vapor to be absorbed through the lungs. The dissolved vapor is oxidized primarily in erythrocytes into mercuric ions by the hydrogen peroxide-catalase pathway (i.e., $Hg^o \rightarrow Hg^{1+} \rightarrow Hg^{2+}$) (6). The oxidation process

[1] Correspondence: Battelle Centers for Public Health Research and Evaluation, 4000 NE 41st St., Seattle, WA 98105, USA

[2] Abbreviations: NES, Neurobehavioral Evaluation System; Hg^o, mercury vapor; CNS, central nervous system; DMPS, sodium 2,3-dimercapto-propane-1-sulfonate; HgU, urinary mercury levels; POMS, Profile on Mood State.

is dose dependent in that low doses result in a higher proportion of Hg^{2+} in blood than do higher doses approaching saturation (7). Blood Hg levels in dental personnel populations range from 1.2 to 14 μg/l (8), well below saturation levels, assuring that the oxidative pathways in the kidney and brain are also below saturation. Thus, HgU varies with occupational Hg^o exposure and provides an effective measure of current dose. With respect to the CNS, the rate of oxidation is slower than circulation time from the lung to the brain, allowing unoxidized Hg^o to pass through the blood–brain barrier, where it is then oxidized to the divalent form (Hg^{2+}), complexed, and retained. Controlled radioactive Hg^o inhalation studies in humans indicate that the brain retains Hg for approximately 21 days (9), providing for CNS accumulation and stabilization over approximately 1 month. This compares well to the 2 month half-life of Hg in urine. These factors have collectively supported the validity of using prechelation HgU as an indicator of CNS dose associated with recent subchronic Hg^o exposure.

Behavioral studies that rely on prechelation HgU are necessarily limited to evaluations of recent exposure because of the relatively short residence or half-life of Hg in the urine compared with that accumulated in soft body tissues. Aposhian et al. (5) have demonstrated that administering DMPS at a dose of 300 mg p.o after an 11 h fast effectively mobilizes Hg from soft tissues, which is then excreted in urine over the subsequent 0–6 and 6–24 h periods. The postchelation HgU level reflects the decrease of Hg in both the kidney and cellular fraction of blood, suggesting that DMPS reduces the renal whole-body burden of mercury in humans (10). Examining CNS effects in relation to both pre- and postchelation measures in this study permitted differentiating CNS effects associated with recent subchronic Hg^o exposure from those associated with body burden, which are attributable to more persistent long-term exposures.

Studies assessing CNS preclinical effects among subjects with urinary Hg concentrations in the range of 50–200 μg/l support four aspects of frank mercurialism (11): *1)* psychosomatic symptoms (salivation, insomnia, and loss of appetite); *2)* alterations in affect or emotional liability [mood swings, irritability, fatigue, loss of interest, withdrawal, and sweating and blushing (erethism)]; *3)* motor effects (in the arms, progressing to uncoordination, imbalance, and cerebella ataxia and tremor in muscles that are highly enervated and perform fine motor control of extremities such as fingers, eyelids, and lips); and *4)* insidious loss of mental capacity (progressively affecting memory, logical reasoning, or intelligence).

Occupational studies assessing urinary Hg^o levels between 2 and 200 μg/l have demonstrated impressive consistency with the four aspects of mercurialism summarized above. Alterations of emotional state, mood, and symptoms have been the most frequently reported effects at HgU levels ranging between 30 and 100 μg/l (12–18). Six dental studies have previously examined mood (19–24) where scores on the Profile on Mood States (POMS) (25) and aggression were higher than controls, supporting our choice of using POMS in this study.

Deficits in motor function were first reported as finger tremor among felters and later as hand tremor among chloralkali workers (26). However, losses in hand steadiness, finger tapping (27, 28), and manual dexterity (29, 30) have also been reported at lower levels of exposure. Among dentists with mean urinary levels of 26 μg/l, statistically significant losses in performance in hand steadiness (known to be correlated with tremor) were also found (30). These studies support a comprehensive evaluation of motor function at even lower levels of exposure as a threshold level of effect remains to be determined.

Determinations of a lower threshold for cognitive effects are complicated by mixed results among several chloralkali worker studies at low exposure levels ranging between 0.025 and 0.076 mg/m^3 (10–19.9 μg/l in blood) (31–33). These conflicting results may be better addressed by studying more uniform subjects, such as dentists, who have similar economic and educational backgrounds. For example, our own pilot dental studies have detected a reduction in cognitive skills (22–24) similar to that seen in other dental studies (19–21). The largest dental study (19) conducted in Singapore examined 98 dentists and 54 nondentist controls, where mean exposures of 16.7 $\mu g/m^3$ Hg^o in air were associated with differences in trailmaking, digit-symbol, digit span, logical memory delayed recall, and visual reproduction. Two other dental studies (20, 21) also found associations between chronic exposure with visuographic memory deficits by using the Bender-Gestalt (21) (one of four tests) and Rey's recurrent figures (20) tests (one of six tests), which also included the PASAT, Rey's AVL, finger tapping, and the grooved peg board. These findings support placing emphasis on the cognitive domain.

The evidence for potential impairment among the four domains provides the basis for test selection on an anticipated continuum between preclinical effects and clinical deficits. Along this continuum, a preclinical effect is defined as a subtle adverse change in performance not usually detected by clinical examination because the observed effect falls within the range of normal performance on tasks. However, preclinical effects can be demonstrated by showing that the variation in cognitive task performance, though well within the normal range, is correlated with exposure to Hg. Preclinical effects range between 3 and 18% when compared to a zero or low-exposure group. Deficits

exceeding 18% are likely to border on clinical significance, and effects of less than 2% are not likely be occupationally relevant (34).

The broad diversity in clinical effects coupled with the evidence from epidemiologic studies indicates more than one mechanism of toxicity is involved, covering several areas of the brain. Consequently, we base our behavioral hypothesis on the results discussed above, which suggest that low-level Hg^o exposures may increase symptoms, alter mood, decrease manual coordination, increase tremor, and cause deterioration of cognitive skills requiring visual-spatial memory and attention. We consider adverse effects on these four domains to be selective, leaving language and retrograde memory largely intact. This justifies the use of each subject's vocabulary score as an available measure of premorbid intelligence or a 'hold test' not expected to be adversely affected by exposure. Our test battery was designed to cover the four domains with adequate redundancy to detect subtle effects and to discriminate between areas resistant to Hg insult. Tests were selected for their sensitivity to Hg^o; their ability to be adapted for joint human/animal assessments, which provides a broader understanding of the results; previous validation by the World Health Organization (35) and the Agency for Toxic Substances and Disease Registry (ATSDR) (36); and use in quantifying neurotoxic effects attributable to low-level exposures.

MATERIALS AND METHODS

The study population and test procedures

Thirty-four practicing dentists and 15 dental assistants were selected to participate in this study. The study group was administered a pretest questionnaire that medically screened subjects for preexisting clinical disorders that may interfere with performance on the test battery such as physical injury, diabetes, epilepsy, alcoholism, multiple sclerosis, encephalitis, manic depression, and use of medications that produce drowsiness or otherwise affect performance. Two subjects were eliminated: one was diabetic, the other was an alcoholic. The study population was predominantly male (69%), Caucasian (92%), middle-aged (mean age=49), native English speaking, right-handed (88%), and consumed a moderate number of alcoholic beverages per week (n=3, SD=4.5). This dental population is an ideal study population, with characteristics that improve detection of subtle preclinical effects, as they have Hg^o exposures within the range of interest (prechelation HgU < 4 μg/l), are highly educated, have excellent test-taking skills, and have well-developed motor skills.

Prior to the DMPS challenge, participants signed a consent form in accordance with the Declaration of Geneva of the World Medical Assembly, and completed a questionnaire covering occupational and medical work histories and work practices (37). Subjects also completed an assessment of symptoms and the POMS. This process was followed by a 1 h test battery described in greater detail below. After the test session, participants were administered DMPS (300 mg, p.o.) after an 11 h fast. Urine was collected from −11 to 0 h prechelation and from 0 to 6 h postchelation. Test administrators were blind with respect to subjects' HgU status.

The behavioral test battery

The test battery described below evaluated symptoms, mood, motor function, and cognition, using the Neurobehavioral Evaluation System (NES) vocabulary score (38) as an estimate of premorbid intelligence.

Symptoms (22)

The symptom questionnaire was adapted from several previous questionnaires that were designed to evaluate potential CNS effects of mercury. Responses to persistent symptoms that last for more than a year were collected on a continuous scale that permits evaluations of both the extent and severity of symptoms among subjects.

Profile of mood states (25)

The self-administered mood scales include 65 mood descriptors, which are rated on a 5-point scale from 'none at all' to 'extreme'. The items comprise six mood scales: total mood (sum of all mood scales except vigor), tension, depression, anger, fatigue, and confusion.

The hand steadiness battery (39)

This task requires subjects to place pins in a series of holes with decreasing diameters in a prescribed manner as quickly as possible, where the number of hits and latencies for eight holes are recorded.

Simple reaction time NES (38, 40)

This computerized NES task requires subjects to press a button with the index finger of the right and left hands every time a stimulus appears on the screen.

Finger tapping NES (38)

This computerized NES task measures motor quickness and accuracy. It requires subjects to tap a button as many times as possible in 10 s under three conditions (dominant index finger, nondominant, and alternate two-button tap with dominant index finger).

Tremor Analysis Test System: acceleration finger tremor (41)

The subject was asked to keep still for three 10 s trials, with a 15 s rest period between trials. Resting tremor (of the dominant and nondominant hands) was recorded by a two-axis microaccelerometer embedded in the tip of a 12 cm × 0.8 cm pencil. The accelerations are normalized by using Fourier analysis to get the power distribution in the frequency band of 0.9–15 Hz. The tremor spectra show absolute and relative power over the range in 0.2 Hz bands, encompassing the 6.5 Hz region previously found to be affected by mercury. Tremor intensity was determined by the root-mean-square of accelerations over the data collection period. The accelerometers have a sensitivity of 85 mV per Gauss and a frequency response rate of 1–830 Hz. A sample rate of 60 Hz reduces noise from power lines. The fast Fourier transform used a sample size of 512. At 60 Hz, each sample of 512 requires 8.53 s of data col-

lection, which is amplified, processed, and transferred to a portable IBM-compatible computer.

One-hole pins (pins/min) (42)

This computerized task requires subjects to place pins in a hole in a prescribed manner as fast as possible for five 1 min trials. The posture of the subject is controlled by having the dominant hand pick up pins while the other is resting on a fixed plate. Secondary measures include the time to 'grasp', 'move', 'position', and 'reach' for each pin.

Vocabulary NES (38)

The NES computerized test is a modification of the Armed Forces Qualifying Test. 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 'hold' test was not expected to vary with Hg exposure, and serves as a measure of premorbid intelligence and user schooling.

Recognition memory test (for words) (43)

This is a word memory test in which subjects are asked to correctly recall 50 words that are administered in a fixed sequence.

Trailmaking A and B (44)

This paper-and-pencil test is an executive function task assessing cognitive tracking. In A trails, the subject must track a numeric sequence on a spatial array. In B trails, the subject must alternate meaningful sequences of numbers and letters on a spatial array. Both tasks were scored for the number of errors and response time.

Visual retention test NES (38)

The NES computerized test requires the subject to memorize a picture and then select the correct one of four possible choices. Over the course of 12 trials, the pictures become more complex. This test is a computerized version of the Benton Memory Task.

The switching task (45)

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. Items are presented in apparent random order, but actually follow 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 eight repetitions for the six switching combinations and between 10 to 16 repetitions for the three control conditions.

Symbol-digit NES (38)

This coding task requires the subject to enter the number that matches a symbol by using a matched set printed at the top of the screen. The task requires fine manual dexterity, visual scanning, and motor speed. A visual memory component increases performance speed if used by the subject.

Exposure to urinary mercury

HgU was analyzed by using cold vapor atomic absorption (5). Both occupational and nonoccupational sources of Hg exposure were covered in questionnaires, including the number of amalgam fillings in the subject's mouth and seafood consumption. Hg speciation analysis revealed that organic intake of Hg was negligible. Personal habits and detailed work practices were later used in regression models to determine factors predicting HgU levels.

Statistical methods

Multiple regression was used to evaluate log-linear, dose-effect behavioral relationships for pre- and postchelation urinary Hg values, controlling for age, race, gender, vocabulary, alcohol consumption, and wearing of eyeglasses (46). Regression models for cognitive outcomes also included the log SRT of the dominant hand to control for motor subcomponents of the cognitive tasks. Summary factor scores (30) were created to reduce multiple outcomes to single scores on select tests with enhanced reliabilities (hand steadiness, finger tapping, tremor, and switching attention). Paired *t* tests between pre- and postchelation HgU coefficients were used to evaluate whether the two dose measures differed significantly in predicting specific test performance. Standardized beta coefficients are a partial correlation coefficient, indicating the unique variance associated with each measure of exposure when all other variables in the model have been accounted for (47). Standardized beta is a metric that can be appropriately compared across independent variables and different domains.

RESULTS

Work-related and personal factors (including postchelation HgU levels and number of amalgam fillings in one's mouth) were determinants of prechelation HgU levels

There was an order of magnitude difference between pre- and postchelation urinary Hg concentrations (**Fig. 1**). The correlation between the two samples (r=0.53), although significant, indicated that different parameters were being assessed, given very high reliabilities of urinary Hg assessments across a day in a previous report (27, 28). HgUs of the dentists and dental assistants did not differ pre- or postchelation (prechelation: dentists=0.89 μg/l, SD=0.51; dental assistants=1.07 μg/l, SD=0.93; postchelation: dentists=10.08 μg/l, SD=7.37; dental assistants=8.07 μg/l, SD=5.99). Several work-related factors that are amenable to intervention were significantly associated with prechelation HgU as well as one personal source of exposure (**Table 1**). These factors include the number of restorations placed per week, the use of dispensers vs. capsules, and the irregular use of a mask while handling Hg, as well as the number of

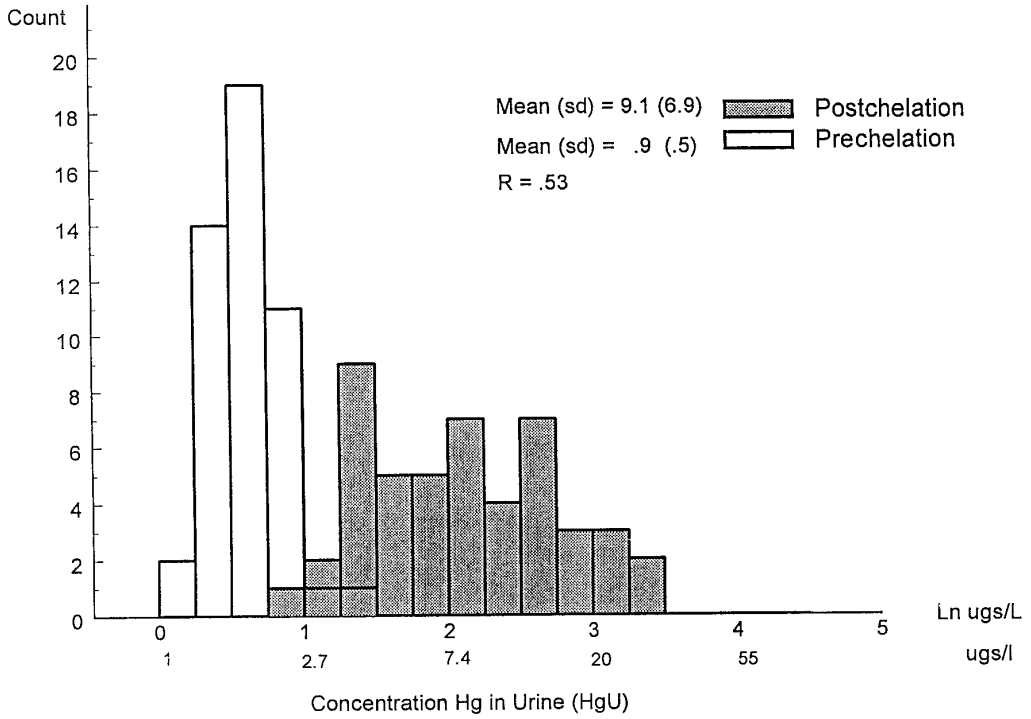

Figure 1. Histograms of pre- and postchelation urinary mercury levels (HgU).

amalgam fillings in one's mouth. All personnel wore gloves.

Pre- and postchelation HgU levels result in distinct patterns of preclinical effects, providing evidence of associations with recent exposure and chronic body burden

The patterns of association between symptoms, mood, motor function, and cognition with pre- and postchelation urinary Hg measures were distinct. Standardized beta coefficients for pre- and postchelation urinary Hg levels showed that all four domains were associated with Hg^o exposure (**Fig. 2**). However, an overview of standardized betas indicates that the relative sensitivity of the two urinary measures differed considerably for individual tests.

Persistent symptoms involving memory and headaches, lightheadedness, and dizziness were selectively associated with postchelation HgU (**Table 2**). In contrast, transient mood scores were associated with both pre-and postchelation HgU values, where the associations for all five mood scales were robust and uniformly associated with prechelation HgU (exceeding all other test scores).

The motor function results showed that finger tapping is also more strongly associated with prechelation HgU, but has a lesser statistically significant association with Hg body burden. Hand steadiness had mixed associations with pre- and postchelation HgU levels, but differences in the strength of the association between recent exposure and Hg body burden were less pronounced (beta=0.39 vs. beta=0.30, respectively). Resting tremor, a clinical measure frequently found affected in cases of severe neurodegenerative disease, was not affected as anticipated.

In contrast, cognitive function as measured by switching attention, trailmaking A and B, and visual retention memory was selectively associated with prechelation HgU. One exception to this trend was the number correct in the Word Recognition Memory Test, the only test dependent on words and memory, which was associated with postchelation HgU. Symbol-digit response time was not associated with either measure of exposure to Hg.

The log-linear relationships throughout the range of postchelation HgU values are illustrated by finger tapping (right/left/alternate number of taps) and the total number of symptoms associated with postchelation HgU (**Fig. 3**). No evidence of a threshold of effect appeared across the body of our results; relationships were smooth and generally conformed to a log-linear trend (i.e., there was no evidence of subpopulation clustering).

DISCUSSION

This is the first behavioral study to distinguish recent Hg exposure from Hg body burden when examining subtle changes in preclinical behavior associated with very low levels of Hg^o exposure. The results are striking in that statistically significant dose-effect relationships were found with prechelation HgU (ranging

TABLE 1. *Work-related and personal factors associated with prechelation HgU*[a]

Factors	Mean	SD	b_{preHgU}	SE	β
Postchelation HgU	9.1	6.9	0.16	0.04	0.43***
No. of amalgams placed/wk	16.1	8.2	0.01	0.00	0.34***
Do not wear a mask (7/49)	15%		0.38	0.12	0.32***
No. of amalgams in own mouth	1.6	0.8	0.08	0.03	0.25**

[a] The mean and standard deviation for each factor in the model is accompanied by the regression coefficient for prechelation HgU, the standard error, the standardized Beta coefficient that presents the unique partial correlation for each factor in the model, and the level of significance. Even controlling for Hg body burden does not eliminate the importance of associations between recent exposure, current work-related factors, and the number of amalgams in one's mouth. *** $P < .005$, ** $P < .05$; full model $R^{21} = 0.61$.

from 0 to 4 μg/l) and postchelation HgU (ranging from 1 to 32 μg Hg/l). These prechelation HgU concentrations were previously thought to be trivial in terms of potential health risks. The modest correlation between pre- and postchelation HgU measures (6) supports the view that the Hg present in body tissues may contribute to apparent associations with recent exposure, as measured by prechelation HgU.

Similar increases in symptoms (12–23), alterations in mood (19–24), reduction in speed and accuracy in motor function (20, 21, 24, 30), and subtle losses in memory and visuospatial cognitive skills (19–23) have been reported in studies of dental professionals (highly consistent with our present findings). However, mean urinary Hg levels among subjects in these studies was higher (>20 μg/l) than found among

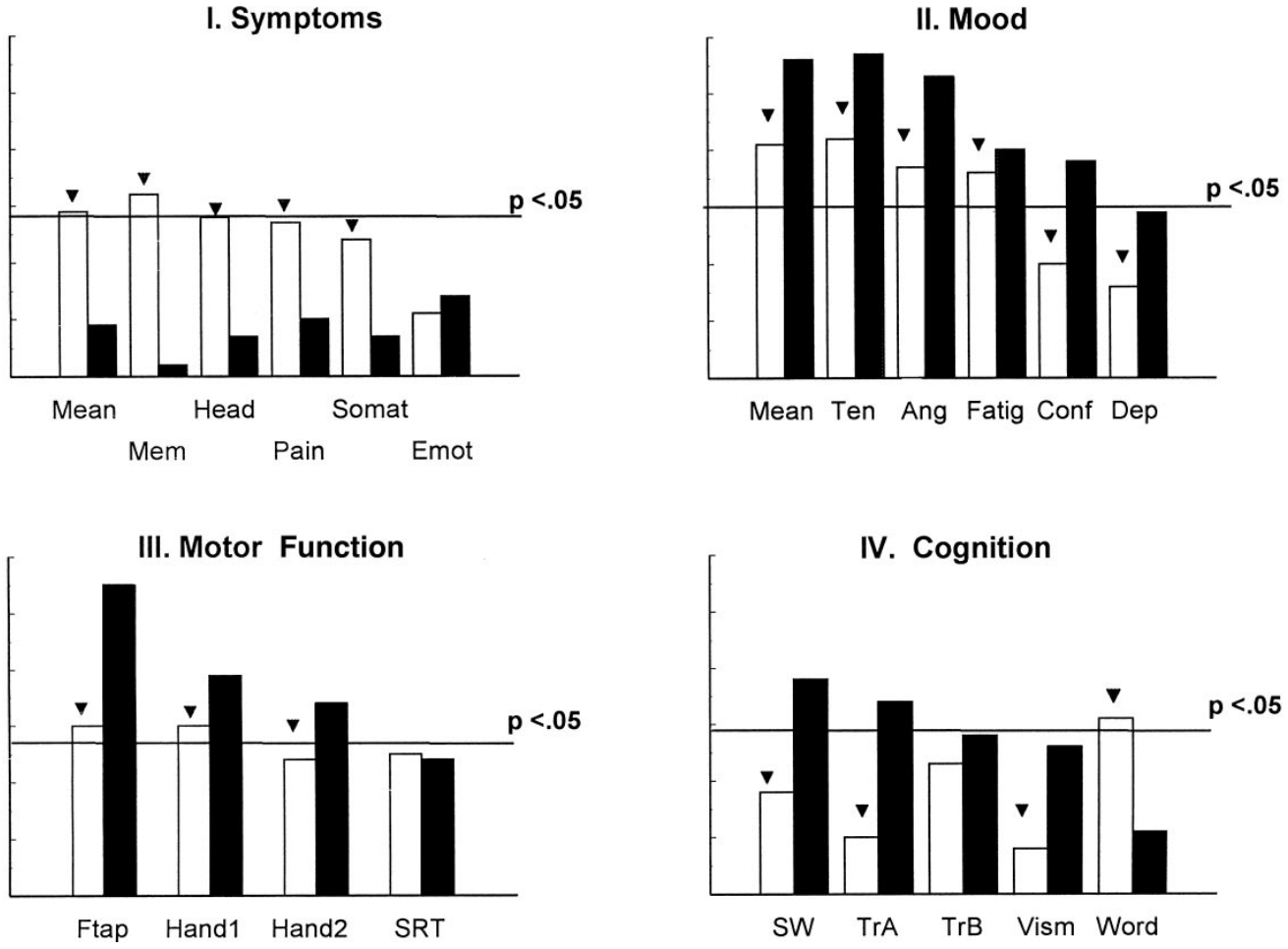

Figure 2. A comparison of standardized beta coefficients across regression models (a partial correlation coefficient) for pre- and postchelation HgU levels in regression models evaluating symptoms, mood, motor function, and cognition. Statistical differences between the two urinary measures are noted by (▼) where the dark bars represent postchelation HgU levels; the horizonal line reflects the significance level at a $P < 0.05$ for each association for both pre- and postchelation HgU levels (n=48).

TABLE 2. *Associations for pre- and postchelation urinary mercury (HgU) values with symptoms, mood, motor function, and cognition*[a]

	Prechelation HgU					Postchelation HgU		
Test variable	Mean	SD	b_{inHg}	SE	β	b_{inHg}	SE	β
Symptoms								
Mean number of symptoms/subject	0.30	0.13	2.44	4.37	0.09	0.005	0.03	0.29*
Memory	1.39	1.22	2.59	19.52	0.02	14.63	6.91	0.32**
Headaches, lightheaded, dizzy	2.39	1.33	7.12	15.27	0.07	10.88	5.46	0.28**
Mood (POMS)								
Total mood	11.65	4.82	−11.21	2.80	−0.56***	−3.07	1.15	−0.41**
Tension	3.04	1.40	−3.33	0.83	−0.57***	−0.91	0.34	−0.42**
Anger	2.08	0.92	−1.97	0.52	−0.53***	−0.51	0.21	−0.37**
Fatigue	2.15	2.15	−3.57	1.14	−0.40**	−1.18	0.44	−0.36**
Confusion	0.12	0.33	−1.19	0.45	−0.38**	−0.23	0.20	−0.20
Depression	2.02	0.98	−1.14	0.63	−0.29*	−0.24	0.24	−0.16
Motor								
Finger taps[+] (right/left/alternate)	61.1	9.1	−2.66	0.71	−0.55***	−0.49	0.27	−0.30*
Hand steadiness[+,L] (7, 8, 9 hole/s)_1	5.80	13.70	1.08	0.41	0.39**	0.28	0.15	0.30*
Hand steadiness[+,L] (7, 8, 9 hole/s)_2	5.80	13.70	1.15	0.54	0.34**	0.27	0.19	0.24
Simple reaction time[+,L] (R/L s)	0.355	0.039	1.07	0.70	0.24	0.38	0.23	0.25*
Resting tremor[L] (center frequency)	6.98	0.97	0.31	0.29	0.25	8.19	8.85	0.26
One-hole pins (pins/min)	34	6	−0.001	0.17	−0.01	−0.27	1.75	−0.03
Cognition								
Visual processing/attention								
Switching attention[L] (ms)	0.792	0.198	1.58	0.76	0.38**	0.17	0.30	0.18
Trails A (s)	26.20	7.60	10.13	4.56	0.34**	1.14	1.93	0.10
Trails B (s)	66.10	22.32	25.99	15.22	0.28*	7.99	5.84	0.23
Visual retention test[L] (number correct)	5.30	1.50	0.27	0.13	0.26**	0.03	0.05	0.08
Symbol-digit time[L] (s)	22.60	4.20	0.11	0.09	0.16	0.01	0.03	0.03
Verbal processing/attention								
Word memory (number correct)	47.60	2.90	−1.37	1.96	−0.11	−1.38	0.71	−0.31*

[a] Symptom, mood, and motor function regression models include vocabulary, age, gender, race, alcohol, and wearing of glasses; cognitive regression models include simple reaction time (dominant hand), vocabulary, age, gender, race, alcohol, and wearing of glasses. [+] Factored scores; [L] log transformed. * $P < 0.10$, ** $P < 0.05$, *** $P < 0.01$.

subjects with comparable preclinical effects reported here. Likewise, comparable CNS effects have been consistently reported among subjects with urinary Hg levels above 50 μg/l (1, 11). These studies collectively support our prechelation HgU behavioral findings in each domain and point to a potential log-linear continuum of health effects from low to high Hg exposure.

However, questions remain unanswered concerning the lower threshold of Hg^o exposure for behavioral effects, as we found no indication of a lower boundary in any of our subjective or objective results (see Fig. 3). This inability to detect a threshold exposure level strengthens the hypothesis that subtle preclinical effects found at very low levels of Hg^o exposure appear on a continuum with the far more severe clinical deficits. Of equal importance, we also found no evidence of special susceptibility within a subset of this dental population. Behavioral responses typically increased with exposure in a fairly uniform manner, indicating a more general response, as illustrated in Fig. 3.

The patterns of subjective responses associated with HgU differed. Persistent symptoms that appear over a year were selectively associated with Hg body burden; this finding suggests that symptoms may remain undetected in evaluations that rely solely on prechelation urinary measures of Hg^o exposure. In contrast, the more transient nature of the POMS was found to be more strongly associated with recent exposure, with a smaller but statistically significant contribution from Hg body burden. This pattern suggests that prechelation HgU levels, which are partially dependent on the 2-month half-life, are more strongly associated with mood; one may speculate that the amount of Hg stored as body burden is less associated, as it may be biologically less available to the CNS.

This study comprehensively assessed fine manual speed, accuracy, and coordination, measures of particular relevance for dental professionals who work with handheld tools. Among the five motor function tests, individual and factored performance scores for finger tapping and hand steadiness were also associated with recent exposure, as measured by prechelation HgU. The standardized beta of −0.55 for finger tapping was comparable to a beta of −0.56 for total mood, indicating a relatively strong association. Similar to the results for mood, performance on both motor tests also had smaller but detectable associa-

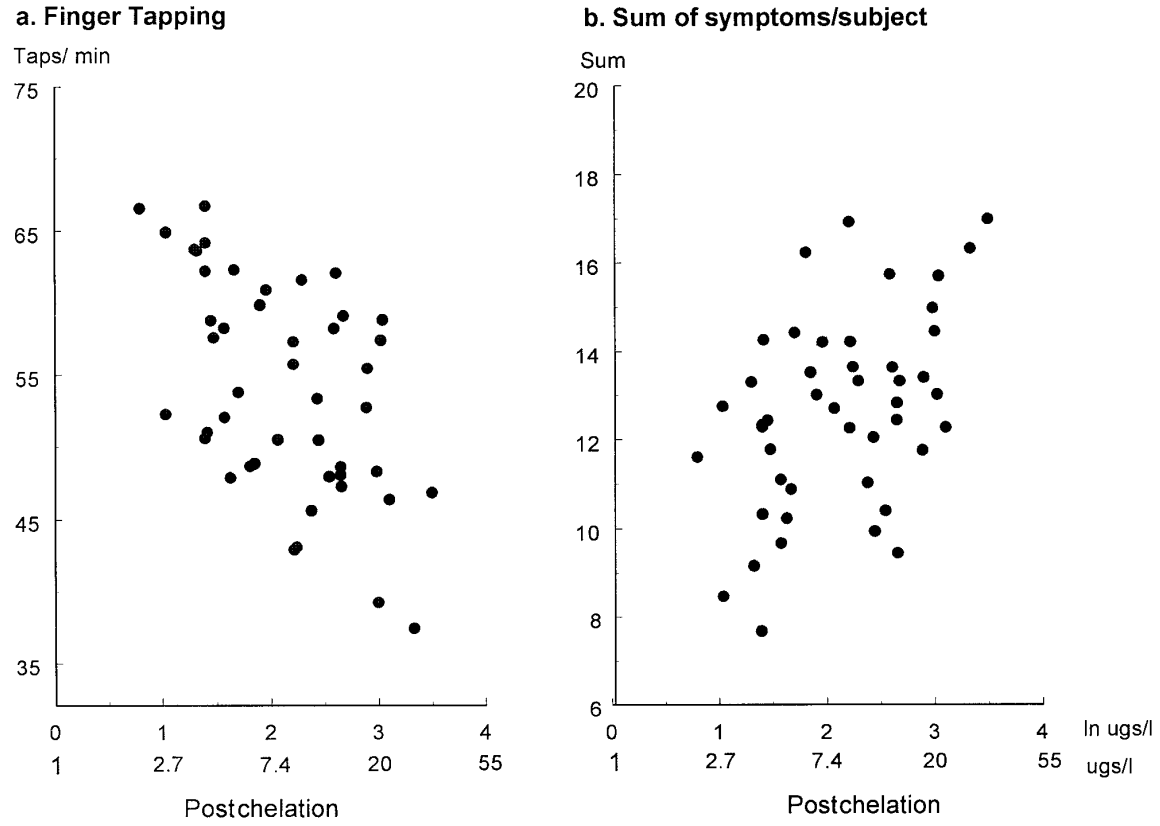

Figure 3. Exposure-effect relationships between postchelation HgU levels and the number of taps/min for finger tapping (*a*) and the sum of symptoms reported per subject (*b*).

tions with Hg body burden. These results suggest that distinctions between recent and long-term exposure could prove even more relevant at higher levels of exposure.

Unlike the other three behavioral domains, the cognitive regression models included the log SRT of the nondominant hand to control for potential misinterpretation associated with slowed motor response. Only one association was observed between Hg body burden and the number correct for word recognition memory, a measure of verbal memory. Associations with recent exposure were found for switching attention, a measure of selective attention; trailmaking parts A and B, measures of psychomotor speed and cognitive flexibility; and the Visual Retention Memory Test, a measure of visual memory. The subtle impairments across this set of cognitive tests suggest diffuse nonspecific alterations in task performance that are potentially associated with aspects of attention. These cognitive effects were selectively associated with recent exposure, with no detectable contribution from Hg body burden, whereas verbal memory was associated only with long-term exposure or Hg body burden, as measured by postchelation Hg levels.

The observed pattern of statistically significant results for prechelation HgU had coefficients that were relatively strong for POMS mood scores (median beta=0.45, range 0.29–0.56) and moderate for the more objective domains of motor function (median beta=0.42, range=0.30–0.55) and cognitive tasks (median beta=0.31, range=0.26–0.38) (see Table 2). Associations with postchelation HgU showed the standardized beta coefficients in each domain were also moderate for symptoms (median=0.31, range 0.28–0.32), relatively strong for mood though less pronounced than the prechelation coefficients for mood (median=0.40, range 0.36–0.42); moderate for motor function (median=0.30, range=0.25–0.30; and sparse but moderate for one cognitive task (beta=0.31). One interpretation of these variations in strength of associations between pre- and postchelation HgU is that urinary Hg from recent exposure is more likely to be available to the CNS and should be more strongly associated with preclinical effects, with less contribution from stored Hg as body burden.

Good occupational work practices further reduce urinary Hg levels given the behavioral effects associated with low levels of HgU (see Table 1). The variation of HgU in a national sample of 6925 dentists participating in the American Dental Association Health Screening Program was related to the variation in air Hg levels, and industrial hygiene surveys among dental offices show that 6–16% of dental practices exceed exposure levels permissible by OSHA

(37). This reinforces the need to comply with industrial hygiene guidelines in dental offices.

The distribution of HgU in the national HgU sample had a range of 0 to 104 μg/l and was not distributed normally: 10% had levels >10.4 μg/l, 3% had levels >18.8 μg/l, 2.5% had levels >20.4 μg/l, and 1% had levels >33.4 μg/l. In this study, our mean prechelation urinary Hg concentration of 0.94 μg/l (SD=0.50) corresponds to the lowest 10th percentile of dentists in the United States. Further, as noted earlier, general population HgU levels (P. Factor, personal communication; ref 2) overlap with these occupational levels, supporting a public health concern for very low-level Hg^o toxicity.

Concern for very low-level Hg^o toxicity is supported by our observations of associations at HgU levels well below the proposed biological standard of 25 μg/l (16, 17) and below urinary levels that would be expected at the OSHA permissible exposure limit of 50 μg Hg^o/m^3 in air (48). The low Hg^o exposures between 0 and 4 μg/l were partially attributable to the number of Hg amalgam fillings in the dental group (as seen in Table 1). The apparent association between HgU and personal risk factors argues for future studies to examine the potential for similar adverse effects in the general population from Hg amalgam fillings. Some might argue that the present findings have immediate implications regarding the continued use of Hg amalgam in dental restorations (49). We are divided on this issue, inasmuch as there are currently unanswered toxicological questions regarding chronic Hg body burden, dose-rate, and potential differences in modality of exposure derived from amalgam restorations alone. Two clinical trials regarding the safety of dental amalgams among children, a potentially more susceptible population, will not be completed for several years (50). Nevertheless, it is clear from the present study that comparing associations with pre- and postchelation urinary Hg levels revealed patterns of previously unobserved effects. These would not have been identified if they had been evaluated in relation to the traditional prechelation urinary Hg levels alone. Thus, the DMPS chelation technique enhances interpretation of observed associations with low-level cumulative Hg^o exposure.

In conclusion, by using an approach that distinguishes recent Hg exposure from Hg body burden, we have observed subtle associations between Hg and symptoms, mood, motor function, and nonspecific cognitive alterations in task performance in an occupationally exposed group with HgU levels comparable to the general U.S. population. Application of this approach may be particularly useful in defining thresholds of Hg^o toxicity and for establishing safe limits of exposure to mercury from dental amalgam material, the restoration itself, diet, and other sources. FJ

Supported by National Institutes of Health grants DE11712, ES04696, ES04940, and by the Wallace Research Foundation. Support was also provided by the University of Washington Center for Ecogenetics and Environmental Health (P30 ES07033). We thank Heyl Co., Berlin, Germany, for the gift of DIMAVAL® (DMPS).

REFERENCES

1. Clarkson, T. W. (1989) Mercury. *J. Am. Col. Toxicol.* **8**, 1291–1296
2. Kingman, A. (1994) Correlations between urinary mercury concentrations and amalgam exposure among NIDR Amalgam Study participants. *J. Dent. Res.* Abstract #1644, IADR, Seattle, Washington
3. Aaseth, J., Jacobsen, D., Anderson, O., and Wickstrom, E. (1995) Treatment of mercury and lead poisoning with dimercaptosuccinic acid and sodium dimercaptopropanesulfonate: a review. Analyst **120**, 853–854
4. Aposhian, H. V., Gonzalez-Ramirez, D., Maiorino, R. M., Zuniga-Charles, M., Xu, Z. F., Hurlbut, K. M., Junco-Munoz, P., Aposhian, M. M., and Dart, R. C. (1995) Mobilization of heavy metals by newer, therapeutically useful chelating agents. *Toxicology* **97**, 23–38
5. Aposhian, M. M., Maiorino, R. M., Xu, Z., and Aposhian, H. V. (1995) Sodium 2,3-dimercapto-1-propanesulfonate (DMPS) treatment does not redistribute lead or mercury to the brain of rat. *Toxicology* **97**, 23
6. Halbach, S., and Clarkson, T. W. (1978) Enzymatic oxidation of mercury vapor by erythrocytes. *Biochem. Biophys. Acta* **523**, 522–531
7. Magos, L., Clarkson, T. W., and Hudson, A. R. (1989) The effects of dose of elemental mercury and first-pass circulation time on exhalation and organ distribution of inorganic mercury in rats. *Biochem. Biophys. Acta* **99**, 85–89
8. Henderson, R., Shotwell, H. P., and Krause, L. A. (1974) Analysis for total, ionic, and elemental mercury in urine as a basis for biological standard. *Am. Ind. Hyg. Assoc. J.* **38**, 576–580
9. Hursh, J. B., Cherian, M. G., Clarkson, T. W., Vostal, J. L., and Vander Mallie, R. (1976) Clearance of mercury (Hg-197, Hg-203) vapor inhaled by human subjects. *Arch. Environ. Health* **Nov./Dec.**, 302–309
10. Zalups, R. K. (1993) Influence of 2,3-dimercatopropane-1-sulfonate (DMPS) and meso-2,3-dimercaptosuccinic acid (DMSA) on the renal disposition of mercury in normal and uninephrectomized rats exposed to inorganic mercury. *J. Pharmacol. Exp. Ther.* **267**, 791–800
11. Gerstner, H. B., and Huff, J. E. (1977) Selected case histories and epidemiologic examples of human mercury poisoning. *Clin. Toxicol.* **11**, 131–150
12. Albers, J. W., Kallenbach, L. R., Fine, L. J., Langolf, G. D., Wolfe, R. A., Donofrio, P. D., Alessi, A. G., Stolp-Smith, K. A., Bromberg, M. B., and the Mercury Workers Study Group (1988) Neurological abnormalities associated with remote occupational elemental mercury exposure. *Ann. Neurol.* **24**, 651–659
13. Piikivi, L., and Hänninen, H. (1981) Psychological performance and long-term exposure to mercury vapor. In *Proceedings of the Second Finnish-Estonian Symposium on Early Effects of Toxic Substances* (Hernberg, S., and Kahn, H., eds) pp.165–169, Institute of Occupational Health, Helsinki
14. Camerino, D., Cassitto, M. G., Desideri, E., and Angotzi, G. (1981) Behavior of some psychological parameters in a population of a Hg extraction plant. *Clin. Toxicol.* **18**, 1299–1309
15. Fawer R. F., de Ribaupierre Y., Guillemin M. P., Berode M., and Lob, M. (1983) Hand tremor measurements: Methodology and applications. In *Advances in the Biosciences* (Gilioli, R., ed) Vol. 45, Neurobehavioral Methods in Occupational Health, pp. 137–144, Program Press
16. Roels, H., Gennart, J. P., Lauwerys, R., Buchet, J. P., Malchaire, J., and Bernard, A. (1985) Surveillance of workers exposed to mercury vapour: validation of a previously proposed biological threshold limit value for mercury concentration in urine. *Am. J. Ind. Med.* **7**, 45–71
17. Soleo, L., Urbano, M. L., Petrera, V., and Ambrosi, L. (1990) Effects of low exposure to inorganic mercury on psychological performance. *Br. J. Ind. Med.* **47**, 105–109

18. Piikivi, L., Hänninen, H., Martelin, T., and Mantere, P. (1984) Psychological performance and long-term exposure to mercury vapors. *Scand. J. Work Environ. Health* **10**, 35–41
19. Ngim, C. H., Foo, S. C., Boey, K. W., and Jeyaratnam, J. (1992) Chronic neurobehavioral effects of elemental mercury in dentists. *Br. J. Indust. Med.* **49**, 782–790
20. Shapiro, I. M., Cornblath, D. R., Sumner, A. J., Uzzell, B., Spitz, L. K., Ship, I. I., and Bloch, P. (1982) Neurophysiological and neuropsychological function in mercury-exposed dentists. *Lancet* **1**, 1147–1155
21. Uzzell, B. P., and Oler, J. (1986) Chronic low-level mercury exposure and neuropsychological functioning. *J. Clin. Exp. Neuropsych.* **8**, 581–593
22. Echeverria, D., Heyer, N. J., Martin, M. D., Naleway, C. A., Woods, J. S., and Bittner, A. C. (1995) Behavioral effects of low-level exposure to Hg^o among dentists. *Neurotoxicol. Teratol.* **17**, 161–168
23. Gonzales-Ramirez D., Mairino, R. M., Zuniga-Charles, M. Z., Hurlbut, K. M., Junco-Munoz, P., Aposhian, M., Dart, R. C., Gama, J. H. D., Echeverria, D., Woods, J. S., and Aposhian H. V. (1995) Sodium 2,3-dimercaptopropane-1-sulfonate challenge test for mercury in humans. II. Urinary mercury, porphyrins, and neurobehavioral changes of dental workers in Monterrey, Mexico. *J. Pharmacol. Exp. Ther.* **272**, 264–274
24. Bueller, K. (1994) Motor Effects of Low-level Hg^o Exposure in Dentists. Master's Thesis, University of Washington, Seattle
25. McNair, D. M., Lorr, M., and Droppleman, L. F. (1971) Edits Manual Profile on Mood States. Educational and Industrial Testing Service, San Diego
26. Smith, R. G., Vorwald, A. J., Patil, L. S., and Mooney, T. F. (1970) Effects of exposure to mercury in the manufacture of chlorine. *Am. Ind. Hyg. Assoc. J.* **31**, 687–700
27. Langolf, G. D., Chaffin, D. B., Henderson, R., and Whittle, H. P. (1978) Evaluation of workers exposed to elemental mercury using quantitative tests of tremor and neuromuscular functions. *Am. Ind. Hyg. Assoc. J.* **39**, 976–884
28. Roels H., Lauwerys, R., Buchet, J.P., Bernard, A., Barthels, A., Oversteyns, M., and Gaussin, J. (1982) Comparison of renal function and psychomotor performance in workers exposed to elemental mercury. *Int. Arch. Occup. Environ. Health* **50**, 77–93
29. Williamson A. M., Teo, R. K., and Sanderson J. (1982) Occupational mercury exposure and its consequences for behavior. *Int. Arch. Occup. Environ. Health* **50**, 273–286
30. Bittner A. C. Jr., Echeverria, D., Woods, J. S., Aposhian H. V. Naleway C., Martin, M. D., Mahurin, R. K., Heyer, N. J., Cianciola, M. (1998) Behavioral effects of low-level exposure to hg among dental professionals: a cross-study evaluation of psychomotor effects. *Neurotoxicol. Teratol.* In press
31. Piikivi, L., and Hanninen, H. (1989). Subjective symptoms and psychological performance of chlor-alkali workers. *Scand. J. Work Environ. Health* **15**, 69–74
32. Schuckmann, F. (1979) Study of preclinical changes in workers exposed to inorganic mercury in chloralkali plants. *Int. Arch. Occup. Environ. Health* **44**, 193–200
33. Langworth, S., Almkvist, O., Soderman, E., and Wikstrom, B. O. (1992) Effects of occupational exposure to mercury vapour on the central nervous system. *Br. J. Indust. Med.* **49**, 545–555
34. Echeverria, D., Fine, L., Langolf, G., Schork, A., and Sampaio, C. (1989) Acute neurobehavioral effects of toluene. *Br. J. Indust. Med.* **46**, 483–495
35. World Health Organization (1986) Operational Guide for the WHO Neurobehavioral Core Test Battery. Draft 25.5, Office of Occupational Health, Geneva
36. Agency for Toxic Substances and Disease Registry (ATSDR) (1995) Adult Environmental Neurobehavioral Test Battery: Research Needs ATSDR, U.S. DHHS, PHS, pp. 698–711
37. Martin, M., Naleway, C., McCann, T., and Leroux, B. (1995) Factors contributing to mercury exposure in dentists. *J. Am. Dent. Assoc.* **126**, 1502
38. Letz R., and Baker E. (April 10, 1985) Neurobehavioral Evaluation System (NES) User's Manual (Version 3.11)
39. Hand Motor Steadiness Battery. The Lafayette Instrument Company, Purdue, Indiana
40. Baker, E. L., Letz, R. E., Fidler, A. T., Shalat, S., Plantamura, D. L., and Lyndon, M. L. (1985) A computer-based neurobehavioral evaluation system for occupational and environmental epidemiology: methodology and validation studies. *Neurotoxicol. Teratol.* **7**, 369–377
41. Tremor Analysis Test System. (1995) The Danish Product Development, Denmark
42. Salvendy, G. (1975) Selection of industrial operators: the one hole test. *Int. J. Prod. Res.* **13**, 303–321
43. Recognition Memory Test by Elizabeth K. Warrington. Western Psychological Services, Los Angeles, California
44. Reitan, R. M. (1986) *Neuropsychological Assessment of Neuropsychiatric Disorders*, I. (Grant, , and Adams, K., eds) Oxford University Press, New York
45. Toutonghi, G., Echeverria, D., Bittner, A., and Ronhovde, N. (1991) The use of switching ability to assess central nervous system function. In Advances in Industrial Ergonomics and Safety III (Karwowski, W., and Yates, J. W., eds) London: Taylor and Francis Inc., pp. 629–636
46. SPSS-X, SPSS Inc., 1993
47. Anderson, T. W. (1958) *Introduction to Multivariate Statistical Analysis*, John Wiley and Sons, New York.
48. Part III, Department of Labor. (1989) Occupational Safety and Health Administration OSHA 29 CFR Part 1910, Air Contaminants: Final Rule. *Federal Register* **54**, 2415–2416
49. Dental Amalgam: A Scientific Review and Recommended Public Health Service Strategy for Research, Education and Regulation. (1993) Final Report of the Subcommittee on Risk Management of the Committee to Coordinate Environmental Health and Related Programs Public Health Service, Department of Health and Human Services, USPHS
50. DeRuen, T. (PI) ''The Casa Pia Study of Dental Amalgam,'' University of Washington, and Crawford, S. (PI) ''Health Effects of Dental Amalgam in Children,'' New England Research Institute; two clinical trials supported by the National Institute of Dental Research

Received for publication December 1, 1997.
Revised for publication March 27, 1998.