An X-ray Fluorescence Technique to Measure In Situ the Heavy Metal Burdens of Persons Exposed to These Elements in the Workplace

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Assays of hair and body fluid concentrations may be valuable measurements of acute exposure to a heavy metal, but they do not provide insight into the total heavy metal intake when the intake is low and chronic. The use of an x-ray fluorescence technique (XRF) enables measurement of the long-term retention of various heavy metals in select tissues in vivo. XRF was used to measure the mercury content of head and bone tissue in 298 dentists with long-term exposure to mercury-containing amalgams. It was also used to evaluate the lead burden of persons suspected of having elevated lead exposure at the workplace, and to assay the lead levels in urban and rural children. These studies indicated that the x-ray fluorescence method of assaying heavy metals in vivo is noninvasive, safe, rapid, and sensitive to levels of many heavy metals that accumulate in human tissues.

For over 200 years, exposure to elements such as lead, mercury, and arsenic has been recognized as causing overt clinical disease. In most cases, the disease state was associated with high levels of exposure. At low exposure levels, recent epidemiological, surveys indicate that for many occupational groups, the disease appears to be "silent" or asymptomatic. However, when the health of asymptomatic individuals is followed for long time periods, evidence of dysfunction of various organ systems is observed. For example, chronic plumbism, due to low levels of airborne lead, can cause discernible changes in function of renal, hematological, peripheral, and central nervous systems.[1,2] The clinical changes, however subtle, were frequently expressed as irritability, fatigue, skeletal pain, colic, headache, depression, and apathy.[3]

Clinical diagnosis of disease due to low level exposure to toxic metals is fraught with difficulties in the presence of other confounding health and social factors. A priori, it is necessary to have an accurate assessment of the metal burden and to be able to relate these levels to organ function. At present, it is difficult to evaluate both of these parameters with precision. Because of measurement difficulties, the metal burden is often deduced indirectly using indices of exposure. For example, the concentration of lead in blood is generally accepted as the direct index of lead body burden. However, in common with many other toxic elements, the lead concentration in the blood depends on a number of variables that include time since metal exposure, as well as mode of intake, excretion, and sequestration by different tissues. Considering these variables, the lead blood level measures recent exposure because the presence of lead in blood is transitory and declines once exposure ceases. Because such limitations exist, a number of other approaches have been developed to assess lead intake. The lead burden has been evaluated indirectly by measuring lead-sensitive metabolic intermediates associated with hemoglobin synthesis and degradation, such as free erythroporphyrins and zinc protoporphyrins.[4,5] Although these determinations have proven to be useful clinical guides to exposure, they are still a measure of recent lead intake.

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To overcome the inherent limitations of these analyses, an x-ray fluorescence (XRF) technique has been developed to measure heavy metal concentrations in teeth, bones, and soft tissue in situ.[6-8] The XRF technique permits simultaneous evaluation of the tissue burden of a number of different heavy metals. Assaying of multiple heavy metals in situ is important especially for persons who may be exposed to a range of toxic elements in the workplace. Moreover, the deleterious health effects due to exposure to a number of toxic metals are likely to be greater than those produced by exposure to a single element. To study the clinical manifestations of this type of synergism, data must be collected on body burdens of heavy metals in situ.

The noninvasive XRF procedure requires a minimal x-ray exposure approximately equivalent to one-tenth of a dental radiograph to a small 1 to 2 cm^3 volume. In addition, the technique is sensitive to the levels of elements found in tissues of exposed workers. Moreover, it can be used to measure the metal burden in a number of target tissues. In this study, the XRF technique has been used to ascertain (1) the mercury levels in the head and wrist of nearly 300 dentists and 200 dental auxiliaries who have been exposed to mercury through the preparation of dental amalgam; (2) the lead concentrations in teeth of 500 children living in urban and rural environments; (3) the lead values of 30 children referred from the Children's Hospital of Philadelphia' Poison Control Center; and (4) the lead content in various tissues in industrial workers suspected of having elevated lead intake. Prior to discussing these findings, the XRF method will be described. Note that the approach described for the assay of lead in different tissues in situ can be used for the measurement of any other common heavy toxic element.

X-ray Fluorescence Technique

X-ray fluorescence can occur when an inner shell electron vacancy is created in an atom. The vacant orbital electron shell is filled by an outer orbital electron with the emission of an x-ray with an energy value corresponding to the difference in binding energies of the inner and outer shell electrons. This energy difference is a signature of the element. For lead, the filling of a K shell vacancy by an L shell electron results in two characteristic x-ray lines at 72.0 ($K_{\alpha 2}$) and 75.0 ($K_{\alpha 1}$) keV. Fig. 1 shows the scattered radiation spectrum in the energy interval from 25 to 31 and 68 to 76 keV, for 3 mL of water and for the same volume of water containing 500 ppm of mercury, lead, and iodine. The 122 and 136 keV γ rays from a Co-57 source are used to excite the K shell electrons of these elements. The spectra show the separation in the energy of the x-ray fluorescence from these elements. The figure also shows the scattered x-ray spectrum from photons that have undergone Compton scattering events in water.

The XRF system used for the in vivo assay of metals consists of an intrinsic germanium diode detector (Princeton Gamma Tech Model 1G110-7) with a cross-sectional area of 110 mm^2 and a thickness of 5 mm. The output pulses from the germanium detector are amplified with a Princeton Gamma Tech Model 34 amplifier

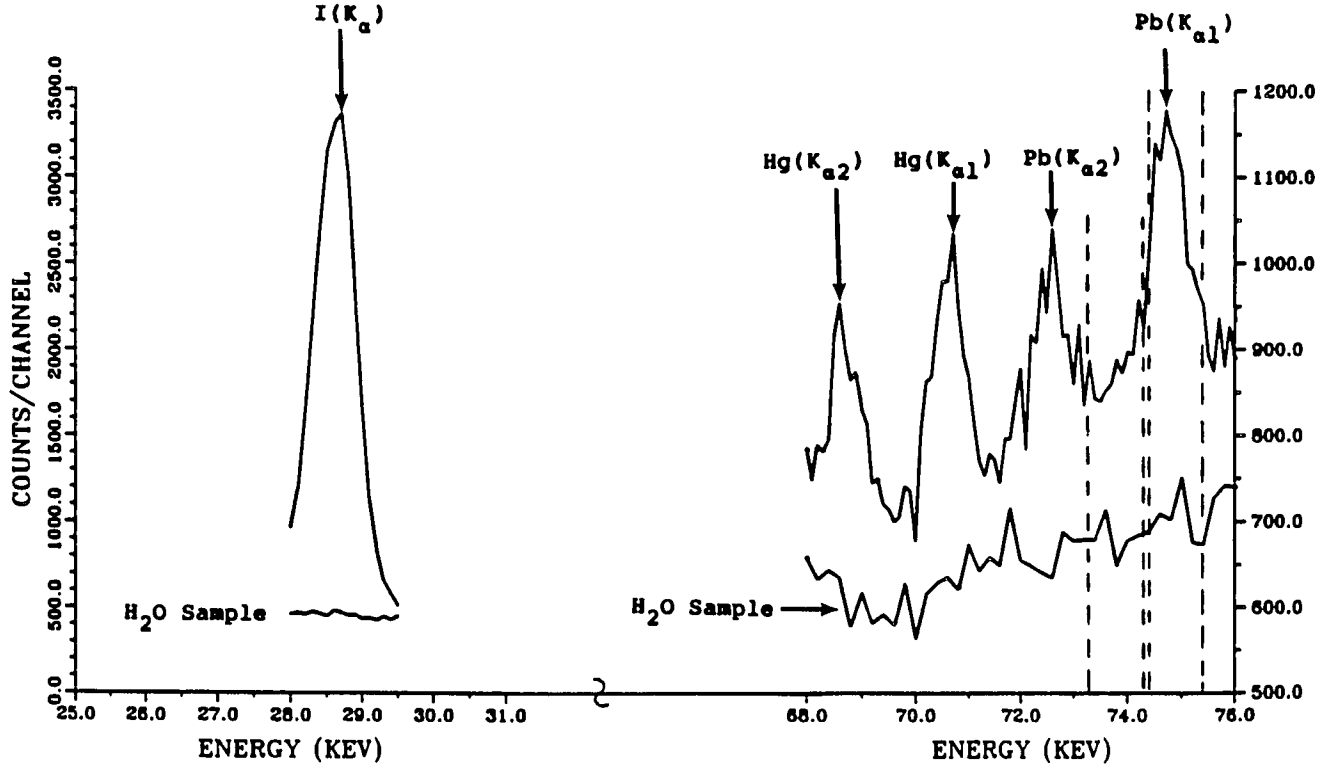

Fig. 1. X-ray fluorescence spectrum from 3 mL of water and same volume of solution containing 500 ppm of iodine, mercury, and lead.

and sorted with a Tracor Northern 4096 channel pulse height analyzer. A sealed 10-mCi Co-57 point source (Amersham Searle Model X.130.5), used to excite the K-shell electron in lead, is housed in a cylindrical tantalum shield 16 mm in diameter and 15 mm long. A borehold 4 mm in diameter and 6 mm deep in the tantalum shield serves to collimate the γ ray source. At 2 cm from the source, the radiation beam is 12 mm in diameter.

In the energy interval 74.5 to 75.5 keV, events are recorded that result from characteristic x-rays from the lead together with Compton scattered x-rays from the water. The number of scattered x-rays recorded in the fluorescence peak of lead is estimated by simultaneously recording the number of events in an adjusted energy interval of 73.3 to 74.3 keV.

The lead concentration in a sample is calculated from the measured number of events in the two energy intervals using signal-to-noise ratio analysis. The signal-to-noise ratio, s/n, is defined as

$$s/n = \frac{(N_f + N'_c) - N_c}{N_c} \cong \frac{N_f}{N_c} \qquad (1)$$

where N_f is the number of fluorescence x-rays recorded in the energy interval 74.5 to 75.5 keV, N'_c is the number of Compton scattered x-rays recorded in the same energy interval, and N_c is the number of events recorded in the energy interval 73.3 to 74.3 keV. Because N'_c is nearly the same as N_c, the signal-to-noise ratio represents the number of lead fluorescence x-rays (signal) to the number of Compton scattered x-rays (noise). The concentration of lead, C, in a sample in micrograms/gram is obtained using the following expression:

$$C = K[(s/n) - (s/n_o)] \qquad (2)$$

where K is the calibration constant, (s/n) is the signal-to-noise ratio from a sample containing unknown amounts of lead, and (s/n_o) is the signal-to-noise ratio from a sample containing no lead. By experiment, the measured signal-to-noise ratio was found to vary linearly with the lead content for aqueous samples containing 0 to 200 μg/g of lead. The calibration constant K in equation 2 is obtained from the slope of the measured signal-to-noise ratio v concentration, and $(s/n)_o$ is the intercept.

To compare XRF with the chemical method using atomic absorption spectrophotometry (AAS), assays of mercury were performed on 1 to 5 g of postmortem tissue samples obtained from a dentist with 30 years of occupational exposure to amalgams containing mercury. Table 1 shows mercury concentration in various tissues measured by XRF and the AAS determinations. The data are presented here to demonstrate the close correlation between the two analytical procedures (r = .98). The in vitro XRF assay shown in the table took approximately 40 minutes of γ-ray exposure from a 10-mCi Co-57 source leading to approximately 1.2 million counts in the fluorescence window.

For determining heavy metals in vivo, the x-ray exposure from the Co-57 source must be minimized. For example, to measure metal concentration of 30 μg/g in situ, with a coefficient of variation of 10%, the number of events recorded in the noise window must be approximately 70,000. Typically, 60,000 to 70,000 counts are collected for an in vivo assay. With a 10-mCi Co-57 source, this number of counts is obtained from tissue in situ in approximately 2 minutes. The x-ray exposure during this time is approximately 50 mR. This level of exposure is approximately one-tenth the exposure required for a dental exam.

TABLE 1
Comparison of X-ray Fluorescent (XRF) and Atomic Absorption Spectrophotometry Analysis (AAS) of Mercury in Human Tissues

Tissue	AAS (μg/g)	XRF (μg/g)
Bone	7.4 ± 0.9	4.7 ± 0.69
Liver	21.4 ± 1.3	25.0 ± 3.1
Kidney	0.9 ± 0.4	<1.0
Lung	1.4 ± 0.2	1.8 ± 0.27
Spleen	3.7 ± 0.6	6.3 ± 0.82
Sternum	7.1 ± 0.8	7.2 ± 0.99

Mercury Exposure and Associated Health Deficits in Dental Profession

Mercury Burdens in Dentist

The XRF technique was used to evaluate the mercury concentration in the temporal region and the wrist of 298 dentists.[7] Fig. 2 shows that 70% of the dentists had mercury levels in the temporal region that were below the XRF detection limit (20 μg/g). Thirteen percent of the dentists had temporal mercury values in excess of 40 μg/g. In nearly all cases, the temporal mercury levels were higher than the wrist mercury levels. This finding probably reflects the difference in the metabolic activities and tissue composition at the two sites.

Urine and hair mercury levels were also measured in the dentists with detectable mercury determined with XRF. In most of the subjects studied, these levels were below those commonly associated with elevated exposure. Only two dentists had elevated mercury levels in their urine, and three had minimal levels in their hair. Moreover, those dentists with elevated urine mercury levels were not those with elevated hair mercury levels. Clearly, the XRF measurements were evaluating a different body pool of mercury than that found in urine and hair. From what is known of the metabolism of mercury, the mercury values in urine and hair probably represent the short-term exposure, whereas the XRF concentrations indicate longer term retention.

Mercury Burdens in Dental Auxiliaries

Mercury burdens were examined for 207 dental auxiliaries with a mean age of 34 years and who had been in dental practice for 10.7 ± 7.1 years. A total of 89% of the subjects had mercury burdens below the detection limit (20 μg/g) of the XRF technique. Six percent of

the auxiliaries had temporal mercury levels in excess of 40 μg/g. In this group, determination of the mercury level in urine and hair indicated low levels of current exposure. The mercury concentration in the temporal region was lower for the dental auxiliaries than for the dentists studied. This result might be explained by the greater number of years that the dentists (average age, 59) had handled the mercury-containing amalgams. The lower mercury concentration in the dental auxiliaries may also represent recent improvements in mercury hygiene.

Health Deficits Associated With High-Mercury Exposure to Dentists

Neurological Deficits. Peripheral nerve function in 23 dentists with high mercury levels and 22 dentists with no detectable mercury, who served as control subjects, was measured by standard electrodiagnostic methods.[3] The neurological evaluation was done without knowledge of the XRF assay. Significant differences were noted between the high-mercury and low-mercury (control) group with respect to the mean sural sensory and median motor conduction velocities, the median motor distal latency, and the median F-wave latency. When values obtained from the individual dentists were compared with those from the laboratory control group, further abnormalities were seen. For example, Table 2 shows that five dentists in the group with high mercury levels had electrophysiological abnormalities consistent with the carpal tunnel syndrome (CTS). Patients with the syndrome exhibit a median motor distal latency greater than 4.6 ms and/or slowed median sensory conduction across the wrist, but normal median motor conduction in the forearm. The CTS is not unusual in persons pursuing a manual occupation. It has been reported to be present in 2 of 19 dentists (10%).[9] However, in the study all five cases of CTS came from the high-mercury group. Experiments have shown that neurotoxins predispose peripheral nerves to local damage at common sites of entrapment,[10] so subclinical mercury vapor exposure may also predispose the median nerve to local damage at the wrist.

Consistent with classic symptoms of mercury poisoning was the finding that a number of dentists had polyneuropathies. Polyneuropathies, defined as reduced motor or sensory conduction velocities or response amplitudes in two or more nerves, were found in seven dentists, all in the group with high mercury levels (Table 2). Three of these dentists manifested their polyneuropathy as localized slowing of conduction at the wrist consistent with CTS.

Electrodiagnosis is a sensitive technique that often detects subclinical entrapment neuropathies in apparently "normal" individuals. Thus, caution should be exercised when conduction studies are used as the criteria for screening a population for neurotoxic exposure.[11,12] Nonetheless, in this study the remarkably high incidence of electrodiagnostic abnormalities was confined to those subjects with raised tissue mercury levels.

Neuropsychological Deficits. Neuropsychological tests were also administered to 26 dentists in the high-mercury group and 17 in the control group. These tests included the Wechsler Adult Intelligence Scale (WAIS), the Bender-Gestalt, finger-tapping, and grooved pegboard tests. Symptoms were assessed by the use of the symptom checklist (SCL-90-R).[13] Scoring for the Bender-Gestalt was done blind (without knowledge of subject's mercury level) by two independent judges. Table 3 summarizes the neuropsychological test results of the two groups of dentists.

The full-scale intelligence quotient scores (WAIS) of the two groups are nearly identical (Table 3). However, the Bender-Gestalt test values of the high-mercury group were significantly different from those of control subjects. The finger-tapping rate and the grooved-pegboard tests did not indicate differences between the high-mercury and the control groups. The median T-value of the general distress index (not shown), an overall measure of distress levels, was 58 (range 33 to 73) for the high-mercury group and 53 (range 41 to 67) for the control group. Fourteen dentists in the high-

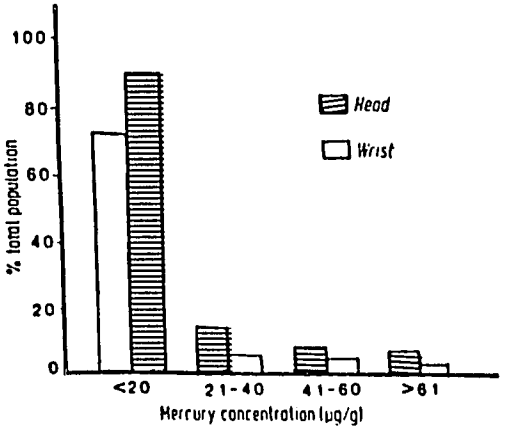

Fig. 2. Histogram showing head and wrist mercury values of a population of 298 dentists.

TABLE 2
Neurophysiological Findings in Dentists

	Group with High Mercury Levels (n = 23)	Control Group (n = 22)
Polyneuropathies	7 (18%)	0
Carpal tunnel syndrome	5 (13%)	0

TABLE 3
Neuropsychological Tests in Dentists

Test	Group with High Mercury Levels (n = 26)	Control Group (n = 17)
WAIS full scale	123 (1.5)	124 (1.4)
Bender-Gestalt (errors)*	65 (1.8)	58 (2.9)
Finger-tapping (rate)		
Preferred hand	70 (1.5)	73 (1.7)
Nonpreferred hand	64 (1.3)	64 (2.0)
Grooved pegboard (sec)		
Preferred hand	67 (1.3)	64 (1.8)
Nonpreferred hand	72 (1.7)	70 (1.4)

* $P < .01$. Results given as means; SEM in parentheses.

mercury and three in the control group had T-scores greater than the normal range (40 to 60). This difference between the number of raised scores in the two groups was significant.

Mild neuropsychological impairment in the group with high mercury levels was indicated by more visuographic alterations (numbers of errors by the mercury group in copying the Bender-Gestalt designs) and higher distress levels (measured from symptom self-reports on a checklist) than in the control group. In general, these findings are in accord with previous descriptions of the effects of mercury exposure in man.[14–17] Nevertheless, all of the participants in our study were performing their professional tasks adequately and showed no intellectual impairment.

Data on frequency of contact with amalgam, as well as social factors, habits, and previous illnesses were also obtained. Analyses of such information for factors predisposing the dentist to neurophysiological-neuropsychological deficits did not show differences between the group with high mercury levels and the control groups.[18] Thus, no single factor seemed to account for the differences in the health status of the two groups other than variations in mercury levels.

Lead Body Burdens in Children

Lead is accumulated by the calcified tissues, and hence either the bone or the tooth lead concentration can be used to indicate intake. The advantage of measuring the tooth lead concentration is that the tooth does not undergo remodeling; hence, "turnover" is very slow. Moreover, as Strehlow[19] demonstrated, the tooth lead level is dose dependent. Shed deciduous teeth have been used to study the extent of lead exposure in a large urban population.[20] These studies indicated that tooth lead levels were highest in children exhibiting pica.[21] In addition, lead exposure was related to societal urbanization.[22]

The disadvantages of measuring lead in shed deciduous teeth are self evident. To gain information on the current lead status of children suspected of suffering from plumbism, the XRF technique was used. The lead values of these children are shown in Table 4. The values shown here are similar to those published by Needleman et al[23] using shed teeth of children living in urban lead-polluted environments.

TABLE 4
Measured Tooth Lead Levels for 30 Children from the Poison Center of Children's Hospital of Philadelphia

Tooth Lead Levels (μg/g)	No. of Children with Tooth Lead Levels in this Interval
0–9	0
10–19	2
20–29	8
30–39	12
40–49	5
50–59	3

These data demonstrated that the XRF technique is sensitive enough to measure the lead level commonly associated with environmental samples. In this study, the XRF technique was used to measure tooth lead levels in 300 children in urban Philadelphia and 200 children in a more rural setting in Bennington, Vermont. As might have been expected, no tooth lead (<20 μg/g) was detected in the children living in Vermont, whereas 6% of the children in Philadelphia had tooth lead levels in excess of 20 μg/g.

The tooth lead values of the 30 children with high levels of exposure were related to their bone lead concentration. A close correlation between tooth lead and bone lead was noted ($r = .88$). In view of this correlation and because of the ease of positioning the small γ beam from the Co-57 source relative to the subject, we now routinely evaluate bone for its lead content in preference to teeth.

Tooth lead levels have also been correlated with other, more conventional, measures of plumbism. The correlations of tooth lead concentration with blood lead ($r = .35$) and the free erythroporphyrin ($r = .51$) concentration are statistically significant.[6] The weak correlation between these measures of lead exposure and the tooth lead levels is an indication of the different effects of lead in the body. From a screening viewpoint, the XRF measurement of tissue metal burdens can be used to select exposed populations. Thereafter, information relevant to existing systemic lead effects can be obtained through more conventional clinical and chemical measurements.

XRF in a Medical Setting

When physicians refer patients suspected of having metal toxicity for XRF analysis, the patients usually have a variety of symptoms/signs or organ dysfunction consistent with chronic lead intoxication. The information gained from the XRF analysis helps the physician understand the etiology of the patient's disease.

Recently, for example, we evaluated the lead burden for three workers who were employed in a secondary lead smelting plant prior to March 1981, when the company suspended activity. These workers were showing deterioration in renal function over the past several years. Their blood lead levels are now normal; however, historic blood lead levels obtained between 1974 and 1981 indicated lead exposure. Typically, the blood lead levels were 50 μg/dl with spikes of 80 μg/dl recorded. X-ray fluorescence measurements of the temporal bone, wrist, knee, and teeth demonstrated lead levels between 40 and 120 μg/g. Usually, adult patients with elevated bone lead levels do not receive chelation therapy; however, to avoid further deterioration in the renal function of these patients, the physicians are considering chelation therapy to reduce the lead body burdens. If such a course of therapy is agreed upon, changes of bone lead levels will be measured using XRF. Monitoring the changes in the lead body burdens permits the physician to tailor the chelation therapy to each patient.

Conclusion

To investigate the deleterious health effects of various heavy metals, it is important to evaluate multiple heavy metal body burdens in persons occupationally exposed to these elements. The XRF technique permits a safe, rapid, and noninvasive procedure for assaying multiple heavy metals simultaneously in various regions, including bone, teeth, and soft tissue. The heavy metal concentration in bone and/or teeth is associated with body retention of these elements; thus, its assay is related to the total body burden. This study has demonstrated in a dental professional population that the mercury concentration in the tissues of the temporal region of the head correlates with neurological and neuropsychological dysfunction associated with mercury exposure.

In numerous other occupations where persons are exposed to heavy metals, monitoring both the health of these workers and their metal body burdens will increase knowledge of metal toxicity and may result in improved safety in the workplace.

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