# MERCURY POISONING: I

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# MERCURY POISONING: I

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# TABLE OF CONTENTS

## CREDITS AND ACKNOWLEDGEMENTS


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# PREFACE

Published during 1970-1972, the papers in this volume consider the following topics: analysis and detection of mercuric compounds, occurence of mercury in the environment and in living organisms, human exposure to mercury and mercuric compounds in man, and metabolism of mercuric compounds. First in a two volume set, this collection includes the most recent advances in this important area of ecological control and toxicology.

Volume II of this collection considers the toxic effects of mercury, the reactions of mercuric derivatives with enzymes and other proteins, reaction of mercury and mercuric compounds with nucleic acids, and mercuric compounds as antimicrobial agents. Articles also describe the latest procedures for detecting trace levels of mercury in various ecosystems.

# Analysis and Detection of Mercuric Compounds

# Determinations of Mercury in Air at University Facilities

EUSEBIO MAYZ, MORTON CORN, Ph.D., and GENE BARRY

## Introduction

THERE IS A LONG history of recognition and assessment of hazards stemming from mercury in the air of various occupational environments. The U.S. Public Health Service studied exposure of workers to mercury in the felt hat industry during the late 1930's.[1,2] There have been reports of the concentrations of mercury found in air of the following facilities: mines,[3] jewelry molding,[4] analytical petroleum laboratories,[5] scientific laboratories,[6] chemical laboratories,[7] hospitals,[8] university laboratories,[9,10] and dental offices and laboratories.[11] In a study performed in five laboratories of the University of California at Berkeley, concentrations of mercury in air in one laboratory exceeded 0.1 mg/m$^3$.[9] In the other study[10] at a university in New York State, a white male chemical laboratory assistant in a metal purifying laboratory was reported to have mercury intoxication: concentrations of mercury in air exceeded 0.1 mg/m$^3$.

The toxicology of mercury is also well known.[12,13] Despite our continuous association with the problems of mercury utilization, the widespread usage of this material demands constant vigilance and reassessment of control methods and philosophy. The current recommendation by the Threshold Limit Values Committee of the American Conference of Governmental Industrial Hygienists to lower the TLV from 0.1 mg/m$^3$ to 0.05 mg/m$^3$ substantiates the dynamic nature of the industrial hygiene aspects of mercury.

The above considerations, together with the very rapid increase in student enrollments at institutions of higher learning in the United States, led to this study. The purpose of this work was to assess airborne concentrations of mercury vapor in facilities at a large university campus. A secondary purpose of the study was to evaluate the degree of awareness of the population at risk at these facilities to the hazards of mercury poisoning.

TABLE I
Airborne Substances Which Interfere with Beckman Mercury Vapor Meter Performance

| Contaminant | Amount Required for Reading of 0.1 Mg/M³ Mercury in Air (ppm) |
|---|---|
| Aromatic hydrocarbons | 500–1000 |
| Sulfur dioxide | 80 |
| Ozone | 20 |
| Nitrogen dioxide | 2000 |
| Acetone | 6000 |

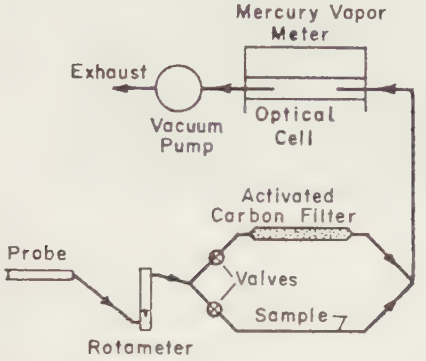


FIGURE 1. Schematic drawing of the mercury vapor meter after modification.

Also, to facilitate assessment of mercury concentrations in air at several facilities in different campus areas, it was necessary to modify a commercially available instrument for mercury assessment. We shall report on these three areas of effort in the present paper.

## Experimental Procedures

### *Instrument Modification*

A variety of detectors have been used to evaluate mercury vapor in air.[1,2,14-19] For this investigation, the Beckman Model K-23 meter, which utilizes the ultraviolet absorption principle, was selected as the instrument of choice on the basis of rapidity of response, portability, and reliability. It was anticipated that a large number of measurements would be required at numerous sites at various times. The instrument was modified in accordance with principles recommended in the literature.[3]

The instrument response is nonspecific. It is known that the air contaminants listed in Table I will yield an instrument response equivalent to 0.1 mg/m³ of mercury vapor in air. In addition, cigarette smoke or other aerosols will absorb ultraviolet light and cause an instrument deflection. Movement of the instrument in areas of light shadows or reflections from polished surfaces will cause fluctuations of the indicator needle on the sensitive range of the instrument (full scale reading: 0.1 mg/m³).

The modifications to the instrument included the addition of a charcoal trap to remove organic vapors and a membrane filter to remove aerosol. Figure 1 is a schematic drawing of the modified apparatus. Figure 2 shows the apparatus disassembled to expose to view the modifications made.

The membrane filter is located at the entry to the activated charcoal filter. It should be noted that the diffuser grille usually associated with this instrument was replaced by an optical cell made of aluminum and having fused quartz end windows and sample inlet and outlet ports. The carbon-filter housing was 9.25 inches long and 1.25 inches in diameter. The cocoanut-activated charcoal was 6 to 14 mesh. A ⅙-hp, 115-volt, 60-cycle pump was used to draw sample through the system. The rotameter indicated that an instrument reading was not dependent on sampling rate for flows between 2 and 4 liters/min. Components of the bypass filter system required for zeroing the instrument were located in an aluminum housing, as shown. In practice, the operator manually turned the indicated valves to zero the instrument by drawing sample either through the filter circuit and then to the optical cell, or directly to the optical cell. During field usage, the instrument was connected to a 200-foot electrical extension cord to avoid frequently disconnecting the apparatus from the electrical outlet. It was found that a warmup period of approximately 20 minutes was required for instrument stabilization following each disconnect–connect cycle. The filters were required to zero the instrument and to balance phototubes at the site of sampling, a procedure which should be performed

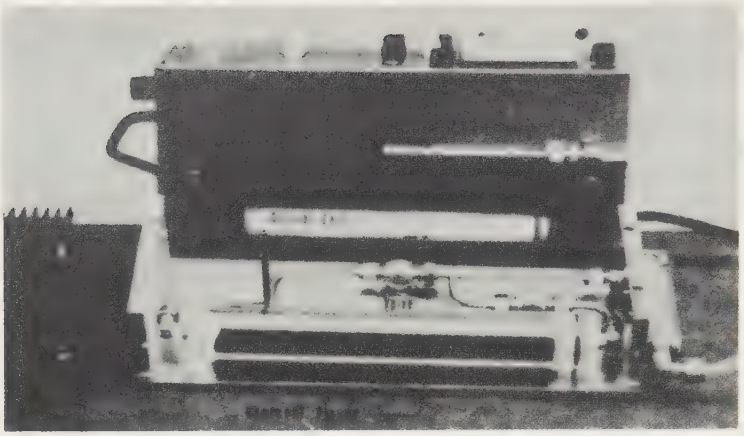

FIGURE 2. Photograph of the modified and disassembled Beckman K-23 instrument.

every 5 or 10 minutes during operation. Prior to the modifications shown, a zero check of the apparatus necessitated moving the apparatus to an environment free of mercury vapor.

*Instrument Calibration*

Mercury vapor in air concentrations for instrument calibration were generated by evaporating weighed droplets of mercury in a 9.055-cubic meter chamber equipped with recirculating fan and exhaust fan. The instrument was located within the chamber, but was positioned to enable one to view the indicating meter from a position outside a chamber window. Figure 3 indicates the close agreement between calculated and measured concentrations in the chamber, particularly in the concentration range less than 0.2 mg/m$^3$. Four calibration points are included for the lower scale range (0 to 0.1 mg/m$^3$) and seven for the upper scale range (0 to 3.0 mg/m$^3$). It was judged that the instrument was sufficiently accurate for field survey purposes.

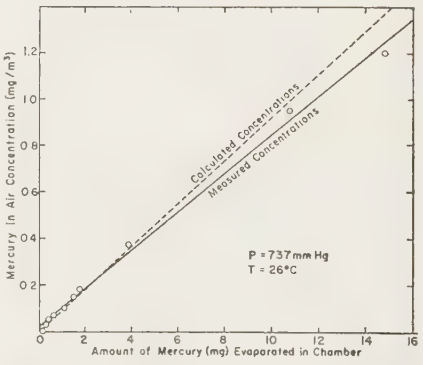


FIGURE 3. Calibration curve for the modified mercury vapor meter.

## Experimental Results and Discussion

Potential mercury hazards at a university facility are manifold. In the survey reported here, mercury in air concentrations were measured in the following facilities: Department of Chemistry, School of Medicine, School of Dental Medicine, Van de Graf Accelerator Laboratory, Physics Department, Engineering School, School of Public Health, Space Research Center. The sources of mercury vapor and measured concentrations in air at each of these sites will be discussed in turn. Measurements were made at each location on at least two separate occasions. In all cases, the guideline used to formulate an opinion relative to the existence of a hazard was the proposed new ACGIH threshold limit value for mercury vapor in air—0.05 mg/m$^3$.

*Department of Chemistry*

Mercury is used in mercury diffusion pumps, Toepler pumps, McLeod gages, polarographs, conductance cells (for low-temperature measurements, particularly), barometers (extensively in magneto-thermodynamic and magnetochemistry laboratories), hydrogenators, and manometers. In addition to these sources, mercury is extensively used in vacuum lines for producing and measuring gas pressures less than atmospheric in systems for studying chemical reactions under controlled conditions. Pressures are controlled by establishing differences in mercury levels in system capillary tubing.

Mercury was present in the air of all laboratories surveyed in this facility. "General" air in various laboratories contained mercury vapor at concentrations in the range 0.020 to 0.035 mg/m³. Concentrations were higher at breathing zones in the vicinity of many of the sources cited above. In close proximity to a magnetometer, near polarographs, and close to several vacuum lines, concentrations consistently exceeded 0.05 mg/m³. At locations where spills had occurred, such as areas beneath sinks or near waste cans, concentrations greater than 0.15 mg/m³ were measured. These sources were responsible for the background or "general" air concentrations, but should not be confused with breathing zone concentrations.

*School of Medicine*

In addition to standard apparatus such as barometers, manometers, diffusion pumps, etc., Scholander gas analyzers and Van Slyke blood gas instruments are extensively used in this facility, particularly in the Department of Anesthesiology. At the latter site, general air concentrations were 0.026 to 0.038mg/m³. In the vicinity of sinks and tables supporting Scholander and Van Slyke units, breathing zone concentrations were in the range 0.06 to 0.14 mg/m³. Personnel are in these rooms for an entire working day, five days per week. Once again, sources of mercury vapor were identified as the residues of spillage on tables, in sinks, on the floor, or in the bottom of wastebaskets.

*School of Dental Medicine*

The major usage of mercury in this facility is in the preparation of amalgams. Mercury is transferred from large containers to smaller containers, as needed. In the Restorative Department, crowns or associated tooth structures are replaced. In the Pedontic Clinic, students provide dental care for children and adults. In both areas, mercury transfer operations occur prior to amalgams preparation in mechanical units which shake mercury and the alloy, both of which are contained in a plastic capsule. Other amalgams are prepared in a mortar and pestle by hand rotating.

General air concentrations in these facilities ranged from 0.010 to 0.020 mg/m³. In the vicinity of amalgamator tables or machines, as well as in air above working sinks, concentrations were as high as 0.070 mg/m³. High concentrations were found under sinks, tables, and other spillage sites.

*Van de Graaf Accelerator Facility*

The major sources in this facility are mercury diffusion pumps. General air concentrations were 0.012 to 0.20 mg/m³. Concentrations in the vicinity of diffusion pumps and near floor level were as high as 0.070 mg/m³. It should be noted that this was the only site in the university where an ultraviolet mercury vapor alarm unit operated on a continuous basis.

At the following facilities, sources of contamination are standard laboratory apparatus, primarily manometers and barometers.

*Physics Department, Engineering School, and Space Research Coordination Center Laboratories*

General air concentrations in the Physics Department were 0.018 to 0.028 mg/m³. Concentrations at selected breathing zone locations were as high as 0.088 mg/m³. Contamination of floor, work tables, and wastebaskets contributed to high concentrations 6 to 12 inches above these surfaces. The highest measured concentration in this facility was 0.12 mg/m³.

General air concentrations in laboratories of the Engineering School and Space Research Center were in the range 0.010 to

TABLE II
Summary of Survey Results

| Site of Survey | Mercury Vapor in Air Concentrations (mg/m³) General Air | Vicinity of Local Sources |
|---|---|---|
| Department of Chemistry | 0.02 –0.60 | 0.15 |
| School of Medicine | 0.026–0.038 | 0.14 |
| School of Dental Medicine | 0.010–0.020 | 0.070 |
| Van de Graaf Accelerator | 0.012–0.20 | 0.070 |
| Physics Department, Engineering School, Space Research Center | 0.018–0.028 | 0.12 |
| School of Public Health | 0.01 | 0.028–0.068 |

0.022 mg/m³. Concentrations in the vicinity of local sources were in the range 0.024 to 0.052 mg/m³.

*School of Public Health Laboratories*

General air concentrations were less than 0.01 mg/m³. Concentrations in close proximity to local sources were in the range 0.028 to 0.060 mg/m³.

Table II is a summary of survey findings.

Mercury vapor in air is thus seen to be ubiquitous in laboratories of the university surveyed. Certain work areas are hazardous with respect to this contaminant. General air concentrations were found to be less than the suggested new TLV of 0.05 mg/m³, but certain breathing zones in the vicinity of apparatus containing mercury were found to exceed the TLV. In general, personnel employed at the sites surveyed were almost totally unaware of the potential hazards of mercury vapor. At some locations, spilled mercury was visible in sinks, on table surfaces, and on floors. Personnel did not appreciate the need to remove these sources of mercury vapor immediately after spillage. The concentrations reported here were measured, with few exceptions, in ventilated laboratories designed for eight to ten air changes per hour.

We conclude that in addition to a program of instruction for personnel in the potential health hazards associated with usage of mercury, administrative units within the university must adopt proven practices for minimizing vaporization of mercury from apparatus and spillage areas. These practices include, but are not restricted to, the use of plastic containers instead of glass containers, locating apparatus containing mercury at a distance from heaters and ovens, supplying vacuum pumps for cleanup of spills, use of flowers of sulfur as an absorbent, and adoption of seamless plastic tabletops. The concentration of mercury vapor in air at any location in these facilities should not exceed 0.02 mg/m³ if these practices are initiated.

## Acknowledgment

We express our appreciation to Mr. Frank Willis, Machinist, Department of Occupational Health, for fabricating the needed modifications to the instrument.

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# The Analysis of Mercury in Urine, Blood, Water, and Air

LEONARD A. KRAUSE, Sc.D., RICHARD HENDERSON, Ph.D.,
HENRY P. SHOTWELL, and DALE A. CULP

## Introduction

IN THE PAST 45 YEARS there have been numerous publications on the analyses of mercury in biological fluids, water, and air. The purpose of this study is to show the feasibility and use of equipment that will accurately determine the concentration of mercury in urine and blood specimens as well as effluent water and work atmospheres where mercury is used. The procedure has the advantages of being simple and quick, and it yields accurate reproducibility.

The extraction of mercury from a liquid medium by chelation with dithizone is the most common method employed today. Another method[1] has used di-$\beta$-naphthylthiocarbazone in place of dithizone. Ethylenediamine-tetraacetic acid sodium salts[2,3] have been used for pre-extractions to remove ions which may interfere with the mercury dithizonate. Following the extraction, the resulting mercury dithizonate is read quantitatively on an appropriate colorimeter. Cold atomic absorption also has been used successfully for analyses of mercury in micro quantities.[4]

The analysis of atmospheres containing mercury is generally limited to two basic procedures. Air containing mercury is drawn through an appropriate impinging device containing a solution of potassium permanganate and sulfuric acid. The permanganate, in turn, is reduced with hydroxylamine hydrochloride, extracted with dithizone, and read on a colorimeter. The second procedure is to use a direct-reading instrument which utilizes an ultraviolet source and a photocell receptor. This method is based on the absorption of the 2537-Å mercury resonance line by mercury vapor, and the mercury is read directly as a quantitative atmospheric concentration.

In 1960 Jacobs *et al.*[5] modified the mercury meter by placing an optical tube or cell between the ultraviolet source and the photocell. The tube, having inlet and outlet ports, had the advantage of utilizing small volumes of air at a steady rate of flow, resulting in micro analyses of mercury in digested and dithizone-extracted blood specimens. This too, then, is principally cold atomic absorption.

Recently several methods have been reported which have eliminated the use of the dithizone procedure. Smith (personal communication, Wayne State University) has

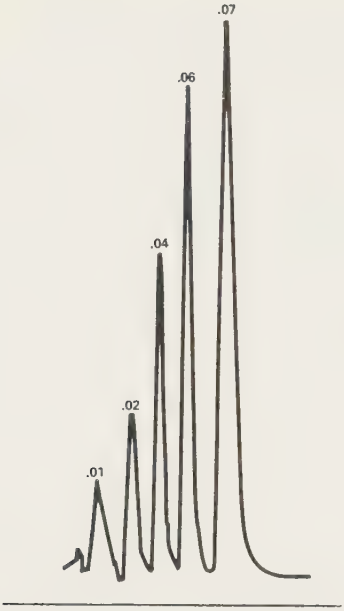


FIGURE 1. Micrograms of mercury versus peak height response.

pyrolized tissue and passed the resulting vapors through a condenser, a chemical filter, and finally the optical-cell mercury meter. For urine specimens, Rathje[6] uses nitric acid digestion followed by reduction of the mercury to the elemental state, with the addition of stannous chloride instead of pyrolysis. The elemental mercury is driven through chemical filters to remove moisture and is again read on an appropriate ultraviolet mercury meter. Lindstedt[7] has used a 12-hour wet digestion of urine with a potassium permanganate-sulfuric acid mixture, reduction of mercury with stannous chloride, and sweeping of the mercury vapor from the solution by an air current at room temperature. In any case, micro quantities of mercury are determinable.

The method presented here is a further refinement of the procedure with less manipulation and an apparatus which allows for possible field determinations of both liquid and gaseous samples. With 1-ml urine specimens, 0.03 $\mu$g of mercury can be determined

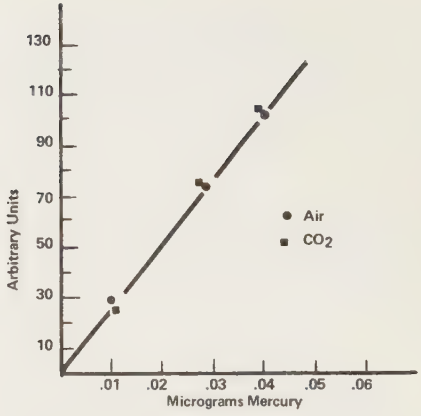


FIGURE 2. Effect of carbon dioxide atmosphere on absorption peak height.

easily. If appropriate aliquots are used, the sensitivity can be reduced to parts per billion in water. Precise volumetric measurements of reagents are not necessary; however, some care should be exercised to ensure that sufficient quantities of the reagents be in the system.

The greatest advantage of this method is the elimination of the chemical filter following liberation of the mercury. The chemical filtration was proposed to remove $CO_2$, water, and other interferences. Our experience indicates that this chemical filter is unnecessary.

By passing the released mercury containing atmosphere through water, the resulting atmosphere is at a constant relative humidity, reducing the necessity for the periodic changing of a chemical filter. A typical response curve for varying concentrations is depicted in Figure 1. No interference from water vapor at room temperature (68° to 70°F) can be noted. Carbon dioxide, as indicated in Figure 2, does not interfere with optical transmissions.

## Equipment and Reagents

### *Apparatus*

The apparatus, as shown in Figures 3, 4, and 5, consists of an all-glass reaction vessel with a receiving funnel insert, connected to a

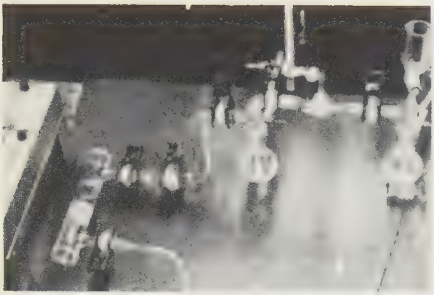

FIGURE 3. Assembled apparatus for installation into ultraviolet light path of mercury detection meter.

water scrubber by means of a three-way stopcock. From the scrubber, glass fittings attach to the optical cell. At point $x$ (Figure 5) a disk of No. 1 Whatman filter paper is inserted, sealed by an O-ring joint. This removes possible droplets of moisture. Aspiration is through coarse frits positioned to fit near the bottom of the flasks as shown. All glass joints are ball-and-socket held by appropriate pinch clamps. The dimensions are critical but only for the volume of the system, since oversized flasks can lead to greatly diluted mercury vapor concentrations and reduce the sensitivity of the system. A satisfactory size for the reaction flask is 100 ml. A flow meter calibrated for flow rates between 1 and 4 liters/min is inserted downstream of the optical cell and before the vacuum pump.

The Beckman Model K-23 double-scale mercury vapor test meter used in these experiments was altered by removing the grille, and mounting and positioning an all-glass optical cell 22.5 cm in length by 4 cm in diameter (fitted with inlet and outlet ports) and with quartz glass windows fused into the ends.

*Reagents*

Nitric acid, reagent grade, concentrated.

Sulfuric acid, reagent grade, concentrated.

Stannous chloride solution; prepare a 20% w/v stannous chloride solution in 6$N$ hydrochloric acid.

*Standard Solutions*

*Standard Stock Mercury Solution.* Two procedures may be use for preparing stock mercury standards: (1) Dissolve 0.1354 gm of mercuric chloride in 100 ml of 1$N$ hydrochloric acid. One milliliter contains 1 mg of mercury. (2) Dissolve 1 gm of elemental mercury in 20 ml of concentrated nitric acid. When dissolved, dilute to 1 liter with water. One milliliter contains 1 mg of mercury.

*Working Stock (10 μg/ml):* Dilute 1 ml of the stock mercury solution to the mark with distilled water in a 100-ml volumetric flask. Each milliliter contains 10 μg of mercury. Working stock should be prepared fresh

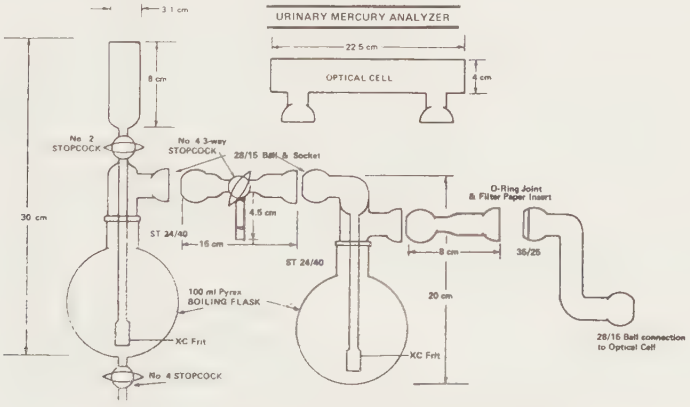


FIGURE 4. Dimensional scheme for mercury analyzer.

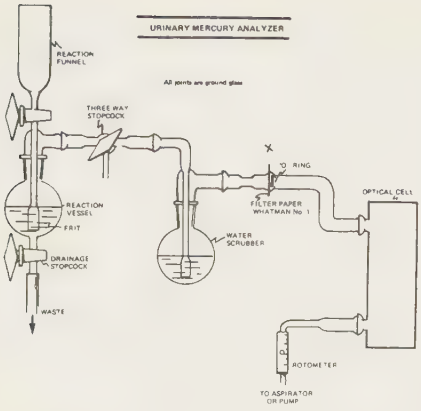


FIGURE 5. Urinary mercury analyzer connection to optical cell.

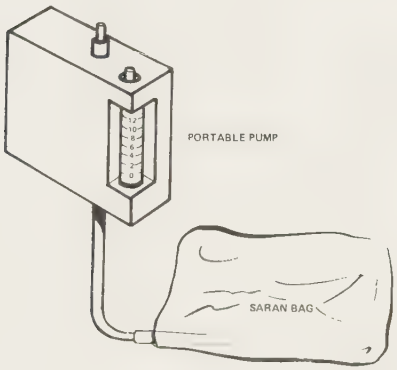


FIGURE 6. Collection arrangement for portable air sampling.

weekly. From the working stock, convenient standards may be prepared.

*Stock Antifoam Solution:* A 1% stock solution of antifoam is made by suspending 1 gm of Antifoam 60 in 99 ml of distilled water (Silicone Products Department, General Electric Corporation, Waterford, New York).

*Working Antifoam Solution:* Add 1 ml of the antifoam stock solution to 500 ml of distilled water.

## Preparation of Standard Curve

Zero the ultraviolet meter with clean air and with the vacuum pump pulling at a known rate between 1 and 4 liters/min. A flow rate of 2 liters/min is recommended.

TABLE I

Recovery of Mercury from Urine

| Micrograms Added | Micrograms Recovered |
|---|---|
| 0.05 | 0.045 |
| 0.10 | 0.110 |
| 0.15 | 0.140 |
| 0.25 | 0.250 |
| 0.50 | 0.520 |
| 0.75 | 0.751 |
| 0.85 | 0.840 |

In a 50-ml beaker, add a measured amount of the standard working mercury solution (range should be between 0 and 10 $\mu$g) and 5 ml of concentrated nitric acid; allow to stand for 2 to 3 minutes, and then add 20 ml of the working antifoam solution. With the three-way stopcock in the bypass position, transfer the beaker contents to the 100-ml reaction vessel, followed by 1 ml of the stannous chloride solution. Rinse the beaker with 20 ml more of distilled water and add to reaction vessel.

If the total quantity of liquid does not cover the entire frit in the reaction vessel, a minimal amount of distilled water may be added to submerge it. This will afford maximum aeration of the sample.

Turn the three-way stopcock so that all air must be pulled through the reaction vessel at a rate of 2 liters/min. Observe the detector for response to the mercury vapor as it passes through the optical cell. Read directly or connect to recorder and establish curve values.

After peaking of the meter, turn the three-way stopcock to the bypass position, allowing the system to air flush. Open the reaction vessel stopcock, and drain by an appropriate water aspirator. Rinse once, close, and refill with next standard solution.

Occasionally, the flow of the sample from the funnel to the reaction vessel may not start and be continuous. This may be overcome by turning the three-way stopcock to a position in which airflow is drawn through both the reaction vessel and the bypass position. By placing a finger over the bypass port, flow can be started gently into the reaction vessel. Once the reaction vessel is filled to the ap-

Table II

Recovery of Mercury from Blood

| Micrograms Added | Micrograms Recovered |
|---|---|
| 0.003 | 0.004 |
| 0.010 | 0.009 |
| 0.025 | 0.029 |
| 0.000 | 0.001 |

propriate mark, the stopcock can be returned to its full bypass position.

## Procedures

*Urine or Water Samples*

As with the preparation of the standards, add 1 ml of urine to a 50-ml flask. To this add 5 ml of concentrated nitric acid; allow to stand 2 to 3 minutes. Add 20 ml of working antifoam reagent. Transfer to the reaction flask, and add 1 ml of stannous chloride solution. Wash the beaker with 20 ml of distilled water, and add this to the reaction vessel. Turn the three-way stopcock from the bypass position to the aspiration position. Read full peak response on meter or recorder. Record peak readings and prepare standard curve.

*Blood Samples*

Place 1 ml of heparinized or citrated blood in a 50-ml beaker. To this add 5 ml of concentrated nitric acid and 5 ml of concentrated sulfuric acid. Allow this mixture to digest for 20 minutes, or until amber color is completely developed. Low heat may be applied. Of this solution, treat a 1-ml aliquot similar to a urine specimen; that is, add 5 ml of concentrated nitric acid, 20 ml of the antifoam working solution, and 1 ml of stannous chloride. Transfer to the reaction vessel, rinse the beaker with 20 ml of distilled water, and proceed as for urine. In some cases, one or two additional drops of the 1% stock antifoam solution may be added to keep down excessive foaming.

*Air Sampling*

Several means can be employed to test the atmosphere suspected of containing mercury. In each case, the air is fed through the three-way stopcock bypass port, bypassing the reaction vessel. A polyethylene tube can be used to sample air directly from a suspected area. The use of Saran bags, 5- and 10-liter sizes, offers ease of handling large grab samples from the contaminated area and permits them to be fed directly to the bypass port. A small portable pump can be used to fill the bags, as shown in Figure 6.

Table III

Retention of Atmospheric Mercury in Saran Bags

| Mercury Concentration in Air Collected in Saran Bags (ppm) | Analysis of Air at Indicated Time Intervals | | |
|---|---|---|---|
| | 30 min | 60 min | 120 min |
| 0.10 | 0.10 | 0.10 | 0.10 |
| 0.50 | 0.50 | 0.50 | 0.50 |
| 1.00 | 1.00 | 1.00 | 1.00 |

## Results

Our results indicate that recovery of mercury from both urine and blood specimens is good. Tables I and II show values for the appropriate samples with known amounts of mercury added and the amounts recovered. Additionally, our experience with the use of a separate collecting apparatus for air analysis has been favorable. The retention time of elemental mercury vapor in air in Saran bags is excellent. Note Table III. However, polyethylene, while not satisfactory for retaining samples because of its porosity, can be used if samples are read within 10 to 15 minutes. The large food-preserving bags can be quickly filled and sealed off and finally attached to the bypass for analysis.

Using this procedure for air analysis, preliminary studies on breath blown into these bags indicate that this is a simple means for determining the amount of mercury retained by individuals exposed to a mercury-containing atmosphere.

## Discussion

The majority of methods currently being used for the determination of mercury in body fluids require a considerable amount of the specimen. With the employment of an optical cell to confine and concentrate the mercury atmosphere, and the ease with which it can be released from a digested specimen,

very small quantities (1 ml or less) may be used. Digestion procedures have been reduced to a minimum.

All equipment should be constructed with glass joints. Low concentrations of mercury can be absorbed easily into Tygon and gum rubber tubing, resulting in low values.

We prefer the use of beakers for digestion rather than digesting directly in the reaction vessel itself. This allows the analyst to set up a series of samples ahead of time and to merely drain the reaction vessel, rinse, and add the next specimen, reducing the time needed to analyze multiple samples.

The reading of peak heights affords one minor advantage: the time for analysis can be speeded up be stopping the forward motion of the recorder; then the reaction vessel can be flushed and rinsed. By this time, the recorder pen should be at the base line ready to record the next sample.

Where the concentration of mercury is very low in water, the aliquot of sample can be increased to approximately 50 ml. This reaches the practical limit of the system, since not more than 60 ml can be used easily in the 100-ml reaction vessel. Standards may be made in urine, although the differences between water and urine are not significant, as shown in Figure 7.

The mercury meter can be read directly using an arbitrary scale, or it can be connected to a recorder where peak height in millimeters is plotted against concentrations of mercury in micrograms per liter of water, urine, or micrograms of blood (Figure 8). A simple jack can be attached to the mercury meter (as shown in Figure 9).

Since peak heights are used as a measure of concentration, the response of the system must be rapid and constant. It is essential that the airflow be maintained at a steady rate. As reported by Rathje,[6] any appreciable increase in the temperature of the contents of the reaction vessel can cause an increase in response of the system to mercury. This effect has been controlled to some degree by the use of the water scrubber which maintains a constant temperature of the air flowing to the optical cell. It is recommended that several standards be run with each new set of specimens, and when new reagents or glassware are used.

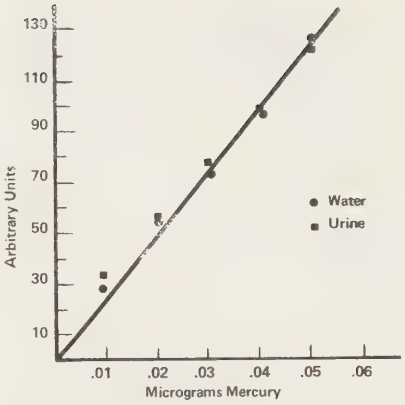


FIGURE 7. Mercury recovery from standards in urine compared to mercury recovered from standards in water.

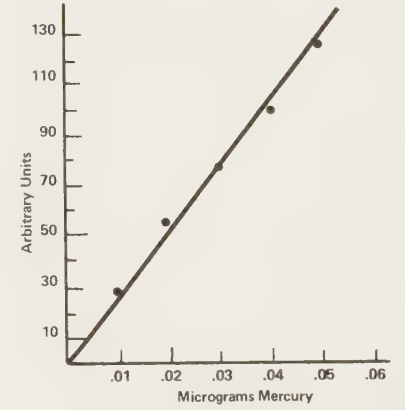


FIGURE 8. Mercury standards against peak height response.

Antifoam is necessary for both urine and blood samples. Occasionally the blood sample may require one additional drop of 1% stock antifoam. We have found that excessive amounts of antifoam (that is, more than 2 drops of the 1% solution) may slightly depress the values; this has been reported by Rathje.

From time to time, depending on the type

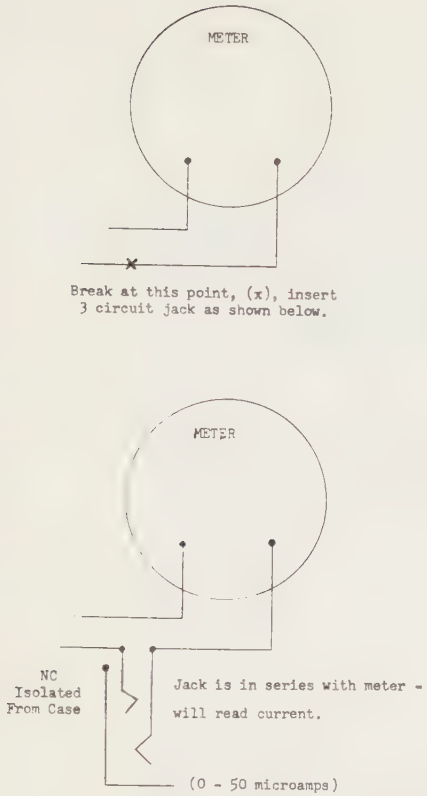


FIGURE 9. Sketch for installing jack to recorder, using the Model K-23 Beckman Mercury Analyzer.

of sample run and the frequency of use, the frit on the end of the sample funnel may become partially clogged with carbonaceous material. From mechanical aspects, this layer of material may interfere to some degree with analyses by reducing the airflow. To clean such a frit of carbonaceous matter, heat the frit (or the entire funnel) to 560°C for one-half hour, then allow to cool slowly.

The analyst may desire to re-orient the reaction vessel to allow for use of smaller volumes. By using a fine-tipped pipet in place of the frit inserted into a deep-well reaction vessel, the sensitivity and rapid release of mercury may be enhanced.

Effects of highly caustic solutions have been studied. By using 1-ml aliquots, 50% caustic solutions do not affect the analysis.

With this procedure, work areas where mercury is present can be monitored; urinary and blood mercury levels determined; and direct evaluation of respiratory mercury retention carried out on site easily, quickly, and economically.

With the increased sensitivity of this and other procedures, all reagents and new glassware, including pipetes, should be tested for mercury. Many calibrations of glass equipment are done with mercury. Some analytical-grade chemicals do contain, as contaminants, heavy metals, which can include mercury.

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1. CHOLAK, J., and J. M. HUBBARD: Micro-determination of Mercury in Biological Material. *Ind. Eng. Chem. Anal. Ed. 18:* 149 (1946).
2. CAMPBELL, E. E., and B. M. HEAD: The Determination of Mercury in Urine—Single Extraction Method. *Amer. Ind. Hyg. Quart. 16:* 275 (1955).
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6. RATHJE, ARNOLD O.: A Rapid Ultraviolet Absorption Method for Determination of Mercury in Urine. *Amer. Ind. Hyg. Assoc. J. 30:* 126 (1969).
7. LINDSTEDT, G.: A Rapid Method for the Determination of Mercury in Urine. *Analyst 95:* 264 (1970).

# Determination of Mercury in Biological and Environmental Samples by Neutron Activation Analysis

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James A. Sondel, and Carolyn M. Hyche

MERCURY IN THE BIOSPHERE is a very unique pollutant because of its apparent indestructibility and its unusual ability to transform into highly toxic compounds by biological methogenation in nature. The sampling and the analysis of mercury in the environmental and biological samples offer some extremely challenging problems. The minute quantities of mercury present in these samples as well as the volatile nature of mercury and its compounds only add to the problems associated with the complexity of the matrices themselves. The current concern over the environmental contamination by mercury brought out several reviews, reports, and bibliographies on mercury (*1–4*). A bibliography prepared by the

(1) "Chemical Fallout," Proceedings of the Rochester Conferences on Toxicity, M. W. Miller and G. G. Berg, Ed., Charles C Thomas, Springfield, Ill., 1969.

(2) "Mercury Contamination in the Natural Environment," U. S. Department of Interior, Office of Library Services, Washington, D. C., 1970.

(3) R. A. Wallace, W. Fulkerson, W. D. Shults, and W. S. Lyon, "Mercury in the Environment—The Human Element," ORNL-NSF-EP-1, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1971.

(4) G. Löfroth, "Methylmercury—A Review of Health Hazards and Side Effects Associated with the Emission of Mercury Compounds into Natural Systems," University of Stockholm, Stockholm, Sweden, 1969.

U. S. Department of Interior (*2*) lists about 60 papers describing various modifications of mercury determinations using atomic absorption, colorimetry, dithizone titration, X-ray fluorescence, pyrolysis, isotope exchange technique, and neutron activation analysis. The recent edition of the U. S. Atomic Energy Commission publication (*5*)—"Radiochemistry of Mercury"—lists about 80 references describing various applications of neutron activation analysis to mercury determination. However, only a limited number of these procedures can be reliably adapted for the determination of mercury in biological and environmental samples to monitor pollution. The procedures described here were developed for the investigation of the mercury pollution of Lake Erie and its aquatic life. Therefore, the examples presented here are primarily samples from the lake.

Neutron activation analysis is a highly specific and sensitive method for the determination of mercury, provided adequate precautions are taken in aliquoting, handling, storage, and pre-irradiation processing of the samples. The determination of mercury in biological and environmental samples by nondestructive neutron activation analysis is mostly of theoretical interest because there is hardly any environmental sample of interest to pollution studies that could be reliably analyzed by this technique. This is because of the extremely low concentrations of mercury in these samples and the interferences due to radioactivities produced by other components of the matrices.

The mercury present in biological tissues is mostly organic mercurials, while the mercury in other environmental samples is often composed of metallic and ionic mercury with varying amounts of bound organic mercury compounds. The mercury analysis of these samples, therefore, requires the degradation of the organic materials and/or the careful extraction of mercury from the insoluble matrices. Because of the high volatility of mercury compounds, ordinary combustion processes are not suitable to decompose the bound mercury and to collect them for analysis. Wet oxidation processes are often used to degrade these materials for trace analysis. An excellent discussion of the release of mercury due to volatilization under a variety of wet oxidation conditions was made by Gorsuch (*6*) through his study on the

(5) J. Roesmer, "Radiochemistry of Mercury," Nuclear Science Series NAS-NS-3026, rev, USAEC Division of Technical Information, Oak Ridge, Tenn., 1970.
(6) T. T. Gorsuch, *Analyst*, **84**, 135 (1959).

recovery of trace elements in organic and biological materials.

The common procedures using a radioactive tracer to determine recovery and analytical accuracy are not well suited for the analysis of mercury in biological and environmental samples. This is because of the limited knowledge of, and possible variations in the chemical form of mercury present in these samples. Experiments using one particular chemical form of radioactive tracer can only infer that the procedure may work, but it does not necessarily mean that the procedure does work for the forms of mercury actually present in the sample. Recognizing these limitations, an attempt was made to develop a reliable neutron activation analysis procedure to determine trace levels of mercury in a variety of biological and environmental samples.

**Table I. Loss of Mercury from Samples during Freeze-Drying[a]**

| Sample identification | Initial levels of mercury (natural form) in ppm | Loss of mercury, % |
|---|---|---|
| A. Fish homogenates | | |
| Fish D-21 | 1.77 | 16.4 |
| Fish E-21 | 0.12 | 18.3 |
| Fish G-21 | 4.56 | 38.8 |
| B. Fish homogenates spiked with radioactive mercury ($Hg^{2+}$ form) | | |
| Fish G-20 | ... | N.D.[b] |
| Fish G-40 | ... | N.D. |
| Fish G-60 | ... | N.D. |
| C. Human brain tissues | | |
| Pons | 0.43 | 56.5 |
| Carona radiata | 0.15 | 24.3 |
| Cerebellar cortex | 0.72 | 18.0 |
| D. Plankton/algae (Lake Erie) | | |
| PL-Cx | 17.86 | 50.0 |
| PL-Cy | 17.86 | 42.1 |
| PL-Cz | 17.86 | 64.3 |
| E. Sediment/silt (Lake Erie) | | |
| s/s-EE | 2.30 | N.D. |
| s/s-EF | 2.35 | N.D. |
| s/s-EG | 2.05 | N.D. |

[a] VirTis Manifold type and VirTis Model 10-100 freeze-dryers were used.

[b] Not detectable.

**Sample Preparation.** In neutron activation analysis using reactor neutrons, it is highly desirable to have the sample compacted, as well as free from excessive amounts of moisture. Since biological samples usually are not too dense and they contain a significant amount of moisture, processes such as oven-drying, freeze-drying, ashing, etc. are often used to prepare samples for reactor irradiation and subsequent handling. During this investigation, a low temperature asher using oxygen plasma for oxidizing tissues, two freeze-dryers (without mercury gauges) and an ordinary laboratory oven were used to investigate the possibility of using them for pre-irradiation sample preparation.

A set of tissue homogenates were mixed with radioactive mercury ($Hg^{2+}$ form) and were homogenized to form a slurry. These samples were aliquoted into freeze-drying flasks and the mercury activity was measured by gamma ray spectrometry using a 10 cm × 10 cm NaI(Tl) detector. The samples were quick-frozen using a mixture of crushed dry ice, liquid nitrogen, and ethyl alcohol. The freeze-drying continued using a similar cold trap. The radioactivity from the mercury was again measured and compared with standards used prior to freeze-drying. The results shown in Table I (Section B) indicate that there is no significant loss of radioactive mercury ($Hg^{2+}$ form) from the fish homogenate.

The freeze-drying processes described above were repeated using a set of fish homogenates, human brain tissues, plankton/algae, and sediment/silt samples previously analyzed for their mercury content by the neutron activation analysis procedure detailed below without any pre-irradiation preparation. The residual samples from the freeze-drying processes were again analyzed for their mercury content by neutron activation analysis. The results of these analyses shown in Table I (Sections A, C, D, and E) indicate that there is significant loss of mercury from all the samples except sediment/silt during the freeze-drying process. Since the use of radioactive tracer indicated that there was no significant loss of $Hg^{2+}$ mercury during freeze-drying, the losses observed here may be attributed to volatile forms of mercury present in the samples.

The investigation of a low temperature asher (Tracerlab Model 505), for preparing analytical samples for neutron activation, involved the use of fish homogenates spiked with radioactive mercury ($Hg^{2+}$ form) as described earlier. The mercury content of the aliquots was measured by gamma ray spectrometry before and after ashing. The results shown in Table II clearly demonstrate that this technique is not suitable for the preparation of ashed samples for neutron activation.

Assuming that it was the volatile organic mercurials that were lost during freeze-drying of the sample, an attempt was made to convert the mercury compounds in the samples to

Table II. Loss of Mercury ($Hg^{2+}$ Form) during Low-Temperature Ashing[a]

| Sample identification | No. of hours of ashing | Loss of mercury, % |
|---|---|---|
| Fish 1 | 3.5 | 81.4 |
| Fish 2 | 3.5 | 81.9 |
| Fish 3 | 3.5 | 91.8 |
| Fish 4 | 7.0 | 98.0 |
| Fish 5 | 7.0 | 98.0 |
| Fish 6 | 7.0 | 98.0 |

[a] Tracerlab Low Temperature Asher Model No. 505, radio frequency power level = 200 watts (maximum), oxygen flow rate = 100 cc/minute, sample temperature = 110 °C (maximum).

inorganic form by exposure to high radiation doses. The results of these experiments, shown in Table III, indicate that the mercury in biological tissues can be stabilized against loss during freeze-drying by exposure to high doses of high energy nuclear radiation. This procedure, however, is cumbersome for routine application.

An attempt to utilize ordinary laboratory ovens to dry samples of sediment/silt and plankton/algae also revealed that there is significant loss of mercury from these samples even when temperatures of the order of 60 °C were used. The results shown in Table IV are self-explanatory.

From the above mentioned findings and the reported findings of Greenwood and Clarkson (*7*) regarding storage of samples, it is generally a good practice not to pre-process samples to limit the bulk or to reduce the moisture content and not to store samples in containers that adsorb mercury on their surfaces. The following procedures were used to prepare samples used for reactor irradiation during this investigation:

Solid Biological Tissues. The samples of fish and other biological tissues were kept frozen until ready for use. The tissues were homogenized using a blender and/or a grinder made of stainless steel or borosilicate glass. A convenient analytical sample (about 1 to 3 grams) of the homogenized tissue was carefully weighed into a small polyethylene bag (4 × 12 cm size) made of 0.2-mm thick sheets. The air from the bag was squeezed out and the bag was heat sealed, allowing a void space equivalent to at least twice the volume of the wet tissue sample, which allowed room for the gaseous radiation products produced during reactor irradiation. Wet tissue weights of these samples were used for calculating the results.

**Table III. Effect of Exposure of Fish Homogenates to Radiation Prior to Freeze-Drying**

| Description of radiation exposure and processing | Mercury content of sample, ppm[a] | Loss of mercury during freeze-drying, % |
|---|---|---|
| 1. No radiation exposure. No freeze-drying | 1.77 | None |
| 2. No radiation exposure. Freeze-dried | 1.50 | 15.3 |
| 3. Exposed to 1.2 megarads of X-rays using a Van de Graaff machine. Freeze-dried | 1.51 | 14.7 |
| 4. Exposed to 2.4 megarads of X-rays using a Van de Graaff machine. Freeze-dried | 1.48 | 16.4 |
| 5. Exposed to 2 megarads of gamma rays and $2 \times 10^{16}$ neutrons per $cm^2$ in a reactor. Freeze-dried | 1.68 | 5.1 |
| 6. Exposed to 5 megarads of gamma rays and $5 \times 10^{16}$ neutrons per $cm^2$ in a reactor. Freeze-dried | 1.69 | 4.5 |

[a] The mercury present in these samples was in the natural form. The results given are the averages of more than two determinations by the neutron activation analysis described.

**Table IV. Loss of Mercury from Lake Samples during Low-Temperature Oven Drying[a]**

| Sample identification | Initial levels of mercury (natural form) in ppm | Loss of mercury, % |
|---|---|---|
| A. Plankton/algae (Lake Erie) | | |
| PL-Bx | 17.86 | 51.1 |
| PL-By | 17.86 | 71.7 |
| PL-Bz | 17.86 | 60.6 |
| B. Sediment/silt (Lake Erie) | | |
| s/s-EA | 2.25 | 23.6 |
| s/s-EB | 2.25 | 12.4 |
| s/s-EC | 2.25 | 12.4 |

[a] 50 hours of drying in a laboratory oven at 60 °C.

Plankton/Algae Samples. The plankton/algae samples were collected using a fine mesh (14 meshes to a centimeter) plankton net. The samples collected in glass bottles were frozen as soon as possible. Prior to sampling for analysis, the contents of glass bottles were allowed to partially thaw to separate most of the ice from the plankton/algae samples. After separating the ice, the plankton/algae samples were transferred into tared polyethylene bags described in the above section. The bags were made to fit into standard 50-ml centrifuge tubes. The polyethylene bags containing the samples, supported in centrifuge tubes, were centrifuged using a high speed centrifuge. The supernatant water was poured out, and the final visible traces of water spots in the bag were removed by a cotton swab. A weighed portion of this sample was taken out into a small aluminum foil dish. The remainder of the sample (about 0.2 to 1 gram) in the bag was weighed along with the polyethylene bag to determine the weight of the wet sample. The aliquot of the sample taken in the aluminum foil dish was dried in a laboratory oven at 60° for 50 hours or until it attained a constant weight. These data were used to calculate the dry weight of the wet sample in the polyethylene bag. The sample was sealed in the bag after squeezing out the air as described earlier.

Sediment/Silt Samples. The sediment/silt samples were collected using two kinds of gear. An Eckman dredge which gathers samples from approximately the top 5 cm of sediment and a Peterson dredge which picks up the materials between 3 and 30 cm below the mud–water interface were employed in collecting the lake sediments. The samples collected in large (2-liter) containers were stored at room temperature until use. Since flint glass surfaces are known to adsorb mercury (*7*), the analytical samples were aliquoted from the middle part of the container. They were homogenized before the excess water in the samples was removed by centrifuging. The moist samples were contained in polyethylene bags and their dry weight was determined as described for the plankton/algae samples. The equivalent dry weight of the sediment/silt samples were used in calculating the results.

In preparing soil samples, coal, flour, and plant tissues for mercury analysis, the above mentioned procedures can be readily adapted. However, for the determination of mercury in liquid samples, none of the above mentioned pre-irradiation preparations are suitable.

Neutron Activation. The samples encapsulated in heavy duty polyethylene containers along with mercury standards (contained in thin quartz vials) were irradiated at a thermal neutron flux of about $5 \times 10^{12}$ neutrons $cm^{-2}$ $sec^{-1}$ for 2

(7) M. R. Greenwood and T. W. Clarkson, *Amer. Ind. Hyg. Ass. J.*, 31, 280 (1970).

hours using a PULSTAR research reactor at the Western New York Nuclear Research Center. Because of the high capture cross section for thermal neutrons (*8*) [3092 barns for $^{196}Hg(n, \gamma)\ ^{197}Hg$, and 107 barns for $^{196}Hg(n, \gamma)\ ^{197m}Hg$], caution should be exercised to limit the size of the standard to avoid self-shielding and flux pertubation. The samples were allowed to decay for at least 1 hour prior to processing in order to allow the short-lived activities from the matrix to decay.

**Wet Ashing and/or Extractive Digestion.** The apparatus we have used for the wet oxidation of biological tissues and extractive digestion of soils and sediment samples is a simple version of the apparatus described by Bethge (*9*) and used by Sjöstrand (*10*) for oxidation of biological tissues. The apparatus shown in Figure 1 can be put together with readily available parts from U. S. distributors.

The irradiated sample contained in the polyethylene bag was removed and the excess bag material around the heat sealed area containing the sample was trimmed to minimize the amount of polyethylene to be ashed. About 200 to 300 mg of bag material that contained the sample was usually ashed along with the sample to prevent any loss of mercury adsorbed or recoiled on to the surfaces of this container. The sample in the polyethylene encapsulation was inserted into the 200-ml distillation flask containing an accurately known amount of mercury carrier (50 mg of Hg/ml as $Hg^{2+}$) and 10 ml of concd nitric acid. The apparatus shown in Figure 1 was assembled and through the top of the condenser 5 ml of concd sulfuric acid and 5 ml of 70% perchloric acid were added. A volume of 2*M* hydrochloric acid sufficient to cover the lower bent portion of the splash head trap was placed above the condenser. The contents of the flask were simmered (<100 °C) for about half an hour and the temperature was then allowed to rise to 120 °C. The two-way stopcock on the reservoir was closed and the distillate was allowed to accumulate in the reservoir of the reflux column. The temperature rises rapidly from 130 °C, and caution should be exercised to ensure that heating is moderate and the temperature rise is not rapid. A violent reaction can result if the heating is excessive and the polyethylene bag catches fire by the action of perchloric and sulfuric acids. When the temperature reached 150–160 °C, the heating mantle was dropped and the flask was allowed to cool down to below 90 °C.

The condensate in the distillation column was carefully drained into the flask and heating continued. The stopcock

(8) W. Seelmann-Eggebert, G. Pfenning, and H. Munzel, "Nuklidkarte," 3rd ed., Der Bundesminister Fur Wissenschaftliche, Forschung, Bonn, 1968.

(9) P. O. Bethge, *Anal. Chim. Acta*, **10**, 317 (1954).

(10) B. Sjöstrand, ANAL. CHEM., **36**, 814 (1964).

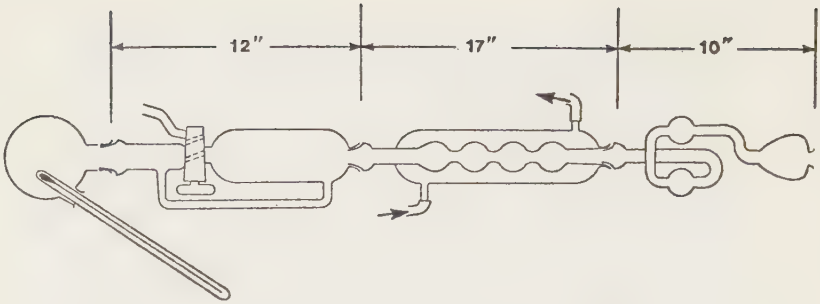


**Figure 1. Apparatus used for controlled wet-ashing and/or digestion of samples for mercury analysis**

was left open to the flask and refluxing continued for another 15 minutes. By this time, all the organic materials including the polyethylene bag had dissolved completely and the solution was clear and free from oily substances from biological tissues. If traces of undissolved or charred tissues and oily suspension remained, the process of heating to 150 °C and subsequent refluxing was repeated. In the case of soils and lake sediment samples, all the organic constituents dissolved leaving aside mostly insoluble silicates. Plankton/algae samples readily dissolve to form a clear solution.

The contents of the flask were allowed to cool. During the cooling period, the stopcock on the reflux column was closed and the splash head trap and the condenser were washed down with one or two 10-ml volumes of water, added to the reservoir on the trap. The washings and the contents of the distillation flask were transferred to a 250-ml beaker. In the case of soil and sediment samples, the insolubles were separated by centrifuging prior to transferring the solutions into beakers.

**Separation of Mercury.** The excess acids were carefully neutralized using ammonium hydroxide. The solution was then made acidic (about 2*M*) with hydrochloric acid. This solution was warmed and treated with hydrogen sulfide gas until the precipitation was complete. The contents of the beaker were warmed and were allowed to coagulate before centrifuging. The sulfide precipitates were washed with warm 2*M* hydrochloric acid to remove most of the iron, aluminum, chromium, manganese, nickel, cobalt, etc., that may have precipitated along with mercuric sulfide. The precipitates, after washing with warm water, were mixed with 5 ml of ammonium polysulfide and 1 ml of 2*M* sodium hydroxide and were kept warm in a water bath, to allow the dissolution of antimony, arsenic, and tin sulfides, if any. The precipitates were then washed with warm water, followed by 3*M* nitric acid, to remove copper, cadmium, and probably traces of other elements such as gold, platinum, molybdenum, selenium, etc.

The mercuric sulfide precipitate in the centrifuge tube was then mixed with 5 to 10 drops of freshly prepared aqua regia and was kept warm on a water bath for 10 to 15 minutes. It is important here to use the minimum amount of aqua regia and also to remove most of the excess acid by evaporation. Caution should be exercised not to allow the sample to go dry. The mercury salts were then diluted with about 30 ml of water and warmed to coagulate the sulfur. The solution was filtered into a 250-ml beaker using a coarse filter paper. The centrifuge tube was washed and this was also poured through the filter. The solution in the beaker was diluted to about 200 ml and used for electrolysis.

**Electrolysis.** The electrolytic deposition of mercury was done using platinum anodes and gold foils ($2 \times 2 \times 0.02$ cm) as cathodes. The gold foils were properly marked and weighed before electrolysis. The mercury standard irradiated along with the samples was dissolved and a known aliquot was transferred into a 250-ml beaker containing an accurately known amount of mercury carrier (about 50 mg of mercury as $Hg^{2+}$). Duplicate samples of the standard were also electrolyzed along with the samples. The electrolytic cells were connected in parallel and a dc potential of about 4.5 volts was applied using a constant voltage supply. The total current flow through sixteen cells was usually less than 0.8 ampere. The electrolysis under these conditions allowed an extremely uniform deposition and amalgamation of mercury on the gold surface without too much bubbling and lump formation. Usually the electrolysis was carried out overnight (16 hours or more).

The cathodes, after electrolysis, had a bright silvery appearance. Incomplete chemical separation of other constituents, and excess acidity of the electrolysis solution result in blackened foils and precipitation. The cathodes were identified and rinsed with deionized water before transferring them into 50-ml beakers containing ethyl alcohol, and the excess alcohol adhering to the foils was wiped off with an absorbent tissue paper. The foils were then either air-dried or oven-dried at 60 °C. Oven drying over ten minutes should be avoided as there is a likelihood of loss of mercury from the gold foil. If the foils should be cooled or are to be stored for a short duration, they may be placed in a desiccator containing silica gel.

The mercury deposited on each of the foils was determined by reweighing. The foils were then sealed individually in between very thin polyethylene sheets and were used for counting. Radioactive tracers ($Hg^{2+}$ form) used to investigate the processes have shown a one-to-one correspondence between the weights of mercury deposited and the amount of mercury tracer. Generally, the recovery of mercury was in the range of 75 to 90% for an electrolysis period of about 16 hours.

**Counting.** The gamma and X-ray emissions from $^{197}Hg$ and $^{197m}Hg$ were counted using a thin ($0.6 \times 5$ cm) sodium

iodide detector with a beryllium window and a 400-channel pulse height analyzer. The advantages and desirability of using a thin crystal for detecting low energy gamma rays and characteristic X-rays have been previously discussed (*11*). The foils were counted using a special sample mount that places the sample in a reproducible geometry close to the detector. Repeat counting of the foils was made, this time reversing the side facing the detector. The pulse height analyzer data were used to calculate the amount of mercury in the original sample. The data from large numbers of samples were processed using Schonfeld's ALPHA-M computer program (*12*).

**Alternate Separation of Hg**. This procedure is suggested for use only when sufficient time is not available for the electrodeposition of mercury. The solution resulting from dissolution of the mercuric sulfide in aqua regia, evaporation, and subsequent dilution may be used to precipitate mercury as mercuric oxide. The acidity of the solution should not exceed 0.3*M* and it is desirable to have it below 0.1*M*. An excess of 0.5*M* sodium hydroxide solution is added to the solution when mercuric oxide precipitates as a bright yellowish red substance. The precipitate is separated by centrifuging, washed with deionized water and ethyl alcohol, and is collected on a tared filter paper. After drying the precipitate at 60 °C in an oven, it is cooled in a desiccator and weighed as HgO to determine the yield. The sample is mounted face down on a 5-cm plastic ring-disk mount using a thin film of mylar. The radioactivities from the isotopes $^{197}$Hg and $^{197m}$Hg are counted as described above, along with similarly mounted standards. Nondestructive neutron activation analysis of several samples of the precipitates to determine the mercury content confirms the stoichiometric composition of the precipitate as HgO.

## RESULTS

The thermal neutron activation analysis procedures described above were used to survey the mercury levels of the edible tissues of the various fish in Lake Erie. Eleven different species of fish from each of the three basins (Western, Central, and Eastern) caught during the 1970 Fall season were used to prepare analytical samples. Twenty-five individual specimens (or less when sufficient numbers were not available) of each species from each of the three basins of Lake Erie were used

(11) K. K. S. Pillay and W. W. Miller, *J. Radioanal. Chem.*, 2, 97 (1969).

(12) E. Schonfeld, "ALPHA-M—An Improved Computer Program for Determining Radioisotopes by Least-Squares Resolution of the Gamma-Ray Spectra," USAEC Report ORNL-3975, National Technical Information Service, Springfield, Va., 1966.

Table V. Mercury Content of Edible Tissues of Lake Erie Fish (1970 Fall Catch)

| Species | Mercury content of edible tissues, ppm | | |
|---|---|---|---|
| | Western basin | Central basin | Eastern basin |
| Walleye | 0.79 (25)[a] | 0.65 (25) | 0.33 (25) |
| Yellow perch | 0.61 (25) | 0.49 (25) | 0.29 (25) |
| White bass | 0.60 (25) | 0.72 (25) | 0.43 (25) |
| Channel catfish | 0.36 (25) | 0.42 (20) | ... |
| Freshwater drum | 0.67 (25) | 0.62 (20) | 0.30 (25) |
| Carp | 0.23 (25) | 0.35 (17) | 0.36 (14) |
| Coho salmon | 0.69 (20) | 0.58 (10) | 0.51 (13) |
| White sucker | 0.55 (24) | 0.56 (8) | 0.35 (25) |
| Gizzard shad | 0.20 (25) | 0.21 (15) | 0.26 (18) |
| Smallmouth bass | ... | 0.55 (14) | ... |
| Smelt[b] | ... | ... | 0.30 (10) |

[a] The numbers in the parentheses refer to the number of fish samples of a particular species used in preparing the composite.
[b] Mercury content of the whole fish.

to prepare composites of the edible tissues. (Edible tissues here refer to the portions of the fish remaining after removing the head, tail, fins, and all the internal organs.) Large variations in the levels of a given species of fish of the same size and approximate age have been reported by Reynolds and Laarman (*13*). The selection of a sample size of 25 was based on the recent findings (*13*) that an "optimally precise" estimate of the average level in a population of the lake fish can be obtained by preparing a composite of about 25 randomly picked specimens from the group. The results given in Table V are the averages of two or more determinations and are expressed as micrograms of mercury per gram of raw tissue. In general, the fish from the Western Basin of Lake Erie had elevated levels of mercury in their edible tissues when compared with similar species caught from the Central and Eastern basins.

Other lake samples that have been analyzed so far include sediment/silt and plankton/algae samples collected from May 1970 to January 1971 from one location in Lake Erie. The results presented in Table VI are in terms of the calculated dry weights of the samples analyzed. Since the wet weights of the samples were liable to change, it is felt that results expressed in terms of dry weights of sediment/silt and plankton/algae will allow for future comparisons. The major industrial mercury waste discharge into the Buffalo River was stopped in April 1970 by Governmental action. However, the

(13) J. B. Reynolds and P. W. Laarman, "Estimate of Total Mercury in Lake St. Clair Walleyes," Great Lakes Fishery Laboratory, Ann Arbor, Mich., December 1970.

**Table VI. Mercury Content of Lake Erie Samples Collected at the Mouth of the Buffalo River**

| Sample identification[a] | Date of collection | Mercury content in ppm (in terms of dry weight) |
|---|---|---|
| A. Sediment/silt | | |
| E-1 | 7-28-70 | 2.80 |
| E-2 | 7-28-70 | 4.99 |
| P-1 | 7-28-70 | 2.59 |
| P-2 | 7-28-70 | 3.62 |
| P-1 | 9-8-70 | 2.27 |
| P-2 | 9-8-70 | 1.95 |
| P-1 | 10-5-70 | 2.84 |
| P-2 | 10-5-70 | 6.15 |
| E-1 | 1-15-71 | 3.69 |
| E-2 | 1-15-71 | 5.58 |
| P-1 | 1-15-71 | 6.79 |
| P-2 | 1-15-71 | 5.57 |
| B. Plankton/algae | | |
| A-1 | 7-28-70 | 81.0 |
| A-2 | 7-28-70 | 45.9 |
| A-1 | 9-8-70 | 51.5 |
| A-2 | 9-8-70 | 31.2 |
| A-1 | 1-15-71 | 74.3 |
| A-2 | 1-15-71 | 63.6 |

[a] The prefixes E and P for sediment/silt samples refer to samples collected by an Eckman dredge (~5 cm deep) and a Peterson dredge (~30 cm deep) from the water–sediment interface. The suffixes 1 and 2 refer to samples collected from the north and south side of the river, respectively.

samples of sediment/silt and plankton/algae collected periodically from Lake Erie at the mouth of the Buffalo River do not show any significant change in their mercury levels during the sampling period.

The analytical method described here was used to determine the base levels of mercury in human brain tissues. The results of the analysis of nearly 70 tissues selected at random from autopsy specimen are reported separately (*14*). These procedures have also been successfully used for the determination of mercury in coal samples, air particulates, and a variety of food materials.

(14) C. A. Glomski, H. M. Brody, and K. K. S. Pillay, "Distribution and Concentration of Mercury in Autopsy Specimens of the Human Brain," *Nature* (in press).

## DISCUSSION

The main uncertainty in the determination of mercury using neutron activation analysis described here arises prior to the wet ashing stage, in the presence of carrier mercury. In solution, mercury exchanges rapidly no matter what its oxidation state or the solvent (*15*, *16*). Therefore, the losses of mercury after this stage can be accurately accounted for and corrections made in the final results. The sampling, storing, and resampling for analysis still offer problems; however, the procedures described here seem to be a satisfactory solution.

The investigation of oven-drying, freeze-drying, and oxygen plasma ashing procedures suggests that none of these methods can be reliably used for pre-irradiation sample preparation. The use of high energy radiation exposure of samples to convert volatile organics to less volatile inorganic mercury for freeze-drying seems possible. Independent experiments performed using methyl mercury chloride have shown that a reactor irradiation of 30 seconds under the neutron and gamma flux conditions used in this investigation completely decomposes the methyl mercury compound to inorganic forms of mercury which are nonvolatile to freeze-drying. The 4 to 5% mercury losses (shown in Table III) from samples irradiated in a reactor and freeze-dried subsequently, may not be too significant because of the several additional steps involved in handling these particular samples. Routine application of this procedure to prepare samples for freeze-drying is not advisable.

The use of thick polyethylene bags to contain the samples for neutron irradiation and subsequent dissolution of the bag, along with the samples, ensures that there is no loss of mercury from the sample to the surfaces of the irradiation container. The heavy duty polyethylene bags specially chosen and used to prepare the sample containers did not present problems of cross contamination similar to those reported by Bate (*17*) Periodic blank determinations were made to determine the mercury levels of the polyethylene bag. None of the polyethylene sheets we have used so far showed any detectable amount of mercury.

The alternate procedure to precipitate mercury as mercuric oxide suggested here works well only if adequate precautions are taken to ensure that the mercuric sulfide is free from other

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Table VII.[a] Results of Mercury Analyses Methods Evaluation Program Using Fish Homogenates[a]

| Analytical method used | Number of laboratories participated | Range of reported values in ppm Hg | | |
|---|---|---|---|---|
| | | Sample D | Sample E | Sample G |
| Flameless (cold) atomic absorption | 13 | 0.93 to 1.80 | 0.03 to 0.18 | 2.80 to 5.21 |
| Flame atomic absorption | 5 | 0.70 to 1.80 | <0.05 to 0.49 | 2.26 to 5.40 |
| Dithizone colorimetry | 1 | 1.31 | 0.05 | 3.98 |
| Dithizone titration | 1 | 0.09 | <0.03 | 0.09 |
| Pyrolysis | 2 | 0.47 to 1.52 | 0.04 to 0.10 | 2.00 to 4.25 |
| Neutron activation analysis | 6 | 0.95 to 1.77 | 0.04 to 0.19 | 2.83 to 4.60 |
| Cold atomic absorption following acid digestion (Fresh Water Institute, Winnipeg, Canada) | | 1.46 | 0.04 | 4.53 |
| Neutron activation analysis with post-irradiation chemical separation (Western New York Nuclear Research Center) | | 1.77 | 0.12 | 4.56 |

[a] Trace Mercury Analyses Evaluation Program sponsored by the Fresh Water Institute of the Canadian Fisheries Research Board.

Table VIII. Results of Mercury Analyses Methods Evaluation Program Using Soil Samples[a]

| Analytical method used | Number of laboratories participated | Range of reported values in ppm Hg | | |
|---|---|---|---|---|
| | | Sample Hg 1 | Sample Hg 2 | Sample Hg 4 |
| Flameless atomic absorption after acid digestion | 5 | <0.2 to 1.46 | <0.2 to 0.35 | <0.2 to 6.00 |
| Flameless atomic absorption after direct thermal vaporization of mercury | 5 | 1.40 to 1.90 | 0.23 | 1.60 to 5.90 |
| Flame atomic absorption after acid digestion | 3 | 0.56 to 29.0 | <0.1 to 0.23 | 4.87 to 9.40 |
| Dithizone colorimetric | 2 | 2.70 | <0.1 | 4.50 to 8.90 |
| Flameless atomic absorption after acid digestion (Colorado School of Mines) | | 1.46 | 0.35 | 5.93 |
| Neutron activation analysis with post-irradiation chemical separation (Western New York Nuclear Research Center) | | 1.47 | 0.41 | 5.70 |

[a] Trace Mercury Analyses Evaluation Program sponsored by the Department of Chemistry, Colorado School of Mines, Golden, Colorado.

impurities prior to dissolving it in aqua regia. Other methods of precipitating mercury by a variety of reagents are suggested by Roesmer (*5*).

The repeated counting of the gold foils was done to ensure that there was no uneven absorption of the low energy emissions from the deposited mercury because of preferential deposition on one side of the foil. We have observed two such foils during the counting of over 300 foils, and this was recognized as being due to the foil clinging to the side of the beaker during electrolysis.

The analytical procedures described here were compared with other techniques used for the determination of mercury in biological and environmental samples. The results of two interlaboratory comparison studies using fish homogenates and soil samples are summarized in Tables VII and VIII. The fish tissues analyzed contained only natural forms of mercury and the results of their analyses reflect the problems involved in analyzing these samples.

The accuracy of the analytical procedures detailed here was determined by radioactive mercury ($Hg^{2+}$ form) tracers. These tracer studies showed that the errors of this procedure were less than 15% at 0.01 parts per million level and less than 5% at 2 parts per million level of mercury in biological tissues. The precision of the analysis as determined by repeat analysis of fish samples and sediment samples containing natural forms of mercury showed a standard deviation of less than 5% at 5-ppm levels, less than 7% at 1.5-ppm levels, and less than 17% at 0.01-ppm levels. The results of our analysis identified in Tables VII and VIII have this precision and probably the same accuracy.

Although the procedures described here can yield very reliable mercury values, it is recognized that the art of analysis still plays a significant role as is clearly evidenced from the distribution of results obtained using a particular kind of analytical procedure (Tables VII and VIII). With the recognized elusive nature of mercury, it may be necessary to take all the precautions mentioned here and probably more, to accurately determine the mercury content of environmental and biological samples.


## ACKNOWLEDGMENT

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# DETERMINATION OF MERCURY IN NATURAL WATERS AND EFFLUENTS BY FLAMELESS ATOMIC ABSORPTION SPECTROPHOTOMETRY

SVERRE H. OMANG

Considerable work has been done during recent years on the flameless cold-vapour atomic absorption method for determinations of traces of mercury. Kimura and Miller[1] were the first to use the reaction between mercury(II) and tin(II), which produces elementary mercury, as a means of isolating the metal from its matrix. Poluektov *et al.*[2], however, applied the same principle combined with direct light absorption measurements in the evolved vapour for mercury determinations. Since then, numerous methods based on the same principle but differing in sensitivity and type of sample have appeared in literature. Recently, Lindstedt[3] described urine analysis in detail, Magos and Cernik[4] dealt with biological samples and Hatch and Ott[5] and Dyvik[6] with metals and rocks.

Little work has been done for water, effluent and sewage analysis. Igoshin and Bogusevich[7] used the described principle in their work on water analysis but reported the relatively low sensitivity 14 $\mu g\ l^{-1}$. They also described a preconcentration step, in which mercury sulphide was precipitated on cadmium sulphide, and were then able to determine smaller quantities.

The limitation in sensitivity is, according to several authors, caused by the high blank value. To reach a lower detection limit, purification of chemicals would thus be necessary. A means of reducing some of this contamination has been used at this Institute during the last three years and mercury determinations have been carried out in natural waters, effluents, sewage, sodium hydroxide, sulphuric acid and to some extent baby food. The modifications and refinements developed in order to reach a detection limit of 0.02 $\mu g$ mercury per litre are described in this paper.

## FACTORS INFLUENCING THE RESULTS

### *Sample treatment*

For such a simple matrix as water, the interferences mentioned by Poluektov *et al.*[2] and by Lindstedt[3] need not be taken into consideration unless industrial waste water, possibly containing organic solvents, noble metals or halides other than chloride, is to be analysed.

Igoshin and Bogusevich[7] state that after storage for 6–8 days, natural water loses mercury by adsorption to the walls of the container. In an acidic solution, however, in the presence of permanganate, there is no such loss even after boiling. The treatment proposed and used by these authors was therefore adopted in the present

work. There is also another reason for undertaking this treatment. Natural Scandinavian waters are normally acidic and contain very little calcium but considerable amounts of organic humus compounds. These compounds could also contain or adsorb strongly some of the mercury present. The simplest way of ensuring a quantitative determination is thus to digest the sample with permanganate sulphuric acid. This same mixture, but in considerably higher concentrations has also been shown[8] to decompose organic mercurials such as methyl- and phenylmercury.

It is of importance to use as small amounts of reagents as possible in order to reduce the blank value. In the case of effluents and sewage containing relatively high concentrations of oxidizable matter, considerably more permanganate might have to be added to prevent decoloration and thereby incomplete oxidation.

*Stability of standard solutions*

Lindström[9] has discussed the losses caused by evaporation of metallic mercury from extremely dilute neutral standard solutions of mercury compounds. Shimomura *et al.*[10] recommended the use of complex-forming agents, such as iodide or cyanide, or oxidants to prevent this. Other workers prepare dilute standard solutions daily in acidic medium which also tends to reduce volatilization. In this work sulphuric acid and potassium permanganate were added in the same concentrations as used for digestion of samples.

The stability of 0.1-p.p.m. mercury standards in 1 *N* solutions of hydrochloric acid, nitric acid and sulphuric acid, as well as the sulphuric acid-potassium permanganate mixture, were tested by means of a radioactive mercury-203 tracer. None of the four solutions stored in open bottles changed their activity appreciably within one week of preparation.

*Reagent contamination*

When the present method of analysis is used, mercury can be shown to be present in almost any type of chemical. It is important therefore to remove as much of this "blank" mercury as possible in order to increase the signal-to-blank ratio. This should, if possible, be done without time-consuming and difficult reagent purification procedures.

In this laboratory, a simple method was used for eliminating the contribution from the tin(II) chloride solution in hydrochloric acid and from the sulphuric acid added in order to prevent precipitation of tin hydroxides. The two reagents were mixed with known amounts of distilled water and the contaminating mercury present, reduced to the elementary state, was stripped by bubbling air through the solution. The potassium permanganate used for digestion cannot be purified in this manner. If necessary, the traces contained in the sulphuric acid used for digestion and in other reagents not used as oxidants, can be removed in the described way after addition of a small amount of tin(II) chloride. However, the main contamination found in this work, was in the tin(II) chloride and reagent-grade hydrochloric acid.

## EXPERIMENTAL

*Equipment and reagents*

A Perkin-Elmer Model 303 atomic absorption spectrophotometer equipped

with an automatic recorder readout accessory and a Hitachi-Perkin-Elmer Recorder Model 159 together with a Westinghouse hollow-cathode lamp and a 8.7-cm long square gas cell made of PVC of 4.8-$cm^2$ cross-section with quartz windows was used. All chemicals were of reagent-grade quality.

*Mercury solutions.* A 1000 p.p.m. stock solution was prepared from 0.1354 g of mercury(II) chloride dissolved in 100 ml of 0.5 *M* sulphuric acid. A 0.1 p.p.m. standard solution was prepared by dilution of the stock solution and final addition of 1.0 ml of 1 + 1 sulphuric acid and 0.5 ml of aqueous 2 % (*w* v) potassium permanganate solution per 100 ml as preservative.

*Aeration apparatus*

A system similar to that used by Lindstedt[3] was used. Air from a gas cylinder passes through a flow meter at the rate of 0.5 l $min^{-1}$ into a 100-ml wash bottle equipped with a glass sinter of coarse porosity. Through a tube filled with ascarite to absorb water and fumes of acid or sulphur dioxide, the air containing mercury vapors released in the wash bottle, enters the gas cell where the mercury concentration is monitored. The flask is equipped with ground-glass joints on inlet and outlet in order to ensure rapid and air-tight connections and also to make bypassing of the wash bottle possible.

*Procedure*

To a 1-l water sample add 10 ml of 1 + 1 sulphuric acid and 5 ml of aqueous 2 % (w v) potassium permanganate solution. Mix well and let stand for 24 h at room temperature. The solution is then ready for mercury determination. For effluent and sewage add more permanganate if decoloration occurs. Run a blank containing all the reagents used in the same way.

Introduce 30 ml of distilled water, 2 ml of 1 + 1 sulphuric acid and 2 ml of 10 % (*w* v) tin(II) chloride dihydrate solution in 1 *M* hydrochloric acid into the wash bottle. Mix well and connect the flask to the aeration apparatus. After 2 min, or when the recorder pen has returned to the base line, disconnect the flask, add 50 ml of the well mixed sample, close the flask, shake well for 20 sec and connect it to the aeration apparatus. Record the mercury peak, using scale expansion 10, damping 1, and speed low on the recorder. In case of foaming add a drop of tri-*n*-butyl phosphate. For samples with a high mercury content either reduce the scale expansion or use a smaller sample aliquot. In this case take care to adjust the total volume in the fask to 84 ml by adding distilled water, as this would otherwise change the sensitivity. A smaller volume causes a higher absorption peak.

After 2 or 3 min, when the recorder pen has returned to the base line, disconnect the flask, add a suitable small volume of standard solution, *e.g.* 0.1 ml of 0.1-p.p.m. mercury solution which does not change the total volume appreciably. This amount of mercury (0.01 μg) produces about 20 % absorption at scale expansion 10. Repeat the procedure for new aliquots of standard solution as soon as the recorder pen has returned to the base line.

After conversion of the measured peak values of percentage absorption to absorbance, subtract the blank value and evaluate the concentration of mercury in the sample from the calibration graph which is usually a straight line. For absorption values smaller than 10 % at scale expansion 1, the conversion to absorbance is unnecessary.

*Decomposition of methylmercury contaminated samples*

To test the completeness of decomposition of organic mercurials, some samples of lake water known to be contaminated by methylmercury from a fungicide were analysed by the described procedure and by the method of Lindstedt[3], which involves very much higher concentrations of potassium permanganate and sulphuric acid.

By Lindstedt's method, 2.44 p.p.m. mercury was found in one sample, a value which, as discussed above, is considered to be the true analysis. With the described method, decoloration of the permanganate occurred and only 1.1 p.p.m. mercury was measured. Since there was obviously too much oxidizable matter in this sample in relation to the amount of potassium permanganate, five times as much permanganate was added to a new sample aliquot. After 20 h, the analysis showed a mercury content of 2.36 p.p.m. and no decoloration. The excellent agreement between the two procedures was taken as evidence of complete decomposition also by the more dilute digestion mixture.

## RESULTS AND DISCUSSION

The proposed method has been used in the analysis of natural waters, effluent, sewage, sodium hydroxide and sulphuric acid. Results obtained by other workers[3-5,9] have shown good agreement between the flameless atomic absorption technique and dithizone extraction or neutron activation methods. Though the same high sensitivity was not achieved by these authors, there is good reason to believe that their results are also applicable to lower concentrations, an argument strongly supported by the results found by means of the described procedure.

Several standard aliquots could be reduced and measured after each other or after or before sample aliquots in the same way as described in the procedure, without any kind of memory effect or irregularity, and with a relative standard deviation of 4.9% in measurements of 0.01 μg of mercury.

Experiments showed that samples containing organomercurials such as methylmercury were also completely decomposed by the described treatment. The measured mercury content in some contaminated samples of waste water was the same whether the samples were digested or not, indicating that all the mercury was present in an ionic or at least easily reducible form. Some values measured in casual samples of drinking water are listed in Table I.

TABLE I

MEASURED ABSORPTION AND DETERMINED CONCENTRATION OF MERCURY IN LAKE WATER (SCALE EXPANSION 10)

| *Sample* | *Aliquot* (*ml*) | *Absorption* (%) | *Total amount* (*ng*) | μg $l^{-1}$ |
|---|---|---|---|---|
| 0.050 p.p.m. standard | 0.100 | 10.0 | 5.0 | — |
| 0.050 p.p.m. standard | 0.010 | 1.2 | 0.5 | — |
| Blank | 50 | 0.3 | 0.01 | — |
| Gjersjoen, 4 m depth | 50 | 9.0 | 4.4 | 0.088 |
| Gjersjoen 55 m depth | 50 | 6.7 | 3.2 | 0.064 |
| Aurevann | 50 | 3.3 | 1.5 | 0.030 |

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# Selective Atomic-absorption Determination of Inorganic Mercury and Methylmercury in Undigested Biological Samples

By L. MAGOS

Mercury and its compounds are released into the environment as a result of industrial and agricultural activities and of the weathering of mercury-bearing rocks. Because of biological methylation in aquatic organisms[1] mercury accumulates as methylmercury in ecological systems. Contamination of food, particularly fish, with highly toxic methylmercury has prompted toxicological research on this compound and compelled authorities to analyse a large selection of foodstuffs for mercury.

Techniques requiring the digestion of samples do not enable inorganic mercury and methylmercury to be distinguished, so that before digestion organomercurials have to be extracted with benzene and determined either by a titrimetric method,[2] or by gas chromatography,[3] but not by atomic absorption as benzene gives falsely high readings. Gage and Warren[4] avoided extraction and made use of the varying lability of methoxyethylmercury, phenylmercury and ethylmercury in the presence and absence of cysteine with and without a 1-hour digestion. For evaluation they used the atomic-absorption method of Magos and Cernik[5] in which mercury is determined by aspirating the vapour through an ultraviolet absorptiometer after reduction with tin(II) chloride. Unfortunately, methylmercury in the presence of cysteine releases mercury from the carbon bond at a rate of only 0·4 per cent. per day,[6] and it is the experience of the author that not more than one third of the mercury can be released even after digestion for 1 hour in acidic cysteine solution.

In experiments in which $^{203}Hg$-labelled mercury compounds are used, inorganic mercury can be selectively determined in the presence of organomercurials, either by an isotope-exchange method,[6] or by its selective reduction with tin(II) chloride.[7]

The method described here is based on the discovery that the rate of reduction of methylmercury or other organomercurials by tin(II) chloride can be made identical to that of inorganic mercury if the amount of tin(II) chloride is above a certain level and a cadmium salt is added to the reaction mixture. Thus, either inorganic mercury *plus* methylmercury or inorganic mercury alone can be released from the sample for determination by atomic absorption. The difference between the two readings gives the amount of methylmercury in the sample. Further, it is possible to release first inorganic mercury and afterwards methylmercury from the same sample.

## Method

### Apparatus—

The mercury vapour concentration meter was manufactured by the Hendrey Relays Division of Columbia Industrial Development Ltd., Slough, Bucks., but as described elsewhere,[5] a water-pump was substituted for the original fan. The inlet of the gas cell was connected by plastics tubing successively with two midget impingers of 30-ml volume and a 200-ml Quickfit test-tube (B34 socket) fitted with a Drechsel bottle head, the inlet of which was converted into a thistle funnel. The first midget impinger was left empty and served as a liquid trap. The second impinger contained 10 ml of distilled water and was immersed in melting ice to act as a water vapour absorber. The Quickfit test-tube was the reaction vessel. When recordings were made the electric output of the mercury vapour concentration meter was connected to a Servogor Potentiometric Recorder (Goerz Electro GmbH, Vienna).

### Preparation of samples—

Homogenates were prepared in 1 per cent. saline with an Ultra-Turrax* homogeniser. When the preparation of a homogenate is difficult to achieve, as with fishmeal, small animals or skin and fur, the following preparative techniques were used. For fishmeal, 0·5 g was mixed in a test-tube with 1 ml of 1 per cent. cysteine solution, 1 ml of 20 per cent. sodium chloride solution and 1 ml of 45 per cent. sodium hydroxide solution. The contents were heated to boiling-point and washed into the reaction vessel. For the whole rat, the sample was weighed and dropped into boiling 40 per cent. sodium hydroxide solution (the volume in millilitres being twice the animal weight in grams). After boiling for 20 minutes the volume was made up with distilled water to give a 20 per cent. w/v solution (based on the weight of the rat). Further dilutions were made as soon as possible to avoid gelatinisation.

### Reagents—

All the reagents were of B.D.H. analytical-reagent grade unless otherwise stated.

*Mercury standard solutions*—All the standard solutions contained 0·5 mg $ml^{-1}$ of mercury. To prepare an inorganic mercury standard 0·6767 g of mercury(II) chloride was dissolved in sufficient 5 per cent. sulphuric acid to give 1000 ml. From this 1 ml was taken and made up to 1000 ml with a solution of 9·0 g of sodium chloride, 0·7545 g of ethylenediaminetetraacetic acid, disodium salt, and 0·063 g of L-cysteine hydrochloride† in distilled water. If this solution is kept in a refrigerator the mercury concentration remains unchanged for at least 6 months. To obtain a methylmercury standard 62·58 mg of methylmercury chloride‡§ were dissolved in 100 ml of acetone and from this solution a 1 to 1000 dilution was made with distilled water. Alternatively, 36·96 mg of methylmercury dicyandiamide§ were dissolved in 500 ml of distilled water and from this solution a 1 to 100 dilution was made with distilled water. As methylmercury solutions tend to lose mercury owing to either volatilisation (from methylmercury chloride) or precipitation (from methylmercury dicyandiamide) their mercury concentrations were checked frequently by the method of the Dow Chemical Company.[8]

*Cysteine hydrochloride, 1 per cent. w/v solution.*

*Sodium chloride, 1 per cent. w/v solution.*

*Sulphuric acid,* 16 N.

*Tin(II) chloride*—100-mg portions were used.

*Tin(II) chloride - cadmium chloride reagent*—Tin(II) chloride (25 g) and 5 g of cadmium chloride were mixed and heated with distilled water until boiling; the volume was made up to 50 ml with distilled water after cooling.

*Sodium hydroxide, 45 per cent. w/v solution.*

*Silicone MS antifoam*—The use of this material was necessary occasionally. With a glass rod that had been dipped slightly into antifoam a ring of antifoam was drawn on the inner wall of the reaction vessel at about middle height.

* Janke and Kunkel K.G., Staufen i. Br.
† Hopkin and Williams Ltd.
‡ K and K, California, U.S.A.
§ AB CASCO, Stockholm, Sweden.

PROCEDURES—

Approximately 30 minutes before the start of a run switch on the apparatus, adjust the air flow to about 2·5 l $min^{-1}$ and set the filter control to "Sample." Immediately before beginning the determinations select the sensitivity and adjust the full-scale deflection according to the operating instructions.

*Methods* 1 *and* 2—Transfer with a pipette 1 to 20 ml of homogenate or standard into the reaction vessel. Add 1 ml of cysteine solution and make the volume up to 21 to 23 ml with 1 per cent. saline. (If automatic pipettes are used add 20 ml of saline to 1 ml of homogenate and 15 ml of saline to 5 ml of homogenate.) Add 10 ml of 16 N sulphuric acid. In the case of fishmeal, fumes formed by the action of sulphuric acid on the sample must be removed by bubbling air through the sample to avoid falsely high readings. After this procedure, or with other samples immediately after the addition of sulphuric acid, add either 1 ml of tin(II) chloride - cadmium chloride reagent (Method 1) or 100 mg of tin(II) chloride (Method 2) to the sample. Connect the Drechsel head with the reaction vessel, thus starting an air flow through the reaction vessel, and add 20 ml of 45 per cent. sodium hydroxide solution through the thistle funnel. Read the peak deflection and calculate the concentration either by use of an internal standard or of the established factor for the type of sample.

*Method* 3—This is a combination of Methods 1 and 2, the procedure starting as in Method 2. Read the peak height and disconnect the air flow between 1 and 3 minutes after the sodium hydroxide addition. Add 10 ml of 16 N sulphuric acid to the reaction mixture followed by 1 ml of tin(II) chloride - cadmium chloride reagent. Restore the air flow through the reaction vessel and add 20 ml of 45 per cent. sodium hydroxide solution through the thistle funnel. Again read the peak height.

PRACTICAL CONSIDERATIONS—

Linearity between peak deflections on the scale of the instrument and mercury contents of up to 1·0 μg have been well established for the tin(II) chloride reduction method.[4,5] The area recorded under the curve can also be used for the evaluation of the mercury content. However, as recorders usually register absorption and not extinction, the use of peak area requires not only an additional step, that is, the measurement of the area, but also the preparation of calibration graphs.

TABLE I

PEAK DEFLECTIONS* CAUSED BY 0·5 μg OF MERCURY IN DIFFERENT BIOLOGICAL MEDIA

| Biological media | Peak deflection caused by 0·5 μg of mercury as | | Deflection in relation to standard (standard = 100) |
|---|---|---|---|
| | Mercury(II) chloride | Methylmercury | |
| None | 324 | 321 | |
| | 331 | 324 | 100 |
| 1 ml of 0·5 per cent. blood solution | 312 | 313 | |
| | 323 | 323 | 97 |
| 1 ml of 0·5 per cent. kidney homogenate | 325 | 310 | |
| | 320 | 325 | 98 |
| 1 ml of 2 per cent. liver homogenate | 265 | 265 | |
| | 275 | 265 | 82 |
| 5 ml of 10 per cent. liver homogenate | 199 | 209 | |
| | 214 | 224 | 65 |
| 1 ml of 2·5 per cent. brain homogenate | 241 | 141 | |
| | 236 | 231 | 72·5 |
| 5 ml of 20 per cent. tuna fish homogenate | 184 | 174 | |
| | 160 | 152 | 52 |
| 0·5 g of fishmeal | 175 | 174 | |
| | 198 | 201 | 61 |

* Values are corrected for blank.

The rate at which mercury is released from a biological sample after reduction differs from one medium to another. It was noted that mercury(II) chloride, when added to urine, was released at the same rate as from saline, but that the rate of release, and consequently the peak height of deflection, was considerably less if mercury was added to 1 ml of blood.[5] Similarly, it has been found in the present work that the rate of release was hardly changed if 1 ml of 0·5 per cent. blood solution or 1 ml of 0·5 per cent. kidney homogenate was analysed, but that the rate of release was considerably decreased when 5 ml of 10 per cent. liver homogenate, 1 ml of 2·5 per cent. brain homogenate or 5 ml of 20 per cent. tuna fish homogenate (made from tinned fish) was analysed. Consequently, when a single determination is carried out, internal standards must be analysed parallel to the sample. When a series of determinations have to be carried out on the same type of sample the average deflection given by 0·1 $\mu g$ of mercury (from a few measurements) can be used for the whole series. Calculation of the factor is as follows: if, for example, the blank gives a reading of 5, the sample gives a reading of 205 and the sample with 0·5 $\mu g$ of added mercury gives a reading of 455, then the response for 0·1 $\mu g$ of mercury $= \frac{455 - 205}{5} = 50$ units.

Because of its greater concentration stability, inorganic mercury was used to provide factors and inner standards in Methods 1 and 2. Naturally, in Method 3 the use of methylmercury as a standard is indispensable in the evaluation of the second peak.

Changes in the sensitivity of the potentiometer or the emanation from source samples (*e.g.*, fishmeal) of some material that might condense temporarily on the gas cell may affect the instrument, so it is more useful to express the factor not as an absolute value, but in relation to the deflection caused by the standard without added biological sample. Table I shows how different biological media influence the peak deflection.

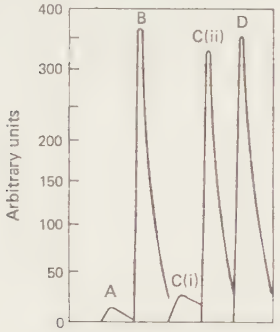


Fig. 1. Release curves given by 0·5 $\mu g$ of mercury as methylmercury [curves B, C(i) and C(ii)] and inorganic mercury (curve D). All the curves were made with tin(II) chloride - cadmium chloride reagent (Method 1) except C(i), which was made with 100 mg of tin(II) chloride (Method 2 or the first part of Method 3) followed by the subsequent release of mercury by tin(II) chloride - cadmium chloride reagent from the same sample [curve C(ii), Method 3]. Curve A constitutes a blank

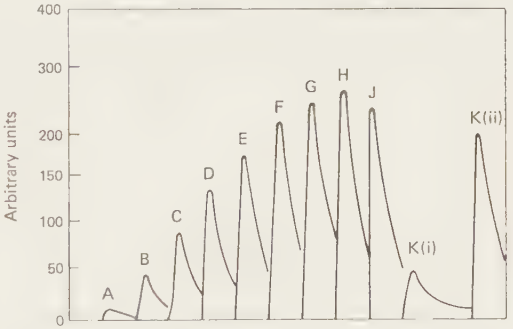


Fig. 2. Release curves given by methylmercury from 5 ml of 10 per cent. liver homogenate. Curve A constitutes a blank. Curves B to G show the effect of adding 0, 0·1, 0·2, 0·3, 0·4 and 0·5 $\mu g$, respectively, of mercury, as methylmercury, to the homogenate (Method 1), curves H and J that of adding 0·5 $\mu g$ of mercury as inorganic mercury with Method 1 and Method 2, and curves K(i) and K(ii), 0·5 $\mu g$ of mercury as methylmercury with Method 3

## Results and discussion

Method 1 releases all the mercury from the sample irrespective of the presence of a carbon - mercury bond. Method 2 releases inorganic mercury at a fast rate and mercury from the methyl bond at a very slow rate. The release of mercury from methylmercury

when Method 2 is used produces a peak or an increase in the existing peak that corresponds to less than 5 per cent. of the methylmercury present in the sample. This release is partly caused by the fact that tin(II) chloride slightly splits the bond between carbon and mercury and partly by the presence of inorganic mercury, even in highly purified methylmercury preparations.[6] The results in Table I and curves B and D in Fig. 1 show that there was no difference in the height of the peak caused by methylmercury or inorganic mercury. Curves H and J of Fig. 2 also show that the rate of release of mercury was the same whether inorganic mercury was released by Method 1 (Fig. 2, curve H) or by Method 2 (Fig. 2, curve J). It can also be seen from Fig. 2 that when mercury was released from 5 ml of 10 per cent. liver homogenate without added mercury (curve B) and with methylmercury added to the homogenate in amounts of 0·1, 0·2, 0·3, 0·4 and 0·5 $\mu$g of mercury (curves C to G), the increase in concentration showed a linear relationship with the increase in peak height read on the scale.

Methods 1 and 2 make possible the determination of total mercury or of inorganic mercury. The difference between values obtained by Method 2 and Method 1 gives the methylmercury content of the sample. The combination of Methods 1 and 2 also makes possible the separate determination of inorganic mercury and organomercury in the same sample (Method 3). In Fig. 1 curves C(i) and C(ii) were made by using Method 3 with a standard solution containing 0·5 $\mu$g of mercury as methylmercury. The reaction vessel was disconnected 1 minute after the first addition of sodium hydroxide. In Fig. 2 curves K(i) and K(ii) illustrate a similar determination with 5 ml of 10 per cent. liver homogenate (air flow disconnected after 2 minutes). The height of the second reading depends on the time lapse between the first addition of sodium hydroxide and the disconnection of the air flow. If the air flow is disconnected too soon the second peak might be even larger than a peak given by Method 1 as atomic mercury is left in the sample after the first reduction. If enough time is left for aspirating out all the inorganic mercury, the second peak will be lower than it should be according to the common factor for Methods 1 and 2, as some mercury escapes from methylmercury during the first phase of the reaction. Consequently, if Method 3 is used the time taken must be standardised and a separate factor established.

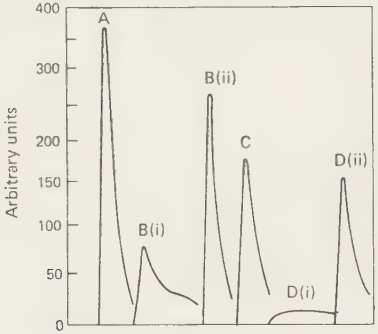


Fig. 3. Analysis of 1·0 ml of 0·5 per cent. kidney homogenate [curves A, B(i) and B(ii)] and 1·0 ml of 2·5 per cent. brain homogenate [curves C, D(i) and D(ii)] of a rat treated with 33 consecutive doses of 0·85 mg $kg^{-1}$ of mercury as methylmercury. Curves A and C were obtained with Method 1, curves B(i), B(ii), D(i) and D(ii) with Method 3

It is shown in Fig. 3 that methylmercury added *in vitro* can be released by use of Method 1 not only from homogenates but also from organs of rats treated with methylmercury dicyandiamide. Curves A, B(i) and B(ii) resulted from 1 ml of 0·5 per cent. kidney homogenate,

and curves C, D(i) and D(ii) from 2·5 per cent. brain homogenate of a rat treated with 33 daily doses (5 doses per week) of 0·85 mg $kg^{-1}$ of mercury as methylmercury dicyandiamide. Curves A and C were obtained by use of Method 1, curves B and D by use of Method 2. It can be seen from the peak heights that in kidneys approximately 75 per cent. of the mercury was in the organic form while in the brain this rose to more than 95 per cent.

The recovery obtained with Method 1 from tuna fish and fishmeal was compared with results obtained by using the method described by the Analytical Methods Committee[9] and carried out by the Laboratory of the Government Chemist. Table II shows that the differences between the two methods were not significant and that the variations were not one-sided.

The recovery from whole rats of 0·85 mg $kg^{-1}$ of mercury as methylmercury 24 hours after its administration was tested in four animals. Faeces and urine were also analysed and it was shown that less than 2 per cent. of the dose was excreted during this period. Recoveries of the remaining dose were 95, 98, 93 and 95 per cent. Homogenisation of the whole animal probably caused slight splitting of the methylmercury molecule as inorganic mercury usually made up from 5 to 8 per cent. of the total mercury content.

TABLE II

MERCURY CONTAMINATION OF TUNA FISH AND FISHMEAL DETERMINED BY THE METHOD OF THE ANALYTICAL METHODS COMMITTEE AND BY THE DESCRIBED METHOD I

| Sample number | Weight of sample analysed by Method I | Mercury contamination, p.p.m. | |
|---|---|---|---|
| | | A.M.C. Method | Method I |
| 1 | 1 g of tuna fish | 0·13 | 0·15 |
| 2 | ,, | 0·15 | 0·20 |
| 3 | ,, | 0·22 | 0·17 |
| 4 | ,, | 0·29 | 0·26 |
| 5 | ,, | 0·55 | 0·58 |
| 6 | 0·5 g of fishmeal | 0·01 | 0·06 |
| 7 | ,, | 1·20 | 0·84 |
| 8 | ,, | 1·60 | 1·48 |
| 9 | ,, | 3·4–4·0 | 3·64 |

The method is not specific in the sense that phenylmercury behaves like methylmercury and about 30 per cent. of the mercury is released from ethylmercury even when tin(II) chloride alone is used. However, in food that has not been directly contaminated with phenyl- or ethylmercury the mercury contaminant is always either inorganic mercury or methylmercury. Consequently, mercury that is released by 100 mg of tin(II) chloride is probably all inorganic mercury while that which is released by the tin(II) chloride - cadmium chloride reagent is either inorganic mercury, methylmercury, or a mixture of both. The situation is similar in animal experiments when methylmercury is administered.

A great advantage of the method is its simplicity together with its adaptability. In serial investigations, when only those samples having mercury contamination above the safety limit are of interest, a standard sample weight can be selected to give a good deflection at the critical concentration. In this instance, if separate internal standards are not used for each sample the same factor can be used for the whole series of determinations.

The error of the method is the same as that previously reported for tin(II) chloride evolution methods[5,10] and depends upon the height of deflection. If the peak height is less than 20 on the scale and this deflection is caused by 0·05 $\mu$g of mercury in 5 ml of 20 per cent. tuna fish or 10 per cent. liver homogenate, the error can be 25 per cent. If the peak deflection is more than 40 the error is not more than 10 per cent. The sensitivity of the method can be increased in two ways, firstly by increasing the sample weight and secondly by decreasing the volume of saline added to the sample. Because of the increased temperature of the reaction mixture after it was made alkaline, mercury is removed more rapidly from the sample by the air flow. Although this last effect had been tested and proved, it is thought that the described procedure satisfies the demands of sensitivity for most routine and experimental investigations.

I acknowledge with thanks The Hendrey Relays Division of Columbia Industrial Development Ltd. for lending the mercury vapour concentration meter, Dr. D. C. Abbott of the Laboratory of the Government Chemist for supplying analysed samples of tuna fish and fishmeal, Mrs. A. Green for excellent technical assistance and Mr. J. A. E. Jarvis for planning and constructing the glassware.

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# A Micromethod for Mercury

E. L. KOTHNY, Ph.D.

## Introduction

TRACES OF MERCURY in biologic and other natural materials including air are useful guides for locating sources of contamination, surveying health hazards,[1,2] and geochemical prospecting.[3,4] Available methods generally involve time-consuming sample preparation and separation of interferences. A single extraction dithizone procedure has been described for vegetation.[5] Very small amounts cannot be processed directly by instrumental techniques. Therefore, colorimetric dithizone methods are still in wide use. The disadvantage of these methods is manipulative in nature. Instrumental techniques bear the same inconveniences in the preparation of organic samples; that is, after wet ashing the mercury is separated from interfering elements and concentrated.[1,6] Inorganic materials and soils low in organic matter have been successfully analyzed by simple evaporation of the mercury by heat and measuring the metal in the gas phase by atomic absorption.[7,8] Mercury can also be separated by distillation from acid solutions as a halogenide[9] or determined by isotope exchange reactions of metallic vapor from solutions containing cysteine.[10] Neutron activation analyses have been extensively described elsewhere.[11] High accuracy is attained, but analyses are costly. To reduce the time for analysis and to facilitate the evaluation of traces, a sensitive and fast method is needed.

Before 1945, methods using triphenylmethane dyes for mercury determination were known to be useful for concentrations higher than 1 mg/ml.[12] In many places, mercury was mentioned as an interference in these methods.[12,13] These observations led to investigation of the details of interfering cations in a method for gold which used methyl violet as the dye and benzene as the extractant.[14] Other triphenylmethane dyes were similar in reactivity,[12] and methyl violet methods have been described for many different cations and anions.[13,15-18] This paper describes a solvent extraction method for mercury using crystal violet as the preferred colorimetric reagent.

## Principle

A wet-ashing method best suited to the properties of the sample is chosen to oxidize mercury to the mercuric state. The volatility of mercury precludes evaporation techniques, especially if halogens or halogenic acids are present. Cr, Fe, Mn, V may be added as catalysts to samples containing organic material. Following the oxidation, the insoluble material is filtered off.

In the absence of organic material, the mercury is complexed directly with 0.003 to 0.015*M* iodine in 0.5 to 0.6*M* HCl. Excess oxidant is destroyed with sulfite, and the complex mercuric tetraiodide ion is converted to the disulfitomercurate diiodide which is resistant to reducing and oxidizing compounds.

A 0.5% excess of sulfite over oxidants must be present. Five milligrams of EDTA for every 10 ml of solution is added to complex interfering elements. Ethyleneglycol monomethyl ether (10% to 20% by volume) is added to desorb and coagulate colloids which may form during complexation or extraction. The solution is made 0.01% in crystal violet (CV). When the concentration of mercury is high, the complex $(CV)HgI_3$ precipitates. This compound is soluble in many organic solvents. However, aromatic solvents, such as toluene, are selective and dissolve only the mercury complex and not the crystal violet from the aqueous solution. The toluene is separated, filtered to remove water droplets and colloids, and the absorbance of the blue color is measured by spectrophotometry at the absorption maximum in the range of 590 to 610 nm. The dye we used absorbed at 605 nm.

## Experimental Procedure

*Urine*

*Reagents:* (A) 20% sodium metabisulfite or bisulfite. Discard unused solution after one month. (B) Dissolve 0.5 gm of crystal violet in 490 ml of ethyleneglycol monomethyl ether and add 10 ml of 25% KI. (C) Sulfur-free toluene.

*Procedure:* The method of Miller and Swanberg[19] was used for sample preparation. After 100 ml of urine is digested with 10 ml of 6N HCl containing 2% $KCr(SO_4)_2 \cdot 12H_2O$ and 1% $FeCl_3$, 50% $H_2O_2$ is added to complete the mineralization. The method is followed up to the point of the peroxide test.

Use the acid solution without filtration. Transfer the cooled solution to a 250-ml separatory funnel, add 1 ml of reagent A, mix, then add 10 ml of reagent B, mix, then add 5.00 ml of toluene, shake, and spin gently five times to avoid emulsifying. Let stand for 2 to 5 minutes, and discard the aqueous layer. Prepare dry test tubes with funnels and folded filter papers (Whatman No. 40, 7 cm). Put a small swab (0.5 $cm^3$ approx.) of filter paper pulp in the filter paper apex and transfer the toluene layer onto it. The filtered toluene extract is stable for 20 minutes when stoppered and kept in subdued light. Transfer the filtrate to 1-cm cells and read the absorbance in a spectrophotometer at 605 nm.

Prepare a blank for each series of determinations or batch of reagent. Always use the same volumes for extraction. Avoid fading by not exceeding the recommended time. The samples can be read against the blank, or the value of the blank can be deducted from the readings of the samples when toluene is used as the zero comparison.

Determine the concentration of mercury from a calibration curve which has been obtained from readings using mercury-free urine spiked with known amounts of a standard solution of mercury. In this case the blank is made with urine.

*Air*

*Reagents:* (A) Mix 1.12 gm of NaI, 0.83 gm of $KBrO_3$, and 2.6 gm of NaBr, with 300 ml of distilled water. When dissolved, add 200 ml of ethyleneglycol monomethyl ether and mix. (B) 2*N* HCl. (C) Absorbing solution: Mix equal parts of A and B and store the 0.0075*M* $IBr_3$ solution in a dark bottle for not more than 2 weeks. (D) 20% sodium metabisulfite or bisulfite. Discard unused solution after one month. (E) 1% crystal violet in ethyleneglycol monomethyl ether. (F) Sulfur-free toluene.

*Procedure:* Punch glass fiber paper* to fit into a plastic disposable holder.† Connect the filter assembly with a short piece of polyvinyl tubing to a 12/5 standard taper socket joint. Then clamp the joint to the inlet of a midget impinger containing 10 ml of absorbing solution C. Follow the impinger by an absorbing tube filled with coarse (8 to 12 mesh) soda lime. The glass fiber filter has a low pressure drop and is inserted to remove dust and cinnabar particles. For field analysis, air is drawn by a Freon-powered sampler,‡ which has a built-in rotameter and control valve. Sample at 2 liters/min for 20 minutes or less. Store the solution in polyethylene-capped test tubes or in the same impinger. Add 0.5 ml of solution D, swirl, then add 0.2 ml of solution E and swirl again. Add 5.00 ml of toluene

*Gelman Instrument Co., 600 S. Wagner Road, Ann Arbor, Michigan 48106. Type E (Binderless) Glass Fiber Filter 37 mm (500 in a box).

†Millipore Corporation, Bedford, Massachusetts 01730. Disassembled Tenite Monitor, Cat. No. M 000 037 PO.

‡Union Industrial Equipment Corp., 150 Cove St., Fall River, Massachusetts 02720. Uni-jet air sampler #B 5465.

and mix gently by inverting the capped tube twenty times. Pipet off the toluene layer and proceed with the toluene as described for urine.

*Vegetation*

*Reagents:* (A) 10*N* $HNO_3$: Mix 630 ml of concentrated $HNO_3$, $d = 1.42$, with 370 ml of distilled water in which 10 gm of $CrO_3$ has been dissolved. (B) 5% $KMnO_4$. (C) 30% $H_2O_2$. (D) 5*N* HCl. (E) 2.5% KI. (F) 20% sodium metabisulfite or bisulfite. Discard unused solution after one month. (G) 0.1% crystal violet in ethyleneglycol monomethyl ether. (H) Sulfur-free toluene.

*Procedure:* Mix 2.5 gm of air-dried sample with 5 ml of reagent A. Simmer in a covered beaker for 1 hour. Add 5 ml of water, filter by suction, and wash sparingly. Keep the residue and repeat the procedure on it to this point, to extract any residual mercury. Combine both filtrates, add 15 ml of reagent B, bring to the boiling point, then simmer in a covered beaker for 30 minutes. Dissolve the precipitated $MnO_2$ by dropwise addition of reagent C, and add 2 ml excess. Simmer for 1 hour. If the solution stays yellowish as compared with a reagent blank, add 2 ml more of reagent C and simmer for an additional hour. Then add 5 ml of reagent D and transfer to a stoppered 100-ml volumetric cylinder and dilute to the 50-ml mark with distilled water. Add 1 ml of reagent E. Destroy the excess iodine by the dropwise addition of reagent F, then add an excess of 0.1 ml. Add 5 ml of reagent G and mix. Add 5 ml of toluene and invert the stoppered tube twenty times. After coalescence, pipet off the toluene and proceed as described for urine.

## Results and Discussion

Elemental analysis of the complex crystallized from toluene confirmed the formula $(CV)HgI_3$. It was observed that N-substitution of at least two of the triphenylmethane amino groups was necessary for high absorptivity. Rhodamine,[20] brilliant green, methyl violet, and antipyrine dyes,[21] meet this requirement. However, an attempt to substitute the more stable rhodamine for crystal violet failed because the complex was strongly absorbed on filter paper. The crystal violet complex gave the highest molar absorptivity.

The absorption spectra are illustrated in Figure 1. The maximum absorbance occurred at 605 nm. The spectra for Hg and organic interference differed somewhat but overlapped. This observation confirmed that complete removal of organic material was essential. The interfering material probably was a reaction product from some plant species and their detritus in soil which produced oxonium compounds or chlorinated acids (for example, trichloroacetic acid) during incomplete oxidation. These compounds also complexed with the dye and were coextracted with the Hg complexes into the toluene.

Recovery of mercury added to oxidized vegetation samples (mixed weeds) is summarized in Table I. For very low values (0.4 $\mu$g) the absolute recovery was 89% compared with neutron activation, and the standard deviation was about the same. This difference probably is due to incomplete oxidation of the cellulose fibers which were filtered out before completing the oxidation.

Calibration curves were equivalent when using urine or synthetic isotonic mixtures (Figure 2). Biological samples, vegetation, and soils high in organic material must be thoroughly oxidized. A closed reflux system was not considered necessary, based on Merodios[9] finding that mercury is not volatile in hot oxidizing acids unless excess halogens are present.

Wet ashing of vegetation was accomplished in 5 to 10 minutes by using small amounts

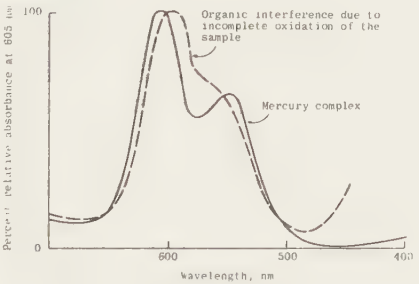


FIGURE 1. Absorption spectra of crystal violet complexes.

TABLE I
Recovery of Mercury from Vegetation

| Hg Present (μg) | Hg Added (μg) | Hg Found (μg) | % of Recovery |
|---|---|---|---|
| 7.0 | 3.00 | 10.0 | 100 |
| 1.0 | 1.00 | 2.0 | 100 |
| 0.33 | 1.00 | 1.33 | 100 |
| 0.53 | 0.33 | 0.83 | 96 |
| 0.73 | 0.20 | 0.91 | 98 |
| 0.44 ± 0.03[a] | — | 0.35 ± 0.02[b] | 89 |

[a] Mean of 23 samples analyzed by neutron activation.[25] Calculated amount in 2.5-gm sample.
[b] Mean of four samples.

of fuming nitric acid and 50% $H_2O_2$ at 60° to 100°C. This procedure was much faster than the acid digestion procedures which require several hours refluxing at 100° to 120°C. Addition of chromic acid helped to complete the oxidation and to destroy excess $H_2O_2$ in a matter of a few minutes. An overnight oxidation procedure for urine with acidified permanganate also worked well.[1,22]

The procedure for air analysis absorbed 99.4% of the mercury vapor and organomercurials in the samples. Separation by dry ashing may be combined with the method for air analysis.

The optimum normality for HCl was between 0.1 and 0.5*N* (Figure 3). An increase in acidity was detrimental because of the formation of CV chloride. Other acids with lower ionization potential may be used at higher concentrations.

The sulfite eliminated free iodine and reduced the anions which give colored complexes with the dye, thus eliminating the interference of $(IBr_4)^-$, $Sb^{5+}$, $Tl^{3+}$, $Pt^{4+}$, $Pd^{4+}$, $Fe^{3+}$, $Mn^{3+}$, and $V^{5+}$. The dye faded faster in acid solution when free sulfite and copper were present.

Concentrations greater than 0.02*M* $I^-$ tended to form predominately $(HgI_4)^{2-}$ and as a consequence less optical absorbance. When the concentration of $Cu^+$, $Pb^{2+}$, $Tl^+$, and $Ag^+$ iodides were higher than their solubility products, the precipitates did not absorb significant amounts of the Hg complex. Concentrations of $Cu^+$, $Bi^{3+}$, $As^{3+}$, $Sb^{3+}$ and $Cd^{2+}$ below their solubility product stayed in solu-

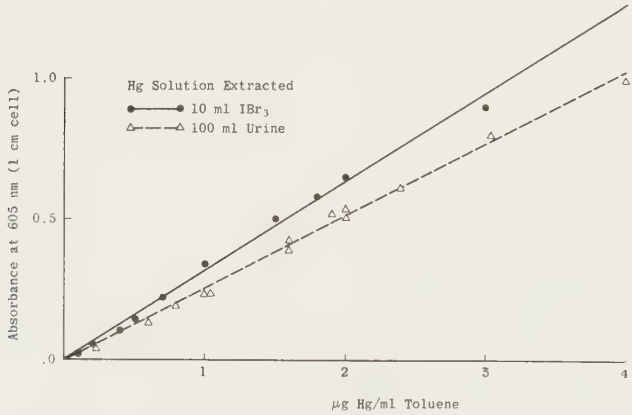


FIGURE 2. Calibration curve for determination of mercury with crystal violet.

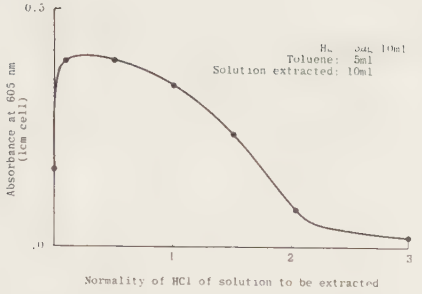


FIGURE 3. Effect of acidity on the extracton of ion complexes.

tion but formed insoluble complexes with crystal violet. The addition of EDTA suppressed this effect to some extent.

Occasionally the crystal violet complex with $Cu^{+}$, $Bi^{3+}$, $Cd^{2+}$, $Mo^{5+}$, and $W^{6+}$ transferred colloidally into the toluene. Filtration of the toluene before measurement of the color obviated these interferences. Most of these complexes, except $Cu^{+}$, tended to absorb some of the mercury complex. Addition of watermiscible solvents corrected these detrimental effects by hydrophilizing and coagulating the colloids. Ethyleneglycol monomethyl ether (10% to 20% by volume) proved to be suitable for this purpose, although other solvents might work as well (for example, ethyleneglycol monoethyl ether, dioxane, acetic acid).

Excessive shaking and insufficient sulfite produced $(IBr_4)^{-}$ and $(ICl_4)^{-}$ which precipitated with the dye immediately and were then coextracted into the toluene.

Stannous ion could have interfered but was oxidized during the preparation of the sample and was not reduced by the sulfite added later. Tungstate was a serious interference when more than 10 mg/liter was present. The insoluble complex which formed with the dye adsorbed the mercury complex to a great extent. However, the amount of W going into solution during preparation of samples was less than this upper limit and posed no problem. Other upper limits were: 100 mg of Mo, 100 mg of Cd, 50 mg of Bi and As, and 30 mg of Sb per liter. At 1 gm/liter, the following ions posed no problem: $NH_4^{+}$ $Li^{+}$, $Na^{+}$, $K^{+}$, $Rb^{+}$, $Cu^{2+}$, $Ag^{+}$, $Be^{2+}$, $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Ba^{2+}$, $Zn^{2+}$, $Al^{3+}$, $Ce^{3+}$, $Ga^{3+}$, $In^{3+}$, $Tl^{+}$, $Ge^{4+}$, $Sn^{4+}$, $Pb^{2+}$, $V^{4+}$, $Nb^{5+}$, $Ta^{5+}$, $U^{6+}$, $Th^{4+}$, $Se^{4+}$, $Te^{4+}$, $Cr^{3+}$, $Cr^{6+}$, $Mn^{2+}$, $Fe^{3+}$, $Co^{2+}$, $Ni^{2+}$, $Pd^{4+}$, $Pt^{4+}$, $BO^{3-}$, $SO_4^{2-}$, $Cl^{-}$, $Br^{-}$, $NO_3^{-}$, $ClO_4^{-}$, $PO_4^{3-}$, $F^{-}$. Some interactions of anions with cations interferred rather seriously. This was the case for $SCN^{-}$ with Zn and Mo, and for $CN^{-}$ with $Ag^{+}$, which gave a stable complex in strongly basic medium,[14, 16,23] and was destroyed at the low pH where the mercury complex formed. Fluoride interferred only when $BO_3^{3-}$ and $Ta^{5+}$ were also present.[24,12] The interference of $BF_4^{-}$ was small, however, and could be eliminated by raising the normality to 1*N* HCl.

Gold remained the chief interference. However, for most cases, removal was not necessary. Complete removal without loss of mercury in the ppm range could be accomplished directly in the sulfite-iodide step by adding a solution containing 0.5 mg of Se and 5 mg of Te per 50 ml of digest, and boiling for 5 minutes. After 15 minutes, the solution was filtered and the procedure was continued with addition of crystal violet and extraction. The filtered, dry toluene solution of the complex was stable for about 30 minutes if protected from light.

The partition between toluene and aqueous phases varied with extraction ratio and salt concentration from 13 to about 200. Therefore standardization of the volumes for the extraction and the preparation of calibration curves is important.

To avoid high results due to accidental contamination, only acid-washed glassware was used.

This method offers an alternative to available instrumental methods. The advantages include high sensitivity, specificity, simplicity, and speed. The method is easily adapted for field use.

## Acknowledgments

The help of Mr. Dan Uchimoto in the preparation of the calibration curves and analysis of urine samples is greatly appreciated. The editorial help of Dr. Peter K.

Mueller and his staff deserves a special mention at this point.

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# A cold vapour mercury atomic fluorescence detector

by K. C. Thompson

## Introduction

Many non-flame atomic absorption methods for the determination of mercury in solution have been published. Manning (1970) has produced a comprehensive review of such methods. The principle of the technique is that mercury in solution is reduced to the elemental state, using a suitable reducing agent. The mercury is then expelled from the solution by a current of air, passed through a suitable long path absorption cell and the absorption of the 253.7nm mercury line from a low pressure mercury source is monitored.

The absorption method has several disadvantages: the system will respond to any vapour that absorbs at 253.7nm (e.g. acetone) and this will then be registered as mercury, an enclosed cell is required which can lead to memory effects or fogging of the cell windows, and the sensitivity and the range of linearity of the calibration curve of an absorption technique are limited.

Previous studies by Thompson and Reynolds (1971) have shown that the cold vapour atomic fluorescence technique is potentially a selective and sensitive method for the determination of mercury in solutions. The fluorescence signal is monitored by a conventional atomic absorption-emission spectrophotometer. The three sigma limit of detection was quoted as 0.002 μg.

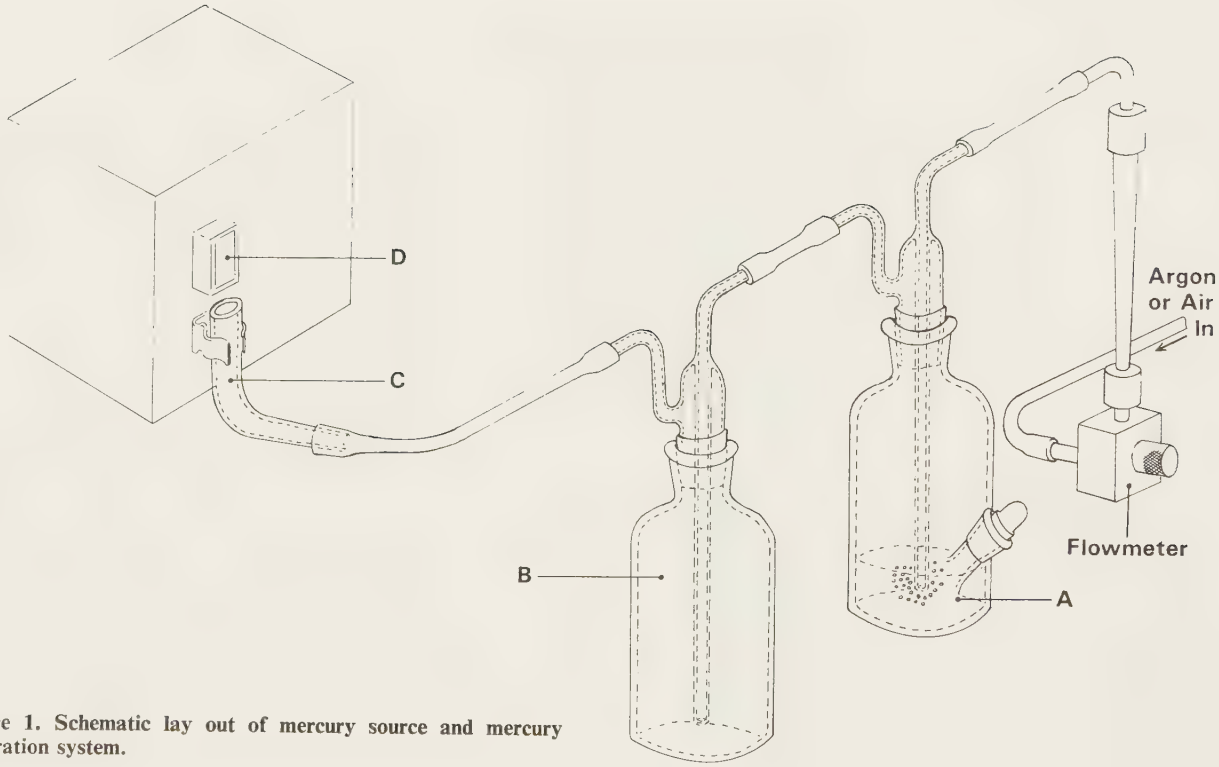


Figure 1. Schematic lay out of mercury source and mercury generation system.

Muscat and Vickers (1971) have also applied this technique to mercury determinations using an enclosed system incorporating a recirculating pump, a drying column and an enclosed fluorescence cell. Their quoted detection limit was 0.003 μg.

This communication describes a simple, sensitive and specific atomic fluorescence apparatus for the determination of mercury in solution.

The fluorescence radiation is detected using a side window solar blind photomultiplier which is insensitive to radiation above 320 nm (i.e. near u.v. and visible radiation). The use of such a photomultiplier instead of the usual combination of monochromator plus conventional photomultiplier results in very efficient light collection of the fluorescence radiation and also considerably simplifies the system. The complete mercury detection system comprises a low pressure mercury source, a mercury generation system, a solar blind photomultiplier, a stabilized d.c. EHT supply, a simple direct coupled amplifier and an RC damped pen recorder. It is possible to monitor readings on an RC damped galvanometer. A drying column is not required.

## Experimental

A Shandon Southern low pressure mercury source and mercury generation system were used. This unit was designed as an attachment for a conventional atomic absorption-emission spectrophotometer for the determination of mercury by the cold vapour fluorescence technique (Shandon Southern Instruments Bibliography, 1971) and is depicted in Figure 1. The system comprises a rotameter, a cell (A) with a side arm, an expansion chamber (B), a 10 mm i.d. Pyrex tube (C) and a low pressure mercury source. The sample was added through the side arm of cell (A) into an acidic solution of stannous chloride. The liberated mercury in the solution was carried by a gas stream through the expansion chamber (B), to the Pyrex tube (C). The low pressure mercury source was positioned such that the light was directed over the top of the Pyrex tube (C). The fluorescence radiation produced from the mercury vapour in the region (D) above the tube (C) was detected using a R166 Hamamatsu side window solar blind photomultiplier. This was mounted in a simple housing positioned to the side of, and above tube (C) so that the photomultiplier did not receive radiation directly from the mercury lamp. It was essential to minimize the specular reflection signal which constituted the constant blank signal. The fluorescence detector head is depicted in Figure 2. The solar blind photomultiplier is almost completely insensitive to radiation above 320 nm, but it will respond to direct sunlight and mercury fluorescent lighting at high EHT settings. The photomultiplier was shielded from these sources by simple non-reflective screens. The EHT to the photomultiplier was supplied from a 200–1000 volt stabilized d.c. power supply. (The maximum EHT used in this study was

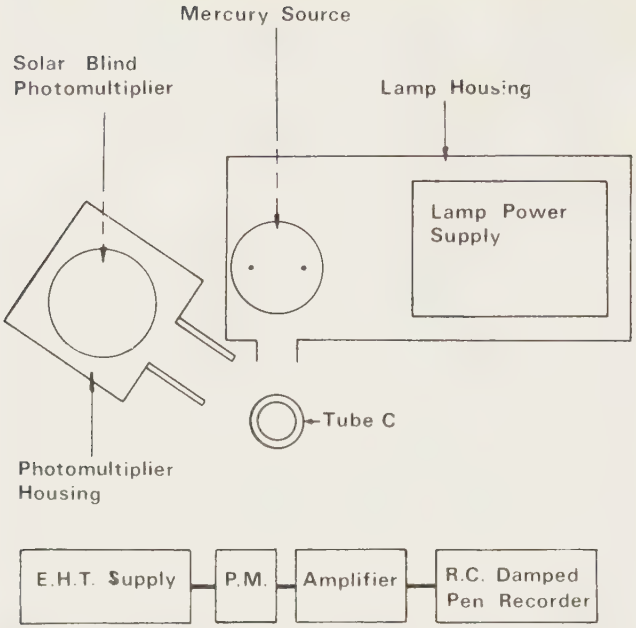


**Figure 2. Schematic lay out of mercury fluorescence detector head.**

600 volts). The output from the 1 megohm photomultiplier load resistor was fed to a Shandon Southern MO4–200 high input impedance amplifier, set at the 50 mV input range, the output of which was directly displayed on an RC damped Bryans 27,000 series pen recorder used on the 1 volt range. The optimum recorder damping was found to be an RC network connected to the input terminals of the Bryans recorder ($R = 12\ K\Omega$, $C = 1000\ \mu F$, time constant 12 seconds). The output voltage swing of the amplifier was 10 volts, hence saturation of the amplifier should not occur.

## Reagents

*Solution* 1: 20 g of stannous chloride dihydrate was dissolved in a mixture of 40 ml of hydrochloric acid and 80 ml of 8 M sulphuric acid. A small piece of pure tin was added to facilitate dissolution. The resulting solution was then diluted to 200 ml with distilled water.

*Solution* 2: 2 M sulphuric acid.

The mercury solutions were freshly prepared from a 1000 ppm master solution, made up by dissolving 1 g of mercury in 50 ml of nitric acid and diluting to 1 litre with distilled water.

All dilute mercury solutions were prepared prior to measurement and contained 1% V/V of nitric acid, the blank solutions contained the same amount of nitric acid.

All reagents were of analytical grade.

## Choice of carrier gas

For equivalent quantities of mercury, the peak heights obtained using argon as the carrier gas were approximately 16 times greater than when air was used. This was attributed to the quenching of excited mercury atoms by nitrogen and oxygen molecules. In a previous study by Thompson and Reynolds (1971) using a monochromator, the peak heights with argon were found to be 35 times greater than those with air. The smaller increase observed in this work was attributed to the larger aperture of the present detector, viewing more of the region where air entrainment occurred. Possibly the use of an argon sheath around tube (C) would improve the limit of detection, but the limit obtained without a sheath was considered to be adequate. The response was slightly dependent on the gas flow rates, the optimum flow rate being 1.5 litres/min for both argon and air. The optimization of the gas bubbling arrangement has been previously described (Thompson and Reynolds, (1971).

## Measurement procedure

Ten ml of solution 1 and 30 ml of solution 2 were added to cell (A) and argon bubbled through the solution at a flow rate of 1.5 litres/min until a stable baseline was obtained on the recorder. (At high sensitivities it was possible to detect mercury in the reagents used). The gas flow was reduced to 0.5 litre/min, the test solution (containing 0.001–2 μg mercury) quickly added through the side arm of cell (A), and the argon flow increased back to 1.5 litres/min. The argon was not turned off during the addition to minimize air entrainment in cell (A). The fluorescence signal was monitored on the pen recorder; a typical trace is shown in Figure 3. The recorder pen returned to the baseline within 5 minutes and the next sample could then be added as before. This procedure could be repeated until the volume of solution in cell (A) reached


**Figure 3. Typical mercury peaks. 0.05 μg (1 ml 0.05 ppm) mercury argon flow 1.5 litres/min. EHT 420 volts.**

approximately 60 ml. At this point, the solution in cell (A was discarded and fresh reagents added. The effect of dilution of the contents of cell (A) on the peak heights was checked by the addition of 1 ml samples of a 0.1 ppm mercury solution to the cell. A volume increase from 40 ml to 54 ml produced no change in peak height, but a further volume increase to 65 ml resulted in a 6% decrease in the peak height obtained. The use of argon prevented oxidation of the stannous chloride in cell (A). Peak height was found to be almost proportional to mercury concentration over the range 0.001–2 μg (see Figure 4).

The constant blank signal due to specular reflection corresponded to 0.03 ug of mercury when using argon. The noise on the baseline gave a two sigma detection limit corresponding to 0.0005 ug of mercury when using argon and 0.003 ug of mercury when using air as the carrier gas. The blank obtained from 1 ml of 1% V/V nitric acid was found to be negligible. For the determination of organically bound mercury an initial oxidation with potassium permanganate (Manning 1970; Lindstedt 1970) would be required. Alternatively the addition of cadmium chloride to the stannous chloride solution has been reported to break down organomercury compounds (Magos, 1971).

It was possible to increase the sample turnover rate when using air by utilizing an auxiliary air supply in conjunction with another cell base. The acid stannous chloride reagent was added to both cells and the air flow turned on. The stannous chloride in the stand-by cell base was degassed by bubbling the auxiliary air supply through a 3 mm i.d. glass tube at a flow rate of 3 litres/min. When a stable baseline had been obtained, 1 ml of sample solution was added to the in-line cell and the peak recorded. It can be seen from Figure 3 that the mercury peaks are asymmetric and that the apex is reached within 45 seconds of sample addition. Once the apex had been reached, the cell head and auxiliary air supply were interchanged, the 1000 μF damping capacitor across the

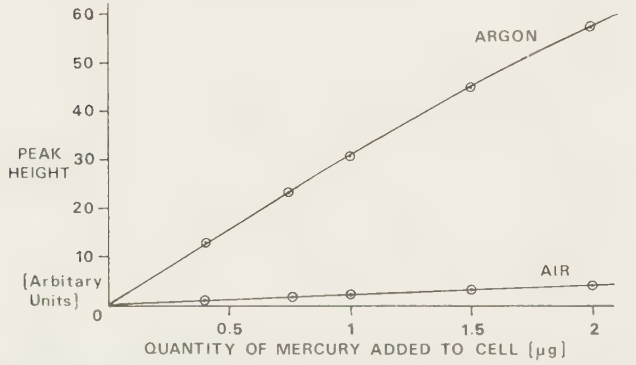


**Figure 4. Mercury calibration curves. Gas flow 1.5 litres/min. EHT 230 volts.**

recorder terminals was shorted by pressing a push contact and after the baseline had stabilized (approx. 30 sec) the next sample could be added to the new cell. The remaining mercury in the original cell was meanwhile removed by the auxiliary air supply. With this simple system a sample turnover rate of 1.5 minutes per sample could be attained. After approximately six samples had been added to each cell the peak heights began to show a steady decrease, this was thought to be due to oxidation of the stannous chloride by the air. Further work is being performed on this type of system using argon as the carrier gas and a switching arrangement that avoids air entrainment into the system.

## Precision

Ten ml of solution 1, 30 ml of solution 2 were placed in cell (A) and argon bubbled through the solution until a stable baseline was obtained. After reducing the argon flow to 0.5 litre/min, 1 ml of an 0.05 ppm mercury solution (0.05 μg mercury) was quickly added, the argon flow increased to 1.5 litres/min and the peak height recorded. When the recorder ahd returned to the baseline the mercury addition was repeated and the peak height again recorded. Fifteen consecutive readings were thus obtained. The relative standard deviation calculated from the peak heights was 4%. Further work is being performed using a modified side arm to cell (A) such that the bottom of the side arm is below the solution level. This allows addition of the sample with minimum air entrainment, prevents loss of liberated mercury through the side arm and thus obviates the need to reduce the argon flow during the sample addition.

## Effect of organic solvents

The effect of organic solvents (acetone, benzene, chloroform and ethanol) in the cold vapour fluorescence technique has been found to be less than in the corresponding absorption technique (Thompson and Reynolds, 1971). These findings were confirmed using the present system.

Using air as the carrier gas, the addition of 0.5 ml of acetone or methanol to cell (A) did not affect the baseline and the peak height obtained from 1 ml of a 0.1 ppm mercury solution containing 50% V/V of acetone or methanol was within 7% of that obtained from an equivalent aqueous mercury solution. (The readings for the aqueous solutions were made prior to adding acetone or methanol to the cell).

Using argon as the carrier gas 0.5 ml of acetone or methanol had no effect on the baseline, but caused a reduction of 35 and 29% respectively in the peak heights obtained from the corresponding mercury solutions. This reduction in peak height was attributed to quenching of excited mercury atoms by the organic solvent molecules in the gas stream. With air as carrier gas the additional quenching effect due to organic solvent molecules is negligible compared with that caused by nitrogen and oxygen molecules.

If relatively large quantities of organic solvents are known to be present the use of air is to be recommended although this results in a reduction of sensitivity compared with argon.

### Mercury in air

Some preliminary results were obtained using the above type of system as a mercury in air detector. The apparatus was calibrated by diluting air saturated with mercury with mercury free air. The two sigma limit of detection was approximately 0.003 ug/litre (mg/m$^3$). The presence of acetone, chloroform or benzene vapour in the airstream caused far less interference in the fluorescence technique than in the corresponding absorption technique. However, specular reflection of the source radiation caused by the presence of particulate matter (e.g. dust, cigarette smoke, ammonium chloride fumes, etc.) in the sample airstream was far more serious. Further work on this system is being carried out.

## Conclusions

The cold vapour atomic fluorescence technique using a solar blind photomultiplier detector is a simple and very sensitive method for the determination of low concentrations of mercury. The interference effects of vapours which absorb at 253.7 nm are considerably less than in the corresponding absorption technique. Moreover, an enclosed cell and recirculating pump are not required.


## Acknowledgements

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# Visualization of the Atomic Absorption of Mercury Vapor by Use of a Fluorescent Screen

**Robert J. Argauer** and **Charles E. White**

The hazard caused by mercury vapor has been recognized for a long time and was reviewed in detail by Steere (*1*). Somewhat more recently interest in environmental protection coupled with the realization that metallic mercury can be converted into dimethylmercury and methyl mercury compounds that appear in the food chain has led to accelerated development of rapid and accurate quantitative methods for the analysis of mercury in organic and inorganic substances (*2*, *3*). Atomic absorption spectroscopy is rapidly becoming the method of choice for analysis of mercury. In this method, the oxidized sample is reduced to metallic mercury, and the mercury vapor is transported through the absorption cell in the optical beam of the spectrometer.

The fact that mercury exhibits a finite vapor pressure at room temperature is not generally evident. We have used the following demonstration in the classroom and in governmental and industrial laboratories to visualize the presence of metallic mercury vapor. The demonstration also illustrates the principle basic to atomic absorption spectroscopy.

Several drops of mercury are placed in a 250 ml polyethylene squeeze bottle, and the bottle is positioned between a light source and a fluorescent screen and squeezed (see the figure). The invisible puffs of

mercury vapor absorb the 2537 Å radiation emitted by the lamp, and dark shadows appear across the fluorescent screen. A Mineralight Model UVSL lamp (Ultraviolet Products, Inc., San Gabriel, Calif.)[1] is used as the source of 2537 radiation. Thin-layer chromatographic plates containing a 254-nm fluorescent phosphor make excellent fluorescent screens (*4*). Since the concentration of mercury in air saturated at 25°C with mercury vapor is approximately 20 μg/l (*5*), **one or two squeezes of the plastic bottle, assuming somewhat less than equilibrium conditions, releases about 1 μg of vapor.** Because the vapor is diluted by the air in the room, the concentration of mercury is well below the limit currently recommended by the American Conference of Governmental and Industrial Hygienists.

To demonstrate that as little as 1 μg of mercury in the plastic bottle will produce the effect prepare a 10 μg/ml mercury solution by dissolving 17 mg of mercuric nitrate, $Hg(NO_3)_2 \cdot H_2O$ in 100 ml of distilled water containing 10 ml of concentrated nitric acid, and dilute to 500 ml with water. Next, dissolve 5 g of $SnCl_2 \cdot 2H_2O$ in 10 ml of warm concentrated hydrochloric acid, and when all the stannous chloride has dissolved add 40 ml of distilled water. Add 2 ml of the stannous chloride solution to the plastic bottle, and squeeze in front of the screen as shown in the figure. No absorption should take place. Add one or two drops of the 10 μg/ml mercury solution to the stannous chloride solution in the plastic bottle and swirl the mixture. Again position the bottle, squeeze, and note the effect. Acetone used to clean the plastic bottles will cause a similar, but diminished absorption effect. Compensation for broad band absorption interferences such as those produced by acetone is provided in an atomic absorption spectrometer by alternately passing the light from a deuterium arc and that from a mercury hollow-cathode lamp through the vapor and taking the ratio between the two beams (*6*, *7*).

[1] Mention of a proprietary product is for identification only and does not necessarily imply endorsement of the product by the U.S. Department of Agriculture.

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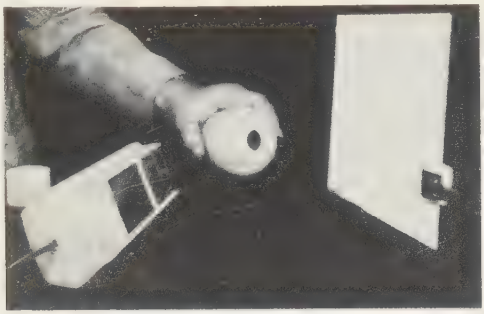

Apparatus used to visualize atomic absorption of mercury vapor.

M. Mazumdar
S. C. Shome

**Gravimetric and spectrophotometric determination of mercury with thiosalicylamide**

Thiosalicylamide has been found a useful analytical reagent for the determination of certain metal ions[1,2]. In the present investigation the reagent has been employed for the gravimetric and spectrophotometric determination of mercury(II). In dilute hydrochloric acid medium, the metal forms a light yellow precipitate with thiosalicylamide which can be weighed as $Hg(C_7H_7ONS)_2Cl_2$ after drying at 110–120°. The mercury precipitate is soluble in 50% ethanol; the ethanolic solution obeys Beer's law at 355 nm in the concentration range 3.90–23.40 $\mu g\ Hg^{2+}\ ml^{-1}$.

*Apparatus*

All the spectral transmittance measurements were carried out with a Carl Zeiss spectrophotometer Model PMQ II with 1-cm quartz cells. The pH values were measured with a Cambridge pH meter (Bench Model).

*Reagents*

*Standard mercury solution.* Dissolve an appropriate amount of mercury(II) chloride in 0.1 *M* hydrochloric acid and standardize by the periodate method.

*Solutions of diverse ions.* Standard solutions were prepared in distilled water, hydrochloric acid being added, where required, to prevent hydrolysis of the metal ions.

*Thiosalicylamide solution.* For the gravimetric method, prepare a 1% solution of thiosalicylamide in 20% ethanol. For the spectrophotometry, use a 0.02 *M* ethanolic solution of the reagent.

All the reagents used were of A.R. grade.

*Gravimetric determination of mercury*

The light yellow precipitate obtained by adding the thiosalicylamide solution to a hot solution of mercury(II) chloride in 1 *M* hydrochloric acid medium was slightly soluble in chloroform and carbon tetrachloride. It was stable towards nonoxidising

TABLE I

DETERMINATION OF MERCURY BY DIRECT WEIGHING OF MERCURY–THIOSALICYLAMIDE COMPLEX

| *Hg taken* (*mg*) | *Wt. of ppt.* (*mg*) | *Hg found* (*mg*) | *Error* (*mg*) |
|---|---|---|---|
| 4.95 | 14.3 | 4.951 | +0.001 |
| 4.95 | 14.2 | 4.930 | −0.020 |
| 9.90 | 28.5 | 9.896 | −0.004 |
| 9.90 | 28.6 | 9.924 | +0.024 |
| 19.80 | 56.9 | 19.740 | −0.060 |
| 19.80 | 57.0 | 19.770 | −0.030 |
| 29.70 | 85.6 | 29.700 | 0.000 |
| 29.70 | 85.7 | 29.730 | +0.030 |
| 39.60 | 114.1 | 39.590 | −0.010 |

TABLE II

SEPARATION OF MERCURY(II) FROM FOREIGN IONS

(19.80 mg of mercury(II) were used in each experiment)

| *Foreign ion added* (*mg*) | *Hg found* (*mg*) | *Error* (*mg*) |
|---|---|---|
| $Mn^{2+}$ (200) | 19.81 | +0.01 |
| $Co^{2+}$ (200) | 19.81 | +0.01 |
| $Ni^{2+}$ (200) | 19.81 | +0.01 |
| $Zn^{2+}$ (150) | 19.84 | +0.04 |
| $Cd^{2+}$ (100) | 19.81 | +0.01 |
| $Cr^{3+}$ (200) | 19.81 | +0.01 |
| $Ga^{3+}$ (200) | 19.81 | +0.01 |
| $In^{3+}$ (150) | 19.81 | +0.01 |
| $Al^{3+}$ (100) | 19.84 | +0.04 |
| $Fe^{3+}$ ( 50)[a] | 19.84 | +0.04 |
| $Ti^{4+}$ ( 50) | 19.77 | −0.03 |
| $Th^{4+}$ (100) | 19.81 | +0.01 |
| $Mo^{6+}$ ( 50) | 19.84 | +0.04 |
| $U^{6+}$ (100) | 19.81 | +0.01 |
| $Bi^{3+}$ ( 50) | 19.81 | +0.01 |
| $As^{3+}$ ( 50) | 19.81 | +0.01 |
| $Sb^{3+}$ ( 50) | 19.84 | +0.04 |
| $Pb^{2+}$ ( 20) | 19.84 | +0.04 |

[a] In presence of phosphoric acid.

acids but reacted readily with alkali. On analysis the pure complex was found to contain 34.70% Hg, 4.75% N, 11.12% S and 12.31% Cl (required for $Hg(C_7H_7ONS)_2Cl_2$: 34.72% Hg, 4.85% N, 11.08% S and 12.29% Cl). It decomposed at 204°.

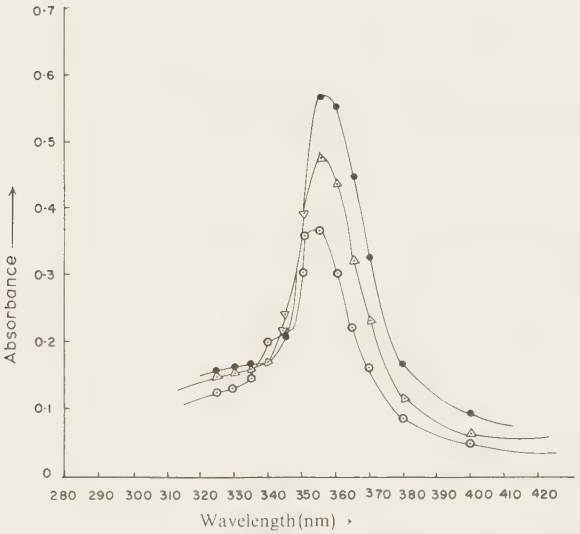


Fig. 1. Absorbance curves of mercury(II) -thiosalicylamide complex in 50% ethanol. (⊙) $[Hg^{2+}] = 11.70$ μg ml$^{-1}$; (△) $[Hg^{2+}] = 15.60$ μg ml$^{-1}$; (●) $[Hg^{2+}] = 19.50$ μg ml$^{-1}$.

For complete precipitation of mercury in 0.1–2.0 *M* hydrochloric acid medium, 1.5–3.5 times the theoretical amount of thiosalicylamide was required. At pH 4.0–5.0, mercury(II) formed a bright yellow precipitate with the reagent, which decomposed on standing. Mercury(I) produced a very unstable yellowish-white precipitate.

*Procedure.* Dilute the mercury(II) chloride solution to 120–150 ml and adjust the acidity to 0.2–1 *M* in hydrochloric acid. Heat to 50–60° and add 10–15 ml of the reagent solution. Digest on a hot water bath for 30 min with occasional stirring. Filter the precipitate on a no. 4 sintered glass crucible, and wash with hot 1% hydrochloric acid. Dry the precipitate at 110–120° to constant weight. Typical results are given in Table I.

*Effect of diverse ions.* Mercury was determined as above, without interference, in the presence of known amounts of Mn, Co, Ni, Zn, Cd, Fe(II), Cr(III), Ga, In, Al, Mo(VI), U(VI), As(III), Sb(III), Pb(II), Bi(III), Th or Ti (Table II). Addition of phosphoric acid was necessary to mask the interference of iron(III). Palladium, platinum and copper interfered.

*Spectrophotometric determination of mercury*

The absorbance curves of mercury–thiosalicylamide complex of varying concentrations of metal in 50% ethanol at pH 2.5 are shown in Fig. 1. The complex showed maximum absorbance at 355 nm.

*Procedure.* Place an aliquot of the mercury(II) solution in a 25-ml flask, and

adjust the pH to 2.5 with sodium acetate solution and dilute hydrochloric acid. Add 2.0–3.5 ml of 0.02 *M* thiosalicylamide solution, thoroughly mix and make up the volume with water and ethanol so that the final solution contains 50% ethanol. Measure the absorbance at 355 nm after 30 min against a reagent blank.

*Study of variables.* Solutions containing 78 μg of mercury(II) were separately mixed with 2 ml of 0.02 *M* solution of the reagent and the mixtures were adjusted to different acidities with dilute hydrochloric acid and sodium acetate solution, before

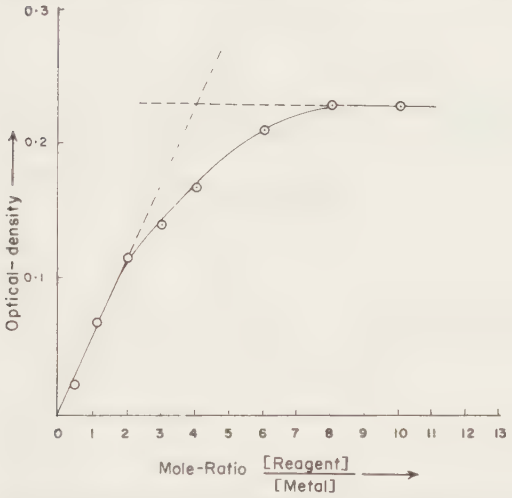


Fig. 2. Composition of the mercury(II)–thiosalicylamide complex in 50% ethanol (mole-ratio method).

dilution to 25 ml with ethanol and water. Maximal colour formation occurred between pH 2.0 and 2.95.

The absorbance of the mercury-thiosalicylamide complex in 50% ethanol was stable upto 12 h.

Beer's law was obeyed at 355 nm over the range 3.90–23.40 μg $Hg^{2+}$ $ml^{-1}$. According to Sandell's[3] recommendation, the optimal range for the determination was 6.83–23.40 μg Hg $ml^{-1}$. The sensitivity of the colour reaction and the molar absorptivity of the mercury thiosalicylamide complex in 50% ethanol were found to be 0.034 μg $cm^{-2}$ and $5.878 \cdot 10^3$, respectively.

*Nature of the complex and its dissociation constant.* The empirical formula of the mercury complex was determined by the mole-ratio method[4]. The results (Fig. 2) indicated that mercury forms a 1:4 complex with thiosalicylamide.

The dissociation constant of the complex in 50% ethanol was found from the mole ratio curve (Fig. 2) to be $6.5 \cdot 10^{-18}$. The degree of dissociation was calculated from the curve as described by Harvey and Manning[5].

*Effect of diverse ions.* The tolerance limits for various diverse ions (Table III), are those concentrations of foreign ions which caused errors of less than ±2%. The interference of iron(III) was again avoided by adding small quantity of phosphoric acid.

TABLE III

EFFECT OF DIVERSE IONS ON THE SPECTROPHOTOMETRIC DETERMINATION OF MERCURY(II)
(190 $\mu$g of mercury(II) was taken)

| *Foreign ion added* | *Amount tolerated* ($\mu$g) | *Foreign ion added* | *Amount tolerated* ($\mu$g) |
|---|---|---|---|
| $Mn^{2+a}$ | 2000 | $Ti^{4+a}$ | 100 |
| $Ni^{2+a}$ | 2000 | $Th^{4+d}$ | 500 |
| $Co^{2+a}$ | 2000 | $W^{6+e}$ | 500 |
| $Zn^{2+a}$ | 1500 | $Mo^{6+e}$ | 500 |
| $Cd^{2+a}$ | 2000 | $UO_2^{2+d}$ | 400 |
| $Cr^{3+a}$ | 1000 | $Bi^{3+d}$ | 500 |
| $Ga^{3+b}$ | 500 | $As^{3+}$ | 500 |
| $In^{3+a}$ | 500 | $Sb^{3+b}$ | 600 |
| $Al^{3+a}$ | 600 | $Pb^{2+b}$ | 200 |
| $Fe^{3+b,c}$ | 50 | | |

[a] As sulphate. [b] As chloride. [c] In presence of phosphoric acid. [d] As acetate. [e] As sodium salt.

*Discussion*

Thiosalicylamide is an important addition to the numerous available organic reagents used for the determination of mercury(II). The reagent possesses some advantages over others such as thionalide[6], monalzine[7] and N-benzoyl-N-phenylhydroxylamine[8]. The procedure for the gravimetric and spectrophotometric determination of mercury with thiosalicylamide is simple. The mercury–thiosalicylamide complex is stable, can be obtained in a directly weighable form and has a high molecular weight. Thiosalicylamide is very soluble in hot water, so that the excess of reagent can be removed easily from the precipitate. Moreover, mercury can be determined with the reagent from dilute hydrochloric acid medium and a large number of foreign ions do not interfere with the procedure. The colour reaction between mercury(II) and thiosalicylamide is of high sensitivity.


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# Printing of mercury distribution on the surface of dental amalgams

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Wallace W. Johnson, DDS, MS,

When a dentist mixes silver alloy and mercury to produce the resultant plastic mass amalgam, he may think that the union of these metals is a permanent and never changing one. This is not so. As important as mercury is to dental amalgam, it is not the stable material it appears to be. Mercury has the property of vaporizing at ordinary temperatures, a property that has never been fully appreciated by the dentist. Mercury vapor is released from newly placed restorations as well as from those that have hardened and aged. It is also released from amalgam scrap, squeeze cloths, and many other instruments and articles used in amalgam preparation and placement.

Several articles have appeared concerning the impairment of health as a possible consequence of mercury evolution from all aspects of the amalgam restoration.[1-5] Though many contradictory opinions have been expressed about the hazards of amalgam treatment, favorable experience associated with its long and extensive use tends to subdue the concern.

Many sophisticated methods have been devised for use in mercury detection, both quantitative and qualitative. However, Nordlander[6,7] described a simple but accurate method with use of selenium sulfide as a detector for mercury vapor as it diffused through pinholes in plastic films. Selenium sulfide is an orange-yellow stable powder that readily reacts with mercury vapor to form a black precipitate. When placed in close proximity to surfaces yielding mercury vapors, selenium sulfide-coated materials display a black print, varying in intensity, that enables the observer to make a clear visual qualitative determination of the mercury concentration of the area. Selenium sulfide can be applied to many materials such as paper, wood, or celluloid to produce a surface that will be reactive to mercury vapor.

The orange-colored modification of selenium sulfide specified by Nordlander is often not readily available. However, it is the active ingredient of Selsun*, which contains 2½% SeS. A satisfactory material can be prepared by diluting 1 part Selsun with 15 parts water, shaking the mixture in a separatory funnel, and using the settled slurry in the preparation of the printing paper. An alternate method is to filter the slurry and use the filtered solids for spreading on paper.

The purpose of this study is to show how Nordlander's method of mercury detection may be modified and used in dental research to indicate areas of high mercury content on the surfaces of dental amalgams. It is used in this investigation to show the release of mercury vapor from amalgam surfaces that have been subjected to simple surface treatments such as carving, burnishing, and polishing.

## Materials and methods

Selenium sulfide† powder was applied to a sheet of bond paper and rubbed thoroughly with a cotton pad until the paper was uniformly coated. Uniformity of the film was considered achieved when

a homogeneous orange-yellow color was obtained. The paper was then cut into strips of predetermined sizes.

Two series of eight circular cavities, each 4 mm in diameter and 4 mm deep, were cut in Plexiglass blocks. Four Class V cavities of equal size and depth were also prepared in separate, freshly extracted teeth.

A commercial fine-cut alloy‡ was mixed with mercury in a 1:1 ratio. With use of a mechanical amalgamator, the mix was triturated 20 seconds with pestle, and 2 seconds without pestle in a plastic capsule. Immediately after trituration, the amalgam was condensed into the cavities. Condensation was done by conventional hand-pressure techniques.

The cavities in the plastic blocks were slightly overfilled and then carved back to the material surface with a razor blade. After carving was completed, four of the restorations were burnished with a Hollenbeck no. 6 burnisher.

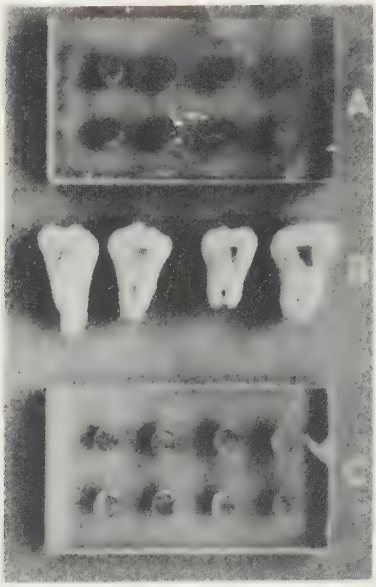



Fig 1 ■ A: Amalgam test samples, lower row burnished. B: Class V amalgam test samples. C: Alloy-zinc phosphate cement control samples.

The four Class V cavities in the extracted teeth were also slightly overfilled with amalgam and carved back to the marginal surface with a Hollenbeck no. 3S carver. Two of the Class V restorations were burnished with the Hollenbeck no. 6 burnisher.

Eight cavities in the last Plexiglass block were filled with the commercial alloy mixed with zinc phosphate cement instead of mercury. These were control specimens (Fig 1).

## Obtaining the mercury printing

Silver and other metals will also react with selenium sulfide and form a black precipitate. However, because of the low vapor pressures of these metals at ordinary temperatures, such a reaction would take a long time unless the sulfide and the metals were in actual contact.

To prevent the selenium sulfide and the metal components of the specimens from contacting each other, a single layer of filter paper was placed between the specimens and the sulfide coated papers. With this filter paper separation, any observable precipitate of the sulfide can be considered a reaction specific for mercury.

As mercury vapor diffuses through the filter paper it reacts with the selenium sulfide to form a black precipitate or print. The degree of blackening is a function of mercury concentration, time of exposure, and temperature.[6]

The prepared specimens were placed in an oven at a temperature of 37C. After 12 hours of exposure, the coated papers were removed for examination of the printing. (The amalgam specimens were replaced in the oven for controlled temperature storage.) Twenty-four hours after condensation, all the amalgams were polished. Freshly coated selenium sulfide papers were again placed by the methods previously described, and the prepared specimens were replaced in the 37C oven. After 12 hours of exposure, the second printings were removed for examination.

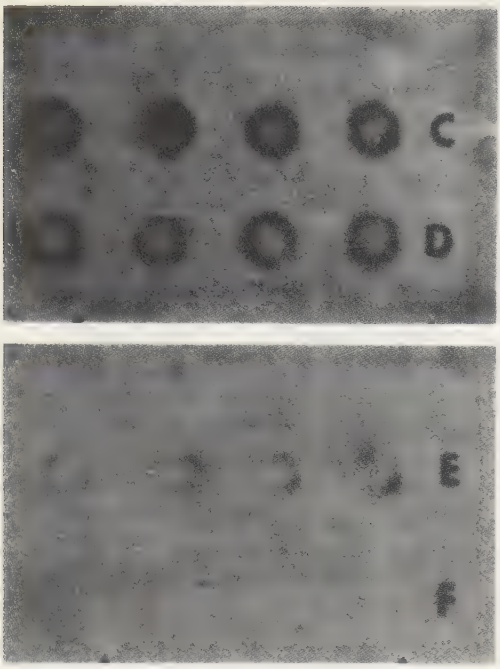



Fig 2 ■ Top: C: Printings from amalgam samples, not burnished; D: Printings from amalgam samples, burnished. Bottom: E: Printings from polished amalgam samples, not burnished; F: Printings from polished amalgam samples, burnished.

## Results and discussion

Mercury printings were obtained from all the amalgam specimens. No printings were obtained from the alloy-zinc phosphate control specimens. A heavy blackening or intense printing was obtained from the unburnished specimens. The intensity of the printing from the burnished specimens was noticeably less, particularly at the marginal areas of the samples. These results seemed to confirm the finding of Kanai[8] concerning the decrease of mercury content at the margins of amalgam restorations caused by burnishing procedures.

Polishing greatly reduced the intensity of the printing from all samples. The effect of burnish-

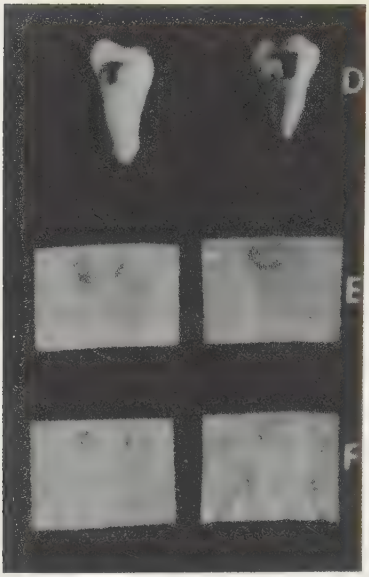
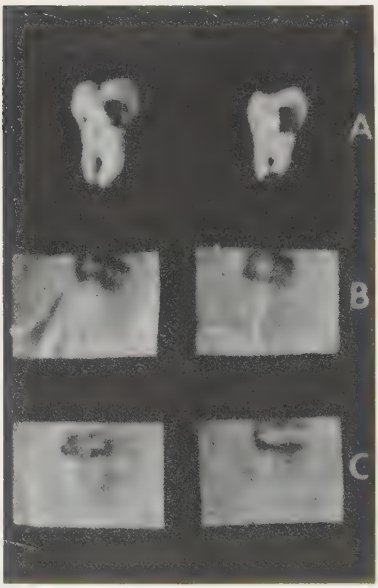



Fig 3 ■ Left: A: Class V amalgam restorations, not burnished; B: Printings obtained after carving only; C: Printings obtained after polishing. Right: D: Class V amalgam restorations, burnished; E: Printings obtained after burnishing only; F: Printings obtained after polishing.



ing was again apparent however, as the specimens that were burnished after carving caused little or no printing after polishing. The unburnished specimens did give a printing after polishing, though it was of a low intensity (Fig 2).

The results from the test specimens placed in extracted teeth were much the same as those placed in the plastic blocks. The effects of burnishing and polishing were also similar. However, these printings did show a noticeable variation. They were more diffuse and did not show the exactness of those specimens placed in plastic blocks (Fig 3).

An explanation for this result might be that the relative difficulty we experienced in adapting the coated papers to the curved surfaces of the tooth allowed the mercury vapor to dissipate more diffusely.

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# Occurrence of Alkylmercury Compound in Caustic Soda Factory

*Seiya Yamaguchi, MD, PhD; Hisao Matsumoto, PhD; Michiyo Hoshide; Sachiko Matsuo; and Shunsuke Kaku, MD*

MINAMATA disease has furnished tragic illustration of the danger of environmental pollution that may follow upon rapid and improvident industrial development. Studies of acceptable limits of mercury in biological and environmental milieus[1] disclose several problems which urgently call for clarification.

It is established that fishes caught in a natural environment contain a certain amount of methyl mercury,[2] though knowledge of the origin and potential toxicity in these circumstances is still insufficient. From the viewpoint of public health, an evaluation of the biological significance of methylmercury as it appears in the food chain is imperative.

This article describes recovery of an alkylmercury compound from the sludge pit of a caustic soda factory in which only metallic mercury has been used in the electrolysis of sodium chloride. The compound has chemical and toxicologic properties that seem to identify it as methyl mercury. This is the first available evidence for alkylation of inorganic mercury in an apparently totally inorganic system.

## Measurement of Mercurials in Caustic Soda Factories

Samples of water and of sludge were collected at several sites at two factories. Measurement of total mercury has been carried out by cold-vapor atomic absorption method.[3] Identification and determination of alkylmercury have been done by gas chromatography and thin-layer chromatography.[4,5] The results obtained are shown in the Table.

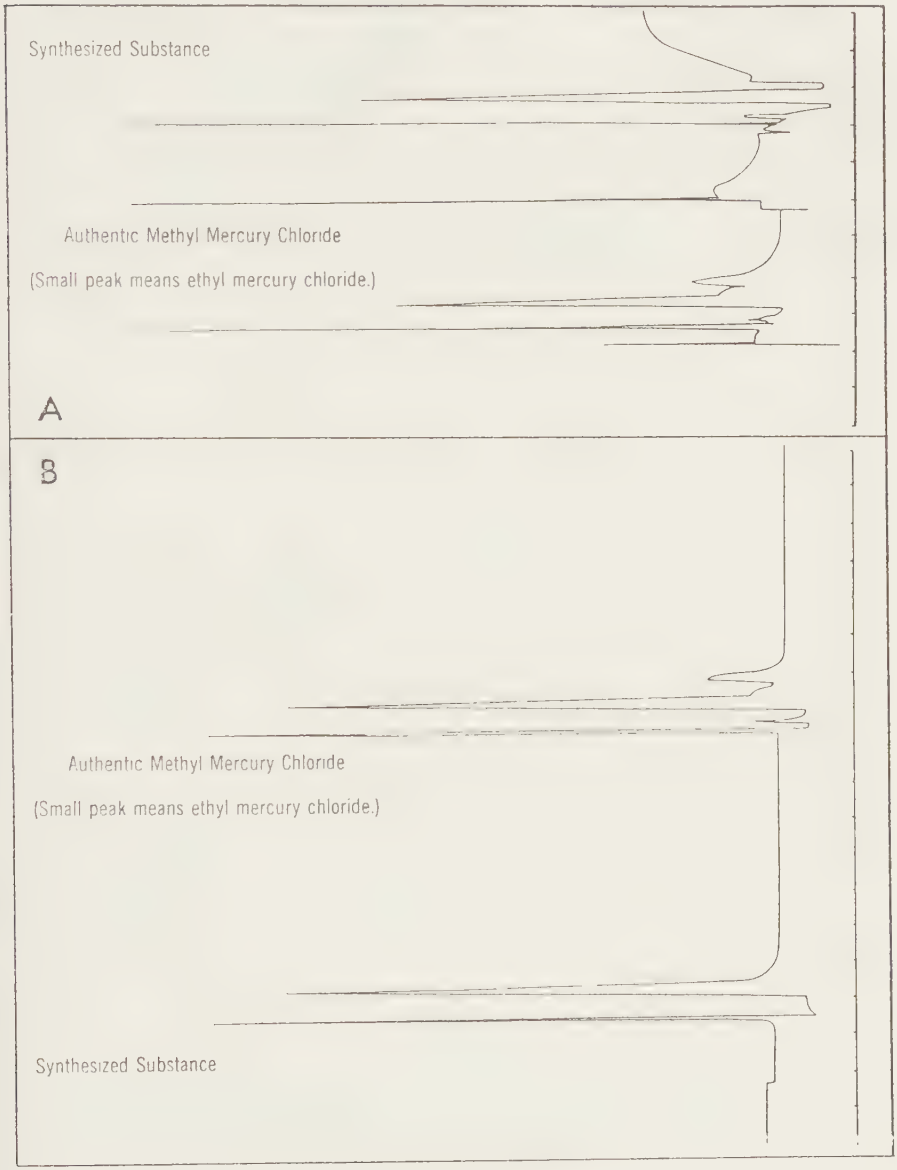
## Chemical Production of Substance Similar to Methyl Mercury in an Inorganic System

To confirm the production of methyl mercury chloride in the process of electrolysis of sodium chloride or thereafter, the following experimental studies were carried out:

1. Measurement of methyl mercury chloride in water containing metallic mercury.
2. Measurement of methyl mercury chloride in mercury bichloride (mercuric chloride) solution.
3. Measurement of methyl mercury chloride in a solution of inorganic mercury (mercury bichloride, mercury bisulfate [mercuric sulfate]) mixed with amorphous carbon (carbon black, a mixture of creosote and acetylene carbon black) (Fig 1, *top*).
4. Measurement of methyl mercury chloride in a solution of inorganic mercurials (mercury bichloride, mercury bisulfate) mixed with calcium carbide or precipitates of calcium carbide (Fig 1).

A substance similar to and thought to be methyl mercury chloride was recognized in the last two experiments (3 and 4). The production of a similar substance thought to be methyl mercury chloride was also identified in effluent water from the electrol-

Fig 1.—Retention times of two experimentally synthesized substances and authentic methyl mercury chloride. **Top,** Synthesized substance of experiment 3. **Bottom,** Synthesized substance of experiment 4.



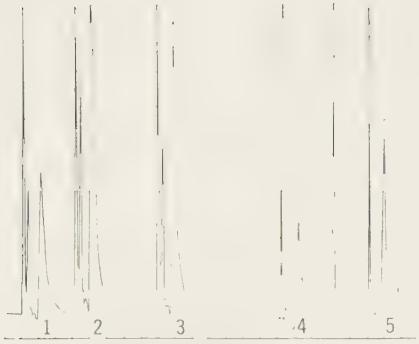


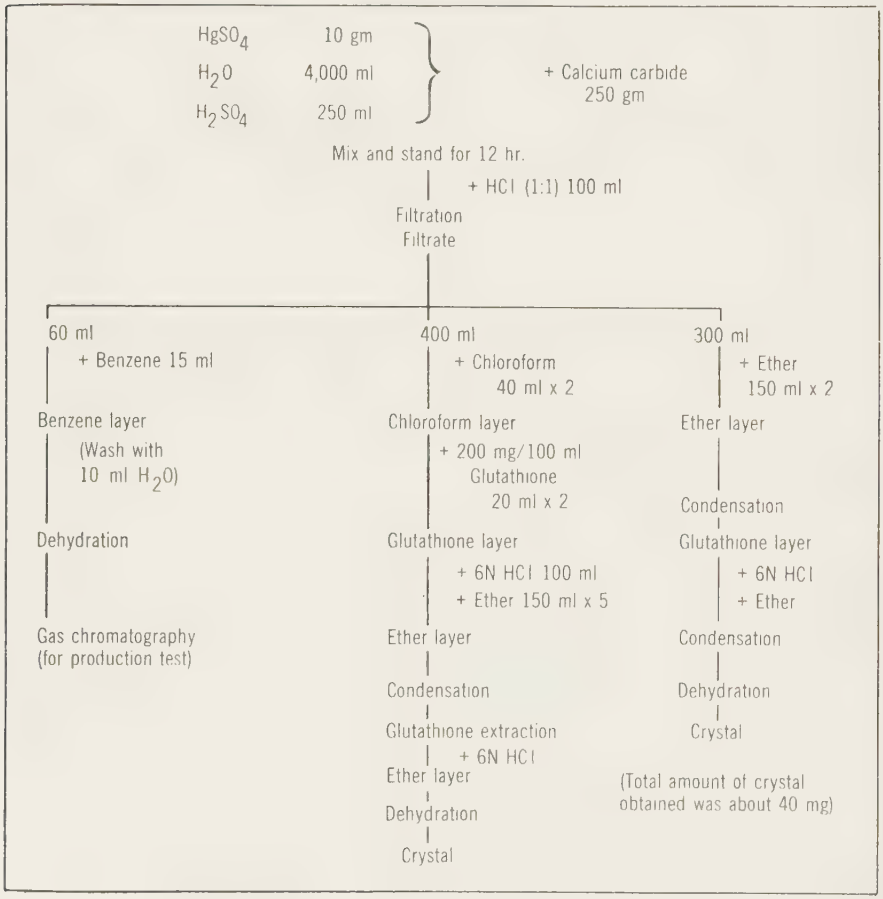

Fig 2.—Retention times of a similar substance produced in effluent water and authentic methyl mercury chloride. **1, 2,** and **3** are the substance produced; **4** and **5** are authentic methyl mercury chloride.

ysis plant when calcium carbide or its precipitate was added and mixed with the effluent. The retention time of the product measured by gas chromatography (Fig 2) corresponded to that of methyl mercury chloride.

### Crystallization and Chemical Properties of Substance

The substance which was obtained in experiment 4 was collected and crystallized by the method shown in Fig 3. All reagents were carefully checked in advance to eliminate contaminants. The crystalline material

Fig 3.—Method of extraction and crystallization of alkylmercury compound.



obtained had the characteristic odor of methyl mercury chloride, melted and evaporated at 173 C, and had 788 $cm^{-1}$ and 1,190 $cm^{-1}$ absorption by infrared analysis as shown in Fig 4. These findings almost corresponded with those of authentic methyl mercury chloride (Fig 5). A solution of the crystalline material was spotted and developed on a thin-layer chromatograph, and the $R_f$ portion was scraped off and analyzed for mercury by the cold-vapor atomic absorption method. The locus where mercury was found showed the same $R_f$ value as that of methyl mercury chloride.

### Toxicological Identification of Crystalline Substance

The characteristic symptoms of methyl mercury chloride poisoning in the rat are ataxia and crossing of the hind legs when the rat is hung by the tail with the head down. The crystalline material was dissolved in water with the aid of propylene glycol and administered subcutaneously to rats once every two days (except Sundays) in a dose of 0.5 mg/day. The total amount of the crystalline material administered to the rat was 13 mg by the end of the experi-

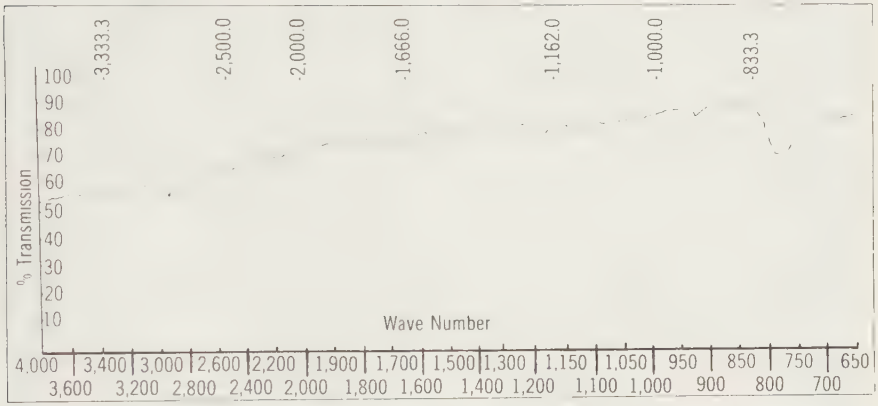
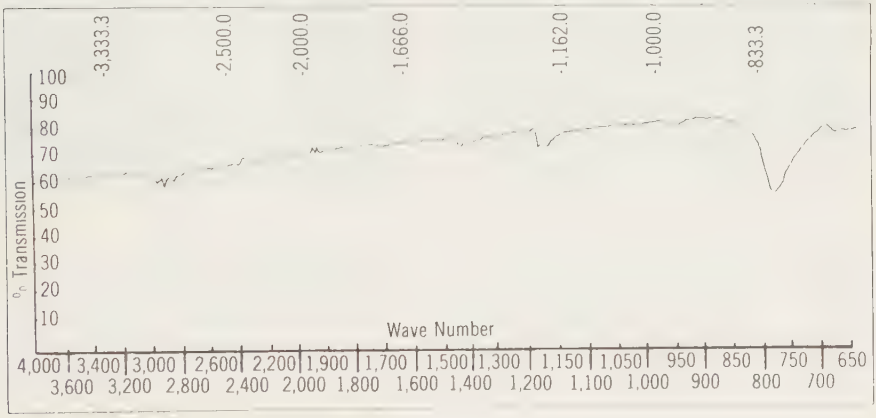



Fig 4.—Absorption of synthesized substance by infrared analysis, using potassium bromide-disk method.

Fig 5.—Absorption of authentic methyl mercury chloride by infrared analysis, using potassium bromide-disk method.



*Total Mercury and Methyl Mercury Chloride Found in Effluents From Two Caustic Soda Factories*

| Sample | Factory A | | Factory B | |
|---|---|---|---|---|
| | Total Hg, ppm | Methyl Mercury Chloride, ppm | Total Hg, ppm | Methyl Mercury Chloride, ppm |
| Electrolysis plant | | | | |
| Small pit (water) | 4.17 | ND* | 4.90 | ND |
| Large pit (water) | 4.13 | ND | 3.50 | ND |
| Large pit (sludge) | 359.90 | 0.014 | 273.60 | 0.311 |
| Effluent water | | | | |
| (Water) | 0.29 | ND | 0.03 | ND |
| (Sludge) | 19.96 | ND | 16.12 | ND |
| Sedimentation pond | | | | |
| (Water) | 0.11 | ND | 0.05 | ND |
| (Sludge) | 25.39 | 0.001 | 20.56 | 0.079 |
| Calcium sludge depot | 28.27 | 0.003 | 254.45 | 0.018 |

* ND, not detected.

Fig 6.—Crossing of hind legs in rat which received authentic methyl mercury chloride.

Fig 7.—Crossing of hind legs in rat which received experimentally synthesized crystal.

ment, ie, 30 days after the first injection.

Figures 6 and 7 show, respectively, the crossing of hind legs in rats which received authentic methyl mercury chloride and the crystal obtained experimentally. The normal leg reflex of a control rat which received only propylene glycol is shown in Fig 8.

The rat was killed after the recognition of ataxia, and the amount of methyl mercury chloride in various organs was determined. The following tabulation shows the amount of methyl mercury chloride, expressed in

Fig 8.—Normal leg reflex of control rat.

parts per million, found in the organs of a rat to which the synthesized material was administered:

| | |
|---|---|
| Brain | 0.78 |
| Liver | 6.58 |
| Kidney | 33.40 |
| Heart | 1.68 |
| Lung | 6.50 |
| Stomach | 1.12 |
| Blood | 18.48 |

## Comment

Several indications of occurrence of methyl mercury in a natural environment have been reported previously.[6]

The discovery that methyl mercury chloride may be produced in an inorganic system similar to that used industrially in caustic soda plants raises problems of major importance. In the industrial situation the reaction has occurred mainly in a sedimentation pit where a precipitate of calcium carbide is added to neutralize water from the electrolysis plant. These observations may indicate the main source of the methyl mercury in the effluent material from caustic soda plants.

This statement does not imply that other possibilities may be neglected. A trace amount of methyl mercury has also been produced experimentally in reaction of inorganic mercury with amorphous carbon—the material of which electrodes in the electrolysis plant are fabricated.

The demonstration of the possible production of methyl mercury in a commonly used inorganic process raises serious questions. These apply to both the amounts of methyl mercury produced in such a fashion and to the potential entry of this material into biological systems. If these processes occur, they may have significant effects on human health, especially if methyl mercury should enter the human food chain or if some biological forms concentrate the substance.

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# Occurrence of Mercury in the Environment and in Living Organisms

# Mercury Pollution of Lake Erie Ecosphere

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The existence of mercury as a widespread pollutant has gained considerable recognition in recent months. The substantial mercury pollution of the Great Lakes became apparent in early 1970 when significantly high levels of mercury were reported in certain fish species of the Great Lakes. Lake Erie became the focus of mercury pollution because of its close proximity to several large mercury consuming industries and its already established notoriety as the most polluted of the Great Lakes. Since the sources of mercury in the ecosphere are not only from industrial discharges, this investigation has attempted to examine a variety of samples collected from the Lake Erie region.

## Materials and Methods

### Sample Collection

The air particulate samples used in this investigation were collected from four different sampling stations in the Buffalo, New York area with sequential air samplers (Gelman Model 24001) using 47 mm diameter filter papers (Millipore EHPO4700 or Dexter X-1215). The filter media employed had a filter efficiency of nearly 100% for particulate matter 0.1 micron or greater (Pillay *et al.*, 1971a).

The coal samples used in this study were from some of the major producing areas of Pennsylvania and Ohio. These samples were obtained by the U. S. Bureau of Mines, Pittsburgh, Pennsylvania, and were homogenized prior to analysis. The samples used in the actual mercury analysis were aliquots of stock samples, although their equivalent dry weights were determined separately by oven drying the samples at 110°C and determining the weight losses.

The various samples of plankton/algae and lake sediments were collected from the sampling points in Lake Erie shown in Fig. 1. All the samples were collected by the research vessel of the Great Lakes Research Laboratory of the State Uni-


FIG. 1. Lake Erie sampling points.

versity College at Buffalo between July and December of 1970. The sediment samples were collected using a Peterson dredge which collects samples from 5 to 30 cm below the mud–water interface and an Ekman dredge which gathers approximately the upper 5 cm of the sediment. The plankton/algae samples were gathered using a fine mesh (14 meshes/cm) tow net that had a 500 $cm^2$ opening. All the fish samples used in this investigation were caught from Lake Erie during the fall of 1970 by both commercial fishermen and by the Bureau of Commercial Fisheries, Sanduski, Ohio. Most of the analyses performed were of the composites of the edible tissues of the fish samples.

The human brain tissues analyzed were collected from the Buffalo area hospitals by the faculty of the School of Medicine, State University of New York at Buffalo. Twenty autopsy specimens were selected at random for this study. The subjects had lived in the Lake Erie region for several years and were not known to have any pathological exposure to mercury or its compounds.

## Analytical Method

The sampling and analysis of mercury in the environment offer some extremely challenging problems. The minute quantity of mercury present in the samples as well as the volatile nature of mercury compounds only add to the problems associated with the complexities of the matrices. A variety of analytical techniques are being employed to detect and determine mercury in both environmental and biological samples. With the growing appreciation of the complexity of analytical problems involved in the determination of trace levels of mercury, investigators are continuously reexamining their analytical procedures and are providing more and more reliable data. The neutron activation analysis procedures used in analyzing samples for this study were described in detail (Pillay *et al.*, 1971b) including preirradiation sample preparations and postirradiation chemical separations. Briefly, the method consists of: (i) encapsulating the samples and exposing them to thermal neutrons in a reactor at a flux level of about $5 \times 10^{12}$ neutrons $cm^{-2}$ $second^{-1}$ for two or more hours; (ii) the radio-

active samples are wet-ashed with nonradioactive mercury carrier using a mixture of nitric, sulfuric, and perchloric acids under good refluxing conditions; (iii) mercury is chemically isolated from the digest and is eventually electrodeposited as elemental mercury on gold foils; (iv) after determining the chemical yield, the radioactivities ($^{197}$Hg and $^{197m}$Hg) are measured using scintillation gamma ray spectrometry to quantitate the mercury values.

## RESULTS

In order to facilitate comparison of results by other investigators, the results of this study are reported as micrograms ($\mu$g) of mercury per gram of raw tissue for all the fish samples and brain tissues. The mercury content of the air particulates are reported as nanograms ($10^{-9}$ g) of mercury per cubic meter of air sampled. Since the moisture contents of lake sediments, plankton/algae, and coal samples tend to vary significantly from sample to sample, their mercury contents are reported as micrograms of mercury per gram of the dry sample. The equivalent dry weights of these samples were independently determined although the analyses were performed on moisture bearing samples. In general, the average loss of weight of plankton/algae samples ranged from 87 to 94% while the loss of weight of sediments was in the range of 15–35%.

### Mercury in Air Particulates

The mercury levels of air particulates collected from four different air sampling stations around Buffalo, New York between November, 1968 and October, 1969 are presented in Table I. These results represent the mercury content of air particulates greater than 0.1 $\mu$ and have been corrected for the trace levels of mercury present in the filter media. The values range from 1 ng/m$^3$ of air sampled to 30 ng/m$^3$. The large variations in mercury levels are due to variations in atmospheric conditions which determine the residence time of particulates in air. Similar data reported for Chicago Metropolitan area (Brar *et al.*, 1969) showed a range of 3–39 ng/m$^3$ of surface air. Another study conducted in the

TABLE I
Mercury Content of Air Particulates from Buffalo, New York Area
(0.1 Micron or Greater)

| Air sampling station | Date of collection | Mercury content ($10^{-9}$ g/m$^3$) |
|---|---|---|
| SUNYAB Campus | November, 1968 | 2.9 |
| SUNYAB Campus | April, 1969 | 27.2 |
| Buffalo Museum of Science | November, 1968 | 5.9 |
| Buffalo Museum of Science | December, 1968 | 15.0 |
| Lackawanna, New York | April, 1969 | 7.0 |
| Dingens Street Station, Buffalo | April, 1969 | 4.3 |
| Dingens Street Station, Buffalo | May, 1969 | 4.6 |
| Dingens Street Station, Buffalo | August, 1969 (1) | 1.3 |
| Dingens Street Station, Buffalo | August, 1969 (2) | 1.1 |
| Dingens Street Station, Buffalo | October, 1969 (1) | 6.1 |
| Dingens Street Station, Buffalo | October, 1969 (2) | 17.0 |

TABLE II

Mercury Content of Coal Samples from Pennsylvania and Ohio

| Bureau of mines no. | Mine, county and state | Mercury content[a] ($\mu$g/g) |
|---|---|---|
| G-58404 | Gem. No. 255, Athens, Ohio | 0.54 |
| G-96943 | Florence, Belmont, Ohio | 0.34 |
| G-90167 | Swisher, Gallia, Ohio | 0.52 |
| G-62668 | Low Ash No. 2, Muskingum, Ohio | 0.55 |
| H-13165 | Aurora No. 5, Clearfield, Pennsylvania | 1.20 |
| G-93727 | Benjamin No. 3, Clearfield, Penssylvania | 0.45 |
| G-94578 | Benjamin No. 3, Clearfield, Pennsylvania | 0.41 |
| G-96138 | Hamilton, Jefferson, Pennsylvania | 0.35 |
| G-42749 | Pilgrim No. 1, Lawrence, Pennsylvania | 1.00 |
| G-21637 | Legal, Schuylkill, Pennsylvania | 0.32 |
| G-30208 | Oakwood, Schuylkill, Pennsylvania | 0.35 |

[a] The values reported are in micrograms of mercury per g of moisture-free coal sample. The moisture levels of powdered coal used here ranged from 7 to 12%.

San Francisco Bay area (Williston, 1968) reported mercury levels of 0.5–50 ng/m$^3$ of air, with higher mercury concentrations observed during the summer months. However, data on atmospheric mercury levels collected on clear days by the U. S. Geological Survey (U. S. G. S., 1970) reported concentrations in air over "nonmineralized areas" varied from 3 to 9 ng/m$^3$ of air. An airborne survey conducted recently by the Committee for Environmental Information (1971) revealed that in Missouri and Illinois coal burning power plants, municipal incinerators and several industrial plants are emitting large quantities of mercury into the environment. The concentrations of mercury in the smoke stack emissions are reported to be in the range of 50–10,000 ng/m$^3$ of the stack plume.

## Mercury in Coal

Mercury in coal has not been monitored systematically or in detail, although the presence of mercury in fossil fuels is now well recognized. Our analyses of the mercury contents of some of the coals from the producing areas of Pennsylvania and Ohio are shown in Table II. The mercury content of these samples ranges from 0.3 to 1.2 $\mu$g/g of moisture-free coal with an average value of about 0.5 $\mu$g/g. A recent survey of 36 American coals (Joensuu, 1971) showed a range of $<$0.1 to 33 $\mu$g of mercury per gram of coal. Very few samples from Montana, Wyoming and West Virginia showed mercury above 10 $\mu$g/g level. Another survey of 53 coals from Illinois (Kennedy *et al.*, 1971) showed a range of 0.04–0.49 $\mu$g of mercury per gram of coal with an average value of about 0.2 $\mu$g/g.

## Mercury in Sediments and Plankton/Algae

Samples of sediments and plankton/algae collected from sixteen sampling points around Lake Erie during the fall of 1970 were analyzed using neutron activation analysis. The results presented in Table III show higher levels of mercury in the sediments and plankton/algae from the Southern Shore of the Western Basin of Lake Erie, the exception being the Black Rock Channel in the

TABLE III

MERCURY CONTENT OF SEDIMENTS AND PLANKTON/ALGAE SAMPLES COLLECTED FROM LAKE ERIE (FALL 1970)

| Station no. | Approximate location | Mercury content in μg/g[a] | |
|---|---|---|---|
| | | Sediments[b] | Plankton/Algae |
| 01 | Buffalo River | 2.0 | 31.2 |
| 02 | Cattaraugus Creek | 1.2 | 25.1 |
| 03 | Barcelona | 0.6 | 2.8 |
| 04 | Ashtabula | 4.6 | 7.4 |
| 05 | Fairport | 1.5 | 12.8 |
| 06 | Cleveland | 12.0 | 33.5 |
| 16 | Toledo | 10.4 | 20.5 |
| 08 | Detroit River | 4.5 | 26.1 |
| 09 | Mid. Bass Island | 1.5 | 20.1 |
| 10 | Port Crewe | 0.5 | 12.4 |
| 11 | Port Stanley | 1.5 | 12.0 |
| 12 | Long Point | 7.0 | 14.7 |
| 13 | Long Point Bay | 1.0 | 23.7 |
| 14 | Port Maitland | 1.8 | 15.4 |
| 15 | Mid-Lake | 1.5 | 9.6 |
| 17 | Black Rock Channel | 12.4 | 27.8 |

[a] In terms of the equivalent dry weight of the sample.

[b] Sediment samples from 3 to 30 cm below the water-sediment interface.

Niagara River. There are generally increased levels of mercury in the plankton/algae from a sampling point when the sediment levels are also high, although this is not consistent. The reason for higher levels of mercury in plankton/algae while sediment levels are low may be due to the increased levels of mercury in the water and the flow pattern of the water in a particular area.

TABLE IV

MERCURY CONTENT OF LAKE ERIE ENVIRONMENTAL SAMPLES COLLECTED AT THE MOUTH OF BUFFALO RIVER

| Date of sample collection | Mercury content in μg/g (in terms of dry weight) | | |
|---|---|---|---|
| | Sediment E[a] | Sediment P[a] | Plankton/Algae |
| 7-28-70 (N) | 2.8 | 2.6 | 81.0 |
| 7-28-70 (S) | 5.0 | 3.6 | 45.9 |
| 9-8-70 (N) | — | 2.3 | 51.5 |
| 9-8-70 (S) | — | 2.0 | 31.2 |
| 10-5-70 (N) | — | 2.8 | — |
| 10-5-70 (S) | — | 6.2 | — |
| 1-15-71 (N) | 3.7 | 6.8 | 74.3 |
| 1-15-71 (S) | 5.6 | 5.8 | 63.6 |
| 7-28-71 (N) | 5.6 | 3.0 | 1.2 |
| 7-28-71 (S) | 1.4 | 1.8 | 6.6 |

[a] Sediment E gathered by an Eckman dredge from upper 5 cm of sediment, while Sediment P gathered by a Peterson dredge from 3 to 30 cm below the mud-water interface.

One location in Lake Erie was sampled over a long period and the findings of the mercury levels in sediments and plankton/algae are reported in Table IV. The variation in the sediment levels are not very significant from July, 1970 to July, 1971 although there is a considerable difference in the level of mercury in the plankton/algae. This may be due to the decreased levels of mercury in water due to the curtailment of major industrial discharges, or it could be due to the effect of freezing the surface of the lake, thereby causing changes in mercury levels in water. A third possibility is that a change in the types or distribution of the types of plankton/algae could cause such changes.

Sediment mercury levels of less than 1 up to 560 $\mu$g/g have been reported in several regions of industrial discharges into the Great Lakes and associated waterways (Chem. Eng. News, 1970). However, the mercury concentrations of bottom sediments of Palos Verdes Peninsula and from La Jolla, California (Klein *et al.*, 1970) showed mercury levels of $<0.1$ up to 1 $\mu$g/g of dry sediment. Another recent survey (Kennedy *et al.*, 1971) of the mercury levels of lake sediments from 31 sampling points in Lake Michigan showed mercury concentrations in the range of 0.03–0.38 $\mu$g/g of sediment. This survey of Lake Erie sediments showed mercury levels of 0.5–12.4 $\mu$g/g of dry sediment.

## Mercury in Fish

Fish composites prepared from the edible tissues of eleven different species from each of the three basins of Lake Erie were analyzed to determine their mercury levels. The results reported in Table V indicate (i) there are generally increased levels of mercury in the fish from the Western Basin of Lake Erie, and the mercury levels are found to decrease going from the Western Basin to

TABLE V
Mercury Content of Edible Tissues of Lake Erie Fish
(1970 Fall Catch)

| Species | Mercury content of the composites of edible tissues (in $\mu$g of Hg per gram of raw tissue) | | |
|---|---|---|---|
| | Western Basin | Central Basin | Eastern Basin |
| Walleye | 0.79 (25)[a] | 0.65 (25) | 0.33 (25) |
| Yellow Perch | 0.61 (25) | 0.49 (25) | 0.29 (25) |
| White Bass | 0.60 (25) | 0.72 (25) | 0.43 (25) |
| Channel Catfish | 0.36 (25) | 0.42 (20) | — |
| Freshwater Drum | 0.67 (25) | 0.62 (20) | 0.30 (25) |
| Carp | 0.23 (25) | 0.35 (17) | 0.36 (14) |
| Coho Salmon | 0.69 (20) | 0.58 (10) | 0.51 (13) |
| White Sucker | 0.55 (24) | 0.56 (8) | 0.35 (25) |
| Gizzard Shad | 0.20 (25) | 0.21 (15) | 0.26 (18) |
| Smallmouth Bass | — | 0.55 (14) | — |
| Smelt[b] | — | — | 0.30 (10) |

[a] The numbers in the parentheses refer to the number of fish samples of a particular species used in preparing the composite.

[b] Mercury content of the whole fish.

TABLE VI

MERCURY CONTENT OF WALLEYES FROM THE CENTRAL BASIN OF LAKE ERIE (1970 FALL CATCH)

| Sample identification | Mercury content in μg/g of tissue[a] | | |
|---|---|---|---|
| | Edibles | Nonedibles | Whole fish |
| A. Young of the year | | | |
| 318 | 0.60 | 0.46 | 0.55 |
| 319 | 0.64 | 0.59 | 0.62 |
| 320 | 0.62 | 0.49 | 0.58 |
| 321 | 0.44 | 0.33 | 0.41 |
| B. Yearlings | | | |
| 327 | 0.75 | 0.24 | 0.58 |
| 328 | 0.92 | 0.30 | 0.69 |
| 329 | 0.93 | 0.46 | 0.76 |
| 330 | 0.75 | 0.31 | 0.59 |
| C. Two years and over | | | |
| 333 | 1.03 | 0.51 | 0.85 |
| 334 | 0.67 | 0.37 | 0.56 |
| 335 | 0.78 | 0.28 | 0.57 |
| 336 | 0.98 | 0.35 | 0.67 |
| Average of 12 specimens | 0.82 | 0.54 | 0.64 |

[a] All the results expressed are in terms of the weight of raw tissues.

the Eastern Basin; (ii) the bottom feeders like gizzard shad and carp do not have higher mercury levels when compared with other species; and (iii) the predators usually have higher levels of mercury than the nonpredators.

The results of mercury analysis of edible and nonedible tissues of a number of walleyes from the Central Basin of Lake Erie are presented in Table VI. The term "edible tissue" here refers to the portions of the fish remaining after removing the head, tail, fins, and all the internal organs. The composite of the tissues removed to obtain the edible tissues are referred to as "nonedibles." These results indicate that the edible tissues of walleyes contain more mercury per unit weight of the tissue when compared with nonedible tissues. There is also an observable increase in the mercury contents of walleyes going from the young of the year to the two years and over group. These results are compared with the selenium and arsenic levels of these fish in Table VII. The analyses of selenium and arsenic were also performed by neutron activation analysis involving chemical isolations. The methods employed have been proved to give excellent precision and accuracy. In comparing the mercury, arsenic, and selenium levels of walleyes, it is apparent that there is a definite tendency for mercury to accumulate with age in the edible tissues of fish, whereas no such tendency is apparent in the arsenic and selenium levels.

During the past twenty months there have been numerous reports of the mercury contents of fish from all the Great Lakes as well as from all the sources of fish coming to the U. S. markets. Mercury has been detected in fish from almost all the regions of the world and its widespread existence is now recog-

TABLE VII

Mercury, Arsenic, and Selenium Contents of the Edible Tissues of Walleyes from the Central Basin of Lake Erie (1970 Fall Catch)

| Sample identification | Mercury ($\mu$g/g) | Arsenic ($\mu$g/g) | Selenium ($\mu$g/g) |
|---|---|---|---|
| Young of the year (10) | 0.39 | 0.10 | 0.34 |
| Yearlings (9) | 0.59 | 0.12 | 0.33 |
| Two years and over (6) | 0.75 | 0.12 | 0.34 |

The numbers in the parentheses refer to the number of fish samples of a particular age group used in preparing the composites.

nized. A large fish caught recently from the Arctic region (an Arctic Charr weighing 5.7 kg and measuring 75 cm with an estimated age of 25 years) was analyzed at our laboratory. The muscle tissues of this fish contained 0.11 $\mu$g of mercury per gram of raw tissue. Although our analysis of the Lake Erie fish does not include any fish of comparable age and size, the differences in the mercury accumulated in the tissues are obvious.

### Mercury in Human Brain Tissues

Nearly two hundred samples of brain tissues collected from twenty randomly selected autopsy specimens were analyzed for their mercury content. Some of these results including their biological significance have been reported earlier (Glomski *et al.*, 1971). The subjects studied in this survey were mostly of the age group 60 years or older and had lived at least part of their life time in the Lake Erie region where there seem to be elevated levels of mercury in the environment. None of the subjects studied were known to have had any industrial or accidental exposure to mercury or its compounds. The mercury levels in the brain tissues ranged from a low of 0.02 $\mu$g/g of raw tissue to a high of 2.27 $\mu$g/g of tissue. Since the analyses were performed using different regions of the brains, these numbers represent the minimum and maximum levels of accumulation among the subjects studied. The average concentration of 193 samples was 0.29 $\mu$g/g of raw tissue. An earlier report (Joselow *et al.*, 1967) on the mercury levels of autopsy specimens of 27 brains range from $<0.05$ to 0.6 $\mu$g/g of tissue, with an average mercury level of 0.1 $\mu$g/g of tissue.

## DISCUSSION

Although mercury pollution of the environment has been known for over fifteen years, in many respects the state of knowledge of how mercury acts as an environmental agent is still in its infancy. The hazards associated with the environmental pollution of mercury have been objectively discussed in two recent reviews (Nelson *et al.*, 1971, and Wallace *et al.*, 1971). Many estimates of the mercury entering the environment have been made, although none of them seem to account for all the mercury presently distributed in the biosphere.

It is estimated (Kolbye, 1970) that in the United States nearly 163 million pounds of mercury have been consumed since the beginning of this century, of which nearly 100 million pounds were consumed during the past two decades.

In addition mercury lost to the environment comes from fossil fuel burning; mining, smelting, and refining operations and a variety of natural redistribution processes. A coal burning plant consuming about 5,000 tons of coal a day with an average concentration of about 0.5 $\mu$g of mercury per gram emits about 4.5 pounds of mercury per day into the air, assuming a 90% volatilization. An annual coal consumption of about 500 million tons can release about 0.45 million pounds annually to the environment. A recent estimate of the total mercury release to the U. S. environment through all forms of fossil fuel burning (Cooke *et al.*, 1971) was about 0.7 million pounds for 1968, while the National Materials Advisory Board estimated a total mercury consumption of 5.6 million pounds by industry and agriculture for the same period. From the details of the specific applications of mercury reported (NMAB, 1969) more than 75% of this 5.6 million pounds is eventually released to the environment. Another estimate made (Lutz *et al.*, 1967) reported that in 1965 the mining, smelting, and refining operation for mercury in the U. S. released about 0.17 million pounds of mercury to the environment, assuming an average 10% loss.

Of the various major sources of environmental mercury, the releases due to mining and metallurgical operations for mercury are nonexistent in the Lake Erie Basin. The releases of mercury from fossil fuel consumption should be similar to other major cities in the U. S. as indicated by the air particulate levels. While the mercury levels in air particulate from Buffalo area are comparable with those of Chicago area, it may be considered lower than that of the San Francisco Bay area, the reason probably being that San Francisco is in the mercuriferous belt and is likely to contain more airborne mercury due to natural distribution processes. Again the mercury levels in the coal mined in the Lake Erie region do not show any unusually high levels of mercury, indicating that the mercury transferred from the pedosphere to hydrosphere may be minimal. While it is possible that the mercury washed from the atmosphere by precipitation can increase the levels of mercury in the hydrosphere, the mercury settling on the soils is readily chelated with humic materials and is not quickly released to the hydrosphere (Cooke *et al.*, 1971). It is, however, recognized (Wood *et al.*, 1968) that bacterial action on the humus slowly releases mercury as methylmercury to the hydrosphere and ultimately to the biosphere through natural processes. Therefore the elevated levels of mercury in the fauna and flora of Lake Erie cannot be accounted for by sources other than intentional usages of mercury in this region.

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# Residues of Total Mercury and Methylmercuric Salts in Lake Trout as a Function of Age

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There have been numerous reports of relatively high concentrations of mercury in fish (*1*). Although many analyses of fish for mercury have been carried out, it is usually difficult to relate concentrations to time of exposure since judging age by scale examination is very difficult, particularly in older fish. In a study of northern pike (*Esox lucius*) Johnels and Westermark (*2*) found the total mercury concentration proportional to the age of the fish but admitted the unreliability of judging their ages by scale examination. In the work reported here residues of mercury have been determined in lake trout of known age.

We were fortunate to have available lake trout (*Salvelinus namaycush*) from Cayuga Lake in Ithaca, New York, of known age since they are tagged and stocked there annually as fingerlings. It was not known what concentrations of mercury might be expected in the fish. Mercury reaching the lake could, however, result from its use in laboratory research, in dental and medical services, in agriculture, in coal burnt in power plants, and from other sources. In October 1970, fish were netted in order to obtain samples of as many different ages as possible. Without evisceration, each was mechanically chopped, ground, and thoroughly mixed. A 1-g

Table 1. Corrected* concentrations of total mercury and mercury as methylmercury in Cayuga Lake trout.

| Fish code | Age (years) | Mercury (total) (ppm) | Methylmercury (calculated in terms of mercury) (ppm) | Percent of total mercury as methylmercury |
|---|---|---|---|---|
| 95 | 1 | 0.24 | 0.074 | 30.8 |
| 99 | 1 | .28 | .098 | 35.0 |
| 101 | 1 | .19 | .066 | 34.7 |
| 59 | 2 | .25 | .108 | 43.2 |
| 78 | 2 | .26 | .096 | 36.9 |
| 89 | 2 | .31 | .121 | 39.0 |
| 80 | 3 | .38 | .208 | 54.7 |
| 82 | 3 | .45 | .271 | 60.2 |
| 112 | 3 | .28 | .157 | 56.1 |
| 104 | 4 | .44 | .375 | 85.2 |
| 105 | 4 | .41 | .288 | 70.2 |
| 151 | 4 | .44 | .346 | 78.6 |
| 155 | 5 | .43 | .349 | 81.2 |
| 10 | 6 | .46 | .412 | 89.6 |
| 11 | 6 | .55 | .479 | 87.1 |
| 13 | 6 | .50 | .445 | 89.0 |
| 2 | 7 | .40 | .283 | 70.8 |
| 4 | 7 | .46 | .403 | 87.6 |
| 5 | 7 | .44 | .349 | 79.3 |
| 1 | 8 | .60 | .534 | 89.0 |
| 6 | 8 | .59 | .519 | 88.0 |
| 8 | 8 | .47 | .479 | 101.9 |
| 19 | 9 | .53 | .433 | 81.7 |
| 3 | 11 | .58 | .407 | 70.2 |
| 15 | 12 | .62 | .415 | 66.9 |
| 16 | 12 | .65 | .503 | 78.2 |
| 22 | 12 | .44 | .389 | 88.4 |

* Corrected for percent recovery (see text).

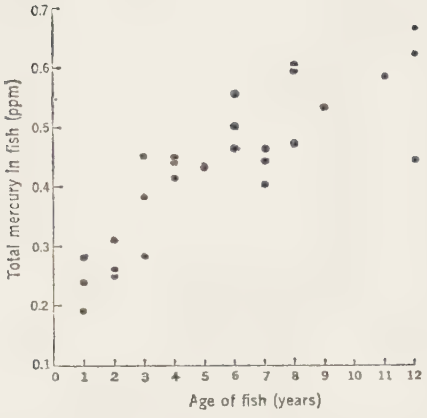


Fig. 1. Total mercury in Cayuga Lake trout as a function of age.

subsample was dried and ashed by Schoniger combustion (*3*). The total amount of mercury in the absorbing solution was determined by flameless atomic absorption spectrophotometry (*4*). This method is easily sensitive to 0.1 part per million (ppm) of mercury in fish. The accuracy of the method was checked by recovery studies in which mercury as mercuric chloride was added to the fish samples before drying, and the samples were then dried, combusted, and analyzed. The percent recoveries of 0.3 ppm of mercury added to four samples of lake trout of ages 1, 2, 3, and 5 years were, respectively, 77, 80, 93, and 83.

Figure 1 illustrates the relation between total mercury in lake trout and age. All mercury concentrations in Fig. 1 were corrected for the average percent recovery (83.25). The length of the fish varied from about 20 cm (for a 1-year-old fish) to about 76 cm (for a 12-year-old fish).

Residues of mercury in fish are often present largely as highly toxic methylmercuric salts (*5*). It was of interest therefore to determine the effect of age on the concentrations of this metabolite in the same fish samples. Westöö has reported a method for the extraction and isolation of methylmercury compounds from fish (*6*). In this method the sample is extracted with hydrochloric acid, the methylmercuric compounds are partitioned into benzene, the bonds linking mercury to sulfur are cleaved with mercuric chloride, the methylmercury is extracted as the hydroxide, and finally the mercury is reconverted to the chloride for gas chromatographic analysis. A microwave-powered helium plasma emission detector (*7*) was used to selectively monitor the emission line of atomic mercury at 2537 Å. The method is sensitive to 0.1 ppm of methylmercuric salts in fish. The percent recovery of 0.174 ppm of methylmercuric chloride added to one 2-year-old and three 3-year-old lake trout samples was, respectively, 56.3, 54.6, 56.3, and 54.6. Westöö found that there is approximately a 30 percent loss of methylmercuric salts in his procedure as a result of unfavorable partition coefficients.

Table 1 presents a list of concentrations of total mercury and methylmercury in fish by age and the percentage of total mercury that was present as methylmercury. The values for total mercury and methylmercury as listed are corrected for the average percent recoveries which were, respectively, 83.25 and 55.4. It is evident that the variation trend in the concentrations of methylmercuric salts with age in fish is, in general, the same as the variation trend of the total mercury with age, although the concentration of total mercury is consistently higher. This relation between total mercury (and methylmercuric salt con-

centration) and age may simply be a reflection of the time during which the fish have been exposed to their environment. It may also be significant that the total proportion of mercury as methylmercury appears to be smaller in the younger fish. Owing to the good reproducibility of analysis for both mercury and methylmercuric salts on several replicated fish samples, the higher total mercury concentrations may be significant and indicative of the presence of mercury in fish in a form or forms other than methylmercuric salts. Another possible metabolite is dimethylmercury, but there is at present no satisfactory method for the determination of this compound in fish. Concentrations of total mercury and methylmercury in the lake trout studied did not appear to be related to the sex of the fish.

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# Fossil Fuels as a Source of Mercury Pollution

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Mercury is toxic to humans and animals and is therefore a very dangerous pollutant to our environment. Its presence in the tissues of wildlife has been studied in Sweden and found excessively high. The eggs of many wild birds do not hatch and numerous animals die because of mercury poisoning (*1*). Especially high mercury concentrations have been found in fish; older fish of larger size have relatively higher concentrations, which indicates that mercury accumulates in tissues (*2*, *3*). This buildup of mercury in the biomass is a recent phenomenon (*2*).

One suspected source of pollution has been mercury-containing fungicides used in treatment of grain seeds. The treated seeds are highly poisonous to grain-eating birds and are available to them abundantly during the seeding seasons. However, the amounts used are much too small to explain high mercury contents in wildlife except in the grain-eating birds (2 to 3 mg per hectare of mercury every 4 to 5 years; 0.5 mg per metric ton of topsoil per year (*4*). A large part of the mercury found in the environment is apparently derived from industrially produced mercury, approximately 10,000 tons per year (*5*), most of which is eventually discarded in waste streams. However, another possible source of mercury could be fossil fuels and ores (other than mercury ores proper). Although the concentration of mercury in fuels is small (*6*), fuels are consumed at an enormous rate; consequently, they must be considered as a possibly significant source of mercury release into the environment.

The amount of mercury in coal is not well known. To obtain a preliminary value, 36 American coals were analyzed (Table 1) by means of a mercury vapor detector (*7*) that had been modified to eliminate organic vapors, which interfere in the detection process.

In this procedure, the sample is burned in oxygen and the fumes are passed through hot (650° to 700°C) silver wire coils to complete the oxidation, after which a heated (150°C) gold amalgamator traps mercury while most of the other fumes pass through. To remove the remaining organics, mercury is released by heating and is re-amalgamated in a second trap; the mercury is again released by heating and is measured in the mercury vapor detector. Some organic fumes that pass through the first amalgamator are not completely oxidized and could cause some loss of mercury; also some mercury could be lost as oxide. Consequently, the values in Table 1 are biased on the low side.

Table 1. Mercury content of 36 American coals.

| Locality (counties) | Hg ($\times 10^{-9}$ g/g) |
|---|---|
| Letcher (Ky.) | 250 |
| Knox (Ill.) | 230 |
| Saline (Ill.) | 240 |
| Northumberland (Pa.) | 595 |
| Northumberland (Pa.) | 245 |
| Northumberland (Pa.) | 120 |
| Richland (Mont.) | 33,000 |
| Pierce (Wash.) | 510 |
| Campbell (Wyo.) | 18,600 |
| Washington (Pa.) | 240 |
| Franklin (Pa.) | 1,200 |
| Franklin (Pa.) | 10,500 |
| Franklin (Pa.) | 6,400 |
| Franklin (Pa.) | 4,900 |
| Somerset (Pa.) | 540 |
| Somerset (Pa.) | 340 |
| Cambria (Pa.) | 1,460 |
| Nicholas (W. Va.) | 6,540 |
| Clay (W. Va.) | 22,800 |
| Clay (W. Va.) | 3,700 |
| Cambria (Pa.) | 1,450 |
| Cambria (Pa.) | 460 |
| Cambria (Pa.) | 90 |
| Cambria (Pa.) | 630 |
| Cambria (Pa.) | 160 |
| McDowell (W. Va.) | 80 |
| McDowell (W. Va.) | 70 |
| Jefferson (Ala.) | 80 |
| Jefferson (Ala.) | 920 |
| Harrison (Tex.) | 380 |
| LeFlore (Okla.) | 110 |
| Pitkin (Colo.) | 220 |
| Parke (Ind.) | 310 |
| Moultrie (Ill.) | 210 |
| Knox (Ill.) | 90 |
| Grundy (Ill.) | 190 |

The analyses were performed on a relatively small number of samples that are not representative; even within one deposit, the mercury distribution is probably not uniform. The mercury content of the Illinois coal samples that were analyzed (average, $194 \times 10^{-9}$ g/g) is in good agreement with analyses done at the Geological Survey of Illinois (average, $180 \times 10^{-9}$ g/g) (*8*). The average of the results (3.3 parts per million) can be hardly applied to the total coal production. If we apply a more conservative estimate of 1 part per million to the yearly world production of coal [$3 \times 10^9$ tons per year (*5*)], we may conclude that 3000 tons of mercury per year are released to the environment by the burning of coal.

The mercury content of oils is not known and remains to be evaluated. The mercury content of ores (excluding mercury ores proper) is most likely higher than in coals. The tonnage of ores mined annually is, however, much smaller than that of coal.

The upper limit for the natural release of mercury due to chemical weathering can be estimated by comparison with corresponding figures for sodium. The sodium leached by weathering is almost completely carried to the sea by rivers, $8 \times 10^7$ tons per year (runoff of rivers, $3.2 \times 10^{13}$ tons per year; noncyclic sodium in river waters, 2.5 parts per million) (*9*). In the weathered rock masses, the ratio of mercury to

sodium can be assumed to be the same as the ratio of their lithospheric abundances ($2.8 \times 10^{-6}$) (*6*, *10*), which yields an upper limit of 230 tons per year for leached mercury. The amount of mercury actually released is probably less than this estimate because proportionally more mercury than sodium is absorbed on clays, hydroxides, organics, and so forth.

The release of mercury into the environment during the combustion of coal is much larger than the amount released by weathering. Detailed studies of the distribution of mercury near power plants and other major users of coal would be useful to determine the level of mercury pollution in the vicinity of such installations.


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# Human Exposure to Mercury and Occurrence of Mercuric Compounds in Man

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# Distribution and Concentration of Mercury in Autopsy Specimens of Human Brain

The dangers of mercury, a pollutant of natural bodies of water and a metal with a strong tendency to accumulate in the nervous tissues, have recently caused considerable concern. We have studied the distribution and concentration of mercury in the brain in order to determine the levels normally present.

Eight autopsy specimens were selected at random for the study. The subjects (Table 1) had lived, at least briefly, in an area where mercurial contamination of the regional inland waters and their fish has been demonstrated, but there was otherwise no known pathological exposure to the metal. The area includes Buffalo, New York, and the adjacent Great Lakes. Six brains were taken from the subjects 6–30 h after death; Nos. 1 and 2 were fixed in formalin before dissection. The tissues were analysed for mercury by neutron activation analysis (presented by S. K. K. P., C. C. Thomas, jun., and J. A. Sondel at meeting of Amer. Chem. Soc., 1971). After neutron irradiation the tissues were wet ashed in a nitric, sulphuric and perchloric acid mixture with mercury carrier in reflux conditions. A preliminary sulphide precipitation was followed by isolation of the mercury by electrodeposition. The radioactivities from $^{197}Hg$ and $^{197m}Hg$ were measured by gamma-ray spectrometry to determine the levels of the metal in the samples. This method readily allowed the quantitation of mercury in the range 0.01 to 2.0 μg/g of wet tissue with a sample size of 1–3 g. The accuracy was better than 15% at mercury levels of 0.01 p.p.m. and exceeded 5% at concentrations of 2 p.p.m.

The relatively small number of patients precluded the definition of absolute normal values but some valid observations can be made. As observed in Table 1, patient No. 6 is remarkable in that he had very large amounts of mercury in all regions of the brain except in the white matter. This patient had been considered a chronic alcoholic with chronic brain syndrome. He had acquired syphilis in 1929 but it could not be determined whether he had been treated with mercurial preparations.

Generally, the highest concentrations of mercury were found in the cerebellum. This led us to consider whether this structure normally contains relatively high levels of mercury or whether this element is preferentially accumulated at this site. The former assumption would appear tenable, at least to some degree, for in those subjects whose cerebellar values were not

**Table 1** Content of Mercury in Various Regions of the Human Brain

| Subject No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Age and sex (yr, M/F) | 70M | 58M | 73M | 68F | 79M | 74M | 33M | Full term stillborn M |
| Area of brain | | | | | | | | |
| Frontal lobe cortex | —— | 0.25 | 0.12 | 0.03 | 0.11 | 1.69 | 0.06 | 0.04 |
| Cerebellar cortex | 0.71 | 0.72 | 0.67 | 0.08 | 0.48 | 1.85 | 0.10 | 0.04 |
| Medulla | —— | —— | 0.12 | 0.04 | 0.18 | 1.29 | 0.19 | —— |
| Pons | 0.43 | 0.30 | 0.19 | 0.05 | 0.25 | 1.66 | 0.05 | —— |
| Mid brain | —— | 0.24 | 0.11 | 0.04 | 0.08 | 1.20 | 0.06 | —— |
| Corpus striatum | —— | 0.11 | 0.09 | 0.03 | 0.06 | 0.61 | 0.06 | —— |
| Thalamus | —— | 0.16 | 0.07 | 0.04 | 0.11 | 1.47 | 0.09 | 0.04 |
| White matter | 0.15 | 0.13 | 0.03 | 0.03 | 0.04 | 0.15 | 0.02 | 0.05 |
| Angular and supramarginal Gyri | —— | 0.23 | 0.07 | 0.03 | 0.15 | 2.00 | 0.04 | 0.04 |

Values are expressed as p.p.m. of wet weight tissue.

maximal, either all the regions of the brain including the cerebellum had very high levels (No. 6) or the cerebellar concentration tended to be relatively prominent (No. 7). Further support for this thesis can be inferred from the data of subject No. 4. The brain of this patient consistently contained the lowest or nearly the lowest concentrations of mercury in all areas studied in comparison with other subjects. The concentration in the cerebellum nevertheless exceeded the other areas in this brain in all instances but one by a factor of at least two. Conversely, the high incidence of clinically demonstrable cerebellar malfunction and gross cellular destruction in this region in patients with documented mercurial poisoning supports the concept of localized preferential accumulation[1,4]. The significance of the mercurial concentration in the foetal cerebellum (0.04 p.p.m.) could not be readily evaluated because of incomplete sampling due to post-mortem autolysis.

The pons is also of interest since in patients Nos. 2, 3, 4 and 5 the pontine concentration of mercury was exceeded only by that of the cerebellar cortex.

Except for the one brain that had a larger concentration of mercury (No. 6), the sampled areas of the formalin-fixed brains (Nos. 1 and 2) contained a greater amount than the other specimens. An analysis of the fixative was not performed.

The white matter of the hemispheres consistently had the lowest or near lowest levels in all individuals. This low profile suggests a correlation between anatomical structure and accumulation of the metal. The white matter is primarily composed of nerve fibres and is devoid of the cell bodies of nerve cells. Thus, this metal seems to be predisposed to localization in the perykarya of nerve cells rather than in dendritic or axonic processes. The ability of mercury to enter enzymatic reactions as well as its presence in microsomal and mitochondrial fractions of mercury-loaded nervous tissue[5] are consistent with this concept.

All regions of the brain examined revealed the presence of mercury. Since the observed levels are within the limits of accuracy of the method used, mercury may well be present as a normal constituent of neuronal tissue. Further studies are necessary to confirm this possibility.

We thank Dr Walter Olszewski, Buffalo General Hospital, for supplying the human brains.

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## Environmental Negligence: The Mercury Problem

EARLY in 1970, analyses of fish caught in Lake St. Clair revealed levels of mercury in excess of the Food and Drug Administration's guidelines of 0.5 ppm. Confirmation and extension of these surprising findings came quickly, and triggered a flurry of meetings, studies, reports, and government hearings that culminated in the unprecedented recommendation by the FDA that consumers no longer eat swordfish because of their consistently high mercury content.

Much of the panic reaction has subsided, but some nagging questions remain. Why did regulatory agencies bearing responsibility for the safety of our food and environment not detect the threat of mercury? Why was mercury, a well-recognized toxic substance, allowed to be discharged, apparently *ad libitum*, into the environment? In short, why did our environmental protection system fail?

### MINAMATA—Tragedy Forgotten

The question becomes all the more difficult to answer satisfactorily when the extensive documentation on mercury as an environmental hazard is examined. The tragedy of Minamata Bay, Japan, involving some 120 individuals from 1953 on, clearly implicated mercury, more specifically the consumption of mercury-contaminated fish and shellfish. A similar outbreak with the same etiology occurred in Niigata during 1964–1965. Both of these episodes, exhaustively studied and described in the technical literature, should certainly have generated respect for the dangers posed by the release of mercury to the environment. Yet their significance apparently was not comprehended widely. Even the finding of inordinately high levels of mercury in shellfish (1–2 ppm) from United States waters, reported in 1960 by Dr. Kurland in his classic report on Minamata Disease, made little impact.

### FAO/WHO—Verbiage Lost

To their credit, the international agencies concerned with food and health; i.e., The Food and Agricultural Organization and the World Health Organization, over the course of many years and many joint sessions, have regularly

addressed themselves to the problems of mercury in the environment. Though the verbiage tends to be overwhelming and often too highly qualified, a clear recognition of the hazard does emerge. Caution and conservatism are the dominant themes. In the 1963 report of the Joint Meeting of FAO/WHO Expert Committees on Pesticide Residues, for example, an estimated acceptable daily intake (i.e., the amount that may be ingested daily over a lifetime without ill effects) for certain mercury compounds—less toxic than the mercury compound that appears in fish—was estimated at 0.00005 mg/kg body weight, a value about ten times lower than the levels used by the FDA in arriving at their current guideline. Though there may be some argument that the FAO/WHO figures represent an overcautious attitude —they were based, incidentally, on toxicity data developed by the FDA—there is little doubt that this estimate of an acceptable daily intake, tantamount to zero, reflected a deep concern for the hazards of mercury. Subsequent FAO/WHO Joint Meetings, in 1965 and 1966, reaffirmed this position. At all of these meetings there were, of course, U.S. representatives.

## SWEDEN—Warnings Unheeded

Environmental contamination by mercury became a severe public health problem in Scandinavia in the 1950s, largely because of the dissemination of mercury to the environment as a fungicide for seed treatment, a slimicide in the pulp and paper industry, and its wasteful discharge from some chlor-alkali plants. The consequences of such applications —drastically diminished bird populations and contaminated foodstuffs— were among the early warning signs of distress. With characteristic vigor, the problem was attacked, after the fact, unfortunately. During the course of their investigations, many eminent Swedish scientists realized that the mercury problem was not limited to Scandinavia and was likely to be found in many industrialized countries, specifically pointing to the United States. This thought was voiced at a special Symposium on the Mercury Problem, held in Stockholm in 1966, at which there were representatives from the United States. In the literature, too, such statements as the following, made by Dr. Berglund of the Swedish National Institute of Public Health, appeared: "I feel personally that the problem exists [in the United States] as it does in other parts of the world, but it is not recognized."

## BATTELLE—Reports Ignored

Perhaps the most telling of the many early admonitions was contained in reports prepared in 1967 for the U. S. Public Health Service by Battelle Memorial Institute, on the evaluation of the environmental hazards of chemicals, including mercury. A considerable amount of mercury was being cycled through the environment, the study reported, although it could not determine where it was going. Recommendations were made for continuing surveillance of all components of the environment to evaluate this special hazard, but these too were apparently ignored.

## Comprehensive Environmental Vigilance Needed

All of the foregoing establish that somewhere in the hierarchy of government science, there was sufficient knowledge of the mercury threat to warrant taking apprehensive action. Why then was such action initiated only, as has happened too often in the past, as a panic response to public alarm?

A full answer would probably require yet another set of Congressional hearings, but some speculations may be made. Inadequate resources, incompetence, misjudged priorities, a general

apathy toward the environment can all be cited. But to a large extent, the former compartmentalized bureaucracy of the agencies charged with watching over the environment must be held accountable. At the time mercury concentrations were building up in the environment these agencies were either nonexistent or were predominantly media-oriented; i.e., concerned specifically with air, water, food, etc. The failure to act in the case of mercury, while knowledge of its potential threat was available in the files of several separate government agencies, may well have been the consequence of the fragmentation of interests and responsibilities in our environmental protection system.

The creation of the Environmental Protection Agency, with centralized authority over several media, was a major step in achieving coordinated control. But today there is still no system with prime responsibility for focusing directly on a toxic substance as it courses through all environmental media, or determining human exposure from all parts of the environment, or evaluating the interactions of toxic substances both within and outside the body. Without such comprehensive approaches, there still exists the possibility of eruption of another environmental scare, with the government again assuming an unseemly defensive posture.

## Alkylmercury Contamination Of Foods

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*To the Editor.*—My attention has recently been drawn to an EDITORIAL entitled "Methylmercury Contamination of Foods" by Eyl (215: 287, 1971), in which the writer refers to the "World Health Organization recommended limit of 0.5 ppm" for mercury in foods.

At no time has WHO recommended a limit for mercury in foods. At the 1967 Joint FAO/WHO Meeting on Pesticide Residues the Meeting *suggested* (but not recommended) practical residue limits of 0.02 to 0.05 ppm of mercury in foods (cereals, fruits, and vegetables) according to local conditions. These levels were suggested as levels which might arise following the use of organomercurial fungicides *in agriculture.*

More recently, the 1970 Joint FAO/WHO Expert Committee on Food Additives made the following statement concerning mercury *as a contaminant:*

> There are no data on which to assign an ADI to mercury: this is urgently required as a guide to levels above which food should be discarded.

# REMOVAL OF MERCURY FROM MEDICAL FACILITIES

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Recently, a great deal of concern has been focused on mercury and its role as an environmental pollutant. An equally disconcerting fact is that many medical facilities are making a direct contribution to the rapid and detrimental redistribution of mercury in the environment. Of interest is the substantial amount of mercuric chloride used by medical facilities in their tissue-preparation solutions. Mercuric chloride use often exceeds 23 kg (50 pounds) a year in large facilities. The mercury compound is particularly detrimental from an environmental point of view, since it is highly soluble in water and is commonly disposed of by pouring down the drain and into the ecosystem. A relatively simple and inexpensive way has been devised to eliminate the mercury from tissue-preparation solutions, such as Zenker's, before disposal. The removed mercury is in a form that can be safely handled and recycled.

The method of mercury removal is based upon the precipitation of the soluble mercuric chloride ($HgCl_2$) as insoluble mercuric sulfide (HgS) by the use of sodium sulfide ($Na_2S$). The procedure is as follows: collect all waste liquids containing mercuric chloride until several liters are available; next, adjust the pH of the waste solution with sodium hydroxide until the waste solution is basic (the pH can be easily checked with litmus paper). It is important that the waste liquid is basic or at least neutral pH. If an acidic condition exists the addition of sodium sulfide will liberate toxic hydrogen sulfide gas. A neutral or basic pH will prevent hydrogen sulfide formation. Next, add 3.5 g of sodium sulfide per gram of mercuric chloride used in the tissue solution. The result should be a dark-black precipitate of mercuric sulfide (HgS). Mix well and let stand several hours. The solution can then be filtered, and the filtrate can be poured down the sink free of mercury. The filtrate can be occasionally checked by the addition of a small amount of $Na_2S$. If no additional precipitate forms, all the mercury has been removed. The filtered mercuric sulfide can be placed in a closed container and collected until a sufficient amount is collected for transfer to a company that processes mercury.

This procedure is a simple, inexpensive and effective way to keep a medical facility from contributing mercuric chloride to the mercury-pollution problem.

# Tempest in a teapot

*Thomas B. Eyl, M.D.*

"Dorian, ridiculing the description of a tempest in the *Nautilus* of Timotheus, said that he had seen a more formidable storm in a boiling saucepan." (Athenaus: *The Deipnosophists* VIII-19) (1)

We are a nation of professional worriers. We worry constantly about cyclamates and cranberry juice; pollution and crime; youth and old age; birth and death; campus unrest and Communism; Cuba and Viet Nam; Russia and China; the Middle East; virility and femininity; money; whether we smell good, sweat too much, have bad breath, smoke too much, are too fat or too thin; whether we are keeping up with the Joneses; what people will think, and even whether we worry enough! As a general practitioner, I see hundreds of people every week whose chief "illness" is worry: that a sprained ankle or wrist may be broken, a simple cold may be "walking pneumonia," a slight sore throat may be "strep throat" (whatever that means!), a lump may be cancerous, a light bump on the head may be a skull fracture or "concussion," blood pressure, cholesterol, or sugar may be too high or too low, and countless other, usually needless, worries. Americans consume an unbelievable 27 million pounds of aspirin a year, enough to treat more than 17,000,000,000 headaches (2), plus enormous quantities of sedatives, tranquilizers, sleeping pills, "pick-me-up's" and other more dangerous (??) drugs.

Frequently, we ban things because we do not understand them, and declare others suspect simply because of our fears of the unknown. The classic example of this irrational attitude is sex: all mention, discussion or even bad thoughts about it have been strictly taboo among decent people. Even today, any joke about sex is automatically a "dirty joke." Kinsey, and Masters and Johnson, initially ridiculed, scorned, and condemned for their efforts to dispel this attitude of ignorance and irrational fear, are now heroes. Our ill-fated experiment with prohibition, with its nasty side effects (rum-running, crooked politics, gangsterism run amok, et cetera) was based on a similar irrational attitude. More recently, such innocent bystanders as tomato juice (3), sugar (4), monosodium glutamate (5), hexachlorophene (6), oral hypoglycemic drugs (7), all "fixed-combination" drugs (8), and, of course methyl mercury (9) in fish and other foods, have all come under fire.

But the case against methyl mercury, as *London Observer News Service* reporter Gerald Leach (10) has observed, "may turn out to be no more than a giant red herring. Marine biologists are becoming increasingly convinced that the 'contaminated' fish contained perfectly normal levels of mercury, picked up from natural levels of mercury in seawater. Either tuna eaters have always been living dangerously, or the United States has set its permissible mercury levels too low." The respected and conservative *British Medical Journal* (11) agrees: "While never forgetting that methyl mercury compounds can be unpleasantly toxic if exposure to them is sufficiently severe, all the available evidence indicates that the traces found in the canned fish imported into this country *provide no basis for the panic banning of their sale. While it is certainly safe to eat 0.5 lb (220 g) of tuna containing 0.5 ppm methyl mercury daily,* there is at present little information on the *magnitude* of the margin of safety. However, many people have undergone long-continued occupational exposure to low levels of methyl mercury without developing signs of neurological lesions, and by no means all the people in Japan who ate contaminated fish became ill. *Thus, there is certainly an amount of methyl mercury which can be consumed regularly without producing damage. The fish are unaffected,* and it is possible that the methyl mercury is firmly and innocuously bound to some tissue con-

stituent, so long as the levels do not exceed a certain amount. The recent work in Sweden leading to the discovery of certain fish in fresh-water lakes with levels of methyl mercury *10 times greater* than in the canned tuna has disclosed no evidence of disease among people who eat a lot of fish. Such people may have concentrations of methyl mercury ranging from 50 to 1,200 ppb of red blood cells, compared with the levels of less than 5 ppb in people who do not habitually eat fish." (Italics supplied.)

Frederick J. Stare, M.D., Chairman, Department of Nutrition, Harvard University School of Public Health, remarks (personal communication): "The discovery of mercury levels in some tuna and much swordfish that exceed the FDA 'interim guideline' (0.5 ppm) has sent a panic wave through the ranks of many *who ought to know better*. Personally, I think this arbitrary guideline could be at least twice as high as it is. Nations such as Sweden, Norway, Denmark and Iceland, whose people eat many times the per capita amount of fish that we do, have longevity and health records that equal or exceed ours. *What is unfortunate about all this* is that 600,000 Americans die annually from heart disease, but there has *never* been a symptom of injury, let alone a mercury-related death in this country, from eating fish." (Italics supplied.)

Leonard J. Goldwater, M.D., Professor of Community Health Services at Duke University (Mr. Mercury), has this to say (12): "Suddenly, almost overnight, mankind has become acutely fearful of mercury in the environment. The alarm is understandable. *Quicksilver has always been regarded as being magical and somewhat sinister*, partly because of its unique property as the only metal that is a liquid at ordinary temperatures. In 1970, alarm rose to a dramatic pitch in North America, following the discovery of mercury concentrations in fish in Lake St. Clair by a Norwegian investigator working in Canada. The current journalistic outcry on the 'mercury problem' has produced a state of public alarm *approaching hysteria*. 'Protective' measures are being proposed and applied *without basis in established knowledge*. The evolutionary evidence suggests that *too little* mercury in the environment might be as disastrous as too much. In the case of mercury, as in all other aspects of our environment, *our wisest course is to try to understand* and to maintain the balance of nature in which life on our planet has thrived." (Italics supplied.)

Aside from accidental poisoning cases by organic mercury fungicides applied to grain seed, never intended as foodstuffs, (13), the only known poisonings by methyl mercury in legitimate foods occurred in Japan, from fish. The world appears to "little note nor long remember," however, certain very relevant details of those cases. 1) The methyl mercury levels in those fish were estimated to average at least 20 ppm wet wt (14). 2) Those poisoned were estimated to have ingested up to 4 mg of methyl mercury daily (14): i.e., four times the estimated lethal dose of 1 mg daily (15). 3) Many of the fish eaten were found floating on the surface of the water (16), nearly dead themselves! 4) The Japanese customarily eat large amounts of fish daily, as one of their chief dietary staples. 5) The whole blood total mercury level found in the one best-studied fatal case was a whopping 1,300 ppb (corresponding to ca. 2,400 ppb in the red blood cells) (17). 6) A recent Swedish report estimates that the lowest whole blood level at which symptoms occurred in Japan was around 200 ppb. The same report suggests, in addition, that the victims may have been abnormally sensitive to methyl mercury (18), much the same as some individuals are abnormally sensitive to pollen, certain drugs, insect stings or bites, et cetera. 7) Kurland (19) of the Mayo Clinic reports that in at least one batch of Japanese fish analyzed, selenium, also a deadly poison in very small doses, was present in amounts equal to methyl mercury. This would appear to support Goldwater's opinion that a "dual action mechanism" may well have been involved, in which methyl mercury played only a partial role. Landner (20) believes that the methyl mercury in fish may have been partly detoxified during its passage through algae from which the fish absorbed it.

The astounding degree of unreliability of many laboratory analyses for mercury in human blood samples has been reported pre-

viously (21). This unfortunate fact has been reinforced by further analytical data since collected by the author. The above-mentioned Swedish report (18) confirms this, by a statement on page 19: "For analyses of blood and certain other types of biological material, one must recognize that the results of individual analyses *may deviate widely* from the true value." (Italics supplied.) Bearing this in mind, and realizing that we must nevertheless begin somewhere, further analyses of blood samples from so-called "heavy fish-eaters" from the Lake St. Clair and St. Clair River areas of Michigan (supposedly among the most mercury-contaminated areas in the United States) do not seem very disquieting (Table 1). These people have, for many years, eaten "contaminated" fish from once every two weeks to four times a week. A certain degree of analytical unreliability notwithstanding, the blood levels appear to correspond well, generally speaking, with those reported under similar conditions from Sweden, and with the relative amounts of fish eaten by the volunteers. In any event, these blood levels are all far below the 200 ppb estimated to be the lowest level at which symptoms appeared in Japan. They are much further below the levels of hundreds of people in Sweden, Finland, Japan, and Canada—up to several hundred ppb (one Swede had 650 ppb!)—who have shown no symptoms whatsoever of poisoning.

Much has been made of the fact that chromosome breakage has been found in lymphocyte cell cultures in vitro taken from Swedes with elevated blood mercury levels, presumably due to high fish consumption. What is usually not mentioned is that the same effect is produced also by a host of other agents: e.g., aspirin, caffeine, colchicine, water, changes in temperature and oxygen pressure, diagnostic or therapeutic radiation, antibiotics, morphine, chloroform, calcium and magnesium deficiencies, theobromine (a diuretic), theophylline (an asthma medication), and many others. Moreover, as geneticists know full well, "the intact human organism differs from cells in the test tube in its ability to detoxify and excrete noxious compounds. Compounds that are toxic in vitro do not necessarily have the same effect in vivo" (22). The authors of the Swedish study (23) are themselves careful to point out: "A large number of chemicals are known to induce chromosome breakage when added to cell cultures in sufficiently high concentrations for a sufficient length of time. The frequency of cells with chromosome breaks showed great variations at the different mercury levels: *this makes the interpretation of the results difficult.* There was no statistically significant difference in the *frequency* of chromosome breaks between the control and the exposed groups. Studies on bone marrow cells should show whether the same correlation occurs also *in vivo*." (Italics supplied.)

Much has been made also (by this author among others) of the fact that methyl mercury readily crosses the placental barrier and accumulates up to 30% higher fetal than maternal RBC levels. (Tejning has recently

TABLE 1

Whole blood total mercury levels reported in 16 "heavy fish-eaters"

| Sample No. | Hg, ppb | Sample No. | Hg, ppb |
|---|---|---|---|
| 1 | 70 | 9 | 22 |
| 2 | 51 | 10 | 21 |
| 3 | 39 | 11 | 21 |
| 4 | 35 | 12 | 21 |
| 5 | 33 | 13 | 21 |
| 6 | 31 | 14 | 18 |
| 7 | 30 | 15 | 17 |
| 8 | 27 | 16 | 16 |

Samples were taken from Lake St. Clair area in Michigan (near Detroit) in October 1970 by the author. Analyses through the courtesy of Dr. Rolf Hartung, University of Michigan School of Public Health. Results are in parts per billion (ppb), or nanograms per gram (rounded to nearest 0.5 ppb).

*Notes: a)* Due to the very small quantities of Hg involved, these levels are not 100% accurate. *b)* Subjects #1 and #2 have eaten fish from Lake St Clair four times a week for many years. Frequency of consumption is not well documented for most of the others. *c)* Subject #13 has been reported in previous papers as having 47, 23, and 52 ppb, respectively (by various laboratories). See references by author. *d)* Methyl mercury portions of these samples have not yet been reliably reported, due to technical difficulties. *e)* Each level reported is the average of several separate analyses. *f)* Levels up to several hundred parts per billion Hg have been reported in many asymptomatic fish-eaters and persons with long-standing occupational exposure to alkyl mercury compounds.

increased this figure to 33% (24).) True enough. But it should be noted that this is very probably true of many other substances in common use, as well, including aspirin. Some authorities (25) go so far as to recommend that "*all drugs* not known to be reasonably safe on the basis of long usage be avoided in women of childbearing age" (whether known to be pregnant or not!).

In a sense, I have deliberately chosen to play the devil's advocate by presenting the "flip side" of the methyl mercury coin. It is not my intention to minimize the risk of this highly toxic substance, which must be studied in far greater detail and in a more sane fashion than heretofore. As Goldwater (12) puts it, we must "apply the techniques of epidemiology, preventive medicine, public health, and industrial hygiene that have been effective in meeting hazards in the past." But I sincerely believe that our sense of proportion is somewhat out of balance. Both state and federal governments have examined countless tons of fish, but have apparently done very little to determine the actual effects of methyl mercury on people. Sensationalistic, even fantastic, stories have appeared in many publications, including at least one highly reputable one (26), from which an article was reprinted in a pictorial newspaper under the glaring banner headline: "Everyone in the U.S. Is Being Poisoned by Mercury!" Another writer in a popular men's magazine even invented a nonexistent disease called "the St. Clair Shakes," purported to be caused by mercury in the water of Lake St. Clair! Surely such publications would do far better to present a cooler, more rational view of the situation, rather than attempting to terrify us with visions of unseen ogres.

There are certainly other things deserving of more attention than methyl mercury in fish and other foods. Most of us think nothing of climbing behind the wheel every day of a lethal missile that last year alone killed 56,000 people, largely because of inadequate driver education and highway safety measures, not to mention irrational behavior. Drinking drivers accounted for approximately one-half of those fatalities. Ten thousand people died last year by gunfire (so we propose to ban guns, which would only serve to take them away from legitimate, law-abiding owners, and leave them only in the hands of criminals). Twelve thousand died in fires, most of which could have been prevented. Nicotine, which most of us use daily, has a higher toxicity rating than methyl mercury; codeine, many other drugs, insecticides, and rodenticides have the same toxicity rating (27).

The May 1971 issue of *The Scientific American* (28) carried a short article entitled "The American Way of Death (cont'd)." The picture it paints is appalling. "Americans under 65 are twice as likely to die of heart attack as (those) living in Norway, Sweden or the Netherlands. The World Health Organization has described (this) as potentially 'the greatest epidemic mankind has faced.' The risk of a heart attack is increased by such factors as hypertension, excessive body weight, elevated cholesterol in the blood and cigarette smoking. Epidemiological studies have marshalled impressive evidence that the development (of coronary heart disease) is controlled by the local habits and conditions of life. It seems highly plausible . . . that modifications of our present way of life could be specified *today* that would . . . dramatically reduce the premature toll of this disease and lower overall mortality in persons before age 65."

*1970 scorecard:* heart disease 600,000; methyl mercury 0.

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# Organic Mercury Identified as the Cause of Poisoning in Humans and Hogs

AUGUST CURLEY, VINCENT A. SEDLAK, EDWARD F. GIRLING, ROBERT E. HAWK
W. F. BARTHEL, WILLIAM H. LIKOSKY, PAUL E. PIERCE

In 1961 Uchida *et al.* (*1*) showed that methyl (methylthio) mercury, traced to the ingestion of shellfish, was the cause of an unusual illness in humans in the Minamata Bay area, Japan. In 1966 Ordonez *et al.* (*2*) reported similar unusual illnesses that involved the central nervous system in humans in Guatemala. Because of the nature of the symptoms, this disease was thought to be encephalitis; however, autopsy samples sent to our laboratory were found to contain high concentrations of mercury. The deceased had ingested wheat seed that had been treated with Panogen (*3*) and contained 17 parts per million (ppm) of mercury. Also in 1966 Borg *et al.* observed mercury poisoning in birds in Sweden (*4*), and Takizawza and Kosaka reported methyl mercury poisoning in humans resulting from the ingestion of fish and shellfish in the Niigata Prefecture, Japan (*5*).

In August 1969 a farmer (Mr. H.) and five of his neighbors in the area of Alamagordo, New Mexico, obtained waste seed grain from a local granary. The grain had been treated with an organomercurial fungicide, either Panogen or a formulation of Ceresan (*3*); both fungicides had been used at different times for seed treatment by the manufacturer. The six farmers used this grain in food for hogs. The father of one family (Mr. H.) began feeding his pigs with this grain in late August or early September. After about 2 to 3 weeks, one hog, which had been fed 60 percent more grain than the others, was slaughtered and the family ate the meat during the next 3.5 months. The other pigs were kept on a similar diet but were fed smaller quantities of the grain. By mid-October, 14 of these feeder pigs had developed blindness, lack of coordination, and posterior paralysis. In the next 3 weeks, 12 of the 14 pigs died. Gait disturbances in the surviving pigs improved, but the pigs remained blind and stunted.

In early December one child of Mr. H.'s family became ill. By late December two other members of the family had developed the same illness. Mercury poisoning caused by the ingestion of contaminated pork was suspected. At this time the mother of the children was pregnant. Three months later the baby was born.

Details of the epidemiology, symptomatology and diagnosis, and the clinical history and treatment of the patients for mercury poisoning will be reported by the Center for Disease Control (formerly the National Communicable Disease Center) (*6*). We report here the results of chemical studies associated with the poisoning episode, the first observed case in the United States of indirect mercury poisoning in humans caused by the ingestion of contaminated meat from animals that had consumed mercury in their diet.

Several methods had been developed for the analysis of mercury in either the inorganic or organic form (*7*). We found that modification of the methods of Willis (*8*) for inorganic mercury and of Westöö (*9*) for organic mercury gave satisfactory results.

Samples of hog tissue (brain, liver, kidney, muscle, colon, pancreas, eye, heart, fat, and lymph nodes), human body fluids (serum, urine, cerebrospinal fluid, and amniotic fluid), and seed grain were prepared for analysis by atomic absorption spectrophotometry by modifications of Willis's method (*8*), as follows: 20-g tissue and grain samples in 25 ml of water were refluxed under a 24-inch (61-cm) condenser with 25 ml of concentrated $HNO_3$-$H_2SO_4$ (1 : 1, by volume) for at least 2 hours; in some tissues additional amounts of the acid mixture were added until the sample was free of solids. Fuming acids at lower reflux temperatures were used in order to minimize the digestion time of some tissues and mercury losses by volatilization. Perchloric acid (15 ml) was added to complete oxidation, and reflux was continued until the solution was light amber. After cooling, the *p*H of the solution was adjusted to 2.5 to 3.5. Samples were chelated with 5 ml of a 1 percent (by weight) solution of ammonium pyrrolidinedithiocarbamate (APDC) and extracted three times with 50 ml of methyl isobutyl ketone (MIBK). Emulsions were broken up by centrifugation. The combined MIBK extracts were reduced to between 4 and 25 ml for subsequent analysis. Urine (86.0 to 906.5 g), amniotic fluid (24.0 g), and cerebrospinal fluid (0.96 to 2.25 g) were adjusted to *p*H 2.5 to 3.5 with concentrated $HNO_3$-$H_2SO_4$ (1 : 1, by volume). These samples were chelated and extracted as before. Serum (5.4 to 36.5 g) was refluxed for 1 hour with 50 ml of 1.0*N* HCl. Then 20 ml of concentrated $HNO_3$ was added, and the condenser was rinsed with water. Reflux was continued for ½ hour. The sample was cooled, distilled water was added, and the *p*H was adjusted to 2.5 to 3.5 with 40 percent NaOH. The sample was chelated and extracted as before. The combined MIBK extracts were reduced to between 4.0 and 20.0 ml for subsequent analysis. Organic extracts were analyzed by atomic absorption spectrophotometry (*10*).

In order to observe emission effects or the absorbance of other possible interfering species near the absorption line for Hg at 2537 Å, test solutions containing (i) 20 ppm Hg, 1200 ppm Na, and 810 ppm P; (ii) 20 ppm Hg and 900 ppm Fe; (iii) 14 to 24 ppm Na and 156 to 180 ppm P; (iv) 20 ppm K and 155 ppm P; and (v) 1000 ppm Fe were prepared, and their influences on Hg absorption were recorded. These concentrations were chosen to simulate concentrations reported to be present normally in tissues and body fluids. Blank effects (water-saturated MIBK and other reagents) were also examined.

Phosphorus, sodium, potassium, and iron were not observed to interfere in either aqueous solution or organic solvent at the analytical wavelength for mercury. There was no absorption from

Table 1. Concentration of mercury (in parts per million) in hog tissues and seed grain fed to that particular hog, as determined by atomic absorption spectrophotometry.

| Hog or grain owned by | Hog | | | | | Grain |
|---|---|---|---|---|---|---|
| | Brain | Liver | Kidney | Muscle | Colon | |
| Mr. H. (hog slaughtered) | | | | 29.4* | | |
| Mr. H. (hog slaughtered) | | | | 27.5 | | 32.8† |
| Mr. H. (blind hog)‡ | 36.1 | 25.2 | 12.0 | 14.2 | | 32.8 |
| Neighbor 1 | 13.1 | 17.3 | 12.1 | 9.5 | 24.2 | 2.97§ |
| Neighbor 2 | 15.8 | 21.0 | 25.2 | 23.1 | 6.5 | 1.27 |
| Neighbor 3 | 12.6 | 3.5 | 8.4 | 16.8 | 14.7 | 2.76 |
| Neighbor 4 | 3.5 | 8.8 | 20.5 | 16.8 | 7.9 | |
| Neighbor 5 | 21.0 | 10.5 | 21.0 | 12.6 | 24.2 | 2.54 |
| Range | < 3.5–21.0 | < 3.5–21.0 | 8.4–25.2 | 9.5–23.1 | 6.5–24.2 | 1.27–2.97 |
| Mean | 12.5 | 12.2 | 17.4 | 15.8 | 15.5 | 2.39 |
| Standard error | ± 3.46 | ± 3.05 | ± 3.10 | ± 2.29 | ± 3.81 | ± 0.38 |

* Pork eaten by members of the family. Animal showed no signs of poisoning. † Mixture of waste grain. ‡ Other tissues analyzed and concentrations (in parts per million): spleen, 7.5; heart, 7.5; fat, 9.8; lymph node, 23.1; cerebrum, 9.8; cerebellum, 42.95; eye, 18.9; heart blood, 31.5; lung, 14.2; pancreas, 15.8; thigh muscle, 11.5; gastrointestinal tract, 17.3; stomach, 8.5. § Possible mixture of waste grain or contaminated feeder chow, or both.

the air-hydrogen flame. Sample calculations were corrected for the blank effects of MIBK and the signal-to-noise effects of the flame.

Aqueous standard solutions were prepared from analytical grade cyano-(methylmercuri) guanidine (Panogen) with the mercury content equal to 10, 20, 50, 100, and 200 ppm. Standards were also prepared in organic solvent from digestion with the $HNO_3$-$H_2SO_4$ acid mixture, chelation with APDC, and extraction with MIBK. These standards were compared with the Fisher atomic absorption spectrophotometric mercury reference standard at concentrations ranging from 2 to 200 ppm.

Some samples (20 g) of tissue and grain and of serum and urine were fortified with Panogen to give mercury contents equal to 12.5, 20, and 50 ppm, and 5, 10, 15, and 50 ppm, respectively. The analytical procedure was evaluated with standards at 20, 50, and 100 ppm.

By means of the analytical procedure 83 to 100 percent of the mercury was recovered from Panogen (mean, 95.8 ± 4.25 percent) at 20, 50, and 100 ppm, and 50 to 60 percent of the mercury (mean, 53.8 ± 1.96 percent) was recovered from tissues and grain fortified at 12.5, 20, and 50 ppm. Losses of mercury from tissue or grain samples may be due to volatilization during prolonged reflux, inefficient condensers, too high a reflux temperature, or combinations of these. Solubility effects during the adjustment of *p*H may also be a factor. Recovery from serum and urine was 98 to 100 percent (mean, 98.7 ± 0.67 percent).

Wet tissue, grain, human blood, and urine were analyzed for mercury by neutron activation analysis at the Nuclear Research Center, Georgia Institute of Technology (*11*). The Reinsch test for mercury (*12*) was positive.

A 600-g sample of waste seed grain was prepared for mass spectral analysis by a modification of the extraction and thin-layer chromatographic procedures of Westöö (*9*). An aliquot of the mercury-containing extract prepared by the method of Westöö was placed in a glass insert, the solvent was evaporated, and the insert was placed into the ion source of a mass spectrometer (LKB model 9000) by direct probe and ionized at 20 and 70 ev (*13*).

A 200-g sample of waste seed grain that had been extracted with methanol–diethyl ether (1 : 1, by volume) was highly colored by the characteristic red dye used to identify organomercury-treated grain. The dye was purified by column chromatography (*14*)

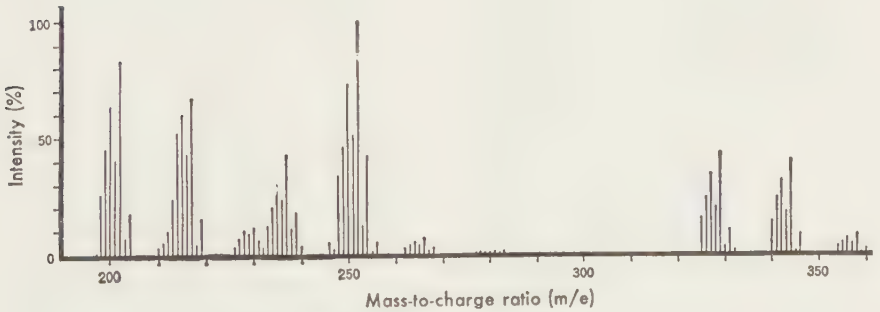


Fig. 1. Mass spectrum of waste grain extract.

for identification by atomic absorption spectrophotometry. Tests for the presence of chlorinated hydrocarbon and organophosphate insecticides were made by gas chromatography (*15*) but the results were negative.

Results of atomic absorption analysis of tissue samples from hogs, waste grain, and feeder chow that might have been contaminated are presented in Table 1. The hog fed large quantities of the waste grain and slaughtered for family consumption contained high concentrations of mercury in the tissues, as did another sick and ultimately blind hog from the same herd; this second hog also had the highest content of mercury (36.1 ppm) in the brain. The waste grain fed to these animals contained 32.8 ppm of mercury. Hogs belonging to the neighbors and fed grain from the same source contained about the same concentrations of mercury in the tissues.

The mixture of grain consisted of floor sweepings and screenings; it contained sorghum, oats, grain, chaff, and rat feces. Some of the grain had been treated with organic mercury compounds as a fungicide. Since there was no homogeneity in any of the grain samples, mercury concentrations varied for each sample. Each hog owner used this mixture in the daily feeding of the animals. The feeder chow might have been contaminated with waste grain. Neighbor farmers transferred feeder chow and waste seed grain by open truck in the rain, which might explain the lower mercury contents (mean, $2.39 \pm 0.38$ ppm) in the neighbors' grain.

Meat products purchased in area food stores also contained mercury. Atomic absorption spectrophotometric measurements indicated that kidney contained $2.2 \pm 1.09$ ppm; neutron activation analysis (*11*) revealed that liver and sausage contained 0.17 and 0.072 ppm, respectively (the detection limit of the atomic absorption spectrophotometer is 0.3 ppm for a 20-g sample of tissue with a volume reduction of 4.0 to 5.0 ml). The present U.S. Food and Drug Administration tolerance limit for mercury in meat products is 0.5 ppm.

The concentrations of mercury in serum, urine, and cerebrospinal fluid were determined in samples from the human victims. Urine samples obtained from Mr. H., his son (age 13), and his two daughters (ages 8 and 20) on 8 January contained, respectively, 0.16, 0.21, 0.20, and 0.06 ppm of mercury. Concentrations of mercury in the urine samples of the neighbors varied from $< 0.05$ to 0.18 ppm (33 samples); the content of mercury in the serum samples of the neighbors averaged $< 0.2$ ppm (38 samples). After treatment with British Anti-Lewisite, the concentrations of mercury in the urine samples of Mr. H.'s son and older daughter (age 20) had increased to 0.50 and 0.49 ppm, respectively [the concentration of mercury in the urine of Mr. H.'s younger daughter (age 8) was $< 0.03$ ppm]. Concentrations of mercury in the serum samples of these children were approximately 16 times those in the urine. The concentrations of mercury in the serum and cerebrospinal fluid of Mr. H.'s son were about the same (3.0 ppm). The urine of Mrs. H., who was pregnant at onset of the children's illness, contained 0.09 ppm of mercury on 8 January and 0.18 ppm on 11 February; her serum contained 2.91 ppm of mercury on 22 January and 0.47 ppm on 11 February. The amniotic fluid contained $< 0.02$ ppm of mercury on 11 February. Concentrations of mercury in the newborn baby's urine ranged from 2.70 ppm at

delivery to 1.56 ppm several days later. These concentrations of mercury indicate placental transfer to the fetus.

The mercury, identified by atomic absorption spectrophotometry, was confirmed as organic mercury by mass spectrometry (Fig. 1); these results substantiated the clinical diagnosis of organic mercury poisoning. The dye coating on the waste seed grain was isolated by column chromatography; it absorbed at 5440 Å. This dye was identical to that in commercial samples of Panogen and Ceresan. The extracts from the waste seed grain prepared by the method of Westöö contained, according to mass spectral analysis, characteristic $Hg^+$, methyl $Hg^+$, methyl $HgCl^+$ (chloride from the analytical procedure), ethyl $Hg^+$, and probably methoxyethyl $Hg^+$ isotopic ion clusters at *m/e* (mass-to-charge ratio) 202, 217, 231, 237, 252, 281, and 296. Other mercury-containing organic ions were observed at *m/e* 329, 344, and 358.

These data clearly show that mercury accumulated in animal tissues and human body fluids and confirm that compounds containing organic mercury were, in fact, the causative agents in the poisoning incident. The changes in the mercury concentrations in the serum and urine of the mother after delivery and the content of mercury in the urine of the newborn baby indicate placental transfer.

### References and Notes


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10. Equipment for analysis of the organic extracts comprised a Beckman model 979 atomic absorption spectrophotometer equipped with a total-consumption, turbulent-flow, burner assembly for an air-hydrogen flame and a Beckman 10-inch (25.4-cm) potentiometric strip chart recorder, operated under the following conditions: wavelength, 2537 Å; lamp, argon-filled, hollow cathode; lamp current, 10 ma; three burners; elevator position, 7.6 cm; three passes (light beam); support gas, air at 20 to 25 pounds per square inch (1.4 to 1.7 atm); fuel gas, hydrogen at 4 pounds per square inch; lean flame; 0.15-mm slit width.
11. We thank M. E. McLain, Jr., Nuclear Research Center, Georgia Institute of Technology, Atlanta, for neutron activation analyses.
12. A. S. Curry, *Poison Detection in Human Organs* (Thomas, Springfield, Ill. 1963), pp. 65–68.
13. Other parameters were as follows: the temperature of the direct probe varied from ambient temperature to 120°C; temperature of the ion source, 290°C; accelerating voltage, 3.5 kv; filament current, 4 amp; trap current, 60 μa; recording oscillograph, 4 cm/sec; scan speed, 5.
14. Glass chromatographic columns, 8 mm in diameter, were packed with 4.0 cm of silica gel (Woelm), grade 1, below 2.0 cm of anhydrous sodium sulfate. The columns were wet with benzene before introduction of the samples. A 4-ml portion of the supernatant from the extract was eluted with about 14 ml of benzene and 5 ml of acetone and then by an additional 1 ml of acetone. The second acetone fraction containing the dye was collected and the acetone was evaporated. The red dye was dissolved in methanol, and its absorbance was determined on a Cary model 14 recording spectrophotometer.
15. The Micro-Tek model MT 220 gas chromatograph was equipped with tritium electron-capture detectors for chlorinated compounds and dual flame photometric detectors for phosphorus and sulfur.


16. We thank Mrs. E. Gray for her assistance with the statistics and receipt of samples.

# Effects of Exposure to Mercury in the Manufacture of Chlorine

R. G. SMITH, Ph.D., A. J. VORWALD, M.D., L. S. PATIL, M.D., and T. F. MOONEY, JR., Ph.D.

## Introduction

IN 1963 9.2 MILLION TONS OF chlorine were produced in the United States and Canada to satisfy the ever-growing demand for this most useful element. Nearly all chlorine is made by brine electrolysis, and in the United States, mercury cells in which the cathode is a flowing sheet of elemental mercury, accounted for about one-third of the production capacity, while in Canada, mercury cells accounted for about two-thirds of production. A typical, modern, 30 square meter mercury cell (Figure 1) may contain up to 12,000 pounds of mercury which is circulated in a closed system and reused indefinitely, but due to circumstances of operation, some losses may occur and exposure of operators to mercury is possible. A cell room may contain a large number of cells in constant operation, and the maintenance of such installations requires the attention of small crews of workers whose exposure to mercury must be evaluated and controlled.

This study was supported by funds from The Chlorine Institute, New York, New York, in the form of grants to Wayne State University.

In recognition of this potential exposure, a study was undertaken several years ago to determine the extent of exposure of cell room workers to mercury, and the effects, if any, of such exposures. The study was under the direction of several members of our Department, but actually was a cooperative effort in which a great many other persons played a significant role.

## Plan of Study

A number of chlorine-producing plants in the United States and Canada, all of whom were members of The Chlorine Institute, were invited to participate in the study, which was designed in its entirety to yield information on both mercury and chlorine. Those plants which did not use mercury cells were concerned only with the chlorine studies, the results of which have been described elsewhere.[1] After extensive planning with medical and industrial hygiene personnel from many of the plants, a plan was agreed upon, the essential features of which were as follows:

1. A "study year" was selected, during which time all required data would be collected. (Although ideally it was desirable that each plant observe the same year, in practice

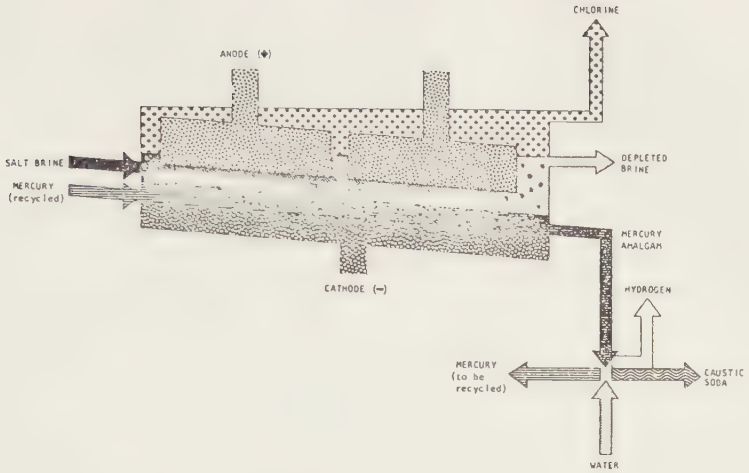


FIGURE 1. Diagram of the mercury-cell used for the production of chlorine from brine.

it was not possible, so the actual "study year" extended over a period of approximately two years.)

2. Every employee potentially exposed to mercury, and engaged in the manufacture of chlorine would be given a thorough medical examination once during the year.

3. At least four times during the year, blood and urine specimens would be obtained from each employee for the purpose of determining mercury levels.

4. Each cell room and adjacent areas in which employees spent working hours would be studied for the purpose of selecting a number of sampling points which would permit characterization of the degree of exposure to mercury throughout the working day.

5. The percentage of time normally spent by each employee at each of the specified points, or areas represented by them, would be determined. In addition, the length of time that each employee was required to wear respiratory protection against exposure to mercury was also to be noted.

6. At least six times during the year, air sampling for mercury was to be performed at each sampling location by a method agreed upon by cooperating industrial hygienists.

7. On the basis of the information derived from items 4, 5 and 6, above, time-weighted average exposures for all employees were to be calculated and used as the basis for estimating exposure to mercury during the year.

8. All data were to be received by our Department and subjected to examination and analysis for the purpose of arriving at conclusions concerning the degree of exposure and the presence or absence of effects.

9. To assist in reaching conclusions, a control population consisting of as large a group as possible of plant employees not occupationally exposed to mercury would be selected and treated in the same fashion as exposed employees.

## Purpose of Study

It is apparent that the principal purpose of the study was to determine whether any adverse effects attributable to mercury could be detected in the exposed employees, but several secondary objectives were also defined, and experimental protocol designed accordingly. In view of the difficulties of diagnosing early mercurialism, for example, particular emphasis was placed on signs and symptoms which could aid the physician in making a positive diagnosis of suspected cases. Related to this problem is the intelligent usage of urine and blood mercury analyses for both diagnostic and monitoring purposes, and it was felt that our study provided an unprecedented opportunity to determine the usefulness of such procedures. The necessity for reliable methods of analysis was obvious, and method selection and development were considered

essential elements of the study. The same can be said of air analysis, of course, for all conclusions were planned to derive from relationships between observed effects and time-weighted average exposures. Finally, it was believed that the substantial volume of data to be generated could serve as a basis for judging the suitability of the present threshold limit value of 0.1 mg/m$^3$ for mercury vapor, and in addition, could suggest values for urine and blood which might serve as "biological threshold limit values", or possibly even as diagnostic aids.

To carry out all of these objectives, an awareness of previously published findings was essential, and a thorough search of the literature was planned and carried out, resulting in a bibliography of nearly 2500 references and abstracts, which is to be published as an aid to those undertaking future research.

## Methods

In recognition of the number of plants involved in the study, their geographical distribution, the need for data throughout a period of one year, and other factors, certain decisions were made which significantly affect the data and the conclusions drawn from them. A brief discussion of some of the more important procedural matters adopted, and the rationale leading to their adoption may be of interest.

### *Medical Examinations*

There was unanimous agreement that the medical examination of all employees could best be performed by a University-selected group of medical specialists who could go to each plant during the study year. There was equally unanimous agreement that this approach was not feasible, and that the required examinations would have to be conducted by, or under the direction of, the respective plant physicians involved. The substance of the examination was designed in close cooperation with physicians from the several plants. The resulting examination was quite thorough, and required the physician to complete an eight-page form on which more than 150 notations could be made. The principal features of the examination included a description of occupational exposure to chlorine and mercury, a detailed medical history, and a complete physical examination which included a neurological and hematological examination, as well as chest x-rays, EKG, and pulmonary function studies. Instructions on how to perform the examination were issued, and particular emphasis was placed on the apparatus and methods for making pulmonary function measurements.

It was recognized that data from a number of physicians, rather than a team, could exhibit some inconsistencies based on individual methods of examination, but it was believed that these would not be sufficient to prevent meaningful interpretations of the data.

### *In-Plant Studies*

For the same reasons just cited, the decision to use plant personnel to make the required in-plant studies of mercury levels and work patterns was agreed upon, and extensive discussions between University and plant personnel resulted in standardized procedures for most aspects of these studies. The essential features will be noted.

The all-important measurements of mercury in the plants were to be made primarily by means of ultraviolet meters made by several manufacturers, and generally referred to as mercury vapor meters. In recognition of the fact that such meters respond only to mercury if present as a vapor, some concern was evidenced that mercury compounds present as dust would go undetected, and accordingly, each plant was requested to sample from time to time in such a manner as to determine total mercury levels, which could be compared to vapor levels. Suggested sampling procedures included absorption in iodine or potassium permanganate solutions, with subsequent determination by a dithizone procedure. It was also suggested that if a filter preceded the absorption vessel, a differentiation between particulate and vapor phase mercury could be made.

Standardization of methods for sampling and analyzing chlorine was required for the evaluation of the effects of chlorine exposure, but in addition, proved to be of importance in relation to mercury as well. A gas phase reaction between chlorine and mercury was

shown to occur by chemists from one of the participating plants, and the reported reaction kinetics were confirmed by Roggenbaum.[2] Although low concentrations of chlorine are capable of removing mercury vapor from the air, and hence can cause a vapor meter to give low results, the ambient levels of chlorine in most cell rooms were, in general, sufficiently low that the effect appeared to be of minor consequence.

According to our data, the extent of diminution of mercury vapor levels by reaction with chlorine depends upon the concentrations of both mercury and chlorine, and the time available for reaction to occur. At an air mercury concentration of 0.1 mg/m$^3$, for example, a concentration of 1 ppm chlorine will cause a meter to read 30% low one minute after mixing, and 37% low after two minutes, the approximate equilibrium time. When the concentration of chlorine is only 0.3 ppm, however, the corresponding figures are 10% and 13%. Most cell rooms were found to contain ambient chlorine levels well below 1 ppm, usually in the 0.1-0.3 ppm range.

Other problems related to determining mercury vapor levels by means of meters are the strong magnetic fields existing within the cell rooms, particularly in the newer plants where amperages are high, and the difficulty of obtaining satisfactory zero readings in large cell room areas. In most plants, the effects of the magnetic fields could be nullified by instrument shielding, but in some of the newer plants, it was reported that no means could be found to assure reliable instrument performance. So far as known, most plants participating in the study were able to obtain reliable data which resulted from solving the various problems involved. In order to minimize sampling errors, calibration procedures for vapor meters were devised, and those plants not wishing to perform the calibrations themselves were invited to send meters to our Department for calibration.

### Blood and Urine Levels of Mercury

In view of previous experiences with determining mercury in biological samples, it was deemed desirable to select methods of proven reliability, and if possible to conduct most, or all of the analyses by the selected methods. A rather large number of procedures thought to be suitable for urinalysis, and a much smaller number of blood procedures were found in the literature, and for several reasons, the urine procedure of Campbell and Head,[3] and the micro blood method of Jacobs[4,5] were selected. Both methods were subjected to extensive testing, and several modifications were made which, in our opinion, made them more reliable.[6] A considerable amount of inter-laboratory checking resulted in a high degree of confidence in the results. Although participating plants were permitted to perform their own analyses, they were encouraged to send samples to our laboratory, and most companies did so. At the conclusion of the study, most of the urine analyses, and all of the blood analyses used in interpreting findings were performed in our laboratories.

### Description of Study Population

A total of 1624 workers participated in the study, but only 642 were employed in mercury cell rooms and constituted the true study population with respect to exposure to mercury and its effects. An additional 600 employed in diaphragm cell plants were the study population for chlorine-only exposure, and 382 workers not exposed to mercury or chlorine constituted the control group. The mercury-exposed group came from 21 different plants located throughout the United States and Canada, and plant populations varied from 12 to 91 employees. Within the group of 642 mercury-exposed workers on whom medical data were obtained, there were 75 for whom no exposure data were forthcoming, hence, the useful study population was 567 workers.

The age distribution of both the study group and the control group is summarized in Table I, and it is evident that the two groups are quite similar to each other. Table II presents a summary of employment histories, showing that more than half of the study group had worked between six and 11 years in the industry, a length of time which would seem to be entirely adequate to result in effects due to mercury if exposure levels were sufficiently high.

TABLE I

Age Distribution of Study Population

| Age Range (years) | Exposed Workers | Control Workers |
|---|---|---|
| 19-29 | 22.4% | 22.5% |
| 30-39 | 36.1 | 24.9 |
| 40-49 | 27.6 | 32.7 |
| 50-59 | 12.2 | 16.5 |
| 60- | 1.7 | 3.4 |

An effort was made to determine to what extent cell-room workers previously employed by the several companies, but no longer in the cell rooms at the time of the study, may have been influenced by exposure to mercury, as evidenced by the reasons for termination of employment. Eight companies provided the information shown in Table III, and although the data are not necessarily conclusive, they do suggest that the principal reasons for termination of cell room employment are promotions, transfers, or other moves, of a conventional nature.

Table IV shows that 63%, or nearly two-thirds of the study population smoked cigarettes, compared to approximately 56% of the controls. When the number of individuals who admitted to consuming alcoholic beverages was compared, the two groups were remarkably similar, with 51.0% of the exposed group and 50.5% of controls consuming alcohol, respectively. No attempt was made to define the quantity of alcohol consumed, nor the frequency of drinking.

Finally, it should be noted that all study employees were males and that no breakdown by race or nationality was made. Most of the members of the control population were workers comparable to cell-room workers, and relatively few office employees were included in the group. Thus, it can be stated that the control group was, in general, similar to the exposed group in most respects, even though the method of interpreting data on the basis of dose response tended to minimize the importance of having a perfectly matched control group.

## Interpretation of Data

### *Air Analyses*

The many thousands of mercury-in-air measurements can be summarized in several ways, but the significant figures with which the study was primarily concerned were the time-weighted average exposures, computed as previously indicated. It should be kept in mind that these values are not the average cell-room mercury levels, but do bear an obvious relationship to such levels. In Table V, the time-weighted average exposures have been grouped as shown for convenience, and when so grouped, 88 employees, or approximately 14% of the total group experienced at least a year of exposure to concentrations of mercury in excess of the present threshold limit value of 0.1 mg/m³. The mean exposure level for the 567 employees for whom data were available was 0.065 mg/m³, with a

TABLE II

Length of Employment in Mercury Cell Rooms

| Years Employed | % of Employees |
|---|---|
| 1 | 13.3 |
| 2-5 | 29.4 |
| 6-9 | 26.3 |
| 10-14 | 25.0 |
| 15-20 | 5.1 |
| 20+ | 0.9 |

(551 employees only)

TABLE III

Termination of Employment of Mercury Cell-Room Workers Previous To Study Period

| Reason for Leaving | % of Employees* |
|---|---|
| Reassigned at own request | 74.1 |
| Reassigned at company request | 7.5 |
| Resigned | 10.9 |
| Retired | 0.3 |
| Accidental injury-disability | 0.0 |
| Medical illness | 0.7 |
| Inability to get along—Co-worker | 0.0 |
| Chronic absenteeism | 0.3 |
| Unsatisfactory job performance | 0.0 |
| Death | 2.4 |
| Other | 3.8 |

*Based on 293 employees over 10 years.

TABLE IV

Cigarette Smoking Habits of Study Population

| Packs Per Day | Exposed % | Controls % |
|---|---|---|
| 0 | 37.2 | 43.7 |
| <1 | 7.2 | 12.9 |
| 1-2 | 55.0 | 42.9 |
| 2+ | 0.6 | 0.5 |

TABLE V

Mercury-Exposed Workers Grouped by Time-Weighted Average Exposure Levels

| Exposure Levels ($mg/m^3$) | Number of Workers | Percentage of Exposed Workers |
|---|---|---|
| <0.01 | 58 | 10.2% |
| 0.01-.05 | 276 | 48.7 |
| 0.06-.10 | 145 | 25.6 |
| 0.11-.14 | 61 | 10.7 |
| 0.15-.23 | — | — |
| 0.24-.27 | 27 | 4.8 |

TABLE VI

Relationship of Mercury Exposure to Blood Mercury Levels
(Expressed as percentage of each exposure level group within designated ranges of blood mercury levels)

| TWA Exposure Level Groups ($mg/m^3$) | Number of Workers | Percentage of Group within Blood Level Range ($\mu g/100$ ml) <1 | 1-5 | 6-10 | >10 |
|---|---|---|---|---|---|
| Controls 0.00 | 117 | 69.3 | 30.7 | 0.0 | 0.0 |
| <0.01 | 27 | 33.3 | 63.0 | 3.7 | 0.0 |
| 0.01-0.05 | 175 | 20.6 | 74.9 | 4.0 | 0.6 |
| 0.06-0.10 | 77 | 10.4 | 81.8 | 6.5 | 1.3 |
| 0.11-0.14 | 53 | 3.8 | 22.6 | 26.4 | 47.2 |
| 0.24-0.27 | 26 | 0.0 | 19.2 | 26.9 | 53.9 |

standard deviation of ± 0.085. In the case of 12 plants, every employee had a time-weighted average exposure of 0.1 $mg/m^3$ or less, while in the remainder some employees were exposed to higher concentrations.

The actual cell-room concentrations of mercury in air ranged from <0.001 to 2.64 $mg/m^3$, (cell-bed grinding) with most values below 0.1 $mg/m^3$. It should be kept in mind that most, if not all plants require workers to wear respirators at certain times when high mercury levels can be anticipated, and although measurements may have been made at such times, they were not to be used in calculations of time-weighted exposure data. It must also be recognized that any attempt to measure air levels of mercury, chlorine, or any substance, for that matter, without a network of continuously recording instruments is admittedly imperfect, and rarely will "usual" conditions be measured and recorded. Nevertheless, as will be shown subsequently, there is strong evidence to support the belief that the sampling program was entirely adequate for its intended purpose.

The assumption that the entire mercury intake of employees is by inhalation may be questioned, and undoubtedly some individuals who are careless in matters of personal hygiene ingest appreciable, but unknown, quantities of mercury. Likewise, it has been stated that cigarettes which may have been carried by workers in shirt pockets can absorb an appreciable quantity of mercury, giving rise to another unknown exposure when subsequently smoked. (Smoking is never permitted in cell rooms because of the hazard of possible hydrogen leaks.) Finally, some mercury may be absorbed through the skin, as has been reported with some frequency in the literature, but an international committee convened in 1968 to consider mercury threshold limit values concluded that, "As the rate of penetration is slow, the practical importance of skin absorption is uncertain. Contamination of skin or work clothes with mercury compounds, however, could cause heavy exposure to mercury vapor by inhalation."[7]

TABLE VII

Relationship of Mercury Exposure to Mercury Levels in Urine Uncorrected for Specific Gravity
(Expressed as percentage of each exposure level group within designated ranges of urine mercury levels)

| TWA Exposure Level Groups ($mg/m^3$) | Number of Workers | Percentage of Group within Urine Level Range (mg/liter) <0.01 | .01-.10 | .11-.30 | .31-.60 | .61-1.0 | >1.00 |
|---|---|---|---|---|---|---|---|
| Controls 0.00 | 142 | 35.2 | 62.7 | 2.1 | 0 | 0 | 0 |
| <0.01 | 29 | 6.9 | 86.2 | 6.9 | 0 | 0 | 0 |
| 0.01-0.05 | 188 | 6.9 | 66.0 | 24.5 | 2.7 | 0 | 0 |
| 0.06-0.10 | 91 | 0 | 62.6 | 30.8 | 6.6 | 0 | 0 |
| 0.11-0.14 | 60 | 3.3 | 18.3 | 31.7 | 16.7 | 23.3 | 6.7 |
| 0.24-0.27 | 27 | 0 | 14.8 | 29.6 | 44.5 | 7.4 | 3.7 |

TABLE VIII

Relationship of Mercury Exposure to Mercury Levels in Urine Corrected to Specific Gravity of 1.018 (Expressed as percentage of each exposure level group within designated ranges of urine mercury levels)

| TWA Exposure Level Groups ($mg/m^3$) | Number of Workers | Percentage of Group within Urine Level Range (mg/liter) | | | | | |
|---|---|---|---|---|---|---|---|
| | | <0.01 | .01-.10 | .11-.30 | .31-.60 | .61-1.0 | >1.00 |
| Controls 0.00 | 138 | 35.5 | 63.8 | 0.7 | 0 | 0 | 0 |
| <0.01 | 26 | 7.7 | 80.8 | 11.5 | 0 | 0 | 0 |
| 0.01-0.05 | 186 | 7.5 | 67.2 | 24.2 | 1.1 | 0 | 0 |
| 0.06-0.10 | 91 | 0 | 68.1 | 28.6 | 3.3 | 0 | 0 |
| 0.11-0.14 | 60 | 3.3 | 13.3 | 26.7 | 20.0 | 31.7 | 5.0 |
| 0.24-0.27 | 26 | 0 | 11.5 | 11.5 | 46.2 | 23.1 | 7.7 |

TABLE IX

Relationship of Mercury Exposure to Mercury Levels in Urine Corrected to Specific Gravity of 1.024 (Expressed as percentage of each exposure level group within designated ranges of urine mercury levels)

| TWA Exposure Level Groups ($mg/m^3$) | Number of Workers | Percentage of Group within Urine Level Range (mg/liter) | | | | | |
|---|---|---|---|---|---|---|---|
| | | <0.01 | .01-.10 | .11-.30 | .31-.60 | .61-1.0 | >1.00 |
| Controls 0.00 | 138 | 35.5 | 60.9 | 3.6 | 0 | 0 | 0 |
| <0.01 | 26 | 7.7 | 80.8 | 11.5 | 0 | 0 | 0 |
| 0.01-0.05 | 186 | 7.0 | 59.1 | 29.0 | 3.8 | 1.1 | 0 |
| 0.06-0.10 | 91 | 0 | 58.2 | 33.0 | 7.7 | 1.1 | 0 |
| 0.11-0.14 | 60 | 3.3 | 18.3 | 30.0 | 23.4 | 25.0 | 0 |
| 0.24-0.27 | 26 | 0 | 15.4 | 30.8 | 42.3 | 3.8 | 7.7 |

*Blood and Urine Analyses*

Useful data regarding urinary levels of mercury were obtained from 627 employees, of whom 67 lacked the necessary air data for computing time weighted averages (TWA). Only 339 persons provided useful blood data, of whom 31 lacked air concentration information. All told, some 2500 urinalyses and 1400 blood analyses were performed, and the results are summarized in Tables VI through IX. In each case, the blood or urine levels cited are the averages for the year, usually based on four samples from each individual. In view of some apparent inconsistencies in the results obtained when urinalyses were conducted elsewhere than in our laboratory, all urine and blood data in the tables derive from samples which were analyzed in our laboratory.

The correlations between TWA's of mercury and blood and urine levels were very strong, exhibiting the highest $t$-values for any variables compared. For air and blood, the $t$-value was 18.1, and for urine 19.2, 13.7, and 18.7 for values uncorrected for specific gravity, and those corrected to 1.018 and 1.024 respectively. All are significant at $P < 0.001$. Figure 2 shows the mean blood levels plotted versus TWA's, and Figures 3-5 show the same relationships for urine uncorrected and corrected to 1.018 and 1.024. The very considerable variability of individual points is clearly observed, but the regression lines which have been drawn, using the method of least squares, are useful in suggesting the responses of groups of employees. Using these lines, it appears that the urine level of 0.25 mg/liter recently suggested by Elkins[8] as a "biological threshold limit value" is confirmed, and from Figure 2, the corresponding blood level would seem to be about 6 $\mu$g/100 ml. A literal interpretation of the regression lines indicates that the air threshold limit value of 0.1 mg/$m^3$ corresponds to a blood level of 6 $\mu$g/100

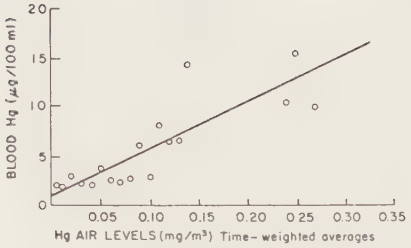


FIGURE 2. Relation of concentrations of mercury in blood to the corresponding time-weighted average exposure levels.

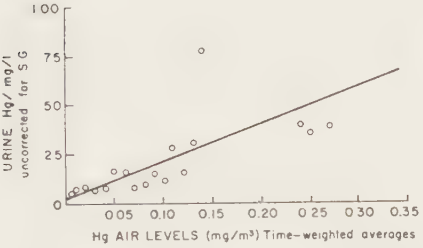


FIGURE 3. Concentrations of mercury in urine (uncorrected for specific gravity) in relation to time-weighted average exposure levels.

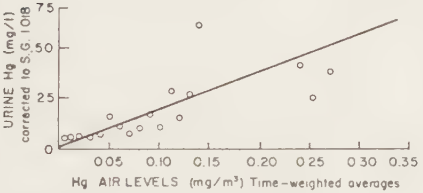


FIGURE 4. Concentrations of mercury in urine (corrected to specific gravity of 1.018) in relation to time-weighted average exposure levels.

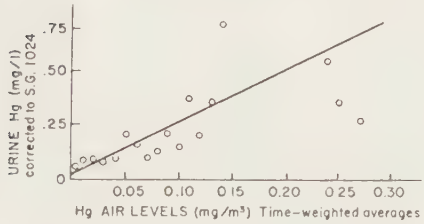


FIGURE 5. Concentrations of mercury in urine (corrected to specific gravity of 1.024) in relation to time-weighted average exposure levels.

ml, and urine levels of 0.22, 0.20, and 0.26 mg/liter for samples uncorrected for specific gravity, and those corrected to 1.018 and 1.024, respectively. All blood and urine data were also compared directly, using mean values for all individuals whose samples were analyzed by our laboratory. The resulting regression lines are plotted in Figures 6-8, and the relationship between blood and urine levels is identical with that deduced from the previous curves comparing TWA's and blood and urine. This rather remarkable agreement would appear to argue strongly for the validity of the air analyses and the TWA calculations, for the blood-urine relationships are completely independent of any air analyses, and yet the levels corresponding to threshold limit value (0.1 mg/m³) exposure are found to be exactly the same as those derived by comparison to air data. In addition, the actual values agree with the data of others, notably Goldwater, et al.[9] and Joselow, et al.,[10] so the probability is great that our blood, urine, and air data are accurate as well as internally self-consistent. A more detailed analysis of the data will appear in a separate paper dealing only with such analytical data.

## Medical Findings

Although diaphragm cell workers exposed only to chlorine failed to show well-defined evidence of adverse effects due to chlorine, mercury cell workers gave considerable evidence of showing some response to mercury.

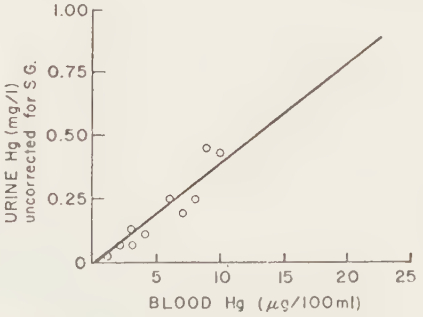


FIGURE 6. Relationship of concentrations of mercury in blood and in urine (uncorrected for specific gravity).

based on the medical findings. (Whenever exposure to mercury is indicated, it is understood that the actual exposure in virtually every case was to mercury plus a low background level of chlorine.) It was possible to compare the medical findings with three different variables: time-weighted average exposure to mercury, blood levels of mercury, and urinary levels of mercury.

All significance levels which follow were computed solely on the basis of time-weighted air exposures to mercury, and these computer-performed calculations determined significance mainly on the linearity of the dose-response relationship. In some instances, this relationship was very strong, in others it was good, but internally inconsistent, while with certain findings the incidence was substantially greater in the mercury-exposed workers than in the control subjects, but was not accompanied by a good dose-response relationship, and hence was computed to be of little significance.

The inconsistencies referred to must be kept in mind when considering our findings, and make it necessary in some instances to conclude that in spite of a relatively high correlation coefficient, certain relationships must be considered meaningful only with appropriate caution.

It may be useful to suggest the possible causes of these inconsistencies, of which the following are judged to be of greatest importance:

1. Normal variations found in any biological measurements, and largely of unknown origin.
2. Inconsistencies or bias unintentionally introduced into the study by the many physicians, industrial hygienists, and others who contributed the original data.
3. Failure to achieve a perfect control group exactly comparable in every way to the study group.
4. Unavoidable variations in the numbers of individuals exposed to the several ranges of mercury-in-air concentrations.
5. The influence of other factors in the work environment (such as extremes of temperature, noise, substances in the air other than chlorine and mercury) capable of exerting as much stress, or perhaps more, than either chlorine or mercury, but completely unknown so far as this study is concerned.

All medical findings can be classified as "signs" or "symptoms", referring either to

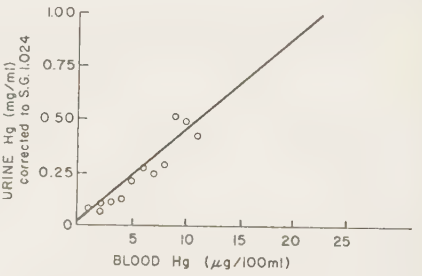


FIGURE 8. Relationship of concentrations of mercury in blood and in urine corrected to specific gravity of 1.024.

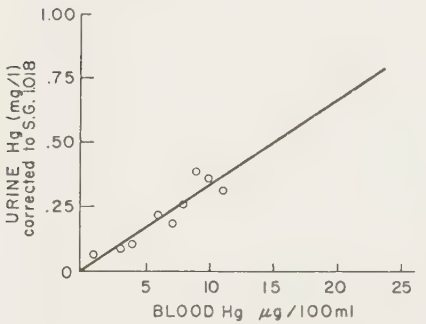


FIGURE 7. Relationship of concentrations of mercury in blood and in urine corrected to specific gravity of 1.018.

TABLE X

Medical Findings Related to Mercury Exposure
(Based on dose-response relationship)

| Findings | Basis | *t*-Value of Correlation Coefficient | Significant at *P*-Level |
|---|---|---|---|
| Loss of appetite | Symptom | 19.55 | 0.001 |
| Weight loss | Symptom | 16.51 | 0.001 |
| Object. tremors | Sign | 7.06 | 0.001 |
| Insomnia | Symptom | 6.98 | 0.001 |
| Shyness | Symptom | 4.54 | 0.001 |
| Diastol. blood pres. | Sign | −3.38 | 0.001 |
| Frequent colds | Symptom | 3.09 | 0.001 |
| Nervousness | Symptom | 2.79 | 0.005 |
| Diarrhea | Symptom | 2.41 | 0.020 |
| Alcohol consump. | Symptom | 2.33 | 0.020 |
| Dizziness | Symptom | 2.08 | 0.040 |

Symptoms—subjective findings, reported by patient.

Signs—objective findings, measured by physician or laboratory.

TABLE XI

Medical Findings not Related to Mercury Exposure
(Based on dose-response relationship)

| Findings | Basis | *t*-Value of Correlation Coefficient | Significant at *P*-Level |
|---|---|---|---|
| Palpitation | Symptom | 1.96 | 0.050 |
| Oropharyngeal | Symptom | 1.83 | 0.070 |
| W.B.C. count | Sign | -1.78 | 0.075 |
| Cardiopulm. illness | Symptom | 1.76 | 0.075 |
| Subj. tremors | Symptom | 1.50 | 0.150 |
| Neurological illness | Symptom | 1.23 | 0.200 |
| Cough | Symptom | 1.20 | 0.300 |
| Hematocrit | Sign | 1.16 | 0.300 |
| Abn. teeth-gums | Sign | 1.07 | 0.300 |
| Tooth decay | Symptom | -0.92 | 0.300 |
| Heart trouble | Symptom | -0.64 | — |
| Chest pain | Symptom | -0.53 | — |
| Systol. blood pres. | Sign | -0.45 | — |
| Fatigue | Symptom | 0.41 | — |
| Abn. EKG | Sign | 0.35 | — |
| Short breath | Symptom | 0.27 | — |
| Sputum | Symptom | 0.19 | — |
| Headache | Symptom | 0.17 | — |
| Constipation | Symptom | -0.17 | — |
| Anxiety | Symptom | 0.16 | — |
| Abn. chest x-ray | Sign | — | — |

some objective measurement made by the examining physician or laboratory, or else to the subjective response to questions asked by the physician in the course of taking a history. It is apparent that objective measurements are preferable to patient responses, particularly when a number of different physicians are performing the interrogations, but it was apparent that certain significant data could result only from patient-volunteered information. In the discussion which follows, the distinction between signs and symptoms is clearly made, and the importance of any particular finding can be judged accordingly. Table X lists significant medical findings presumed to be related to mercury exposure based on *t*-values of correlation coefficients. These findings reflect the dose-response correlation based on time-weighted air exposure to mercury levels of 567 workers exposed to mercury with known exposure levels (thus excluding the no-data group of 75). It should be noted that the computed data were based on calculations at each individual time-weighted exposure level of mercury, from $<0.01$ to 0.27 mg/m$^3$, and that the groupings of exposure ranges in the incidence tables are arbitrary, and meant only for simplicity of tabulation.

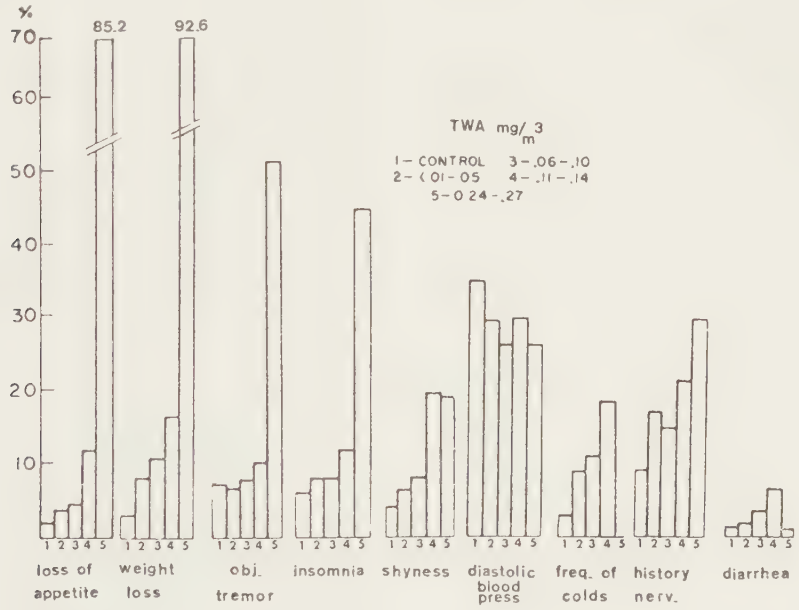


FIGURE 9. Percentage incidence of certain signs and symptoms related to exposure of workers to mercury.

Table XI lists findings which do not appear to be related to mercury exposure. The same data contained in Table X are presented graphically in Figure 9. The tendency of each finding to increase in incidence as the exposure levels of mercury increase is more clearly seen, and in addition, the extent to which the highest exposure group exerts influence on the overall findings may be noted.

*Loss of Appetite and Weight Loss*

Both of these subjective symptoms showed a very strong positive correlation with exposure to mercury. Substantiation with actual weight loss measurements as an objective sign was not performed. The low incidence of both of these symptoms among the control group and the increasingly higher incidence at increasing mercury exposure levels resulted in the significant linear correlation.

*Neuropsychiatric System*

A. Subjective symptoms: insomnia, shyness and nervousness appeared to be related to mercury exposure at high confidence levels, and dizziness at a somewhat lower confidence level. Other neuropsychiatric symptoms failed to show significant relationships, but it is noteworthy that all such symptoms were more prevalent in the mercury-exposed employees than in the control group. Although such an observation may be considered presumptive of mercury causation, the absence of good dose-response relationships must rule out any such conclusions, for numerous other environmental factors could be responsible for group differences.

Alcohol consumption was essentially identical in both the exposed and control groups, but within the exposed group there appeared to be evidence of increasing consumption related to the degree of exposure to mercury, $P = 0.02$, but much of the relationship derived from the highest exposure group (TWA 0.24-0.27 mg/m$^3$), who were unusual with respect to several findings. It is not possible to determine whether mercury exposure was the reason for the higher percentage of employees who consumed alcohol, nor whether mercury or alcohol was responsible for the loss of appetite and other symptoms reported with greater frequency by this group.

B. Objective Signs: in contrast to the poor dose-response relationship of subjective tremors reported by employees, objective tremors of fingers, eyelids and tongue were significantly related to mercury exposure levels ($P = 0.001$). The incidence of abnormal reflexes was the same among controls as among mercury workers as a group, but when exposure was greater than 0.10 mg/m$^3$, there was an appreciably higher incidence of abnormal reflexes, which resulted in an apparent dose-response relationship of questionable significance.

*Cardiorespiratory System*

A. Subjective Symptoms: cough, sputum production, chest pain, shortness of breath, palpitation and history of past cardiopulmonary illness did not show correlation with mercury exposure levels. These symptoms were more prevalent among smokers than non-smokers, but the difference was not statistically significant. Exposed workers did complain of more frequent colds, however, and the dose-response relationship was strong, with the important exception that the highest exposure group was again anomolous, and reported zero incidence with respect to colds, a somewhat improbable occurrence.

B. Objective Signs: diastolic (but not systolic) blood pressure showed a significant negative correlation ($P = 0.001$) with mercury, and it has been speculated that the known diuretic effect of mercury compounds may be related in some manner to this finding.

C. Chest x-rays were furnished by plant physicians, and were interpreted by a panel of Wayne State University radiologists. Eighty-four out of 313 x-rays from control subjects were interpreted as abnormal (26.8%), whereas 159 out of 622 x-rays from workers exposed to mercury were read as abnormal (25.6%). Thus, the incidence of abnormal x-rays among mercury cell workers was lower than that of the controls, but the difference is not statistically significant. There was no increased incidence of abnormal chest x-rays with increasing exposure to mercury.

There were 175 mercury cell workers who had one or more acute episodes of exposure to chlorine, and within this group, there were 48 abnormal x-rays (27.4%) compared to an incidence of 23.8% in those not acutely exposed. This difference was not statistically significant. Because of special concern with the chest x-rays of those who reported acute exposure episodes with chlorine, sufficient information was obtained to enable a classification of exposures as severe, moderate, or other, and although the findings do not relate directly to mercury exposure, there was no observable correlation, partly due to the small number of episodes classified as severe and moderate. A more complete accounting of these findings is presented in the paper dealing with chlorine effects, by Patil, et al.[1]

Most of the findings which caused an x-ray to be considered as abnormal are listed in Table XII, together with the incidence in the various exposure groups.

D. Pulmonary function tests revealed normal values in the vast majority of workers, whether mercury-exposed or controls, and there was no significant dose-response correlation when mercury exposure was compared to vital capacity, maximum breathing capacity and forced expiratory volume (one second and three seconds). Both diminished pulmonary function as well as abnormal chest x-rays appeared to be age related.

E. Abnormal EKG and past cardiopulmonary illness were unrelated to mercury exposure.

F. Smoking Habits: there was a higher percentage of cigarette smokers in the mercury-exposed population than in the control group, but no correlation with the degree of exposure.

*Oropharyngeal Disturbances*

A. Subjective Symptoms: oropharyngeal changes, other than those of teeth and gums, showed some dose-response relation, principally due to the high incidence within the highest exposure group. History of tooth decay showed no such relationship.

B. Objective Signs: abnormalities of teeth and gums were not dose-related, and, in fact, the controls showed a higher incidence of abnormal teeth than did the exposed workers.

*Gastrointestinal System*

Diarrhea was reported more frequently by exposed workers than controls, but again the highest exposure group was negative. The actual incidence was low in all groups, and it is doubtful if the relationship is meaningful. Constipation showed no relation to exposure.

## Correlation of Findings

In spite of the strong correlations between TWA's and blood and urine levels, the correlations between blood or urine levels and the medical findings were in general much weaker, and usually resulted in the definition of clear relationships only in the case of those findings which most strongly correlated with air levels. Thus, loss of appetite, weight loss, and objective tremors retain their apparent relationship to mercury intake, whereas most other findings do not. It would appear that properly computed TWA's are the physician's best index to estimating the probability of ap-

Table XII
Abnormal Chest X-ray Findings

| Time-Weighted Exposure mg/m³ | .01–.05 | .06–.10 | .11–.14 | .24–.27 | Entire Group | Control |
|---|---|---|---|---|---|---|
| No. of Indiv. in Group | 334 | 145 | 61 | 27 | 567 | 313 |
| *Findings:* | % Incidence | | | | | |
| Calcification (lung, hilar, multiple) | 19.2 | 17.3 | 36.1 | 7.4 | 19.9 | 19.5 |
| Opacities (large, small) | 2.1 | 0.0 | 1.6 | 0.0 | 1.4 | 2.6 |
| Tuberculosis | 3.0 | 0.7 | 4.9 | 0.0 | 2.5 | 2.9 |
| Abn. pleura or diaphr. | 1.5 | 3.5 | 1.6 | 3.7 | 2.1 | 4.8 |
| Bronchitis-Bronchiectasis | 0.3 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 |
| Blebs, emphysema, fibrosis | 0.6 | 0.0 | 0.0 | 0.0 | 0.4 | 1.6 |
| Cardiovascular abnormal | 1.8 | 0.0 | 0.0 | 0.0 | 1.1 | 1.3 |
| Histoplasmosis, granuloma indeterminate lesions | 0.9 | 2.1 | 1.6 | 0.0 | 1.2 | 1.3 |
| Other | 0.0 | 0.0 | 1.6 | 0.0 | 0.2 | 0.6 |

pearance of symptoms in exposed populations even though blood and urine readily predict the probable extent of employee exposure.

## Discussion

The physicians, industrial hygienists, and plant personnel within the chlorine industry who were responsible for initiating this study into the effects of mercury and chlorine were motivated by an awareness that mercury is a toxic substance and that over-exposure could be injurious to the employees in their industry. At the same time, the physicians in particular were also aware that, except for a few isolated cases of acute illness, there was virtually no evidence of chronic mercurialism in their employees when the classical symptomatology was sought. This is particularly noteworthy in view of the fact that physicians in this industry, and for that matter all physicians responsible for the health of workers in the chemical industry, tend to have an unusual awareness of toxicological hazards and their consequences. The entire chlorine industry is extremely health and safety conscious, and in fact, The Chlorine Institute is an organization devoted primarily to the safe manufacture and use of a recognized hazardous substance.

In view of these considerations, it is not surprising that the results of our study can be summarized by stating that in all major respects, the employees examined were in good health and in no way distinguishable from the control population with respect to such basic matter as impairment of the cardiorespiratory, gastrointestinal, or hepatorenal systems. Most measurable properties, including laboratory hematological data, chest x-rays, EKG, were found to be completely normal. The clinical picture that does emerge, however, is one of a group of workers who apparently exhibit a dose-related response to mercury exposure by evidencing somewhat higher incidences of a number of neuropsychiatric symptoms. Although the findings are largely based on subjective responses, the initial awareness that the central nervous system was expected to be affected by sufficiently high concentrations of mercury makes it logical to believe the findings. It is easy to see, however, that individuals displaying the symptoms found to be more prevalent with increased mercury exposure would be hardly distinguishable from other employees exhibiting similar symptoms for a variety of reasons unrelated to exposure to mercury.

The implications of the results of this study on the current threshold limit value of 0.1 $mg/m^3$ are to some extent dependent on matters of judgment rather than fact. The data indicate that with respect to most of the symptoms, the dose-response relationship does not exhibit sufficiently high incidence to warrant concern until the present threshold limit value is exceeded. In a few instances, such as weight loss and loss of appetite, there does not appear to be any threshold defined by our data, and in almost every instance the uncertainties attending the observations and the calculations of TWA's make it possible to express some reasonable doubt as to significance.

An international committee which recently met in Stockholm for the purpose of considering maximum allowable concentration (threshold limit value) values for mercury,[7] recommended that the threshold limit value for vapor be set at 0.05 $mg/m^3$, and that for other inorganic compounds it be set at 0.1 $mg/m^3$. Currently, the Threshold Limit Value Committee of the American Conference of Governmental Industrial Hygienists has made a similar reduction and has adopted a tentative value of 0.05 $mg/m^3$ for mercury vapor. The data presented here show no significant signs or symptoms in persons exposed to mercury vapor at or below a level of 0.1 $mg/m^3$. However, the data do raise a question regarding the adequacy of the safety factor provided by a TLV of this magnitude.

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# Effects of Exposure of Workers to Mercury at a Sodium Hydroxide Producing Plant

YASSIN M. EL-SADIK, M.D., and ABDEL-AZIZ EL-DAKHAKHNY, Ph.D.

THIS STUDY WAS CONDUCTED during the years 1969-1970 in a plant producing sodium hydroxide. This plant electrolyzes sodium chloride to obtain sodium hydroxide, using mercury in the electric cells. Workers in this plant are not permanently employed on one job; they may be shifted from a job where there is mercury exposure to another, where there is no mercury hazard.

Mercury had long been recognized to be general cellular poison and effective protein precipitant. Chronic mercurialism may appear after few weeks of exposure, or it may be delayed for much longer periods. Psychic and emotional disturbances are characteristic. Neurological disturbances also appear. Stomatitis is a common manifestation.[1] Theoretically high exposures produce high levels of mercury in urine as well as high frequency of intoxication.[2,3]

Bidstrup and associates stated that the excretion of more than 300 $\mu g$ of mercury in 24 hours is usually accompanied by symptoms and signs of chronic mercury poisoning.[4] Goldwater found that mercury excretion levels are not easily correlated to mercurialism.[5] High urine mercury levels frequently occur without evidence of poisoning, and poisoning may occur with relatively low urine mercury.[6,7] Ladd and his associates in 1966 found that the ranges and averages of urinary mercury of those with and those without clinical evidence of mercury poisoning did not demonstrate any remarkable difference.[8] Measurable amounts of mercury in urine follow occupational exposure, and such amounts may persist for as long as six years after exposure has ended.[9]

The aim of this study was to get data about the effects of short and long duration of exposure, and to determine the effects of mercury exposure interruption on the mercurialism clinical picture. Also a purpose of this study was to prove or disprove a relation of mercury levels in urine and saliva with the degree of mercury toxicity.

## Methods of Study

Each worker exposed to mercury in this plant was examined. The examination included personal and occupational history. Present and past history were obtained regarding mercurialism. Mouth, skin, heart, chest, abdomen, and central nervous system were examined. Blood pressure was measured and the readings were classified according to

TABLE I

Clinical Findings among Different Age Groups
(for those exposed to mercury for less than 6 months)

| Age group years | No. of workers | Average Hg in urine µg/liter | Average Hg in saliva µg | Complaint | | Past stomatitis | | Stomatitis | | Blood Pressure | | Tremors | | Behaviour change | | Deep reflex | | Hb% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | |
| 25-29 | 1 | 96 | 7.6 | 1 | 100 | 0 | 0 | 0 | 0 | V = 1 | 100 | 1 | 100 | 1 | 100 | 0 | 0 | 68 |
| 30-39 | 9 | 48 | 6.6 | 7 | 77.7 | 2 | 22.2 | 4 | 44.4 | N = 6<br>V = 1<br>H = 2 | 66.6<br>11.1<br>22.2 | 1 | 11.1 | 2 | 22.2 | 1 | 11.1 | 75 |
| 40-49 | 3 | 132 | 3.7 | 3 | 100 | 2 | 66.6 | 2 | 66.6 | N = 1<br>V = 1<br>H = 1 | 33.3<br>33.3<br>33.3 | 1 | 33.3 | 0 | 0 | 1 | 33.3 | 71 |
| Total | 13 | 71 | 6.0 | 11 | 84.6 | 4 | 30.8 | 6 | 46.2 | N = 7<br>V = 3<br>H = 3 | 53.9<br>23.1<br>23.1 | 3 | 23.1 | 3 | 23.1 | 2 | 15.4 | 73 |

N = normal blood pressure
V = vague blood pressure
H = hypertension

TABLE II

Clinical Findings among Different Age Groups
(for those exposed to mercury from 6 months to 3 years)

| Age group years | No. of workers | Average Hg in urine µg/liter | Average Hg in saliva µg | Complaint | | Past stomatitis | | Stomatitis | | Blood Pressure | | Tremors | | Behaviour | | Deep reflex | | Hb% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | |
| 20-24 | 2 | 184 | 4.1 | 2 | 100 | 1 | 50 | 2 | 100 | N = 2 | 100 | 1 | 50 | 1 | 50 | 0 | 0 | 72 |
| 30-39 | 10 | 112.0 | 3.6 | 8 | 80 | 6 | 60 | 7 | 70 | N = 4<br>V = 2<br>H = 4 | 40<br>20<br>40 | 5 | 50 | 5 | 50 | 2 | 20 | 70 |
| 40-49 | 1 | 4 | 8.6 | 1 | 100 | 0 | 0 | 1 | 100 | N = 1 | 100 | 0 | 0 | 1 | 100 | 0 | 0 | 68 |
| Total | 13 | 101 | 4.1 | 11 | 84.6 | 7 | 53.9 | 10 | 76.9 | N = 7<br>V = 2<br>H = 4 | 53.9<br>15.4<br>30.8 | 6 | 46.2 | 7 | 53.9 | 2 | 15.4 | 73 |

N = normal blood pressure
V = vague blood pressure
H = hypertension

the report of the Expert Committee on Cardiovascular Diseases and Hypertension.[10] Normal range are those readings below 140/90 mm Hg, abnormal range of hypertensive are those readings 160/95 mm Hg and above, intermediate readings are vague. Workers were asked, in an indirect way, about behavioral changes. Presence of behavioristic changes was considered according to the individual complaint, confirmed by his foreman and the personnel department. Hemoglobin was measured using Sahli Hemoglobinometer.

From each individual a urine sample was taken during his work to determine its mercury level. Saliva was collected from each one by asking him to spit all the saliva content in his mouth into a pyrex container. The volume of the saliva for each individual was about 5 ml. Mercury levels in urine and saliva were determined by the dithizone method.[11]

Environmental assessments for mercury levels in the working environment were carried out. Thirty-six samples were obtained on different days. The air volume for each sample was 50 liters. Mercury levels were determined by the same dithizone method used for both urine and saliva. The mercury levels in the environmental samples ranged from 0.072 to 0.88 mg/cubic meter of air with an

TABLE III

Clinical Findings among Different Age Groups
(for those exposed to mercury for 3 years and more)*

| Age group years | No. of workers | Average Hg in urine μg/liter | Average Hg in saliva μg | Complaint | | Past stomatitis | | Stomatitis | | Blood Pressure | | Tremors | | Behaviour | | Deep reflex | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | Hb% |
| 25-29 | 5 | 66 | 5 | 5 | 100 | 4 | 80 | 4 | 80 | N = 3<br>V = 2<br>H = 0 | 60<br>40<br>0 | 5 | 100 | 5 | 100 | 3 | 60 | 73 |
| 30-39 | 17 | 64 | 5.8 | 16 | 94.1 | 4 | 23.5 | 14 | 82.4 | N = 8<br>V = 5<br>H = 4 | 47<br>29.4<br>23.5 | 14 | 82.4 | 12 | 70.6 | 9 | 52.9 | 75 |
| 40-49 | 4 | 39 | 6.4 | 4 | 100 | 3 | 75 | 4 | 100 | N = 1<br>H = 3 | 25<br>75 | 4 | 100 | 3 | 75 | 2 | 50 | 76 |
| Total | 26 | 61 | 5.7 | 25 | 96.2 | 11 | 42.3 | 22 | 84.6 | N = 12<br>V = 7<br>H = 7 | 46.2<br>26.9<br>26.9 | 23 | 88.5 | 20 | 76.9 | 14 | 53.8 | 75 |

*No case had been exposed to Hg for a duration more than 10 years.
N = normal blood pressure
V = vague blood pressure
H = hypertension

average of 0.3 mg/cubic meter. (The TLV for mercury is 0.1 mg/cubic meter).[12]

## Results

Tables I, II and III show the clinical findings, average mercury level in urine in micrograms per liter, average mercury in saliva samples and the percent hemoglobin among different age groups of workers exposed to mercury, and according to their work duration.

Of the 13 workers exposed to mercury for less than six months, 11 had complaints and nine of these complained of neurasthenia (Table I). Similarly of 13 workers with exposures from six months to three years, 11 reported complaints and 10 of these were neurasthenia (Table II). Table III shows that 26 workers had exposures of three years or more with only one worker not having complaints and 21 complaining of neurasthenia. All cases of tremor noted among these workers were affecting the hands. Hypertension was noted only in workers over 30 years old.

Tables IV and V present data from the examination of workers who had not been exposed to mercury for about three months to four years prior to this examination but who had had previous mercury exposures of less than three years or more than three years. In each of these two groups two workers reported neurasthenia.

The results of examinations of the unexposed control group are given in Table VI. The one case of stomatitis in this group also had tender gums and few ulcers on the cheek. Two cases of tremor of the hands and one case of increase in deep reflex response were noted. These three cases and those having hypertension were all 40 or more years of age.

Blood pressure values for all groups are shown in Table VII. Comparing the exposed with controls of similar age, it is noted that 32 (48.5%) of the exposed persons had normal blood pressures while six of seven controls (85.7%) were normal. Vague readings were met only among the exposed groups. Comparison of hypertensive cases did not show a statistically significant difference between exposed and controls ($x^2 = 1.588$).

## Discussion

Exposure to mercury causes different symptoms and signs. Mercury neurasthenia is a main complaint among cases suffering from mercurialism. The duration of exposure affects the severity of mercury toxicity, and the number of cases having stomatitis, change in behavior, tremors and increase response

TABLE IV

Clinical Findings among Different Age Groups
(for workers previously exposed to mercury for 3 years or less)

| Age group years | No. of workers | Average Hg in urine μg/liter | Average Hg in saliva μg | Complaint | | Past stomatitis | | Stomatitis | | Blood Pressure | | Tremors | | Behaviour | | Deep reflex | | Hb% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | |
| 25-29 | 2[a] | 98 | 6.2 | 2 | 100 | 0 | 0 | 2 | 100 | N = 1<br>V = 1<br>H = 0 | 50<br>50<br>0 | 1 | 50 | 1 | 50 | 0 | 0 | 64 |
| 30-39 | 7[b] | 73 | 3.7 | 7 | 100 | 2 | 28.6 | 3 | 42.9 | N = 5<br>V = 0<br>H = 2 | 71.4<br>0<br>28.8 | 2 | 28.6 | 1 | 14.3 | 1 | 14.3 | 72 |
| Total | 9 | 79 | 4.3 | 9 | 100 | 2 | 22.2 | 5 | 55.5 | N = 6<br>V = 1<br>H = 2 | 66.6<br>11.1<br>22.2 | 3 | 33.3 | 2 | 22.2 | 1 | 11.1 | 70 |

[a] One worker had no exposure to Hg for three months or less prior to examination.

[b] Two cases had no exposure to Hg for three months or less prior to examination.

N.B. Remaining workers were free of exposure to Hg for more than three months and not more than four years prior to examination.

N = normal blood pressure
V = vague blood pressure
H = hypertension

TABLE V

Clinical Findings among Different Age Groups
(for workers previously exposed to mercury for 3 years and more)[a]

| Age group years | No. of workers | Average Hg in urine μg/liter | Average Hg in saliva μg | Complaint | | Past Stomatitis | | Stomatitis | | Blood Pressure | | Tremors | | Behaviour | | Reflex | | Hb% |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | |
| 25-29 | 2 | 32 | 1.4 | 2 | 100 | 0 | 0 | 0 | 0 | N = 0<br>V = 0<br>H = 2 | 0<br>0<br>100 | 0 | 0 | 0 | 0 | 0 | 0 | 81 |
| 30-39 | 2[b] | 46 | 3.8 | 2 | 100 | 1 | 50 | 0 | 0 | N = 1<br>H = 1 | 50<br>50 | 0 | 0 | 1 | 50 | 0 | 0 | 73 |
| 40-49 | 3[c] | 67 | 3.7 | 2 | 66.6 | 2 | 66.6 | 3 | 100 | N = 1<br>V = 1<br>H = 1 | 33.3<br>33.3<br>33.3 | 2 | 66.6 | 0 | 0 | 0 | 0 | 72 |
| Total | 7 | 51 | 3.1 | 6 | 85.7 | 3 | 42.9 | 3 | 42.9 | N = 2<br>V = 1<br>H = 4 | 28.6<br>14.3<br>57.1 | 2 | 28.6 | 1 | 14.3 | 0 | 0 | 75 |

[a] There are cases exposed to Hg for more than 10 years.

N.B. Remaining workers had no exposure to Hg for three months to more than four years prior to examination.

[b] One case had no exposure to Hg for three months or less prior to examination.

[c] One worker had no exposure to Hg for three months or less prior examination.

N = normal blood pressure
V = vague blood pressure
H = hypertension

of deep reflexes increased with the duration of exposure (Tables I, II, III).

The value of mercury level in urine is not a criterion to determine the degree of toxicity, as the average mercury level in urine among the controls was 34 μg/liter, while cases with 4 μg/liter had manifestations of mercurialism (Table II). Not only that but the average mercury levels in urine for those exposed up to three years is less than that for those exposed three years or more. This observation coincides with some previous studies.[5-8] No lower mercury level in urine can be taken to exclude the presence of mercurialism. This is opposed to findings given by Bidstrup and associates.[4]

TABLE VI

Clinical Findings among Different Age Groups without Mercury Exposures

| Age group years | No. of workers | Average Hg in urine µg/liter | Average Hg in saliva µg | Complaint | | Past stomatitis | | Stomatitis | | Blood Pressure | | Tremors | | Behaviour | | Reflex | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | Hb% |
| 25-29 | 2 | 26 | 0 | 2 | 100 | 0 | 0 | 0 | 0 | N = 2 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 69 |
| 30-39 | 2 | 34 | 0 | 2 | 100 | 1 | 50 | 1 | 50 | N = 2 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 81 |
| 40-49 | 3 | 32 | 0 | 2 | 66.6 | 0 | 0 | 0 | 0 | N = 2<br>V = 0<br>H = 1 | 66.6<br>0<br>33.3 | 1 | 33.3 | 0 | 0 | 1 | 33.3 | 74 |
| 50-59 | 3 | 40 | 0 | 3 | 100 | 0 | 0 | 0 | 0 | N = 1<br>V = 1<br>H = 1 | 33.3<br>33.3<br>33.3 | 1 | 33.3 | 0 | 0 | 0 | 0 | 58 |
| Total | 10 | 34 | 0 | 9 | 90 | 1 | 10 | 1 | 10 | N = 7<br>V = 1<br>H = 2 | 70<br>10<br>20 | 2 | 20 | 0 | 0 | 1 | 10 | 70 |

N = normal blood pressure
V = vague blood pressure
H = hypertension

TABLE VII

Blood Pressure among Workers Exposed to Mercury and among the Control Group*

| | Normal Blood Pressure | | Vague Blood Pressure | | Hypertension | | |
|---|---|---|---|---|---|---|---|
| Age Group Years | No. | % | No. | % | No. | % | Total |
| Exposed 20-24 | 2 | 100 | – | – | – | – | 2 |
| Control 20-24 | – | – | – | – | – | – | – |
| Exposed 25-29 | 4 | 40 | 4 | 40 | 2 | 20 | 10 |
| Control 25-29 | 2 | 100 | – | – | – | – | 2 |
| Exposed 30-39 | 24 | 53.3 | 8 | 17.8 | 13 | 28.9 | 45 |
| Control 30-39 | 2 | 100 | – | – | – | – | 2 |
| Exposed 40-49 | 4 | 36.4 | 2 | 18.2 | 5 | 45.5 | 11 |
| Control 40-49 | 2 | 66.6 | – | – | 1 | 33.3 | 3 |
| Exposed 50-59 | – | – | – | – | – | – | – |
| Control 50-59 | 1 | 33.3 | 1 | 33.3 | 1 | 33.3 | 3 |
| Total exposed except 20-24 age | 32 | 48.5 | 14 | 21.2 | 20 | 30.3 | 66 |
| Total control except 50-59 age | 6 | 85.7 | – | – | 1 | 14.3 | 7 |

*Includes workers with past or current exposures.

Mercury level in urine increased with the duration of exposure up to three years, but for those exposed for three years or more, there is a drop in the mercury level in urine. Where the exposure is as high as 0.3 mg/m$^3$, the mercury level in urine may denote severity of toxicity among groups, but not among individual cases, exposed up to three years. The disproportionate mercury level in urine, in relation to the duration of exposure can be explained, as the body tries to eliminate mercury via the urinary system. The kidneys are exposed to the cellular poisoning nature of mercury and by the increase of duration of exposure the kidneys are more affected and likewise the mercury concentration in the urine. This is supported by what had been mentioned before about the toxic effect of mercury on the kidney tissues.[1] For an exposure 0.3 mg/m$^3$, three years exposure can affect the power of kidneys' excretion.

So we can say that mercury level in urine is important as regard early exposures of in-

dividuals, but may be of no value for exposures of long duration.

Mercury in saliva can be of diagnostic importance in case of mercurialism. No individual among the controls showed mercury in saliva, while all mercurialism cases showed mercury. This statement to be accepted, emphasizes the need for studying the effect of dental amalgam filling on mercury excretion in saliva. We can add that mercury level in saliva does not correlate with the degree of toxicity or the duration of exposure.

By comparing the number of cases having stomatitis, tremors, increase in response of deep reflexes and change in behavior among those exposed to mercury, with those previously exposed for a similar duration, we can observe that interruption of exposure to mercury improve all the previously mentioned signs. So this denotes that such clinical findings are due to mercury, and interruption of exposure to mercury improves the clinical picture of mercurialism. Also interruption of exposure affects the rate of mercury excretion in urine and saliva. Stomatitis, tremors and changes in behavior developed among any age, but increase in response of deep reflexes only affects those aged 30 years or more. Mercury has no effect on the hemoglobin concentration. Although hypertensive cases among the exposed are not significantly higher compared with the controls of similar age groups, we cannot ignore mercury as playing a role in the higher tendency in hypertension among the exposed. This may be favored by the tendency to a decrease in normal readings and increase in vague readings with the increase in duration of exposure, if we compare those exposed to mercury up to three years with those exposing for three years or more.

Also comparing the number of hypertension cases among those exposed for a duration less than three years, with that among those exposed for three years or more, there is a tendency to an increase with the increase in duration of exposure.

If we compare hypertensive cases among the currently exposed workers and those previously exposed for similar periods but not currently exposed, there is no statistically significant difference, denoting that hypertension, if it is due to mercury exposure, is a permanent effect. Hypertension due to mercury exposure had been reported in an unusual case involving accidental penetration of metalic mercury into the subcutaneous tissues. Also Barni and his associates mentioned that mercury poisoning causes arteriosclerosis mainly affecting the kidneys which results in hypertension.

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# Significance to health of mercury used in dental practice: a review

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During the past year, the public press and other news media, which provide popularized versions of reports on science and health, have carried many articles emphasizing hazards of mercury poisoning and mercury pollution of rivers and lakes. Current interest has been stimulated also by congressional hearings and a special television program. Several factors have contributed to the present concerns.

- There has been widespread publicity of several dramatic instances of poisoning, including two in Japan in which more than a hundred persons were killed or subjected to serious neurological damage from consumption of fish taken from areas polluted with mercury from industrial wastes. Birth defects also occurred.
- Recent research has provided a better understanding of the mechanisms whereby the mercury discharged into streams and standing water may be solubilized by biological actions and concentrated by a factor of 3,000 or more so that dangerous levels of mercury, especially as methyl mercury, may be found in fish. This subject has been summarized in a recent editorial.[1]

■ Several instances of extensive spillage of mercury into surface waters have been identified and steps have been taken to reduce contamination from these sources.

The total consumption of mercury in 1969 in the US has been estimated to be at least 2,740,000 kg (more than 6 million lb).[2] Largest quantities were reported to have been used in the production of electrolytic chlorine, in electrical apparatus, and in paints, although substantial amounts of mercury preparations have been used also in agriculture for treating seed grains. Almost 100,000 kg (approximately 210,000 lb) of mercury is believed to be used each year in dentistry. Nearly all of this is mercury metal used in the preparation of amalgam restorations. On the average, therefore, each practicing dentist in this country uses more than 1 kg, or about 2.5 lb, of mercury each year.

Dental procedures have not been significantly implicated in the current concerns for mercury exposure. It is important, however, for the profession again to evaluate its procedures as new information becomes available on the physiological significance of the increased exposure to mercury from all sources. The dentist will be concerned, therefore, for both the "body burden" of mercury that may be derived from food and other sources as well as that accumulated from exposure to mercury in his office.

The report of an international committee[3] is the basis for the following classification of mercury and its compounds in the order of their decreasing toxicity: methyl and ethyl mercury compounds, mercury vapor, and inorganic salts of mercury and a number of organic forms, such as phenyl mercury salts.

Exposure to mercury metal, particularly its vapor, is the most significant factor in the consideration of the toxic potential of mercury as used in dentistry.[4] Methyl mercury has no direct significance to dentistry, but it is of incidental interest because of its formation from industrial mercury wastes and because of the subsequent contamination of fresh water fish that may be used as food.

The use of inorganic salts of mercury in the dental office has been abandoned because of the availability of more effective and less hazardous agents. Organic mercurials as disinfectants play a minor role in dental practice also because of the availability of more effective agents.

For convenience, the remainder of this review will deal with three considerations: the exposure of the patient with special reference to amalgam restorations that remain in the teeth for a long time; the long-term exposure of dental personnel and suggestions for minimizing mercury hazards; and the methods of disposing of mercury residues and waste amalgam to avoid any additional contamination of the environment.

## Exposure of patients

Frykholm,[4] in placing 4 or 5 amalgam fillings in each of 5 patients, used a radioisotope of mercury in preparing the amalgam. On the fifth day after the placement of the fillings, the urinary excretion of tagged mercury had gradually increased for each individual and averaged 2.5$\mu$g/liter. After that, the tagged mercury level dropped to zero, in each case, by the seventh or eighth day. The amalgam fillings containing radioactive mercury were all removed to avoid further exposure to radiation when the urinary tagged mercury reached zero. On the day after removal, the urine level of tagged mercury increased to 5$\mu$g/liter and then dropped to zero in two days. This type of evidence shows how little mercury is contributed to the body by dental amalgam.

With use of radioactive mercury, $^{203}$Hg, Frykholm and Odeblad[5] studied the penetration of mercury through dental hard tissues. These observations both in vitro and in vivo in human, monkey, and dog teeth reveal some tenths of a microgram of mercury in the pulp tissue of individual teeth. No tagged mercury was detected in organs remote from the oral region. Massler and Barber[6] reported spectrographic analyses of dentin that revealed the

presence of mercury, silver, tin, copper, and zinc. They also observed that zinc oxide-eugenol and zinc phosphate cement bases blocked these metallic penetrations. Söremark and others[7] used the isotope $^{197}Hg$ in amalgam and observed that it penetrated dental tissue. They too observed the blocking of this penetration by zinc oxide-eugenol cement. In addition, they reported that cavity liners prevent this efflux of metal ions into the dentin.

Absorption of mercury by individuals receiving amalgam restorations has been the subject of additional studies.[8-10] Determination of urinary mercury has been used for most of these observations. Present evidence[11] suggests that mercury is detectable in 20% of normal individuals. Concentrations in these 20% normal urines vary from 0.5 to 100 $\mu$g/liter, or more, but rarely is the concentration greater than 50$\mu$g/liter. "Normal" mercury level in urine refers to the amount found in persons who have had no known occupational, medicinal, or other obvious source of exposure.

An analysis of many urine mercury evaluations collected over several years during extensive studies[10] of individuals in industrial environments disclosed that 80% contained no detectable (less than 0.5$\mu$g/liter) mercury in their urine. No record was kept of the number of these individuals who had amalgam fillings. Because a large proportion of people in these studies received good dental care, it can be estimated that considerably more than the 20%, who had mercury in their urine, had amalgam fillings. The authors,[10] therefore, thought old amalgam fillings contribute little, if any, mercury to the body. This information stimulated a further study that involved 119 individuals not currently undergoing dental treatment. Only six of the 119 showed detectable mercury in their urine, and one of the six was taking a mercurial diuretic. Twenty-four others who were having dental amalgam restorations placed were tested for mercury in the urine before and after the amalgams were placed. There was little or no difference in the urinary mercury content before and after placement of amalgam restorations.[10]

Several individuals in other studies showed slight mercury elevations after dental appointments when no amalgams were placed. To test the theory[10] that this mercury could come from a chemical instrument or skin sterilizing solution, three volunteers permitted their gingiva to be painted with a 1:1 aqueous dilution of Nitromersol ($C_7H_5HgNO_3$). All three had no mercury in their urine before exposure and all three showed mercury during the 24-hour period after exposure. Hoover and Goldwater[10] concluded that, because there are other sources of mercury in the dental office and the total of all sources is not significant, dental amalgams do not appear to be an important source of mercury absorption and excretion.

In an industrial setting where workers breathed air at the threshold limit value (TLV) of 0.1 mg of $Hg/m^3$ of air,[12] sustained for an eight-hour day, their kidneys were able to clear the mercury from the blood. Where the mercury vapor had been maintained in excess of the TLV for a period of time, mercurialism symptoms were found in some workers. At the onset of symptoms, the urine mercury level dropped for each person. This lowering of the urine mercury level accompanying the onset of symptoms has been observed in many similar studies. This onset of symptoms accompanying the drop in urine mercury content is attributed to the injury of the kidneys caused by the high mercury levels. The mercury level in the urine, therefore, is not dependable for toxic determinations.[9]

Recently, Joselow and others[13] measured the concentration of mercury in saliva from the parotid gland as collected with a vacuum cup at the opening of Stensen's duct. The blood mercury level correlated more closely with the salivary level than with the urinary level. Further studies may disclose that the mercury levels in parotid saliva are a reliable indicator.

Mercury in dental amalgam has contributed to allergic responses in the form of dermatitis in susceptible individuals.[4,14–16] These responses cleared on removal of the amalgam fillings. The allergic reactions to mercury from amalgam restora-

tions were on patients, almost without exception, who had been sensitized by previous treatment with mercurial drugs.[4,16] When one considers the millions of patients who have amalgam restorations placed each year, one can see that the proportion of allergic reactions is indeed low.

The danger to the patient of inhalation of mercury vapor has been considered remote because of short periods of exposure to the vapor. Vapor levels have been studied repeatedly and until recently[17,18] found to be in the TLV. This TLV may be lowered to 0.05 mg/m$^3$ if the proposal of the American Conference of Governmental Hygienists is adopted. In this light, the two recent reports[17,18] take on added significance because they show that there is a potential danger to the dentist and coworkers who occupy the dental office for prolonged periods. This will be discussed in relation to the environment of dental personnel.

## Exposure of dental personnel

The second area of interest is the potential hazard for dental personnel through their exposure to mercury used in the office. Two methods of estimating exposure are direct measurement of vapor in the air and indirect measurement through analysis of hair, nails, and urine. Herbst and others[19] and Frykholm[20] reported observations of urinary mercury. Nixon and Smith[21] reported on mercury in hair and nails of personnel working in dental offices. In both studies, the mercury level or concentration was greater than that observed for controls not routinely in the dental environment. The urine mercury of eight dentists[19] averaged 6.3$\mu$g/liter and that of 13 dental assistants (average of 17 years in dentistry) averaged 14.3$\mu$g/liter. The room air concentration of mercury vapor for each of these was less than the TLV of 0.1 mg/m$^3$. These individuals also may have frequent direct contact with mercury. Although the safe level for mercury in urine or in hair and nails is not known, it is desirable to keep these concentrations as low as

possible.

Perhaps the greatest amount of work has been concentrated on mercury vapor in dental offices. The two aforementioned studies[17,18] indicate that some offices do have mercury vapor levels in excess of the recognized acceptable level of 0.1 mg/m$^3$. These recent reports were based on findings made with more sensitive methods of analyzing air for mercury, and they contrast with earlier studies[22,23] that indicated no hazards. Several factors, in addition to more accurate detection, may have led to this change: carelessness, because the teaching for years has been based on the premise of little danger; redecoration of offices to include rugs in the operating room; and use, without due caution, of mechanical amalgamators, ultrasonic condensers,[24,25] and high-speed rotary cutting instruments.[26]

When mercury is spilled, it disperses into small droplets that increase its surface area and its tendency to vaporize. Mercury has significant volatility as revealed by an equilibrium concentration of about 2 mg/m$^3$ of air at 25°C. Its vapor pressure increases rapidly as temperature rises; about an eightfold increase occurs as the temperature rises from 20°C to 50°C. It is fortunate that equilibrium concentrations, which are physiologically intolerable, are not reached under usual working conditions. Several factors, however, can cause the vapor to reach potentially hazardous levels. Some of these factors are: dispersion of mercury into small droplets; movement of droplets, especially by the type of agitation that tends to produce aerosols; use of heated or heating devices near the mercury; and poor ventilation of the working space.

In addition to the hazards of mercury vapor is the danger of external contact with metallic mercury or mercury compounds. Mercury compounds, whether ionic or nonionic, are absorbed through the skin and mucous membranes.[9] For example, one gram of a 10% ammoniated mercury ointment used daily for one month caused an increase of 500$\mu$g of mercury in the excreta during that time.[9]

## Environment

The third principal area of concern is contamination of the environment through disposal of waste amalgam. Each time a dentist places an amalgam restoration, he must prepare an excess to ensure sufficient amalgam to construct the restoration properly. The amalgam removed during shaping of the restoration is usually rinsed from the mouth or aspirated, and it should be caught in a strainer or trap in the waste drain. Unfortunately, appropriate solid waste traps are not present on many cuspidors, aspirators, and evacuators. They should be installed. The dental assistant should recover this scrap and place it in a covered container with the excess amalgam that is prepared but not used. A survey of several geographical areas in the US disclosed that recovery of some amalgam scrap is widespread. The Department of Defense recovered from the Army, Navy, and Air Force dental corps 42,000 pounds of scrap amalgam in 1969.[27] Dentists surveyed in Washington, DC, parts of Ohio, Minnesota, and California save their excess amalgam for collecting groups who contribute the money from the sales of the scrap to charitable organizations. Because at least 50% of the scrap is mercury and 25% is silver, it is quite valuable.

## Physiological response

Mercury in the body has many pharmacological actions, such as the inhibition of urease, invertase, and other enzymes carrying SH groups and the influencing of bioelectric phenomena by altering transmembrane potentials and by blocking nerve conduction.

The most reliable early, objective symptom of chronic mercurialism in man is a fine tremor observable in handwriting or other attempts to perform fine motions.[9] Accompanying these slight muscular tremors may be a loss of appetite, nausea and diarrhea, and a variety of subjective symptoms.

Acute mercurial poisoning generally occurs after prolonged chronic mercurialism or on sensitization. The sudden onset of symptoms, following a protracted chronic condition, may be due to the development of a sensitivity to mercury. This had been proposed as the cause of the sudden onset of symptoms and ultimate death of a dental assistant who for 20 years regularly compounded dental amalgam.[28]

Data on age at death of dentists compared to that of the same age group in the general male population (1961-1966) showed no difference in longevity (71.2 years).[29] Such data for dental assistants are not available.

Many reports of verified sensitivity to the mercury in dental amalgam are in the literature.[4,14–16] The usual response, after removal of a vapor or inorganic mercury contamination, is a complete reversal of the symptoms.[30] This occasional development of sensitivity to mercury emphasizes the need for good mercury hygiene, not only to prevent toxic reaction but also to prevent sensitization.

## Recommended mercury hygiene

The first step in any hygiene program is the recognition of a hazard. Because mercury, its compounds, and its vapor are potential sources of poisoning, continuous care must be exercised.

Although penetration into and through the hard tissues of the teeth is not considered a hazard, it is easily prevented with a cavity liner or an intermediate base. The metallic ions that enter the dentin are credited with the discoloration around silver amalgam fillings. The prevention of penetration of metallic ions into the dentin and the aid in sealing of the space between the amalgam-tooth interfaces are sufficient reasons to require the routine use of a liner under all amalgam fillings.

Mercury vapor is hazardous to dental personnel with prolonged exposure, but it is not hazardous to the patient who is subjected to only brief peri-

ods of exposure. Vapor contamination results principally from spills and the resultant dispersion of many small droplets. Both the mercury containers and the handling area should be specially designed. The containers should be nonbreakable, tightly covered, and easy to handle. A tray or some other surface suitable for containing inadvertent spills should be used for the working or handling area. The containers and working areas should be placed to avoid heat sources, such as radiators, sterilizers, or other heating devices. In addition to controlling the temperature, many units exchange the air and clear transient high mercury vapor concentrations. The use of air conditioners affects the mercury vapor level in the office. Adequate ventilation is extremely important in reducing the hazard of mercury vapor.[17,31] If the air is recirculated after passing through a filter, the filters must be cleaned or replaced frequently.[18]

The use of carpets in the office area for comfort and appearance has added an almost impossible obstacle to the recovery of mercury spills. This factor intensifies the need to prevent any mercury from falling on the floor. If a conventional vacuum cleaner is used on such a contaminated surface, the metallic mercury will not be picked up.[17]

The recovery of spilled mercury is best carried out with suction immediately after the spill, before any additional dispersal or droplets. The existing oral evacuation equipment can be temporarily modified with an extension to a vessel containing some water and serving as a trap. A short tube bearing a tip similar to a medicine dropper should lead to the water trap. The small opening on the tip is held to each droplet to be aspirated into the trap. Most of the spill can be recovered in this manner. Droplets that cannot be reached without causing an intolerable upheaval can be dusted with sulfur powder,[32] or covered with a water slurry of sulfur and calcium oxide.[33] The sulfide layer will prevent vaporization as long as the droplets are not agitated. Rugs that have become grossly contaminated by repeated spills should be replaced by a smooth surface floor covering.[17]

The recovery of scrap amalgam is widespread. No doubt some scrap, however, does go into the waste basket and into an incinerator. If a large amount of such amalgam were disposed of in an incinerator, it might become an air pollutant. Consequently, it is wise to encourage complete cooperation in the collection of scrap to reduce even further this potential source of contamination.

Some capsules used with mechanical amalgamation permit the discharge of fine droplets of mercury during mixing. The recently introduced threaded cap capsules and preproportioned sealed capsules are excellent for containing the mercury during mechanical mixing.

Another potential source of contamination of room air with mercury vapor is the removal of old dental amalgam fillings with high-speed rotary instruments. The operator must use a water coolant to avoid developing sufficient heat to vaporize mercury and to minimize the dispersion of fine particles. The use of a water spray and a high volume evacuator (properly trapped) will hold this hazard to a minimum.

Nossek and Seidel[24] reported that a spray of partially amalgamated mercury is formed during compaction of amalgam with an ultrasonic condenser. They analyzed the room air during the compaction and recorded no elevation of the mercury vapor concentration; they concluded that this is not hazardous. Chandler and others[25] observed and reported this same spray of mercury droplets and also recorded no elevation of mercury vapor concentration. They concluded, however, that this dispersal of the mercury droplets, many of which are less than 5$\mu$m in diameter and, consequently, can be aspirated deep into the lungs, is a serious potential hazard and, in any event, is extremely poor mercury hygiene.

Heat is used in the preparation of copper amalgam. High mercury vapor concentrations have been reported[4] in offices in which copper amalgam is used. Because there is no compelling reason to use the copper amalgam, it can be eliminated as a filling material. In any event, the sale of this material is reported to be negligible in the US.

Because mercury and some mercury compounds may be absorbed from body surfaces, all personnel should avoid direct contact with these materials. If contact is made with metallic mercury or mercury compounds, the area affected should be washed with soap and water to reduce the time that the microscopic particles cling to the skin.

Because some individuals may become sensitized after varying times of exposure to mercury or its compounds, it is especially important for dental personnel to learn of the potential hazards early in their professional education, not only to develop good habits but also to prevent the occasional sensitization. Should this happen, one must completely avoid future contact with mercury or its compounds.[4,18]

## Summary

Mercury is a potential hazard in the dental office as in any other location where the metal or its compounds are used extensively.

Radioactive mercury, used in preparing dental amalgam for restorations, was recovered in extremely small quantities in the urine, for up to eight days after placement of the fillings. Other studies, involving old amalgam fillings, also show little or no contribution of mercury to the body by dental amalgams.

Mercury vapor is dangerous when breathed in sufficient concentrations for a long time. Two recent studies indicate levels of mercury vapor concentration in excess of the currently accepted threshold limit value in some dental offices. This increase over that observed in previous studies should be a matter of concern for personnel working continuously in these offices. Several factors contribute to this higher mercury vapor concentration: carelessness, because teaching for years has been based on the premise of little danger; redecoration of offices to include rugs; and the use, without due caution, of mechanical amalgamators, ul-

trasonic condensers, and high-speed rotary cutting instruments.

Scrap amalgam is usually collected and salvaged, and it should not contribute significantly to the environment.

Chronic mercurialism results from long-term, continuous exposure by workers who are either contacting mercury and its compounds or breathing mercury vapor in excess of 0.1 mg/m$^3$ of air. Acute symptoms generally follow a long-term exposure because the individual becomes sensitized. Urinalysis tests for mercury are reliable for screening evaluations to discover exposure, but because the urinary mercury level drops with the onset of symptoms of mercurial poisoning, this test is not dependable to determine toxic reactions. One recent study indicates that mercury level determinations on parotid saliva may be more reliable than determinations on urine mercury levels.

The most dependable early symptom of chronic mercurialism is a slight muscular tremor. This is observed in handwriting or other fine motor movements. On removal of the mercury contaminant, the symptom generally subsides.

For good mercury hygiene, the user should:

- Store mercury in unbreakable, tightly sealed containers;
- Confine any inadvertent spills to an easily cleaned tray or similar work space;
- Design the dental offices with seamless flooring that extends two inches up each wall;
- Coat the cavity surfaces with a varnish or a base;
- Salvage all amalgam scrap and keep it in a tightly covered container;
- Work in well-ventilated spaces;
- Eliminate the indiscriminate use of mercury-containing solutions;
- Avoid the heating of mercury or amalgam;
- Use water spray and suction when grinding dental amalgam:
- Use conventional dental amalgam compacting procedures, manual and mechanical, but should not use ultrasonic amalgam condensers.

This review of the literature does not suggest a significant hazard to the patient in the use of amalgam restorations. The risks to dental personnel, however, as emphasized by Frykholm,[4] must neither be exaggerated nor neglected.

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