ery studies show the method to be quite acceptable. A comparison of the mercury data using this method and the FDA Official Method shows the two methods to be in agreement within experimental error.

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Submicrogram Level Determination of Mercury in Seeds, Grains, and Food Products by Cold-Vapor Atomic Absorption Spectrometry

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A cold-vapor atomic absorption spectrometric technique has been employed for the determination of total mercury in several biological samples such as seeds, grains, fruits, vegetables, fish, and meat. The biological sample was digested with a mixture of concentrated sulfuric and nitric acids. Organically bound sample mercury was converted to the divalent form by a 3–5 hr 60° digestion with sulfuric and nitric acids using erlenmeyer flasks. Sample digestates were subsequently reduced with stannous chloride for determination by recirculating cold-vapor AAS down to 0.01 μg. The maximum amounts of mercury, 9.45 and 7.15 ppm, were found in hothouse tomato seeds and treated wheat seeds, respectively.

In the United States, in spite of the past extensive use of mercury and mercury compounds, little has been known about the extent of contamination of food and of other biological material (Basely, 1971; Newsome, 1971; Bache and Lisk, 1971; Pillay et al., 1971; Rottschafer et al., 1971; Moffitt and Kupel, 1970; Kalb, 1970; Fishman, 1970; Mayer, 1970; Cranston and Buckley, 1972). In contrast, much work has been done in Sweden, Japan, and Canada on mercury and methylmercury determination in biological materials (Lindstedt, 1970; Lindstedt and Skare, 1971; Malaiyandi and Barrette, 1970; Hatch and Ott, 1968; Takizawa, 1970; Suzuki et al., 1970, 1971; Sumino, 1968a,b; Fagerstrom and Jernelov, 1971; Westoo, 1966, 1967, 1968, 1969; Noren and Westoo, 1968; Johnasson et al., 1970). Although federal regulations establish a zero tolerance for mercury pesticides, no residue at all may appear in food samples sold in interstate commerce. Research in Japan, Sweden, and Canada reveals that the widespread use of these materials may result in some residues in many food products.

It was, therefore, decided that several biological samples, such as fruits, vegetables, meat, seeds, and grains, etc., be systematically analyzed for total mercury content, so that information may be available about the mercury content in various biological samples in Western Kentucky.

Various methods have been employed for the determination of mercury in atmospheric, aquatic, and biological samples (Basely, 1971; Pillay et al., 1971; Rottschafer et al., 1971; Moffitt and Kupel, 1970; Kalb, 1970; Fishman, 1970; Mayer, 1970; Cranston and Buckley, 1972; Lindstedt, 1970; Lindstedt and Skare, 1971; Malaiyandi and Barrette, 1970; Hatch and Ott, 1968; Suzuki et al., 1970, 1971; Sjostrand, 1964; Sandell, 1959; Nobel, 1961; Rathje, 1969; Gage and Watten, 1970; Bucknell, 1951). The colorimetric dithizone method (Basely, 1971; Mayer, 1970; Sandell, 1959; Nobel, 1961; Bucknell, 1951), which is considered to be the classical analytical procedure for the determination of trace amounts of mercury, is not very sensitive and is subject to a large number of chemical interferences. The method of neutron activation analysis (Pillay et al., 1971; Rottschafer et al., 1971; Sjostrand, 1964) has been employed for a variety of samples, but this method is expensive. Recently, several methods involving the use of cold-vapor atomic absorption spectrometry for mercury determinations have been published (Moffitt and Kupel, 1970; Kalb, 1970; Fishman, 1970; Cranston and Buckley, 1972; Lindstedt, 1970; Lindstedt and Skare, 1971; Malaiyandi and Barrette, 1970; Hatch and Ott, 1968; Suzuki et al., 1970, 1971; Rathje, 1969; Gage and Watten, 1970).

Kimura and Miller (1962) were the first to demonstrate the well-known reaction (eq 1) to liberate mercury from a digested sample and transport it to an absorption vessel by an air current in connection with dithizone determination. The same principle was later utilized by Poluektov et al. (1964) in liberating mercury vapor using cold-vapor atomic absorption spectrometry. This principle has since been applied for the analysis of biological samples for total mercury content. Presently, the cold-vapor atomic absorption spectrometric technique is preferred over less sensitive colorimetric dithizone and more expensive neutron activation techniques.

$$Hg^{2+} + Sn^{2+} \rightarrow Hg^0 + Sn^{4+} \quad (1)$$

A relatively simple digestion procedure has been developed in the present study which can be applied to various biological samples. The biological sample was digested for 3 to 5 hr in a mixture of concentrated sulfuric and nitric acids in a water-bath shaker at 60°. The chemically bound

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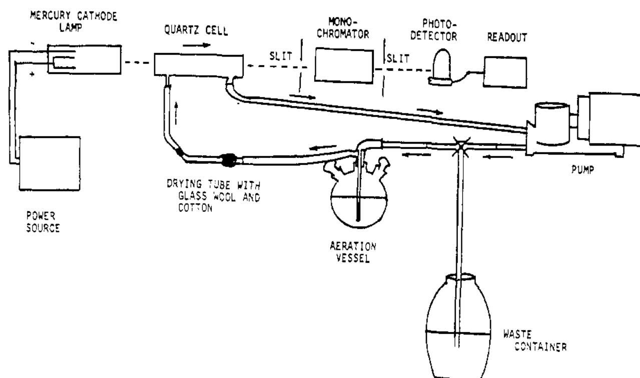

Figure 1.

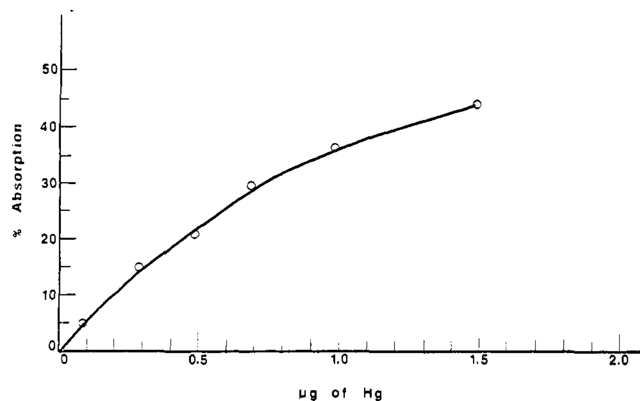

Figure 2.

mercury is thus converted to divalent mercury, which is subsequently reduced to free mercury using a mixture of stannous chloride, hydroxylamine hydrochloride, and sodium chloride, and determined using cold-vapor atomic absorption spectrometry at 2536.5 Å. The method is accurate and rapid, and no constant supervision is required. The recovery with nine different standard mercury compounds was better than 90%. The analytical technique used is similar to that described by Hatch and Ott (1968).

EXPERIMENTAL SECTION

Instrumentation. The instrument used in the determination of mercury was a Jarrell-Ash Maximum Versatility, Model 28-500, atomic absorption spectrometer. A hollow cathode mercury lamp was used as the source of 2536.5-Å radiation. The burner head was replaced by a quartz cell, 10 cm in length and 1.5 cm in diameter. After passing through the quartz cell, the mercury vapor was vented into a beaker filled with dilute nitric acid. A Brooks Sho-Rate "250" Model 1357 flow meter was used to monitor air flow rate. A Pall filter (Model ACB4463EL) was used to filter house air, prior to its use. A Perkin-Elmer digital concentration readout Model (DCR-2B) and a Honeywell Electronik 194 recorder were connected to the atomic absorption spectrometer. All water used was prepared by passing laboratory distilled water through an Illco-Way deionizer. An automatic Eberbach Water-Bath shaker, equipped with a thermoregulator, was used to agitate the samples during the digestion procedure. All instrumental parameters were optimized and consisted of the following: lamp current, 10 mA; wavelength, 2536.5 Å; air flow rate, 800 ml/min; recorder, 1 in./min, 0.25 sec response time; slit width, 100 μ entrance, 150 μ exit.

Reagents. High purity organo-mercury compounds were obtained from Pesticide Research Labs, Perrine, Fla. The mercury standard material was ACS Reagent grade mercuric chloride. All other reagents were of ACS Reagent grade and obtained from J. T. Baker Chemical Co.

Digestion Procedure. The biological sample was weighed accurately in a previously cleaned and dried 125-ml erlenmeyer flask. The size of the sample generally ranges from 0.1 to 10 g depending upon the type of sample to be analyzed. For seeds, grains, and food products, a 5-g sample is recommended. Five drops of Silicon Antifoam, Sag 5441, a Union Carbide product, 100% active and used for the aqueous system, are added to a mixture of 10 ml of concentrated nitric acid and 15 ml of 18 *N* sulfuric acid. The sample is digested in a waterbath shaker at 60° for 3–5 hr, depending upon the type of biological sample. The lipid material is filtered on paper, and the sample is ready for determination using the cold-vapor atomic absorption spectrometer. The digested material is normally a clear solution at this point.

Calibration Procedure. Mercuric chloride solution, with 0.1% mercury, is diluted with 1 *N* H_2SO_4, so that 0.1 μg of Hg/ml is obtained. A known amount of the standard solution is placed in a three-necked flask and 10 ml of concentrated nitric acid and 15 ml of 18 *N* sulfuric acid are added to it. The volume is adjusted exactly to 100 ml. It is important that the total volume of the aeration system be kept constant.

In the second three-necked flask, an amount of deionized water equal to the volume of the standard mercury solution is added. After the addition of appropriate amounts of acids, the volume is adjusted to 100 ml. This solution serves as a blank.

The flask is connected to the instrument by means of Tygon tubing (see Figure 1). Ten milliliters of 10% stannous chloride, 20 ml of a mixed solution of sodium chloride (30%), hydroxylamine hydrochloride (25%), and 5 drops of antifoam are added. The air is passed through the flask through a glass frit into the quartz cell at a flow rate of 800 ml/min. The divalent mercury is reduced to free mercury. As it passes through the quartz cell, it absorbs the light radiation at 2536.5 Å in proportion to its concentration. The present absorption is measured and recorded on the strip chart recorder. Measurements of the recorder peak height are made and the peak height is plotted against the micrograms of Hg. Sample and standard peak symmetries are identical. A typical calibration curve is shown in Figure 2.

Analysis of Biological Samples. The clear solution of a biological sample is similarly placed in a three-necked flask and analyzed in exactly the same way as the standard solutions. The measurement of the recorder peak height is made and subsequently used to determine the total concentration of mercury present in the biological sample. This enables the determination of the amount of mercury in terms of parts per million (ppm), since ppm = total Hg (μg)/wt of biological sample in grams. The minimum detectable quantity is defined as the one for which the peak height is twice the height of the noise level. In the present case, the minimum detectable quantity of mercury is 0.01 μg.

RESULTS AND DISCUSSION

Several biological samples, most of which were from the Western Kentucky area, were analyzed for total mercury content. Initially, six different seed samples were analyzed. The results obtained seemed consistent and were easily duplicated within experimental error. Several standard additions were then made to new samples along with their analysis, and excellent recoveries (better than 90% in all cases) were observed, as shown in Table I. The same procedure was then applied to various types of biological samples. The results obtained are shown in Tables II and III.

As can be seen from Tables I and II, the mercury content in most seeds and grains varied from 0.01 to 9.45 ppm. Trace amounts of mercury can, however, be expected in al-

Table I. Analysis of Seeds and Grains with Known Amounts of Mercury Compounds[a]

Sample	Hg compd added	Total Hg found, μg	Total Hg found, ppm	% recovery
Clover, Ladino	None	0.200	0.040	
	$Hg(SCN)_2$	0.650		92.8
	$Hg(OAc)_2$	0.705		100.7
	$Hg(NO_3)_2$	0.640		91.4
Corn	None	0.205		
	$MeHgC_2N_3H_4$	0.540		103.9
	C_6H_5HgCl	0.710		100.7
Lespedeza (c)	None	0.167	0.033	
	C_6H_5HgOAc	0.670		100.8
	C_6H_5HgOH	0.660		99.25
	CH_3HgCl	0.655		98.5
Soybean	None	0.215	0.043	
	$HgCl_2$	0.690		97.2
Wheat	None	0.080	0.016	
	$HgCl_2$	0.565		97.5

[a] A 5-g sample was used in each case. Addition of the mercury compounds was made so that 0.5 μg of Hg was added, except for the case of methylmercury dicyanodiamide, for which the amount of Hg added was 0.315 μg. Duplicate runs were made in each case. Samples were obtained from local seed and grain stores.

Table II. Analysis of Seeds and Grains for Mercury Content[a]

Sample	Total Hg, ppm	Sample	Total Hg, ppm
Barley	0.019	Lespedeza (B)	0.018
Beans, lima	0.013	Oats	0.012
Beans, pole	0.058	Okra	0.043
Beans, red eye	0.025	Parsnips	0.035
Beans, wax	0.017	Peas, Alaska	0.012
Beans, white kidney	0.035	Peas, blackeye	0.022
Beets (A)	0.026	Pepper, banana	0.218
Beets (B)	0.042	Pepper, pimento	0.051
Cabbage	0.048	Pepper, sweet	0.020
Cantaloupe	0.029	Radish, icicle	0.013
Carrot (A)	0.027	Radish, scarlet	0.016
Carrot (B)	0.030	Sorghum	0.022
Chard	0.103	Spinach	0.076
Clover, red	0.017	Squash	0.015
Cucumber	0.021	Sunflower	0.012
Fescue, Ky. 31	0.018	Tomato, hothouse	9.45
Kale	0.019	Tomato, Oxheart	0.090
Lettuce, black	0.044	Tomato, Ponderosa	0.050
Lettuce, Great Lakes	0.030	Watermelon	0.064
Lespedeza (A)	0.017	Wheat (A)	7.15

[a] Sample sizes ranged from 0.1 to 5.0 g. Duplicate analyses were run in each case and the numerical value for total mercury represents an average of the two experimental values obtained. In no case did the two separately determined values differ by more than 0.004 ppm.

most all samples due to natural conditions. It is, therefore, necessary to pay greater attention to the samples containing abnormally high amounts of mercury. Hothouse tomato seeds, obtained from a local seed and grain store, were found to contain an average of 9.45 ppm of mercury. As mentioned earlier, delicate hothouse tomato plants are often sprayed with mercury fungicides. An abnormally high content of mercury in hothouse tomato seeds was, therefore, not entirely unexpected. Low contents of mercury in two other types of tomato seeds, viz. Oxheart and Ponderosa, were, however, remarkable. Treated wheat was found to contain 7.15 ppm of Hg, an abnormally high value, suggesting that the use of mercury fungicides in the treatment of wheat is still in practice. Relatively higher values for Swiss chard and banana sweet pepper seeds are also noted.

Depending upon the concentration of mercury in different seed samples, the sample size was adjusted for 0.1 g for hothouse tomato seeds to 5.0 g for most other seed samples. Each seed sample was finely powdered, prior to digestion, using an Eskimo WHIZ-MIX Blender, Model 516. The problem of foaming, during the initial stage of digestion, was effectively controlled by use of the silicon anti-foam agent. It is noteworthy that by using these specific digestion conditions excellent recoveries are obtained in open flasks without the aid of a condenser or cold-finger.

As shown in Table III, fresh fruits, meats, and vegetables were analyzed for total mercury content. The amount of mercury found seems fairly normal and within expected limits. Two different tomato samples were analyzed. Sample A was vacuum dried and the weight given corresponds to the weight of the dry tomato. The weight of sample B corresponds to the wet weight of the sample and, as can be seen, the dry weight contains nearly 15 times greater amount of mercury than the wet weight sample. For all other fruits, meats, and vegetables listed in the table, the wet samples were weighed. Relatively higher mercury contents in lemons and limes and relatively lower mercury contents in onions and grapefruit have also been noticed.

The cooked fish sample was obtained from a local restaurant. The mercury content was found to be significantly higher than the rest of the fish samples. The Alaskan silver salmon and Alaskan king crab were found to contain the lowest amount of mercury. The mercury content in all samples, except the cooked-fish sample, was based on wet weight analysis.

Table III. Analysis of Certain Food Products for Mercury Content[a]

Sample	Total Hg, ppm	Sample	Total Hg, ppm
Apple	0.010	Lime	0.048
Bacon	0.072	Onion	0.007
Banana	0.011	Pepper, green	0.011
Beans, green	0.017	Pork, muscle	0.007
Beef, fat	0.020	Pork, sausage	0.047
Beef, muscle	0.007	Potato	0.012
Chicken, gizzard	0.007	Squash	0.010
Chicken, liver	0.009	Steak, ribeye	0.017
Chicken, lungs	0.012	Strawberry	0.019
Crab, king	0.032	Tomato (A)[b]	0.175
Cucumber	0.011	Tomato (B)	0.012
Egg, white	0.112	Tomato, canned	0.009
Fish, cooked	0.375	Tomato, chili sauce	0.013
Fish, bream	0.051	Tomato, juice	0.034
Fish, crappie	0.099	Tomato, ketchup	0.009
Fish, salmon	0.021	Tomato, paste	0.010
Grapefruit	0.010	Tomato, puree	0.005
Ham, cooked	0.010	Tomato, sauce	0.004
Hamburger	0.011	Turnip greens	0.014
Lemon	0.043	Weiner	0.008

[a] Samples were obtained from local food stores. Sample size ranged from 0.250 to 10.0 g. Duplicate runs were made in each case. All samples were wet weights except tomato (A). [b] Sample was vacuum dried.

The mercury content found in various other kinds of

meat samples is comparable with the mercury content in meat observed in Sweden and Denmark (Westoo, 1969). The analysis of meat samples reveals that the mercury content is considerably higher in bacon and pork sausage. The determination was based on the wet sample weight. In 1963, Smart and Lloyd (1963) reported a high content of mercury ($\simeq$10 ppm) in eggs from hens fed with seed containing about 6–14 ppm of methylmercury dicyanodiamide. This compound has been used extensively in the United States for the treatment of seed. The only egg white sample analyzed shows the mercury content to be greater than 0.1 ppm, much higher than the average Swedish value of 0.029 ppm.

In order to check the applicability of this method to most all types of biological samples, several hair samples were digested and analyzed for their mercury content. The hair samples dissolved easily and presented no problems. Values ranged from 0.255 to 1.32 ppm of Hg for the 18 samples analyzed.

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Methylation of Inorganic Arsenic by Mammals

Jolean U. Lakso*[1] and Stuart A. Peoples

Four cows and four dogs were fed sodium arsenate and potassium arsenite daily for 5 days. Their urine was analyzed for methylated arsenic (MA) and inorganic arsenic (IA) during the control, feeding, and feed-off periods. The control values of MA and IA in cow urine were 0.12–0.26 and 0.05–0.17 ppm, respectively. These levels peaked at 3.53 ppm of MA and 1.33 ppm of IA during arsenate feeding and at 4.78 ppm of MA and 1.57 ppm of IA during arsenite feeding. For the dog urine, the MA and IA control values were 0.20–0.31 and 0.11–0.16 ppm, respectively. Feeding arsenate caused a peak in MA and IA values of 6.23 and 10.48 ppm, respectively. Feeding arsenite caused a rise to 5.03 ppm of MA and 5.16 ppm of IA. Values for both arsenic forms reached control levels 5 days after feeding stopped in both mammalian species. It was concluded that both species produced methylated arsenic when given either trivalent or pentavalent inorganic arsenic.

McBride and Wolfe (1971) have given evidence, using laboratory cultures, that methanogenic bacteria (*Methanobacterium* strain M.o.H.) can methylate arsenic. Braman and Foreback (1973) pointed out that because suitable analytical procedures were lacking, it has been difficult to establish if such methylation or its by-products existed in nature. Braman et al. (1972) described a spectral emission system for arsenic. Braman and Foreback further refined the procedure to analyze for inorganic trivalent and pentavalent arsenic as well as several methylated forms of arsenic. They subsequently analyzed samples taken from "natural water, bird eggshells, seashells, and human urine" (Braman and Foreback, 1973). They found dimethylarsenic acid to be the "major and ubiquitous form of arsenic in the environment". Methanearsonic acid was also present, but in lower concentrations.

In two previous reports the authors (Peoples et al., 1971; Lakso et al., 1973) have shown that the principal form of arsenic in mammalian urine and plants was a methylated form of arsenic (MA). The presence of MA in the normal background of plants suggested the possibility that the MA in the mammalian urine could have its origin in the vegetative portion of the diet and not from the metabolism of the animal. However, the findings of McBride and Wolfe (1971) that bacterial methylation occurred under anaerobic conditions suggested arsenic methylation could occur in the rumen of the cow, a site of intense anaerobic bacterial action (Hungate, 1966).

The experiments reported here were done to ascertain if

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