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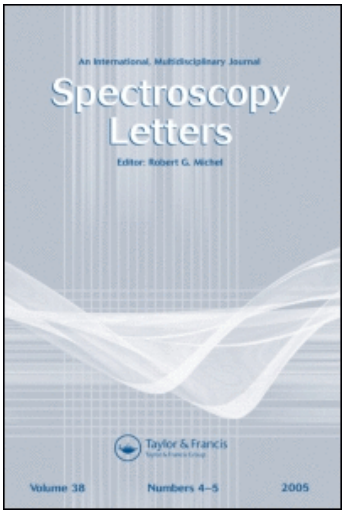

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THE DIRECT DETERMINATION OF MERCURY IN BREATH AND SALIVA BY CARBON BED ATOMIC ABSORPTION SPECTROSCOPY

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ABSTRACT

The direct determination of total mercury levels in breath and saliva was accomplished by atomic absorption spectroscopy using a carbon bed atomizer. The Robinson quartz "T" atomizer permitted the analysis of breath samples by directly trapping the constituents on the activated carbon bed of the atomizer. Analysis of saliva was performed by direct injection of sample onto the hot carbon bed. This direct analysis eliminated errors caused by both loss of volatile mercury compounds during sample pretreatment and contamination of the sample by added reagents.

The average mercury concentration in the breath of a non-occupationally exposed population was found to be 2.65 $\pm$ 0.47 ng Hg/L. The average mercury concentration in saliva from the same population was found to be 0.27 $\pm$ 0.02 ppm Hg. No correlation was found between mercury levels in breath, saliva and scalp hair for a normal population. Mercury levels in breath and saliva were shown to reflect recent mercury exposure.

INTRODUCTION

1. The Use of Breath and Saliva as Biological Indicators of Exposure to Mercury

Symptoms of both acute and chronic mercury intoxication include a variety of oral and respiratory problems. Examples are salivary gland

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swelling, excessive salivation, metallic taste, foul breath, loose teeth, soft, spongy gums, a blue-black line around the gums due to a mercury-sulfyhydryl complex and rapid respiration.[1,2,3,4] Consequently, breath and saliva appear to be likely biological indicators of exposure to mercury.

a) Breath Analysis

A breath sample possesses many advantages for clinical analysis or occupational health studies as discussed at length in a review by Dubowski.[5] Breath is a convenient sample to monitor since it can be collected rapidly with no inconvenience to the subject. Gaseous samples require little or no pretreatment. Portable detectors are available for many gaseous substances, so breath sampling can be used to provide real-time "field" measurements. Breath samples can also be collected and stored in gas sampling bags, or the component of interest trapped on an adsorbant for later analysis.

Breath samples reflect the concentration of substances transferred across the alveolar-capillary membranes. A continuous equilibrium is established for gases and volatile substances between the alveolar air and pulmonary circulation. Therefore, breath analysis reflects the blood level of substances such as CO_2, alcohol, CO, anesthetic gases, sulfides and volatile metals.

It is well known that elements like Se and Te can be volatilized from the lungs as methylated compounds following oral ingestion of inorganic Se and Te compounds. It is also well known that inorganic mercury can be methylated by bacteria. It is feasible that mercury or methylated mercury could be excreted from the body in the breath.

Breath has been studied by a number of researchers, to determine its suitability as an indicator of exposure to mercury and to elucidate the metabolism of mercury in the body.

Several animal studies indicated that rats can volatilize intracardially or intravenously injected mercury from the lungs and body surface.

Clarkson and Rothstein[6] found that a volatile mercury compound was exhaled by rats after injection with radioactive $^{203}Hg(NO_3)_2$. Ten percent of the total mercury excreted on the first day was through the lungs and body surface.

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Magos[7] found mercury in the breath of rats injected with either radioactive elemental mercury or mercuric ion, but the amount exhaled varied with the chemical form of the mercury injected. About 20% fo the Hg dose was exhaled within 30 seconds of injection. Only 1.8% of the Hg^{2+} dose was exhaled. Ostlund[8] investigated the metabolism of dimethylmercury in mice after inhalation or intravenous exposure. The major part of the dose was rapidly exhaled as dimethylmercury, with 80-90% of the dose eliminated in 6 hours.

No evidence of volatilization of inorganic mercury through the lungs has been found for humans. Studies of mercury levels in human breath are limited and most involve exposure of humans to elemental mercury vapor.

In 1941, Shepard et al.[9] reported the almost quantitative removal of Hg vapor from respired air by measuring Hg^0 in ambient air and exhaled breath. This can be explained in terms of the lipid solubility and high diffusibility of Hg^0. It has been calculated that at 40°C the partition coefficient of mercury between air and body lipids is about 1:20, in favor of the body.[10] The results of Shepard et al. have been confirmed by other workers[11,12,13] who found that 75-85% of inspired Hg^0 (concentrations ranging from 50-350 $\mu g\ Hg/m^3$ air) was retained in the human body.

Hursh et al.[14] conducted an experiment in which 5 humans inhaled stable and radioactive mercury vapor for periods of about 20 minutes. Seventy-four percent of the inhaled dose was retained by the subjects, with retention occurring almost entirely in the alveoli. Breath samples were taken for 3 days following exposure. On the average, 7% of the mercury retained was exhaled in the breath, with a half-life of 18 hours. A plot of mercury exhaled versus time consisted of two components. The first component, including mercury flushed out of the lung dead space, was lost very rapidly. The second component showed a more gradual loss of mercury. The authors postulated that mercury could be retained in, and subsequently released in, the alveolar air space over a period of several days following exposure. This was thought to give rise to the second component. Whole-body counting experiments gave an average half-time of 2 days for clearance of mercury from the lungs.

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Reinhardt et al.[15] measured mercury vapor in the breath of dental patients. Large amounts of mercury vapor were exhaled by patients following the removal of old amalgam fillings by drilling. A plot of mercury exhaled versus time showed two components, in agreement with Hursh et al.[14]

It is evident that recent dental work can contribute significantly to mercury vapor levels in breath. Goldwater[3] stated that amalgam dental fillings contributed to daily absorption of mercury by the body. No references could be found in which "normal" mercury levels in breath were measured.

Mercury in breath can reflect recent exposure, as shown in the above studies. Breath mercury levels should be considered in any discussion of elimination of mercury from the body. The suggestion has been made by Stopford[4] that an equilibrium exists between inhaled Hg^0 and Hg^{2+} in blood, so that Hg^0 is always available to cross the lung membrane and be exhaled.

b) Saliva

Saliva has not been extensively investigated as a biological indicator of exposure to mercury. The usefulness of saliva for this purpose is a matter of some debate. Studies performed in the 1920's found no significant amounts of mercury in saliva.[16,17] Berlin[18] stated that Hg^{2+} is accumulated in salivary and sweat glands, and that saliva, tears and sweat are routes of excretion for Hg^{2+}. Joselow et al.[19] found that saliva appeared to reflect the mercury concentration in blood and therefore could be a physiological fluid of great diagnostic importance. They noted, however, that most saliva is reswallowed and the mercury reabsorbed, so that saliva is not an effective route of excretion for mercury. Stopford[4] stated that no mercury was found in saliva unless an exposure to mercury vapor had occurred, but that such mercury levels could be much higher than mercury levels in blood.

Excessive salivation and salivary gland enlargement are symptoms of mercury poisoning, but are more common on exposure to inorganic mercury than to organomercury compounds.[4] Work in the 1920's on patients injected with Hg^0, Hg_2Cl_2 and $HgCl_2$ as treatment for syphilis revealed very small quantities of mercury in saliva.[16,17] In a study of industrial mercury

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workers, Joselow et al.[19] analyzed the saliva of exposed workers and normal adults. The concentration of mercury in the saliva of normal adults was < 0.5 μg/100 mL. Mercury concentrations in saliva of exposed workers ranged from 1 - 15.5 μg/100 mL with a mean of 5 μg/100 mL. In the exposed group, saliva levels were about one-tenth of the urine concentrations. A better correlation was found between mercury levels in blood and saliva than between mercury levels in urine and saliva.

Stopford[4] reported finding an average salivary mercury level of 59 ppm (range: < 1 - 436 ppm) saliva from workers at a chemical plant producing mercury compounds

2. Analytical Difficulties in the Analysis of Breath and Saliva

a) General

Breath and saliva samples contain only trace (< ppm) amounts of mercury. Care must be taken to prevent contamination of the sample in the collection process. Loss of volatile mercury compounds must be prevented during storage and analysis of the sample. Preconcentration of the mercury in the sample is necessary for many analytical techniques and loss of mercury or contamination can occur during this process.

b) Breath

There is a difference in chemical composition between lung dead-space air and alveolar air. This difference has been used to explain the inflection point in plots of exhaled mercury versus time from exposure.[14,15] Only substances which pass through the circulatory-alveolar bounday can be determined in breath. The sample must be collected under conditions of known temperature, pressure and flow rate to determine accurately concentration of analyte and sample volume.

Breath has been shown to contain a wide variety of organic and inorganic gases.[5] Potential interferences from these molecules (e.g., molecular absorption of an atomic spectral line) must be considered in the analysis.

Collection and storage of breath samples[5] in foil containers, gas bags, glass gas pipets or by adsorption onto activated charcoal, silica gel, etc., can lead to loss of mercury by adsorption onto container walls, by diffusion out of the container or by inefficient trapping.

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c) Saliva

The analysis of saliva presents many of the same analytical problems as other biological fluids: limited sample availability, low concentration of analyte and a complex matrix. Sample digestion and concentration are usually required, with the concomitant risks of mercury loss or contamination.

Saliva is produced by the parotid, submaxillary and sublingual glands. The chemical composition of saliva depends on the gland producing it and on the intensity, duration and type of stimulation.[20] Table 1, containing data reported by Afonsky,[20] presents some of the compounds and elements found in unstimulated saliva and their typical concentration ranges. Saliva also contains such things as epithelial cells, leucocytes, red blood cells and bacteria which add to the complexity of the matrix and change the chemical composition.

Various methods have been used to collect saliva for analysis.[21] Spitting, suction or simple drainage from an open mouth have been used to collect whole saliva. Special suction devices are used for collecting saliva from a particular gland.[21]

3. Common Analytical Methods for the Determination of Mercury in Breath and Saliva

a) Breath

Methods used for the determination of mercury in breath include radiotracer methods, UV-photometry and vapor-phase atomic absorption spectroscopy.

Breath samples from subjects exposed to radioactive mercury were usually collected on Hopcalite (active MnO_2/CuO) or activated charcoal. The adsorbant was counted in a well-type scintillation counter.[6,7,14] Ostlund[8] used inorganic mercury and dithizone solutions to absorb exhaled radiolabelled organomercury compounds. The solutions were analyzed by low-temperature TLC and gamma counting. The detection limit was 2 - 80 pg Hg.

Nielsen Kudsk[12,13] used a commercial UV photometer to measure mercury in respired air. Two condensers at 11°C were used to remove water vapor from the breath before it passed into the photometer cell. A sensitivity of 3 μg Hg was reported.

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TABLE 1

Selected Components of Unstimulated Saliva[20]

Component	Typical Concentration
Total Solids	240-1500 mg %
Organic Solids	130-380 mg %
Ash	55-370 mg %
Ca	2.5-11 mg %
P(total)	15-25 mg %
Na	1.0-65 mg%
K	30-95 mg %
Cl	30-145 mg %
Mg	0.1-0.7 mg %
citrate	0-2.0 mg %
thiocyanate	0-0.31 mg %
Fe	0-0.6 ppm
Cu	10-47.5 µg %
Co	0-12.5 µg%
F	0.08-0.25 ppm
S	3-20 mg %
Br	0.2-7.1 ppm
total protein	140-640 mg %
cystine	0-.45 mg %
glutamic acid	.2-12.5 mg %
methionine	0-0.1 mg %
gluthathione	15.4 mg %
Vitamin B_{12}	0.15-5 ppb

Reinhardt et al.[15] passed breath samples through a drying tube and over a silver wool collector. Mercury was released from the silver wool by heating and the vapor was drawn through a quartz-cell atomic absorption system.

b) Saliva

Joselow et al.[19] determined mercury in saliva with a UV photometer. Saliva samples were digested using a cold digestion and then extracted with dithizone. The solvent was evaporated and the residue heated in a furnace. The released mercury vapor was drawn through the photometer cell.

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Stopford[4] gave no details of the analytical method used in measuring saliva mercury levels in industrially-exposed workers.

4. Speciation of Mercury Compounds in Breath and Saliva

Very little work has been done to identify the chemical form of mercury in breath. Most studies have measured the fractional amount of Hg vapor retained by the lungs as a function of mercury vapor in inspired air or have measured only the amount of expired radioactive mercury, not the chemical form. Only Ostlund's TLC method[8] allowed identification of the chemical form of mercury exhaled by mice. By varying the absorbing solutions and TLC conditions, he was able to separate a number of inorganic, alkyl and aryl mercurials.

Speciation of mercury compounds in air was reported by Johnson and Braman.[22] They used a series of selective adsorption tubes to separate mercuric compounds, methylmercuric compounds, elemental mercury vapor and dimethylmercury. It seemed feasible to us to combine this selective adsorption technique with our atomic absorption system in order to determine the chemical form of mercury in breath. Our attempt is discussed below.

No reports of speciation of mercury compounds in saliva were found.

5. Goals of this Study

The goals of this study were to develop a direct method for the determination of mercury in breath and saliva, and to develop a method for speciation of mercury compounds in breath and saliva.

Atomic absorption spectroscopy using the Robinson quartz "T" atomizer fulfilled the requirements for direct determinations of mercury in breath and saliva. The quartz "T" atomizer has been previously described.[23] The system has been used for the direct determination of mercury in hair and for the analysis of breath and biological fluids for cadmium.[23-25]

EXPERIMENTAL

1. Equipment

a) Atomic Absorption System. The atomic absorption system used for these studies has been described.[23] Minor modifications were made as follows:

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For breath sampling, the carbon bed was composed of activated National carbon[26] in order to adsorb mercury compounds from breath.

In addition, the top of the quartz "T" atomizer was fitted with a one-holed rubber stopper. A short piece of glass tubing was inserted through the stopper and a 30-cm length of Tygon tubing was attached to the glass. Subjects exhaled through the Tygon tubing directly onto the atomizer carbon bed.

For liquid sample introduction with the Drummond microdispenser, the top of the atomizer was fitted with a one-holed rubber stopper to center the microdispenser over the carbon bed.

b) Drummond Scientific Microdispenser, 1 - 5 microliter capacity. The microdispenser was unsatisfactory as delivered, due to excessive drop hangup on the end of the glass barrel. The dispenser was modified by Dr. D. K. Wolcott,[27] formerly of this research group. By drawing a fine capillary tip on the end of the disposable glass barrel and transferring the travel-limiting sleeve from inside the dispenser body to the plunger, the microdispenser was converted to an air-displacement device. (Figure 1) In the modified device, an excess volume of air was retained between the plunger and the sample. When the plunger was depressed, the excess air assured that the entire volume of liquid was ejected from the barrel.

c) Graphoil, pyrolytic graphite-coated graphite, PT 101, 0.005 in. x 3 in. x 3 in. (Ultra Carbon Corporation, Bay City, Michigan).

d) Hamilton Microliter syringe no. 701-N, 10 microliter capacity.

e) Hamilton Gastight syringe no. 10001, 1 mL capacity.

f) Precision Sampling Corporation "Pressure-Lock" gastight syringe, 10 mL capacity.

2. Chemicals

a) 1000 ppm Hg^{2+} solution. Prepared from mercuric chloride (Matheson, Coleman and Bell) and deionized distilled water.

b) 45 - 60 Mesh Chromosorb W

c) 45 - 60 Mesh Chromosorb W with 5% (W/W) SE-30

3. Analytical Operating Conditions

a) Demountable Hollow Cathode Lamp: 3mA current, helium filler gas.

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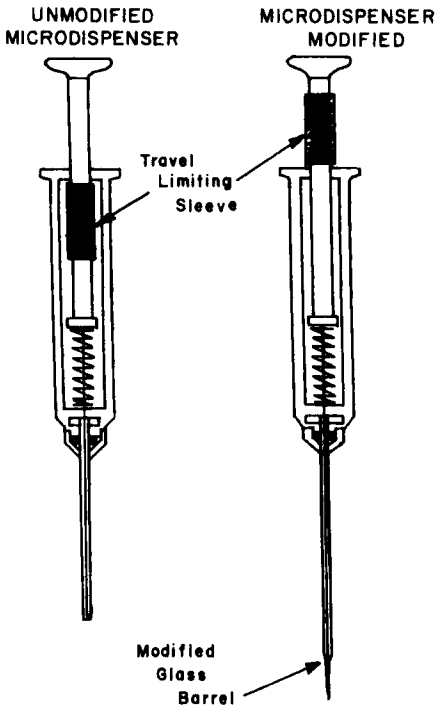

FIGURE 1: DRUMMOND MICRODISPENSER MODIFIED BY DRAWING A FINE CAPILLARY TIP ON GLASS BARREL AND MOVING THE TRAVEL LIMITING SLEEVE AS SHOWN. MODIFICATION ELIMINATED DROP HANG-UP ON THE TIP AND INSURED COMPLETE SAMPLE EJECTION BY AIR-DISPLACEMENT.

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b) Carbon bed temperature: 1450°C

c) Atomizer purge gas: commercial compressed nitrogen was passed through a scrubbing train of silica gel, activated charcoal and resistively-heated copper turnings. The scrubbed nitrogen was supplied to the atomizer when breath samples were not being collected. Purge gas was supplied to the atomizer at 275 mL/min., a rate slightly faster than the usual atomizer pumping rate of 250 mL/min. This provided a positive pressure system and permitted use of the 184.9 nm line by excluding air from the atomizer. Scrubbed air was used as the purge gas for work at 253.7 nm.

d) Light path temperature: 900°C minimum

e) Slit width: 100 um (184.9 nm); 25 um (253.7 nm)

f) Wavelength: 184.9 nm or 253.7 nm

g) Monochromator purge gas: scrubbed nitrogen, 2L/min. for 184.9 nm, none for 253.7 nm.

h) P.M.T. voltage: 500 V

4. Sample Collection and Analysis

a) Breath

A two-liter breath sample was collected as described previously.[24]

For resonance line absorption measurements (253.7 or 184.9 nm), the demountable Hg hollow cathode lamp was used. For measurement of molecular background, the sample collection and analysis procedure was repeated, but a deuterium lamp was used in place of the hollow cathode lamp.

All stages of the sample collection procedure were timed using a stopwatch so that samples could be collected and analyzed in a reproducible manner.

A "blank" sample was collected and analyzed to determine the absorption from air or nitrogen pulled through the atomizer in between breath samples. A two liter aliquot of air or nitrogen was drawn over the bed in place of a breath sample. The absorbance from the blank was subtracted from the absorbance of the samples run that day.

Breath samples were collected from adult males and females in the Louisiana State University population. Chemistry faculty, graduate students, undergraduate students and secretarial personnel constituted the sampled group. None of the sample population was occupationally exposed to mercury (other than normal laboratory exposure). One of the subjects was currently working with elemental, inorganic and organic mercury on a limited basis. Another subject, to be discussed below, was exposed to mercury through ingested paint. Three of the forty-two subjects were cigarette smokers. Most subjects consumed fish (a known source of mercury) two to four times per month. None of the subjects had undergone any dental work within the previous six months.

Speciation of mercury compounds in breath was attempted using the selective adsorption tubes described by Johnson and Braman.[22] One adsorption

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tube was packed with SE 30-coated Chromosorb W treated with HCl vapors and one adsorption tube was packed with NaOH-treated Chromosorb W.[22] These tubes were placed in the Tygon tubing line leading to the atomizer so that the breath sample passed through the adsorption tubes before reaching the carbon bed.

b) Saliva

Saliva samples were collected from fourteen individuals in the sample population discussed above. The subjects rinsed their mouths with tap water to remove any foreign particles and swallowed several times. Subjects then expectorated into nitric acid-cleaned polyethylene vials. It is possible that some dilution of the saliva occurred as a result of rinsing the mouth with tap water. The dilution was not thought to be very significant, but in any case, it was preferable to analyzing saliva contaminated with coffee or chewing gum. Saliva samples were analyzed immediately after collection. Two methods of analysis were used.

I. Carbon Disk Method

Carbon disks were prepared by punching 6 mm disks from sheets of pyrolytic graphite-coated Graphoil with a standard hole punch. The disks were cleaned by heating them in the carbon bed at 1450°C until no mercury absorption signal was seen. After cooling the bed under scrubbed nitrogen, the disks were removed and stored in capped nitric acid-cleaned polyethylene vials. The disks were cleaned about two hours prior to use.

A 1-μL aliquot of saliva was placed on a Graphoil disk with a Hamilton microliter syringe. The disk was dropped onto the hot carbon bed and the absorption signal measured. Samples were analyzed in triplicate. Background absorption was measured with the deuterium lamp on additional aliquots of the sample.

II. Direct Injection with Microdispenser

Two microliter aliquots of saliva were injected directly onto the hot carbon bed with the modified Drummond Microdispenser. Background absorption was measured with the deuterium lamp on separate aliquots.

The glass rod technique developed by the authors for speciation of mercury compounds in solution[28] was used to examine saliva. The end of the

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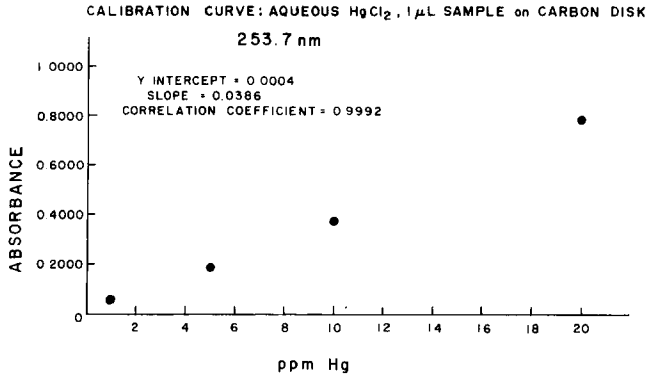

FIGURE 2: CALIBRATION CURVE PREPARED BY PLACING 1 uL ALIQUOTS OF AQUEOUS $HgCl_2$ STANDARD ONTO HEAT-CLEANED 6 mm-DIAMETER GRAPHOIL DISKS WITH A MICROLITER SYRINGE. DISKS WERE DROPPED DIRECTLY ONTO HOT CARBON BED.

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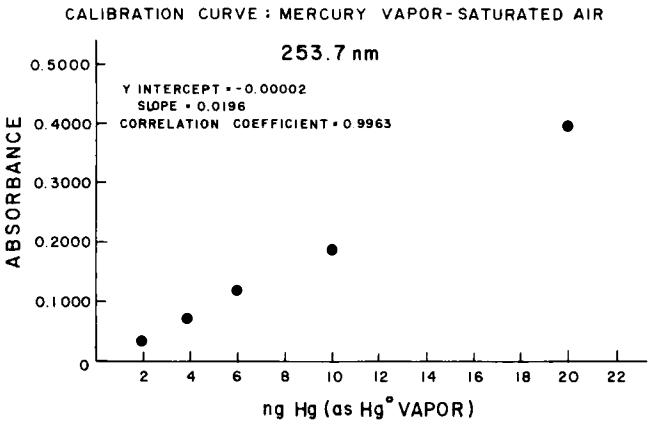

FIGURE 3: CALIBRATION CURVE AT 253.7 nm PREPARED BY INJECTION OF MERCURY VAPOR-SATURATED AIR WITH A GAS-TIGHT SYRINGE. AMOUNT OF MERCURY INJECTED CALCULATED FROM VAPOR-PRESSURE DATA AND THE IDEAL GAS LAW.

glass rod was cleaned in a Bunsen burner flame, allowed to cool and dipped into the saliva sample. The glass rod assembly was placed in the atomizer and the absorption trace measured as a function of time.

5. Calibration

a) Breath

Calibration was accomplished by injecting various volumes of air saturated with mercury vapor onto the cold carbon bed with a gas-tight

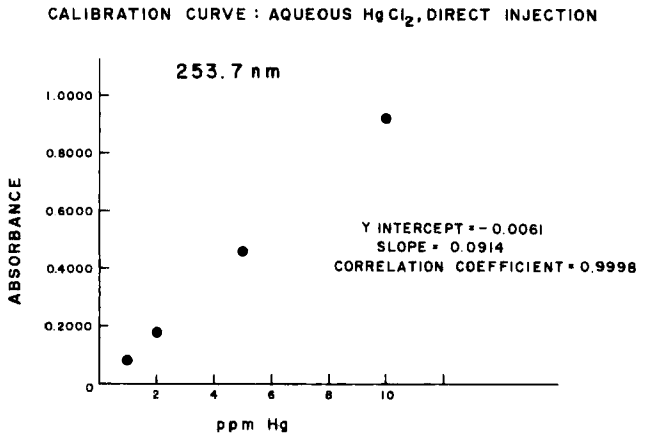

FIGURE 4: CALIBRATION CURVE AT 253.7 nm PREPARED BY DIRECT INJECTION OF 2 uL ALIQUOTS OF AQUEOUS $HgCl_2$ STANDARDS WITH THE MODIFIED DRUMMOND MICRODISPENSER.

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syringe. The amount of mercury injected can be calculated from vapor pressure data.[23] The adsorbed mercury vapor was analyzed as if it were a trapped breath sample.

The sensitivity, defined as that quantity of mercury equal to 1% absorption, was $(1.5 \pm 0.2) \times 10^{-11}$g for the 184.9 nm line and $(1.0 \pm 0.1) \times 10^{-10}$g for the 253.7 nm line.

Calibration curves were linear up to about 70 ng Hg (approximately 3 mL of air saturated with Hg vapor) at the 253.7 nm line. With larger volumes, an absorption signal began to be recorded before the bed was heated. This was probably due to mercury being released from the carbon near the bottom of the bed, which was warmed by the heated optical path.

b) Saliva

Aqueous solutions of Hg^{2+} in the 0.01 to 10 ppm range were prepared fresh daily by dilution of a 1000 ppm stock Hg^{2+} solution. Standards were diluted with distilled deionized water. Calibration curves were run by the two methods described for introduction of saliva samples into the atomizer, the carbon disk method and direct injection with the Drummond microdispenser. The absorbance due to blank carbon disks and deionized distilled water was measured and subtracted from the absorbance of the standards when required. Background absorption was measured with the deuterium lamp.

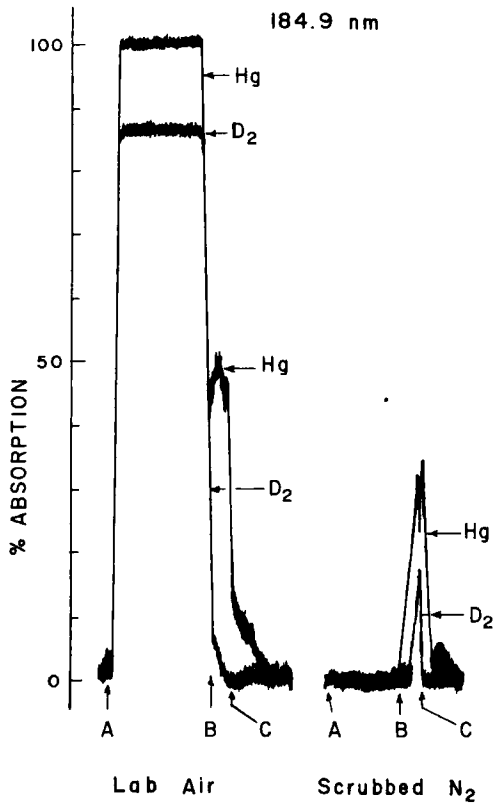

FIGURE 5: ABSORPTION TRACES OF AIR AND N_2 AT 184.9 nm.
POINT A: SAMPLE INTRODUCED TO COLD CARBON BED. POINT B: SAMPLE FLOW STOPPED AND RF GENERATOR TURNED ON TO HEAT CARBON BED. POINT C: ATOMIZED SAMPLE PULLED INTO LIGHT PATH.
RESONANCE ABSORPTION (Hg, 184.9 nm) AND MOLECULAR BACKGROUND ABSORPTION (D_2, 184.9 nm)

Calibration curves were linear up to 10 ppm Hg at the 253.7 nm line.

Typical calibration curves are shown in Figures 2 - 4. Precision was determined by making 20 injections of a 2 ppm Hg solution. The mean concentration found by the carbon disk technique was (2.0 $\pm$ 0.4) ppm; that found by direct injection was (2.0 $\pm$ 0.2) ppm.

RESULTS

1. Mercury Concentrations in Laboratory Air and N_2 Purge Gas

An aliquot of laboratory air or N_2 purge gas was analyzed in order to detect any mercury in these gases which would be trapped by the carbon bed

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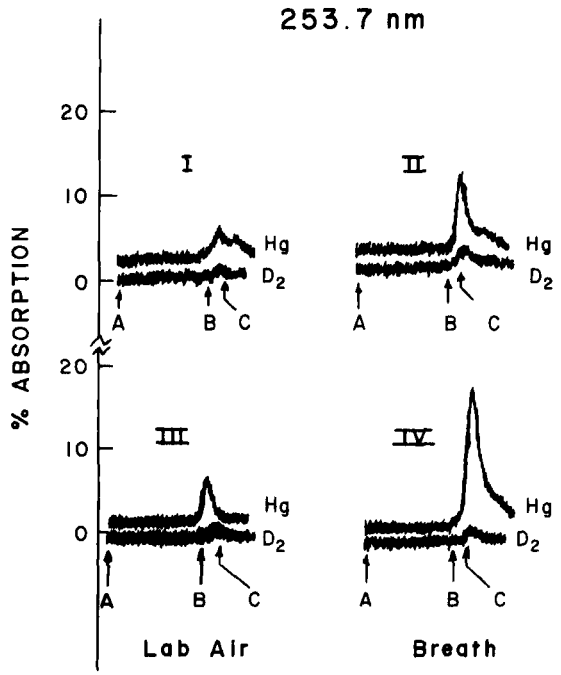

FIGURE 6: ABSORPTION TRACES OF AIR (I AND III) AND BREATH (II AND IV) SAMPLES AT 253.7 nm.
POINT A: SAMPLE INTRODUCED ONTO COLD CARBON BED.
POINT B: SAMPLE FLOW STOPPED AND RF GENERATOR TURNED ON TO HEAT CARBON BED.
POINT C: ATOMIZED SAMPLE PULLED INTO LIGHT PATH.
RESONANCE ABSORPTION (Hg, 253.7 nm) AND MOLECULAR BACKGROUND ABSORPTION (D_2, 253.7nm)

during the cooling period between breath samples. This determination was considered to be a blank for subsequent breath samples.

Absorption traces of laboratory air and nitrogen purge gas at 184.9 nm are shown in Figure 5. An absorption trace of laboratory air at 253.7 nm is shown in Figure 6. Resonance absorption and background absorption are shown.

Mercury concentrations found in laboratory air on various dates are listed in Table 2. All data were measured at 253.7 nm. The mean mercury concentration found was 2.3 μg Hg/m^3 air. The range was from 0.1 to 6.3 μg Hg/m^3 air.

2. Mercury Concentrations in Breath

Absorption traces of breath at 184.9 nm and 253.7 nm are shown in Figures 6 and 7. Use of the 184.9 nm resonance line was investigated in

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TABLE 2

Mercury Concentrations in Laboratory Air

Date	ng Hg/2L air	μg Hg/m^3 air
7/14/81	2.6	1.3
7/15/81	1.8	0.9
7/16/81	11.0	5.5
7/20/81	8.0	4.0
7/24/81	1.4	0.7
7/31/81	4.0	2.0
8/27/81	2.0	1.0
10/26/81	1.2	0.6
10/28/81	0.3	0.1
12/14/81	3.0	1.5
12/15/81	3.0	1.5
12/15/81	11.8	5.9
12/17/81	3.9	1.9
1/18/82	8.5	4.3
1/21/82	0.7	0.3
2/ 2/82	12.5	6.3

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the hope of exploiting the increased sensitivity of this line over the spin-forbidden 253.7 nm line. As can be seen in Figure 7, 100% absorption of the 184.9 nm resonance line occurred at the start of the breath sample collection period. This absorption was due to oxygen, carbon dioxide, water vapor and the molecular constituents of breath. At the end of the ten minute sampling period, nitrogen purge gas was introduced into the atomizer and transmission of the resonance line rapidly increased. However, absorption of the resonance line by mercury released from the carbon bed began before the resonance signal had returned to 100% transmission. This resulted in a sample absorption signal with a non-horizontal baseline which made the peak height difficult to measure reproducibly. In addition, a correction factor had to be applied if the absorption signal began at less than 100% T.

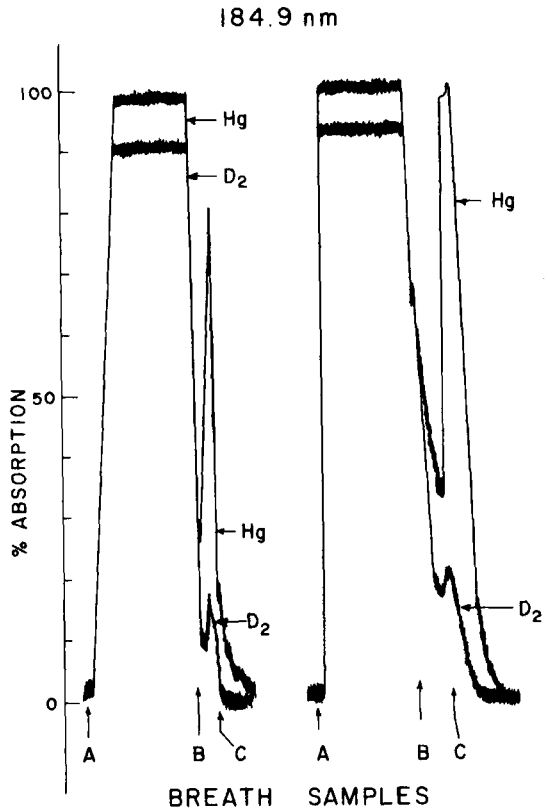

FIGURE 7: ABSORPTION TRACES OF BREATH SAMPLES AT 184.9 nm.
POINT A: SAMPLE INTRODUCED ONTO COLD CARBON BED.
POINT B: SAMPLE FLOW STOPPED AND RF GENERATOR TURNED ON TO HEAT CARBON BED.
POINT C: ATOMIZED SAMPLE PULLED INTO LIGHT PATH.
RESONANCE ABSORPTION (Hg, 184.9 nm) AND MOLECULAR BACKGROUND ABSORPTION (D_2, 184.9nm) ARE SHOWN.

No molecular absorption occurred at 253.7 nm and the sensitivity was adequate for breath analysis. Therefore, no quantitiative analyses were made at 184.9 nm.

Mercury concentrations in the breath of 42 subjects were determined. All determinations were made at 253.7 nm. The results are listed in Table 3. The overall average mercury concentration in breath was found to be 5.3 ng Hg/2L breath; that for males was 4.2 ng Hg/2L breath and for females, 6.9 ng Hg/2L breath.

3. Variation in Mercury Concentration in Breath with Exposure to Mercury

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TABLE 3

Mercury Concentrations in Breath

Female		Male	
Subject no.	ng Hg/2L Breath	Subject no.	ng Hg/2L Breath
1	3.5	21	10.4
2	1.5	22	0.5
3	3.2	23	1.6
4	1.0	24	1.5
5	3.1[a]	25	6.5
6	3.1	26	0.7
7	6.3	27	3.3
8	15.5	28	0.7
9	1.8	29	none detected
10	7.2	30	3.2
11	12.2	31	3.8
12	19.4	32	0.3
13	none detected[a]	33	0.7
14	16.7[a]	34	1.3
15	none detected	35	1.9
16	29.7	36	3.1
17	none detected	37	1.0
18	2.0	38	7.2
19	8.5	39	18.5
20	3.6	40	5.0
		41	4.5
		42	10.0

[a] cigarette smoker

Females	Males
n = 20	n = 22
$\bar{x}$ = 6.9 ng Hg/2L Breath	$\bar{x}$ = 4.2 ng Hg/2L Breath
σ = 7.9	σ = 4.4
range: none detected-29.7 ng Hg/2L Breath	range: none detected-18.5 ng Hg/2L Breath

Overall

n = 42
$\bar{x}$ = 5.3 ng Hg/2L Breath
σ = 6.5
range: none detected-29.7 ng Hg/2L Breath

Two females were studied who were exposed to mercury on a limited basis.

The breath of the first subject was analyzed on nine different occasions over an eight month period. The mercury concentrations found ranged from $< \frac{0.1 \text{ ng}}{2L}$ to $\frac{17.1 \text{ ng}}{2L}$ and are listed in Table 4. Analysis #4 was performed about 3 hours after the subject had handled elemental mercury. Analysis #9 was performed about two hours after the subject had weighed out solid methylmercury chloride. These analyses showed mercury concentrations in the breath of 17.1 and 12.3 ng Hg/2L,

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TABLE 4

Mercury Concentrations in Breath of an Individual Exposed to Mercury

Subject 1

Analysis no.	Date	ng Hg/2L Breath
1	7/14/81	none detected
2	7/16/81	3.8
3	7/31/81	none detected
4[a]	10/26/81	17.1
5	10/28/81	3.6
6	12/15/81	none detected
7	12/17/81	6.7
8	1/21/82	none detected
9[b]	2/ 9/82	12.3

[a]exposed to elemental mercury

[b]exposed to methylmercuric chloride

TABLE 5

Mercury Concentrations in Breath and Saliva of an Individual Exposed to Mercury Through Ingestion of Mercury-containing Paint

Subject 2

Date	ng Hg/2L Breath	ppm Hg in Saliva
12/14/81	44.5	0.80
12/15/81	28.4	---
1/18/82	64.0	---
2/ 9/82	19.0	---
5/ 5/82	11.2	0.08

Note: Exposure ceased 12/31/82

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respectively, which were considerably higher than concentrations found on other occasions, which had an average of 2 ng Hg/2L.

The second subject was exposed to mercury through oral ingestion of mercury-containing paint. She was in the habit of pointing her paint brush in her mouth when painting ceramic figurines. The exposure was discovered when the subject was asked to donate a breath sample for analysis. The mercury concentration found was 44.5 ng Hg/2L breath, the highest concentration measured in this study at that point. A repeat sample confirmed the high level. The subject then ceased putting the brush in her mouth. Mercury levels in the subject's breath decreased as shown in Table 5. The mercury levels found in the breath of these 2 subjects were not included in the data in Table 3.

4. Speciation of Mercury in Breath

An attempt was made to speciate mercury in breath through the use of the two adsorption tubes described previously. The HCl-treated SE-30-coated Chromosorb W was supposed to retain $HgCl_2$-like compounds. The NaOH-treated Chromosorb W was supposed to retain CH_3HgCl-like compounds. Elemental mercury vapor and dimethyl mercury should have passed through the adsorption tubes and been retained on the carbon bed. Air saturated with mercury vapor was used to check the adsorption tube system.

1 mL of air saturated with Hg^0 (about 20 ng Hg) was injected through both adsorption tubes. The sample was treated as though it was a breath sample. No absorption signal was seen when the carbon bed was heated, compared to a 20% absorption signal for the same volume of air injected without the adsorption tubes. 10 mL of air saturated with mercury vapor was injected through the adsorption tubes. A 6% absorption signal was generated, compared to a 93% absorption signal without the adsorption tubes.

Each adsorption tube was tried separately, with the same results. The tubes obviously adsorbed elemental mercury vapor, although they were reported to not adsorb it. The method was therefore unsuitable for the speciation of mercury compounds in breath.

5. Mercury Concentrations in Saliva

Mercury was determined in the saliva of fifteen subjects, most of them from the breath-sampled population. The two mercury exposed subjects were included in the sampled group.

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TABLE 6

Mercury Concentrations in Saliva

Female		Male	
Subject no.	ppm Hg	Subject no.	ppm Hg
1	0.32	5	0.24
2[a]	0.39	6	0.19
3	0.32	7	0.21
4[b]	0.80	8	0.19
4[c]	0.08	9	0.50
5	0.24	10	0.28
		11	0.21
		12	0.15
		13	0.10
		14	0.40

[a]exposed to elemental and organic mercury.

[b]exposed to mercury through ingestion of paint. Sample taken 12/14/81.

[c]same subject as 4[b]. Sample taken 5/5/82.

Results are listed in Table 6. The overall mean concentration was 0.27 ppm Hg with a standard deviation of 0.11 and a range of 0.15 - 0.50 ppm Hg. The paint-exposed individual had a saliva mercury level of 0.80 ppm. This was greater than 4 σ from the mean and therefore was not included in calculating the mean. Her saliva mercury level decreased with time, as can be seen in Table 5.

The female sample population was too small to allow statistical comparison of the male and female average concentrations.

2 µL saliva samples gave resonance line absorption signals of 2 - 8% with no molecular background absorption.

6. Correlation between Mercury Concentrations in Breath, Saliva and Hair

Twelve subjects had mercury levels in both breath and saliva measured in this study. Sixteen of the breath-sampled population had mercury levels in scalp hair determined in an earlier study.[23] Nine subjects had data available for all three matrices.

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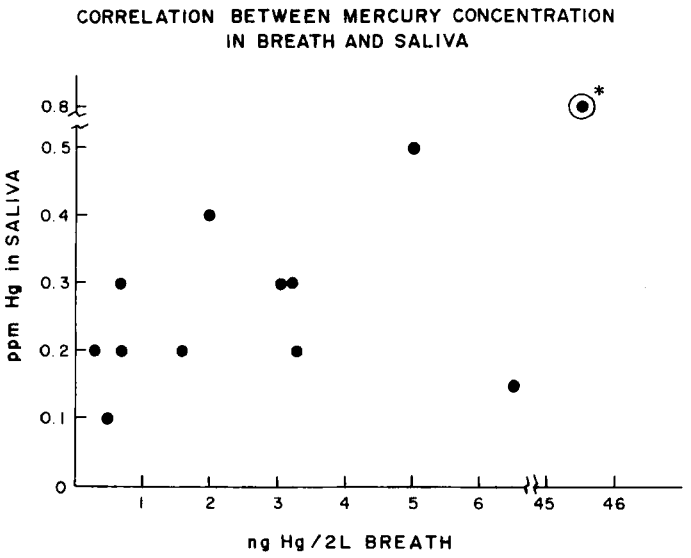

FIGURE 8: NO CORRELATION EXISTS BETWEEN MERCURY LEVELS IN BREATH AND SALIVA FOR A NORMAL POPULATION. (CORRELATION COEFFICIENT = 0.244 EXCLUDING POINT MARKED WITH ASTERISK). RECENT EXPOSURE TO MERCURY THROUGH INGESTION OF MERCURY-CONTAINING PAINT (POINT MARKED WITH ASTERISK) RESULTED IN VERY HIGH MERCURY LEVELS IN BOTH BREATH AND SALIVA.

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Correlation diagrams are given in Figures 8 - 10.

7. Speciation of Mercury Compounds in Saliva

The absorption-time trace of a saliva sample on the glass rod consisted of a single broad absorption peak with a maximum at a retention time of 1 minute.

DISCUSSION

1. Advantages of the Quartz "T" Atomizer

The use of the quartz "T" atomizer in the determination of mercury in breath and saliva has several advantages over other methods of analysis:

a) It permitted use of the more sensitive 184.9 nm resonance line as well as the more commonly used 253.7 nm line.

b) It eliminated the need for sample pretreatment, due to the efficient one-step atomization process. Atomization took place outside of the light path, so that scatter and background absorption were decreased.

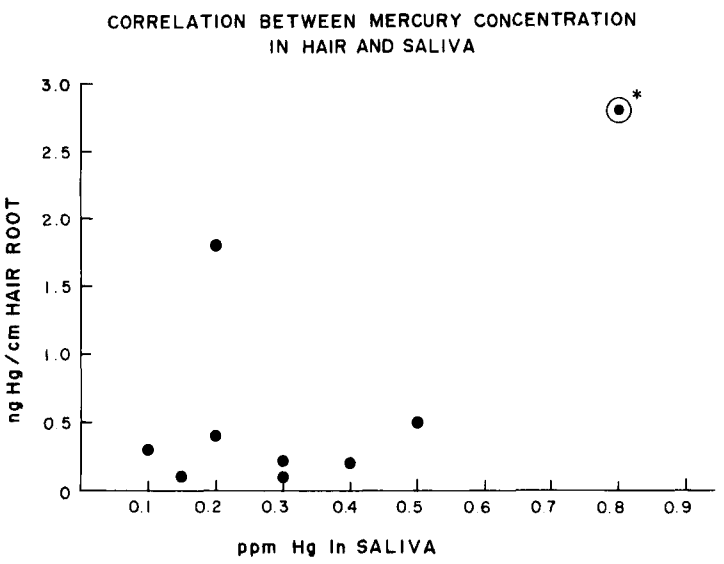

FIGURE 9: NO CORRELATION EXISTS BETWEEN MERCURY LEVELS IN HAIR AND SALIVA FOR NORMAL POPULATION (CORRELATION COEFFICIENT = -0.119 EXCLUDING POINT MARKED WITH ASTERISK). RECENT EXPOSURE TO MERCURY THROUGH INGESTION OF MERCURY-CONTAINING PAINT (POINT MARKED WITH ASTERISK) RESULTED IN VERY HIGH MERCURY LEVELS IN BOTH HAIR AND SALIVA.

c) Mercury from breath samples was trapped directly in the atomizer, so that no transfer of the trapped sample was necessary. This eliminated one possible source of loss.

d) Accuracy was improved for saliva analyses because the direct determination eliminated losses of mercury due to incomplete digestion or incomplete recovery from concentration steps. In addition, no mercury contamination occurred from reagents used for wet ashing.

e) Accuracy for breath and saliva analyses was improved because all of the mercury which entered the atomizer passed through the light path and was detected. No losses such as those due to volatilization during the drying and ashing cycles of commercial graphite furnaces or due to incomplete reduction of mercury during cold-vapor analysis could occur.

2. Analysis of Breath Using the 184.9 nm Resonance Line

As can be seen in Figure 7, the mercury absorption peak occurred on the shoulder of the molecular background absorption. It is clear from

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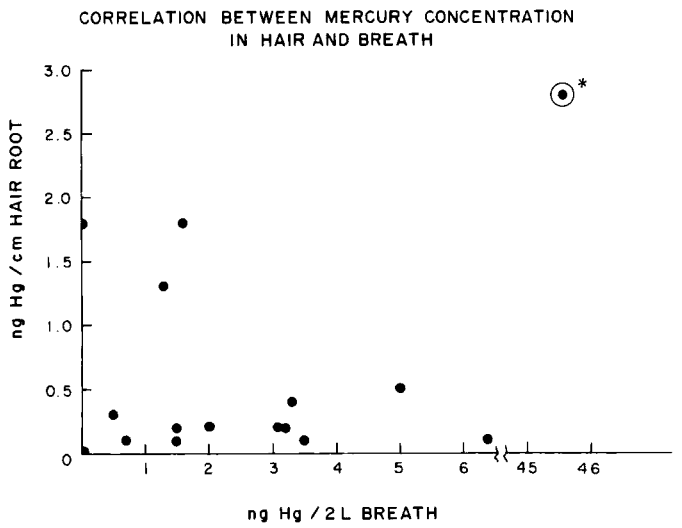

FIGURE 10: NO CORRELATION (CORRELATION COEFFICIENT =-.292 EXCLUDING POINT MARKED WITH ASTERISK) EXISTS BETWEEN MERCURY LEVELS IN HAIR AND BREATH FOR NORMAL POPULATION. RECENT EXPOSURE TO MERCURY THROUGH INGESTION OF MERCURY-CONTAINING PAINT. (POINT MARKED WITH ASTERISK) RESULTED IN HIGH MERCURY LEVELS IN BOTH HAIR AND BREATH.

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the absorption trace that mercury was indeed present in expired air, but it was not known if any volatile mercury compound was eluting from the carbon under the molecular absorption peak. Since the 253.7 nm resonance line proved to have sufficient sensitivity for breath analysis without the problems of molecular absorption, no further work was done at 184.9 nm.

3. Analysis of Breath Using the 253.7 nm Resonance Line

The sensitivity of the 253.7 nm line (defined as a 1% absorption signal) was about 1×10^{-10} g Hg. This was perfectly adequate for breath analysis, since most breath samples gave an absorption signal of between 3% and 20% absorption. Molecular absorption, determined by the absorption signal of a sample measured with the deuterium lamp, was negligible, as can be seen in Figure 6.

4. Range of Mercury Concentrations in Breath of Normal Population

No studies of mercury concentrations in the breath of a normal population were found in the literature. It is not unreasonable to suppose

that breath is a means of excretion of mercury from the body, in a manner analogous to excretion of Se and Te. It must be remembered that possible sources of mercury in the sample include mercury volatilized from the lung, mercury released from the surface of dental amalgam fillings in the mouth, mercury volatilized by bacteria in the mouth, mercury volatilized from saliva or entrained in saliva droplets in the exhaled breath.

The average mercury concentration in the breath of the population sampled was found to be 5.3 ng Hg/2L breath or 2.65 μg/m . It has been estimated that a person exhales 10^4 L breath/day.[8] This would result in excretion of 26.5 μg Hg/day through the breath.

A significant difference exists between the mean concentrations of mercury in breath for the male and female populations sampled.

5. Variations in Mercury Concentration in Breath on Exposure to Mercury

a) Subject 1

The average mercury level of subject 1 was 2.0 ng Hg/2L breath as determined by seven separate analyses over a period of eight months. On two occasions, mercury levels significantly higher than this were found.

After a morning was spent in preparation of elemental mercury-copper foil amalgams, a concentration of 17.1 ng Hg/2L breath was measured. Exposure was to Hg^0, primarily through inhalation, although some absorption through the skin may have occurred. On the second occasion, the subject had weighed out solid CH_3HgCl for preparation of solutions. Exposure was again primarily through inhalation of fine dust with possible minor skin contact. A breath sample taken afterwards contained 12.3 ng Hg/2L breath.

b) Subject 2

This subject was first sampled on 12/12/81 and was found to have a very high level of mercury in her breath. She did not smoke, had had no recent dental work and consumed fish about four times per month. She explained that for about one month prior to the sampling date she had been painting ceramic ornaments and had been pointing the paint brush in her mouth. Three of the ceramic paints (black, white, and gray) were analyzed and were found to contain mercury. The chemical form of the mercury was not known. The subject refrained from putting the paint brush in her mouth from this time on

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and exposure ended about two weeks later. The subject's breath was monitored for two months and the mercury level showed a decrease by the second month. A sample taken 5 months after exposure had a level of 11.2 ng Hg/2L, well within "normal" range.

The subject's husband also had a breath sample analyzed, but the concentration was found to be within one standard deviation of the normal average concentration. It seemed, therefore, that the high mercury levels found in Subject 2's breath were due to her paint exposure and not to an exposure common to her and her spouse, e.g., diet, residence, etc. Moreover, saliva samples from Subject 2 and her husband showed that she had the highest mercury concentration of the population sampled, while his value was within 2 σ of the "normal" mean.

6. Attempt at Speciation of Mercury in Breath

The chemical form of mercury in the breath is not known. Previous studies of animals and man indicate that mercury can be exhaled as Hg^0 and $(CH_3)_2Hg$, but these studies were done after deliberate exposure to mercury. The chemical form of at least some exhaled mercury was the same as the chemical form to which the subject was exposed.

Since several elements can be volatilized through the lungs as dimethyl compounds (e.g., $(CH_3)_2Se$, $(CH_3)_2Te$), dimethyl mercury is a possible form of exhaled mercury. Elemental mercury is also a possible form of exhaled mercury and may also be present in breath due to amalgam dental fillings.

It is of great interest to note that researchers have found mercaptans and dimethylsulfide in breath.[29,30] These are assumed to be metabolic products from sulfur-containing amino acids. The mobile forms of mercury *in vivo* are thought to be mercury-gluthathione and mercury-cysteine compounds.[31,32] It is possible that mercury may be volatilized as a mercury-sulfur compound.

An attempt was made to use a speciation technique reported in the literature[22] which had separated $HgCl_2$, CH_3HgCl, Hg^0 and $(CH_3)_2Hg$ in air by selective adsorption. The first two adsorption tubes in the sampling train were reported not to adsorb Hg^0 or $(CH_3)_2Hg$. Accordingly, if the only chemical forms of mercury in breath were Hg^0 and/or $(CH_3)_2Hg$, the absorption signal from breath should be unchanged on passing through these two adsorption tubes.

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Both tubes, one containing HCl-treated SE-30-doated Chromosorb W and the other, NaOH-treated Chromosorb W, were placed in series in the Tygon tubing leading to the atomizer.

However, no absorption signal was seen upon injection of air saturated with mercury vapor into the speciation train. Complete adsorption of as much as 100 ng Hg by the tubes occurred despite the report that these materials did not absorb Hg^0. Each tube was also tried separately and complete adsorption of the injected Hg^0 was seen for each. Therefore, speciation of mercury in breath was not possible using this technique.

7. Range of Mercury Concentrations in Saliva

Very little work has been done on mercury levels in saliva, especially in a normal population. In the study by Joselow et al.[19], less than 0.005 ppm Hg was found in the saliva of non-occupationally exposed subjects, but in this case saliva was collected from the parotid gland with a suction device, so that saliva did not contact the rest of the mouth.

The average mercury level found in saliva in this study was 0.27 ppm. This value is significantly higher than that reported by Joselow et al.[19]. The higher value could be the result of the improved accuracy of our direct determination. It could also result from increased mercury concentration in whole saliva through contact with amalgam fillings and bacteria in the mouth. All of the subjects had some amalgam fillings but the exact number was not ascertained.

The saliva mercury level of the paint-exposed individual was greater than 4 σ from the mean of the normal population. It is evident that saliva mercury levels do reflect recent mercury exposure, confirming the reports of Joselow et al.[19] and Stopford.[4] The mercury level in her saliva decreased to normal levels over a six-month period following the last exposure. The other female with the two high breath mercury values had a saliva mercury level of 0.39 ppm, well within 2 σ of the mean. Her breath was measured at the same time the saliva samples were taken and was found to have no detectable mercury.

8. Speciation of Mercury Compounds in Saliva

The retention time of the single, broad mercury-containing peak in saliva was 1 minute. Aqueous $HgCl_2$ and CH_3HgCl had a retention time of

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0.5 minute and gave very sharp peaks. The retention time of the mercury peak in saliva is the same as that in urine and less than that in sweat (1.5 minutes). It can be concluded that the mercury compound in saliva is less volatile than $HgCl_2$ and CH_3HgCl. The mercury maybe bound to a low molecular weight sulfur-containing amino acid.

9. Correlation of Mercury Levels in Breath, Saliva and Scalp Hair

As can be seen in Figures 8 - 10, no correlation exists among mercury levels in breath, saliva and scalp hair for a normal population. In light of the findings of Joselow et al.[16] that saliva mercury levels appeared to reflect blood mercury levels, it is somewhat surprising that there is no correlation between saliva and breath mercury levels. Breath should reflect blood mercury levels if Hg^{2+} is the major form of mercury in blood and an equilibrium between Hg^{2+} and Hg^0 is present in blood. Therefore, breath should correlate with saliva mercury levels. It is possible (and probable) that mercury does not exist as Hg^{2+} in the blood, but as a complex with glutathione or cysteine. It is also possible that the major part of mercury in breath does not come from the lungs but from amalgam fillings in the mouth.

A definite correlation exists for mercury levels in all three matrices for recent mercury exposure. The paint-exposed individual had the highest breath, saliva and scalp hair levels of all subjects surveyed. The breath and saliva levels both dropped back to normal within six months of the last exposure.

CONCLUSIONS AND SUMMARY

The use of the quartz "T" atomizer for atomic absorption spectroscopy enabled the development of a simple, sensitive, effective method for the determination of mercury in breath and saliva.

The average mercury concentration in the breath of a normal adult population was found to be 2.65 $\mu g\ Hg/m^3$ breath.

The average mercury concentration in the saliva of a normal adult population was found to be 0.27 ppm Hg.

Breath and saliva were shown to reflect recent exposure to several chemical forms of mercury. Therefore, both matrices can serve as biological indicators of mercury exposure.

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No correlation was found among mercury in breath, saliva and scalp hair for a normal population. Good Correlation was found among mercury levels in all three matrices for recent mercury exposure.

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