MERCURY LEVELS IN SOME SELECTED FOODS AND EVALUATION OF ASSAY TECHNIQUES

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Manel I. Gomez

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ABSTRACT

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AND EVALUATION OF ASSAY TECHNIQUES

By

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The primary objective of the study was to assess total mercury levels in selected foodstuffs representative of the average diet. Foods with no direct exposure to mercury contamination were selected to represent background levels of mercury. The study was confined to foods of Michigan origin, in order to relate background mercury levels to geographic location.

Total mercury measurements were made by flameless atomic absorption spectrophotometry after wet acid digestion of samples. Concentrated H_2SO_4 digestion was applicable to most animal products with the exception of beef and pork liver and high-fat foods, such as cheese. Nitric acid: sulfuric acid digestion mixtures were employed on all plant products. Refined products such as salt and sugar were digested with HNO_3 alone. The major modifications in digestion procedures were the use of 35 percent w/v HNO_3 in place of concentrated HNO_3 and the use of steam bath temperatures throughout the

digestion and subsequent $KMnO_4$ oxidation. The moderation and control of digestion conditions thus accomplished, permitted the use of simple digestion equipment with no attached condenser systems and the handling of a large number of samples at one time.

Digestion procedures were evaluated by recovery studies on mercury added as mercuric chloride and methylmercuric chloride to food samples of known mercury content. A recovery of 83-87 percent of mercury added as methylmercuric chloride in the 0.01-0.2 µg. range and 97 percent of mercury as mercuric chloride in the same range indicated satisfactory efficiencies in digestion. In addition, the analytical procedures were evaluated by inter-laboratory comparative studies on reference samples of fish. The results were in good agreement.

Losses of mercury of 71 and 85 percent were observed in preliminary lyophilization and vacuum drying of egg samples respectively, precluding the use of these methods in the preliminary concentration of samples prior to wet-digestion.

The concentrations of mercury found in major foods indicate that the average diet contains a very low level of mercury in the range 0.01-0.03 ppm. Amounts of mercury found in vegetables and fruits show that residues are barely detectable and are at or below the 0.01 ppm level. The average concentration of mercury in dried plant products such as grains and cereals was 0.02 ppm while those in animal

products was 0.03 ppm. Fish was the only food showing significant levels of mercury with a mean concentration of 0.17 ppm. This level was however still below the F.D.A. guideline of 0.5 ppm. No substantial differences were observed between different strains of plant and animal products except in the case of fish.

Results of this study suggest that mercury levels present in the major foods do not represent overt contamination above a mean background level of 0.01-0.03 ppm. The digestion techniques employed were shown to have merit for the routine analysis of a large number of food samples.

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INTRODUCTION

In the last two decades mercury has come to be recognized as a major and persistent environmental contaminant, as a result of its widespread use in industry and agriculture and its emission into the atmosphere from smelting operations and burning of fossil fuels. The toxicity of mercury had been known and recognized in early times. However, the evaluation of its toxicity in the light of its behavior and interactions in natural systems came to be considered only recently.

Present day large scale uses of mercury have resulted in its widespread dissemination throughout the ecosystem, contaminating the most basic ingredients of the environment, water, soil, air and food. This element and its compounds have been demonstrated to have the unique property of biotransformation into one of its most toxic forms, methylmercury, which is readily magnified in the food chain. Food is a major source of intake and therefore the importance of close and continuous vigilance on the levels of mercury present in foods cannot be overemphasized. Plant and animal products used as foodstuffs by man may contain mercury in quantities detrimental to health.

At present there are few, if any, studies which are monitoring the relative importance of the environmental mercury sources to its total concentration in foodstuffs. Data presently available on the levels of mercury in food pertain mostly to individual components of the diet or single categories of food, such as grains, dairy products, meat or fish. Only limited data are available on mercury contents of total diets or food items representative of total diets.

Natural or background levels of mercury in sea water have been associated with levels of mercury found in marine fish. Background levels of mercury in soils and ground water have been determined to evaluate the extent of environmental contamination. However, no systematic monitoring of the background level of mercury in foods has been reported and the limited data available present wide variations. Such studies have been largely handicapped by lack of methodology involving a minimum of sample preparation and accompanying losses of mercury.

The present study was undertaken to determine total mercury levels in some selected foods of Michigan origin, with no direct exposure to mercury contamination. The primary objective of the study was to determine background levels of mercury in foods. Concurrently, evaluations were made of the assay techniques for determining submicrogram levels of mercury, with a view to developing a simple, rapid and reliable method of analysis for mercury in a variety of plant and animal materials.

REVIEW OF LITERATURE

The current concern over the environmental contamination of mercury has directed attention to its sources, transformations and mechanisms of concentration in the food chain. The two major areas of present day use are industry and agriculture.

Industrial Uses of Mercury

Chlor-alkali production by the mercury cell method has been estimated to contribute the largest measure to the environmental burden of mercury. About 25 percent of the annual U.S. consumption of 6 million pounds of mercury in 1969 (Table 1) was used in mercury cell chlor-alkali production (U.S. Bureau of Mines, 1970). It is estimated that in the process, close to one-third pound of mercury can be lost for every ton of chlorine produced and plants with a daily output of 100 tons of chlorine could have an annual discharge rate of 10,000 pounds of mercury (Bligh, 1972).

Large amounts of mercury are also used in the manufacture of a variety of products such as paints (anti-fouling and marine), electrical apparatus and batteries, cosmetics, fluorescent and neon lights, acetaldehyde and plastics (U.S. Bureau of Mines, 1970).

Table 1. U.S. Mercury Consumption for 1969[1]

Industry	Consumption Thousands of Pounds
Chlor-Alkali Industry	1572
Electrical Apparatus	1382
Paint	739
Instruments	391
Catalysts	221
Dental Preparations	209
Agriculture	204
General Laboratory Use	126
Pharmaceuticals	52
Pulp and Paper Making	42
Amalgamation	15
Other	1082
Total	6035

[1]Source: U.S. Dept. of Interior, Bureau of Mines.

Though organomercurial slimicides were used in the past in the paper and pulp industries, in many countries regulations controlling the use of mercurials in the manufacture of paper, intended for use in food packaging, have led to limited use of organomercurials (Bligh, 1972).

In addition to being intentionally used in industry, mercury is also an unintentional by-product in a number of processes involving natural products containing traces of mercury. Bailey et al. (1961) reported that native mercury and possibly other forms of mercury occur in petroleum, natural gas and crude oils. Many petroleum deposits were shown by Bertine and Goldberg (1971) to contain mercury in the ppm range and thus petroleum is another significant source due to its large-scale use. Weiss et al. (1971) also estimated that significant quantities of mercury could conceivably be discharged into the environment from the heating of shale and limestone components to temperatures of 1,500°C in the manufacture of cement.

The incineration or indiscriminate disposal of many industrial and consumer products containing mercury constitutes still another source of mercury in the environment (D Itri, 1972).

Agricultural Uses of Mercury

Organomercurial fungicides have been used in agriculture since 1914. The three basic types of organomercurials are the alkyl, aryl and alkoxyalkyl derivatives of mercury. Of

these the alkyl derivatives are the most toxic and the alkoxyalkyl derivatives the least. Since the beginning of the '50s mercurial seed dressings were suspected in Sweden as the cause of diminishing bird and wildlife populations (Borg et al., 1966). The most toxic alkylmercurials were taken off the market in Sweden in 1966 (Lofroth, 1969) and recently in the U.S. However though the use of organomercurials has been diminishing and though they constitute only a fraction of the industrial uses of mercury they represent a more direct and significant source of contamination in respect to foods. Smart (1968) observed that in Britain, the officially specified zero levels for residues were exceeded even when crops were treated in accordance with strict agricultural practice. The most widely treated plant products are grains, which constitute an important component of animal feeds and through which route mercury may be carried in the food chain to meats and animal products. In experimental studies, hens fed seeds treated with methylmercury had high mercury content in their eggs (Smart and Lloyd, 1963). A family in New Mexico suffered severe mercury intoxication from the ingestion of pork from hogs fed treated seed (Curley et al., 1971), while a number of epidemics of poisoning have been reported from Guatemala, Pakistan, Iraq and Iran from the direct ingestion of treated seeds by humans (Eyl, 1971).

Translocation

Organomercurial fungicides applied as seed and soil dressings and foliar sprays have been reported to enter the food chain directly as residues on plant material and by systemic translocation within plant tissues. Translocation of mercury from treated rice was shown to produce significant levels of residues in harvested grain by Tomizawa et al. (1966) in Japan. Epps (1966) reported higher levels of mercury in rice from treated seed than from untreated controls. However, Westermark (1967) found comparable levels of 0.008-0.012 ppm in wheat and barley whether or not they were grown from treated seeds. Saha et al. (1970) found significant amounts of mercury in grain from wheat grown in soil treated with methylmercury dicyandiamide.

Translocation of mercury to edible parts of plants from the foliar application of mercurials has been demonstrated in fruits and vegetables. Martin and Pickard (1957) reported mercury residues in the range of 0.02-0.12 ppm resulting from the experimental spraying of apples. Stone et al. (1957) reported measurable traces in the skin and pulp of treated apples. Ross and Stewart (1960) suggested translocation from other parts of the plant (other than from surface residues) to account for the residues of 0.05 ppm at harvest time. They further established a translocation mechanism (1962) by demonstrating the presence of residues in fruit enclosed in plastic bags during spraying. Szkolnik et al. (1965) added credence to the persistence of mercury in biological

systems with evidence of low levels of residues in trees that had received no spray treatment in 10 years or more.

Similar mercury residue accumulations have been observed in potatoes and tomatoes as with apples. Smart (1964) demonstrated translocation of mercury by the root, skin and leaves of potatoes, though soil treatment with inorganic mercury gave rise to only negligible levels in the tubers at harvest.

Furtani and Osajima (1965-1966), investigating the content of mercury in rice, fruits and vegetables, inferred that mercury in food products is partly the residue of fungicides sprayed on crops and partly due to absorption from the soil through the roots. Mercury levels in soils may represent cumulations of direct mercurial applications or fallout from atmospheric sources superimposed on naturally occurring levels.

Aomine et al. (1967) observed that mercury permeates the soil profile in a form more soluble than the sulfide and is retained by the soil for considerable periods of time. Ross and Stewart (1962) found no mercury in apples in an orchard where surface soil contained as much as 1.8 ppm and concurred with Booer (1944) that the insoluble mercuric sulfide was the end product of transformation of mercury compounds in the soil. In disagreement with these observations Furtani and Osajima (1966) found abnormally high mercury contents in brown rice cultivated with no applications of fungicides.

The overall evaluation of the translocation of mercury as a factor contributing to the contamination of foods is difficult on the basis of data involving different mercury compounds, rates of application, dosages and harvesting schedules. However it appears that some of the mercury applied to seeds and foliage is translocated to grain and edible parts of plants though not in significant amounts. The more persistent and important sources of mercury are the soil accumulations resulting from the direct application of fungicides, indirect fallout of atmospheric mercury and run-off from terrestrial sources.

Biomethylation

While the toxicity of organomercurials has been recognized for a long time, the inorganic mercury wastes of industry were until recently believed to be innocuous and safely disposed of in water where they were supposed to remain relatively inert. The epidemics of poisoning in the Minamata and Niigata incidents in the years 1953-1965 were the first indication that industrial wastes were not as innocuous as hitherto assumed.

Japanese workers (Irukayama et al., 1966) were the first to identify the causative factor in Minamata disease as methylmercury present in the shellfish of Minamata bay. It was suspected that the source of mercury was the conversion of inorganic mercury wastes to methylmercury by the action of microbes in the mud or by plankton. Jensen and Jernelov

(1967) demonstrated this conversion using micro-organisms of bottom sediments.

The underlying mechanism of the methylation was worked on by Wood et al. (1968) who showed that methylcobalamine was involved in the methyl transfer, in the synthesis of both monomethyl and dimethyl mercury in enzymatic and nonenzymatic systems. Immura et al. (1971) found supportive evidence for the mechanism and emphasized the essentiality of methyl-cobalamine as methyldonor in the reaction. Landner (1971), in experiments with Neurospora crassa was able to demonstrate methylation by an organism with no known requirement for vitamin B_{12}. While inorganic mercury is essential for the biomethylation reaction, Jernelov (1969) has shown that aryl and alkoxyalkyl mercurials undergo breakdown to intermediate inorganic mercury before conversion to methylmercury.

From the foregoing it is evident that whatever the form of mercury discharged into aquatic and other systems supporting methylating organisms, the ultimate end-product is the highly toxic methylmercury. Studies of aquatic and other food products bear out the widespread presence of methylmercury. In Sweden, regardless of the nature of the mercurial pollutant, only methylmercury has been identified in fish (Westoö, 1967). In addition, Westoo (1967 and 1969) reported that 80 to 100 percent of the mercury was present as methylmercury in a number of animal products as well. In the context of biomethylation it is of interest that the same workers demonstrated in vitro methylation of mercury

by liver homogenates (Westoö, 1968). Kiwimae et al. (1969) showed that hens fed seeds treated with inorganic, alkoxyalkyl or aryl compounds laid eggs containing only methylmercury, although this finding was not confirmed by Stoewsand et al. (1971). What is of greatest concern is the estimate that already existing deposits of mercury in bottom sediments in lakes and rivers can continue to generate methylmercury for many years to come and be a continuing source of contamination of the aquatic food chain (Johnels, 1967). Beasley (1971) identified significant levels of mercury in fish protein concentrates. Since fish is used as a source of protein in animal feeds, it can be a continuing source of contamination of many animal products.

Magnification in the Food Chain

Besides the unique property of biomethylation, mercury has the tendency to undergo concentration in the aquatic food chain. With the aid of labelled compounds Hannerz (1968), studying uptake and accumulation of mercury in fish, concluded that in comparison with other mercury compounds methylmercury had the greatest concentration factor. The ability to concentrate mercury though common to all fish varies in relation to age, species and trophic levels. Larger predatory fish such as pike and swordfish have higher concentration factors (Johnels and Westermark, 1969). Tejning and Vesterberg (1964) reported a similar concentration effect in eggs when hens fed on treated seed laid

eggs containing twice the concentration of mercury as was present in the fodder.

Mercury in Foods

Through all of the mechanisms discussed in the foregoing, aquatic food would be expected to have the highest concentrations of mercury. Fish have been shown in Japan, Canada and Sweden to have comparatively high background levels of mercury. In Minamata and Niigata, mercury levels in fish, of the order of 5-20 ppm were identified (Bligh, 1972). Most of the fish investigated from fresh waters and coastal areas in Sweden were reported to have concentrations in the range of 0.2-1.0 ppm (Lofroth, 1969). These mercury concentrations were mostly related to industrial sources of pollution. For example, Johnels et al. (1967) found that organisms downstream from paper mills had higher levels of mercury than those upstream. Bligh (1972) similarly observed that Canadian fish in the vicinity of every chlor-alkali plant had levels of mercury over 0.5 ppm.

Swedish workers also related the concentration of mercury in other foods and animal tissues with industrial and agricultural uses of mercury. Westoö (1966, 1967) correlated a decline in the mercury content of Swedish eggs with the ban on alkylmercury seed dressings. Johnels et al. (1968) noticed increased concentrations of mercury in feathers of recent Ospreys as compared to those of pre-industrial times.

Many experimental studies have been undertaken to evaluate mercury levels in relation to known and identifiable sources of contamination. Smart (1968) examined a number of grain, fruit and vegetable crops in order to evaluate pesticide residues. Observations derived from such studies are restricted to specific foods or categories of food and "normal" levels of mercury are based on a limited number of samples used as controls. Stock (1934) initiated analysis of mercury concentrations in foods in the 1930s and since then other workers have reported results of mercury concentrations in foods (Goldwater, 1964). There is a dearth of data on more comprehensive surveys of mercury in foods carried out in recent times and unrelated to specific sources of contamination. Jervis (1970) examined mercury levels in over 300 different Canadian foods, many of which were reported to contain mercury levels approaching or above tolerance levels. Some of Jervis' data were questioned by Somers (1971) whose re-examination of some samples yielded results lower by a ten fold factor. The studies of Corneleiussen (1969) on a number of common foods in various U.S. cities serves as the only basis for comparison of results from the present study.

Standards

From Swedish toxicological studies and data from the Niigata incident an upper maximum acceptability limit for mercury in fish was set at 1.0 ppm in Sweden, with a non-enforced recommendation to limit consumption of fish to one

meal a week (Lofroth, 1969). In both the U.S. and Canada, an interim safety limit of 0.5 ppm was adopted as a matter of expediency on the basis of the Swedish standard.

According to the Miller Amendment to the Federal Insecticide and Rodenticide act of 1955, a zero tolerance is specified for mercury residues in foods, resulting from the application of any mercury containing materials (Zweig, 1963). The tolerance limits adopted in many countries range from the 1 ppm limit adopted by Sweden and the U.K. to 0.03 ppm in the Benelux countries (Smart and Hill, 1968). However, no firm basis has yet been established for determining a safe standard for mercury in foods. The only available guideline at present is that of 0.05 ppm as permissible upper limit for mercury in foods, proposed by WHO in 1967 (FAO/WHO, 1967). The latter guideline seems to find justification in the light of recent evidence of c-mitotic effects observed in human leukocytes exposed to methylmercury chloride concentrations as low as 0.25 ppm (Fiskesjo, 1970). No information is presently available on the sub-chromic effects of the ingestion of low levels of mercury in food over long periods of time. Such effects need to be considered in the stipulation of a valid standard for mercury in foods.

MATERIALS AND METHODS

Food Samples

Foods were selected to represent a cross-section of the average diet and included three categories of food, animal products, cereals and legumes and fruits and vegetables. Three basic subsidiary items of diet, sugar, salt and bread were included in the study. Foods of animal origin included dairy, fish, meat and poultry products. Fruit and vegetable samples were representative of market samples as to ripeness and maturity. Vegetables examined included stem and root vegetables.

The survey was intended to cover foods of Michigan origin and to relate mercury levels to geographic location. For purposes of documentation and access to history of samples, nearly all samples were obtained from Michigan State University, Experimental Research Station sources. Some food items not available from the Experimental Station were acquired from known private farms or retail stores in Michigan.

Sampling Procedures

Equal portions of three to four random samples of material were drawn from bulk samples (bins of cereal, carcasses

of animals etc.) and pooled to give representative sub-samples (Tables 2,3 and 4). The quantity and size of samples were as recommended for routine pesticide analysis by Zweig (1963).

Storage of Samples

Fresh produce was packed in polyethylene bags and held in cold storage at 32° F. Pillay et al. (1971) found no detectable traces of mercury in polyethylene stored blanks and recommended the latter as a suitable medium for storage of samples for trace analysis. Fresh produce samples were analyzed within a week to 14 days of collection. However, some samples such as potatoes and apples had already been held for varying periods under refrigeration from time of harvest to time of collection. Refrigeration of these samples was continued up to time of analysis. Dried vegetable products, cereals and legumes were packed in polyethylene bags and stored at 32° F. A representative sample of each (sub-sample of 500 gm.) was ground in a Wiley mill to a consistency which would pass through a 40 mesh seive, and stored in stoppered containers for analysis. Milk was refrigerated at 32° F and analyzed the day following collection. Processed products such as salt and sugar were shelf stored in original containers at room temperature, while bread and cheese were held under refrigeration at 32° F. All animal tissues were packed in cryovac bags and frozen. Eggs were refrigerated at 32° F and fish samples were obtained from the MSU Institute of Water Research Laboratory as frozen fillets or homogenized samples.

Table 2. Food Items of Animal Origin, Assayed for Total Mercury

Food Item	Sample Collected: Quantity Pooled	Sample Collected: Quantity Drawn	Condition	Source
Dairy Products				
Milk (i)	4 milkings	1 qt.	Fresh, Whole Unpasteurized	MSU Dairy Farms
Milk (ii)	2x1 gallon	1 qt.	Homogenized Pasteurized, 2% Fat	Retail Store
Milk Dried		1 lb.	Spray Dried	Commercial Source
Cheese		1 lb.	Processed, Cheddar	MSU Dairy Dept.
Poultry Products				
Chicken Meat (Breast)	3 carcasses	3 lb.		
Chicken Livers	3 livers	10 oz.	Fresh	MSU Poultry Farms
Eggs	24	24		
Meats				
Pork				
(i) Duroc	2x1 lb. portions (2 carcasses)	2 lb.		
(ii) York	2x1 lb. portions (2 carcasses)	2 lb.		
Pork Livers			Fresh	MSU Swine Farms
(i) Duroc	2x1 lb. portions (2 carcasses)	2 lb.		
(ii) York	2x1 lb. portions (2 carcasses)	2 lb.		
Beef	3x1 lb. portions (3 carcasses)	3 lb.	Fresh	MSU Beef Barns
Beef Liver	3x1 lb. portions (3 carcasses)	3 lb.	Fresh	MSU Beef Barns
Fish				
Large Mouth Bass	Portion of Long. Back Muscle	10 gm.	Homogenized or Filleted and Frozen	MSU Institute of Water Resources, Laboratory, Winter Green Lake, Kellogg Bird Sanctuary
Yellow Perch	Portion of Long. Back Muscle	10 gm.		
Sunfish Pumpkin Seed	Portion of Long. Back Muscle	10 gm.		

Table 3. Food Items of Plant Origin, Assayed for Total Mercury

Food Item	Sample Collected		Source
	Quantity	Condition	
Cereals			
Wheat			
(i) Dickson	2 lb.		
(ii) Beizes			
Barley			
(i) Avon		Unhulled	MSU Crop Science Research
(ii) Talbot	2 lb.		
Oats			
(i) Garry	2 lb.		
Rice, long grain white.	2 lb. (2xl lb. pkg.)	Polished Packaged	Retail Store
Legumes			
(i) Navy Beans	2 lb.	Dried	MSU Crop Science Research, Saginaw Valley Bean Farms
(ii) Red Kidney	2 lb.		
Vegetables			
Asparagus	2 lb.	Fresh, washed trimmed	Private Farm Hart, Mich.
Potatoes			
(i) Russet Burbank	3 lb.	Stored under refrigeration	MSU Horticulture Research Center.
(ii) Merrimak 528	3 lb.		
Fruits			
Apples			
(i) Golden Delicious	3 lb.	Stored under refrigeration	
(ii) Johnathan	3 lb.	Stored under refrigeration	MSU Horticulture Research Center.
Tomatoes	3 lb.	Freshly harvested	
Strawberries	2 qt. (2xl qt. pkg.)	Freshly harvested	Retail Produce Market. (grown in Grand Rapids, Mich.)

Table 4. Three Subsidiary Food Items, Assayed for Total Mercury

Food Item	Sample Collected		
	Quantity	Condition	Source
Bread, White	2 lb. (2x20 oz. pkg.)	Day-old	Retail Store
Sugar, White Granulated (i) and (ii)	2 lb. (2x5 lb. pkg.)	Refined, packaged	Retail Store *(Saginaw)
Salt, uniodized	1 lb. (2x1 lb. pkg.)	Refined	Retail Store *(Detroit)

*Source of raw material.

Physical Pre-Treatment of Samples for Analysis

Samples were prepared for analysis as detailed in Table 5. Frozen animal tissues were thawed sufficiently to permit slicing and macerated at low speed in a Waring blender. Frozen tissues were not thawed completely in order to avoid loss of "drip." Hand-chopping and slicing of samples were performed on a glass chopping plate with stainless steel knife. Meat, pork and poultry samples were trimmed of fat as completely as possible to represent residue levels in "lean" meat.

All equipment used in sample pre-treatment was thoroughly washed using

1. Alconox detergent
2. 8-10 rinses in tap water
3. 2 rinses in distilled, de-ionized water

Stringent precautions were observed in handling and transfer of samples to avoid mercury contamination.

Mercury Determination

Mercury in all samples was determined as total inorganic mercury using the flameless atomic absorption spectrophotometry technique. The instrument used in this study was a direct reading Coleman Mercury Analyzer - MAS 50, equipped with closed system aeration described by Hatch and Ott (1968). The range of detection was between 0.0 and 9.0 micrograms total mercury, on two scales of measurement (0.00-0.25 µg. and 0.25-9.0 µg.) and the specified sensitivity of the

Table 5. Physical Pre-Treatment of Samples for Analysis

Food Item	Treatment	Conditions
Milk (i)	-	-
Milk (ii)	lyophilized[a]	10 hours
Milk Spray Dried	-	-
Cheese	-	-
Chicken Meat	Chopped and Macerated	Waring blender (low speed)
Chicken Liver	Chopped and Macerated	Waring blender (low speed)
Eggs (i)	Homogenized	Waring blender (low speed)
(ii)	Vacuum Dried	Room temperature (12 hours)
(iii)	Lyophilized	10-12 hours
Pork, Pork Liver, Beef and Beef Liver	Chopped and Macerated	Waring blender (low speed)
Fish	Homogenized	Ploytron homogenizer
Cereals	Ground	Wiley Mill
Legumes	Ground	Wiley Mill
Vegetables	Chopped, sliced or cubed	Manually
Fruits		
(i) Flesh	Macerated	Waring blender (low speed)
(ii) Peel	Chopped	Manually

[a] Virtis Freeze-mobile - Model 10-145-MR-BA.

[b] Waring blender with glass container.

instrument is given to be 0.01 µg. mercury. Readings were estimated to the closest 0.005 µg. of total mercury.

Instrument calibration was checked at the beginning of the study and thereafter periodically using mercury standards described below. Typical calibration results from four sets of independent readings are shown in Figure 1. All analyses were performed against 3 to 4 standards in each run of samples. Day to day reproducibility of instrument calibration was excellent.

Preparation of Standards. Mercury Standard: Mercuric chloride was dried at 100° C for 3 hours and stored in a desiccator. 1.3538 gm. were weighed out and dissolved in approximately 100 ml. distilled de-ionized water, 55 ml. of 18 N H_2SO_4 were added and the solution diluted to 1 liter (mercury stock solution, 1 mg./ml.--stable up to 1 month).

Intermediate Standard: One ml. of stock solution was pipetted into a 100 ml. volumetric flask, 10 ml. of 18 N H_2SO_4 were added and the solution diluted to volume with distilled de-ionized water. Each ml. of standard contained 10 µg. mercury.

Working Standard: One ml. of intermediate solution was pipetted into a 100 ml. volumetric flask, 10 ml. of 18N H_2SO_4 were added and the solution diluted to volume with distilled de-ionized water to make a solution containing 0.1 µg. mercury/ml. Fresh intermediate and working standards were prepared from stock solution at the time of use.

Figure 1. Instrument Calibration

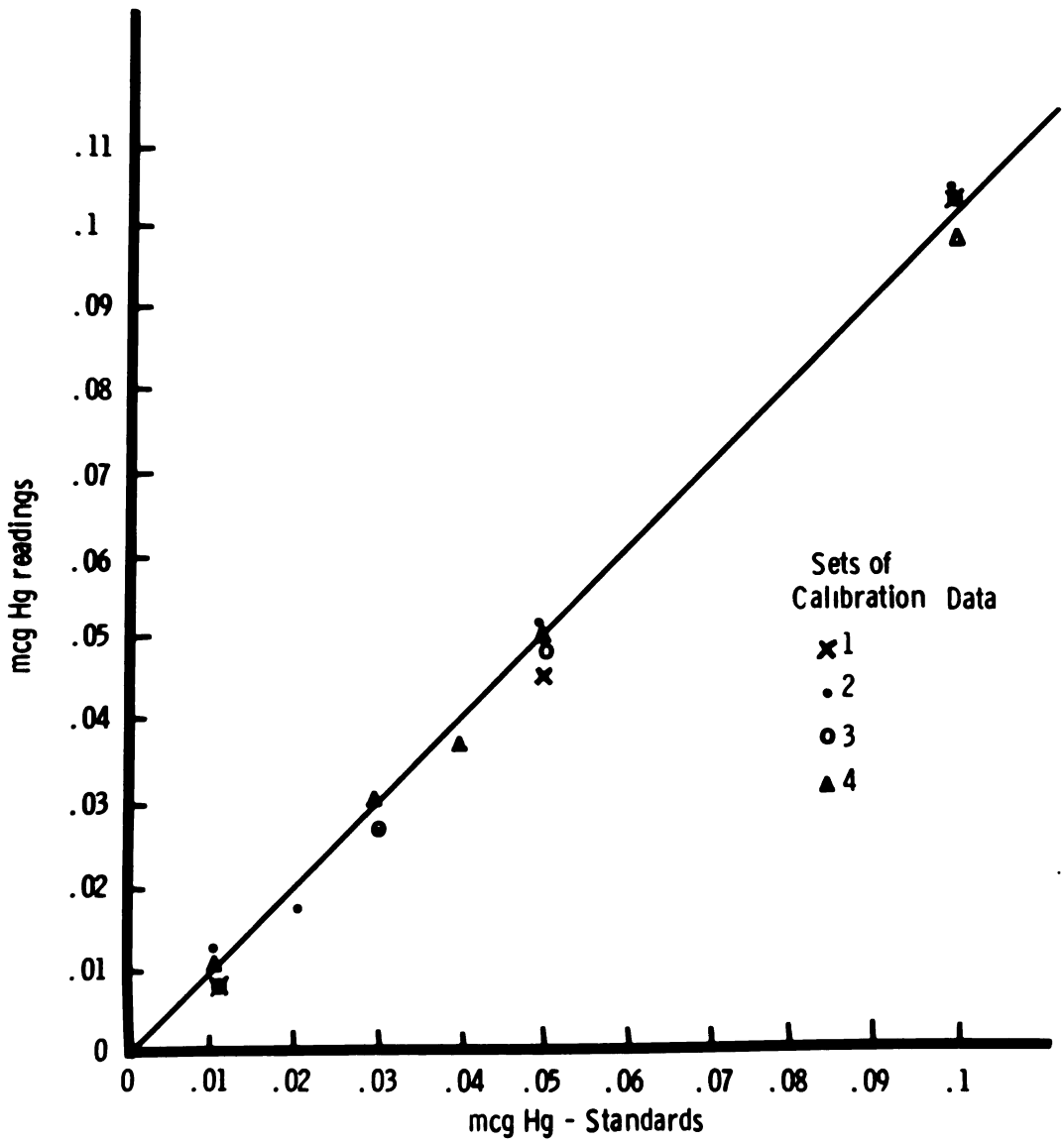

Fig. 1. Instrument Calibration

Calibration of Instrument

Volumes of the working standard ranging from 0.1 to 5.0 ml. were pipetted into aeration flasks to which were previously added 100 ml. distilled de-ionized water and 2 drops of 5 percent $KMnO_4$. Then, 5 ml. of 10 percent hydroxylamine hydrochloride were added and the flasks swirled to effect complete decolorization of $KMnO_4$. The aeration head was inserted into the flask and readings noted at the maximum recording of the meter. Readings were corrected for reagent blanks which were in the range 0.00 to 0.005 µg. mercury.

Analytical Method

Analysis was based on the Hatch and Ott (1968) procedure for determining mercury at the nanogram level.

The sample was acid digested (specific choice of acid or combination of acids were used depending on the nature of the matrix) and the mercury present was oxidized to the mercuric form with a suitable oxidizing agent. Potassium permanganate was the oxidizing agent of choice. The excess $KMnO_4$ was reduced with hydroxylamine hydrochloride and the mercury was reduced to the metallic state with stannous chloride. The pump and aerator system circulated mercury vapor into the absorption cell where absorption at 253.7nm took place and the resulting change in energy was transmitted to the photo detector.

Reagents

The application of the method of analysis at very low mercury levels greatly depends on the precision with which reagents blanks can be reproduced. Reagents were screened in preliminary experiments to obtain reagents which would yield low and constant blank readings. All reagents were of analytical grade (ACS) and were from sources detailed below.

Mercuric chloride (Speciality Chemicals Division, Baker Chemical Company)

Sulfuric acid--Sp. Gr. 1.84 (Speciality Chemicals Division, Baker Chemical Company)

Nitric acid--Sp. Gr. 1.47 (Mallinckrodt)

Hydrochloric acid--Sp. Gr. 1.18 (Mallinckrodt)

Acetone (Baker Chemical Company)

Hydroxylamine hydrochloride (Sigma Chemical Company)

Stannous chloride (Mallinckrodt)

Potassium permanganate (Mallinckrodt)

Methylmercuric chloride--97-99% (K & L Laboratories Inc.)

All aqueous solutions of reagents were made using distilled de-ionized water.

Glassware

Reproducibility of results at low levels of determination is dependent on strict attention to cleanliness of glassware. The following procedure was used in routine cleaning of glassware.

1. Wash in "Alconox" detergent.
2. 8 rinses in tap water.
3. Soak in warm 3 N HNO_3.
4. 4-5 rinses in tap water.
5. 2 rinses in distilled de-ionized water.

Digestion Procedures

In biological samples containing mercury, destruction of organic matter without loss of mercury is a major concern because of the volatility of mercury and its covalent compounds. The moderation of digestion conditions on the other hand could result in incomplete release of mercury. Digestion procedures were modified to obtain a satisfactory compromise between these two factors. Due to the variety of materials analyzed and differences in response of the materials to the oxidizing mixture, the following three different digestion procedures were adopted.

Digestion I.

Sulfuric acid (Sp. Gr. 1.84) digestion, with $KMnO_4$ (5% w/v) oxidation was found applicable to most animal foods with the exception of beef liver, pork liver and cheese. A limitation of this digestion procedure is the incomplete digestion of fats which are reported to contain negligible amounts of mercury (Barett, 1956) and are normally removed by filtration prior to the oxidation procedure. The digestion procedure was based on Uthe and Armstrong's

(1970) modification of the Hatch and Ott (1968) method. A double oxidation was introduced as recommended by Barett (1956) and in the Dow Chemical Co. Method (1970).

One to three grams of sample were placed in the bottom of a tared 100 ml. volumetric flask using a transfer tool of the type recommended by Uthe and Armstrong (1970). Five ml. of concentrated (36N) H_2SO_4 were added, a 10 ml. beaker inverted over the mouth of the flask and the flask heated for 1½-2 hours on the steam bath. Although the dissolution of organic matter appeared to take place in a shorter time, heating periods of less than an hour gave rise to excessive foaming in the $KMnO_4$ oxidation step as well as in the final reduction-aeration step indicating incomplete oxidation of all organic matter. At the end of this period digestion flasks were removed from the steam bath, allowed to stand one hour at room temperature before they were cooled in an ice bath and undigested fats removed by filtration through a pledget of glass wool. The filter was rinsed repeatedly with 1:1 H_2SO_4 and the digest further cooled in an ice bath before gradual addition of 15 ml. of $KMnO_4$. The flasks were allowed to stand at room temperature until the initial reaction subsided, after which heating was continued as before for 20-30 minutes. The flasks were cooled, loosely stoppered and stood overnight for final analysis.

Digestion II.

A modification of the method reported by Jeffus and Elkins (1970) employing a H_2SO_4:HNO_3 digestion mixture,

was found applicable to all the plant foods examined. Some plant materials, notably legumes, rice and apples gave excessive foaming with consequent loss of some of the sample, when concentrated HNO_3 (Sp. Gr. 1.47) was used in the digestion mixture. Use of concentrated HNO_3 also necessitated higher temperatures and prolonged heating to effectively remove all nitrous fumes. The reaction was more easily controlled with 5.6 N HNO_3 (35 w/v).

In addition samples high in carbohydrate tended to char and carbonize if excess HNO_3 was not used. Therefore, instead of the 1:1 digestion mixture recommended by Jeffus and Elkins, a 3:1 (HNO_3:H_2SO_4) mixture was used. Fifteen ml. of HNO_3 were added (in one step or in increments depending on sample reaction) to 1 to 3 grams of sample in a 100 ml. volumetric flask and the flask held at room temperature for 30-45 minutes. To samples of low moisture content up to 5 ml. of water were added prior to the addition of HNO_3 to prevent charring. The flasks were set on a steam bath and heated until dissolution of solids was nearly complete after which the flask was removed from the bath and cooled in ice. Five ml. H_2SO_4 were added slowly and the flask held at room temperature till the initial reaction subsided. Then heating was resumed on the steam bath for an additional 1½ hours until evolution of brown nitrous fumes ceased. The flask was cooled in ice and subjected to the $KMnO_4$ oxidation as described in Digestion I above.

With some plant materials such as unhulled cereals, there was incomplete breakdown of cellulose and evidence of waxy undigested residues. The residues were not filtered since some loss of mercury could occur through entrapment on the cellulose fibers and the whole sample was submitted to aeration and reduction.

Digestion III.

The method of Hoover, Melton and Howard (Hoover et al., 1970) was tried on eggs, fish and some plant materials in preliminary experimentation. Thirty-five percent (w/v) HNO_3 was substituted for concentrated HNO_3 as in Digestion II. To avoid a vigorous initial reaction samples were added in small increments of 0.5 gm. at a time to 10 ml. of HNO_3 in a 125 ml. Erlenmeyer flask, with heating between additions to complete the initial reaction. One to two grams of sample were added in this manner and digestion continued for one to two hours till evolution of nitrous fumes ceased. The digest was cooled, diluted with 20 ml. distilled water being careful to rinse down the sides of the flask, and oxidation carried out as in Digestion I.

With most plant materials, the dissolution of organic matter was much less complete than with Digestion II. With animal tissues more complete dissolution of solid matter was observed, though in comparative digestions higher recoveries of mercury were obtained from eggs and fish, with Digestion I. Digestion III was thus found inadequate for most of the

materials studied, though it was applied to the relatively refined food products, salt and sugar with which Digestion I resulted in precipitation of sodium sulfate and charring respectively.

Reduction-Aeration

The method of reduction-aeration was uniform for all samples. The digested materials were transferred quantitatively to the aeration flask making sure that any adhering manganous oxides in the digestion flask was solubilized with 1 to 2 drops of 10 percent hydroxylamine hydrochloride. Then the final volume was made up to 100 ml. with de-ionized distilled water. Five ml. hydroxylamine hydrochloride was added and the flask swirled briefly to effect dissolution of all the manganous oxides. This was followed by the addition of 2 ml. of 10 percent stannous chloride and immediate insertion of the aeration head. Readings were noted with the "memory" switch on to retain the highest reading recorded.

Recovery Studies

Samples of representative items of the food categories examined were spiked with known additions of mercury as mercuric chloride and methylmercuric chloride and subjected to digestion and subsequent analysis.

Preparation of Standards

1. Mercuric chloride: The same standards were used as for instrument calibration.

2. Methylmercuric chloride: 12.78 mg. of methylmercuric chloride was weighed direct into a stoppered 10 ml. volumetric flask. The reagent was held in an ice bath during weighing to minimize volatilization. Transfer of reagent to the weighing flask was done as rapidly as possible and the flask stoppered immediately. All subsequent operations were conducted under the hood.

Methylmercuric chloride was dissolved in acetone and made up to 10 ml. with acetone to give a primary standard containing 1 mg. mercury/ml. (Magos, 1971). Linstedt (1971) has confirmed that after digestion all interferences (by absorption at 253.7nm) from acetone were eliminated.

Intermediate Standard (10 µg. mercury/ml.)

One ml. of stock solution was pipetted into a 100 ml. volumetric flask and made to volume with distilled de-ionized water.

Working Standard (0.1 µg. mercury/ml.)

A working standard was prepared by appropriate dilution of the intermediate standard.

All standards were stored in the refrigerator and intermediate and working standards prepared at time of use. Volumes of working standard ranging from 0.1 to 2.0 ml. were added to 1 gm. samples of chicken meat and barley and were subjected to digestions I and II respectively. Two to four replicate samples were run at each level and three unspiked blanks of each food material were run with each digestion procedure.

Recoveries of Mercuric Chloride

In routine analysis, 2 to 3 samples of foods from each of the categories examined were spiked with known additions of mercuric chloride in each run of analyses to determine recoveries. Even when mercury levels determined by the different digestion techniques on the same sample differed, good and comparable recoveries of added mercury (as mercuric chloride) were obtained under different digestion conditions.

RESULTS AND DISCUSSION

Levels of Mercury in Foods

Twenty-eight varieties of foods were examined for total mercury content. Mercury content of all samples were expressed on a fresh weight or "as received" basis and results were uncorrected for recovery.

In Tables 6-13, the results of assays for mercury in food are grouped according to the class of foods analyzed. The mercury levels are expressed in ppm and are the mean of four to six replicate analyses.

Table 14 summarizes the base-line levels of mercury in the major food groups examined and compares these results with some recently reported values.

Animal Foods

Results of analysis of the four categories of animal foods studied, Fish, Dairy, Poultry, and Meat are shown in Tables 6, 7, 8 and 9. Of the animal foods studied only fish had levels approaching the action level of 0.5 ppm adopted by F.D.A. If the W.H.O. guideline or tolerance level for mercury in foods of 0.05 ppm is considered, all fish samples examined exceeded this level.

Fish

Fish used in this study were drawn from an isolated inland lake with no known source of direct industrial contamination. However, a "natural" level of mercury may have accumulated through fallout from coal burning power plants and through the ecological chain involving migrant bird populations on the lake. Birds feeding on treated seed in the fields or on fish in contaminated lakes may carry a body burden of mercury which may be transposed to other habitats through droppings, and feathers and other excretory material.

Pappas and Rosenberg (1966) reported background levels of mercury in haddock, in the range of 0.017-0.023 ppm. However, the background level of 0.13 ppm reported by Bligh (1972) for perch from Lake Winnipegosis in Canada, is more consistent with the findings of this study.

Dairy Products

Results of analysis of dairy products are summarized in Table 7. Samples of whole milk from two different sources were analyzed for mercury content and mean levels in both sets of samples were found to be 0.01 ppm. Goldwater (1964) reported values in the range 0.003-0.001 ppm for whole milk in the U.S. while Corneleiussen (1969) reported values ranging from 0.002-0.02 ppm in various cities in the U.S. Similar concentrations were found by Reigo (1970) in Sweden. The levels of mercury obtained on freeze-dried samples of

Table 6. Total Mercury Content of Animal Foods--Fish

Food Item	Mercury Concentration (ppm) Range		Mean
Micropterus salmoides (Large mouth bass)	0.22-0.28	(6)[1]	0.25 ± 0.025
Perca flavencens (Yellow perch)	0.12-0.18	(5)	0.15 ± < 0.005
Lepomis Cyanellus x Lepomis gibbosus (Sunfish x pumpkin seed)	0.11-0.13	(6)	0.12 ± < 0.005
	Mean Value for Fish - 0.17 ppm		

[1] Figures in parentheses refer to number of determinations.

Table 7. Total Mercury Content of Animal Foods--Dairy

Food Item	Mercury Concentration (ppm) Range	Mean
Milk Whole (i) Unpasteurized	0.01 -0.02	(5)' 0.01 ± 0.005
Milk Whole (ii) Homogenized, pasteurized	0.005-0.01	(6) 0.01 ± 0.005
Milk, (i) Freeze Dried	0.005-0.01	(6) 0.01 ± < 0.005
Milk Spray Dried	0.01 -0.03	(6) 0.02 ± 0.01
Cheese Cheddar	0.015-0.030	(6) 0.02 ± 0.01
	Mean for Dairy Products - 0.01 ppm	

'Figures in parentheses refer to number of determinations.

Table 4. Total Pressure Control of Animal Dogs--Daily

Dog Item	Range	Mean
Recovery Corroboration (Dr.)		

whole milk when expressed on a wet-weight basis represented losses of mercury in freeze-drying. These losses were not quantitated since the levels found were generally below the lower limit of detection. Samples of spray-dried milk from a commercial source contained levels close to the upper limit reported by Corneleiussen (1969). Similar values were observed for the cheese sampled in the present study.

Poultry Products

Chicken meat was found to contain higher levels of mercury than pork or beef. Smart and Lloyd (1963) reported 0.01 ppm or less in tissues from hens fed untreated seed, while Westoö (1966) found mercury residues in normal Swedish commercial samples of poultry to be in the range of 0.028 to 0.031 ppm for liver and 0.009-0.022 ppm for breast meat. Results of the present study are consistent with those of Westoö. Liver levels of mercury obtained in this study were not substantially higher than those of breast meat, in agreement with the observation of Jervis (1970) that increased liver levels were not a consistent feature with young birds.

Results of Table 10 indicate that in both vacuum drying and lyophilization of eggs in the pre-treatment for analysis, considerable losses of mercury occur, with greater losses in vacuum drying. Similar results were obtained by Pappas and Rosenberg (1966) in vacuum drying of fish and egg samples prior to their combustion analysis. However, with

Table 8. Total Mercury Content of Animal Foods--Poultry

Food Item	Mercury Concentration (ppm) Range	Mean
Chicken Meat (Breast Muscle)	0.015-0.030	(6)[1] 0.025 ± 0.005
Chicken Liver	0.025-0.030	(6) 0.030 ± < 0.005
Eggs, Fresh, Whole	0.030-0.040	(6) 0.035 ± 0.005
Eggs Lyophilized	0.03 -0.07	(6) 0.05 ± 0.015
Eggs Vacuum Dried	0.005-0.01	(6) 0.01 ± < 0.005

[1] Figures in parentheses refer to number of determinations.

lyophilized eggs these workers obtained values in the range 0.0-0.003 ppm which are lower by at least one order of magnitude than those of the present study. While the results of Table 10 indicate a 71 percent loss of mercury in lyophilization, Pillay et al. (1971) reported even higher losses ranging from 81-98 percent from fish homogenates (using mercury-free vacuum gauges), which in part is thought to explain the low background levels reported by Pappas and Rosenberg on lyophilized eggs. Hens fed grain treated with methylmercury were shown to produce eggs having twice the mercury concentration as was present in the fodder (Westoö, 1966). Tejning (1967) also pointed out that in the female chicken a considerable amount of excretory mercury normally accumulating in feathers is lost to the egg. Due to this capacity for biological concentration, eggs like fish may probably have higher levels than other foods as is suggested by the results of this study.

Meats

Mercury levels in meats are represented in Table 9. Levels were below 0.01 ppm in both pork and beef although corresponding liver samples showed higher values in the range 0.01-0.03 ppm representing some degree of liver accumulation of mercury relative to flesh. The values obtained for pork and beef in the present study were below those reported by Westoö (1966) for Swedish pork (0.06 ppm) and by Somers for Canadian meat (0.04 ppm) respectively.

Table 9. Total Mercury Content of Animal Foods--Meats

Food Item	Mercury Concentration (ppm) Range		Mean
Pork (i)	$\leq$ 0.005	(6)[1]	0.005 $\pm$ < 0.005
Pork (ii)	$\leq$ 0.005-0.01	(6)	0.01 $\pm$ < 0.005
Pork Liver (i)	0.02 -0.04	(6)	0.03 $\pm$ 0.01
Pork Liver (ii)	0.02 -0.03	(5)	0.03 $\pm$ 0.005
Beef	$\leq$ 0.005-0.02	(6)	0.01 $\pm$ 0.005
Beef Liver	0.01 -0.015	(6)	0.01 $\pm$ < 0.005

[1] Figures in parentheses refer to number of determinations.

Table 10. Comparison of Total Mercury Contents of Fresh, Vacuum Dried and Lyophilized Eggs

Treatment	Mercury Concentration (ppm)			
	Range		Mean	
	Wet Weight	Dry Weight	Wet Weight	Dry Weight
Fresh eggs[a]	0.03-0.04	-	(6) 0.035 ± 0.005	-
Vacuum dried[b]	0.00-0.01	0.00-0.01	(6) 0.005 ± 0.005	0.01 ± 0.005
Lyophilized[c]	0.01-0.02	0.03-0.07	(6) 0.01 ± 0.005	0.05 ± 0.01

Percentage loss in vacuum drying --85%

Percentage loss in lyophilization--71%

[a] Representative sample from homogenate of 12 eggs.

[b] Representative sample from above, vacuum dried at room temperature for 16 hours; 1 gm. wet weight = 690 mg. vacuum dried weight.

[c] Representative portion of homogenate (a) lyophilized for 10-12 hours; 1 gm. wet weight = 200 mg. dry weight.

Plant Food

Cereals and Legumes

Amounts of mercury found in cereals and legumes are shown in Table 11. The overall mean of all values for cereals was 0.02 with a range of 0.01 to 0.03 ppm. There was no substantial difference in values between different strains of the same grain. It has been reported that the residues in ears of grain grown from dressed seed are very small. Westermark (1967) found mercury levels in grains of wheat or barley ranging from 0.008-0.012 ppm whether or not they were grown from dressed seed, while Somers (1971) reported lower ranges of 0.005-0.009 in Canadian wheat. Pappas and Rosenberg (1966) analyzed wheat samples from various parts of the U.S. and reported values ranging from 0.013-0.127 ppm. However, it was not ascertained whether the wheat was grown from treated wheat or not.

In Japan, where organomercurials have been extensively used in rice cultivation, Tomizawa (1966) found comparatively high background levels of mercury of 0.227-0.238 ppm in rice from unsprayed fields, while Smart and Hill (1968) reported negligible levels ($\leq$ 0.005) of mercury in rice imported to U.K., occasionally rising to 0.01-0.015 ppm. It was not ascertained whether the rice used in this study was of domestic origin (U.S.) or was imported. However, levels of mercury obtained were in agreement with those reported by Smart. The mean value of 0.03 ppm obtained for legumes was the highest level observed in plant products, but was of

Table 11. Total Mercury Content of Plant Foods--Cereals and Legumes

Food Item	Mercury Concentration)ppm) Range		Mean
Cereals			
Wheat (Dickson)	0.01 -0.025	(6)'	0.02 ± 0.01
Wheat (Beizes)	0.02 -0.03	(6)	0.02 ± 0.005
Barley (Avon)	0.02 -0.025	(4)	0.02 ± < 0.005
Barley (Talbot)	0.02 -0.04	(6)	0.03 ± 0.01
Oats (Garry)	0.01	(5)	0.01 ± < 0.005
Rice, polished Long grain	≤ 0.005-0.01	(7)	0.01 ± 0.005
Mean value for Cereals			0.02 ppm
Legumes			
Beans--Navy	0.025-0.03	(6)	0.03 ± 0.005
Beans--Red Kidney	0.02 -0.035	(6)	0.03 ± 0.01
Mean Value for Legumes			0.03 ppm

'Figures in parentheses refer to the number of determinations.

the same order of magnitude as those reported by Corneleiussen (1969) and Somers (1971) of 0.01 and $\leq$ 0.02 respectively.

Fruits and Vegetables

Table 12 summarizes the results of analysis of fruits and vegetables. Of 39 samples of fruit and vegetables examined in 4 to 6 replicate analyses, 24 gave mercury concentrations less than 0.01 ppm and they did not show significant traces of mercury when considered individually. However, these samples treated as a group showed a mean level approaching 0.01 ppm revealing a detectable trace of mercury in the majority of the samples.

In work on experimentally sprayed apples, residues ranging from 0.02 to 0.12 ppm were reported by Martin and Pickard (1957), while values for unsprayed controls were usually $\leq$ 0.01 ppm. Jacobs and Goldwater (1961) found residues of 0.07-0.06 ppm in sprayed Red Delicious apples while unsprayed controls had only 0.01 ppm at harvest and values as high as 0.16 ppm earlier in the season. The latter values were regarded as being unusually high for background levels of mercury.

Table 12 also shows a comparison of results obtained from unwashed peel, washed peel and pulp samples of potatoes, apples and tomatoes. The consistent difference between unwashed and washed peel of potatoes and apples probably represents surface contamination. The comparable values of mercury in washed peel and pulp of these plant products

Table 12. Total Mercury Content of Plant Foods--Fruits and Vegetables

Food Item	Mercury Concentration (ppm) Range		Mean
Fruits			
Apples (Golden Delicious)			
Flesh	≤ 0.005-0.01	(6)[1]	0.01 ± < 0.005
Peel Washed	≤ 0.005-0.01	(6)	0.01 ± 0.005
Peel Unwashed	0.015-0.03	(4)	0.02 ± 0.01
Apples (Johnathan)			
Flesh	0.01	(6)	0.01 ± < 0.005
Strawberries	0.01	(6)	0.01 ± < 0.005
Tomatoes			
Flesh	≤ 0.005-0.01	(5)	0.01 ± < 0.005
Peel Washed	0.01	(5)	0.01 ± < 0.005
Peel Unwashed	0.01 -0.02	(5)	0.015 ± < 0.005
Vegetables			
Potatoes (Russet)			
Flesh	0.01 -0.015	(4)	0.01 ± < 0.005
Peel Washed	0.01 -0.02	(6)	0.015 ± 0.005
Peel Unwashed	0.02 -0.03	(6)	0.03 ± < 0.005
Potatoes (Merrimack 528)			
Flesh	≤ 0.005-0.010	(6)	0.01 ± < 0.005
Peel Unwashed	0.01 - .020	(6)	0.015 ± < 0.005
Asparagus	0.01 -0.015	(6)	0.01 ± < 0.005
	Mean Value for Fruits and Vegetables-0.01		

[1] Figures in parentheses refer to number of determinations.

indicates the lack of a distribution differential at background levels. Smart (1964) extended this observation to treated crops as well and noticed no difference in residues in peel and flesh of potatoes receiving foliar sprays in the growing season. He further adduced that the residue present in peel was evidence that soil mercury could be taken up by the skin of the tuber as well as by the root system.

Bread, Salt and Sugar

Results of analysis of bread, salt and sugar are shown in Table 13. Of the three subsidiary foods analyzed, salt was the only dietary item found to have levels of mercury higher than most of the other foods examined. This is of further interest since salt was the only mineral food examined and its higher mercury content may be related to geological levels of mercury. It is also probable that the higher values recorded could have resulted from interfering absorption (at 253.7nm) by some inorganic or organic component or additive of the salt. The values obtained, however, also necessarily incorporate a possible negative error arising from incomplete reduction of the mercury in the final reduction step by presence of traces of chlorine liberated from the previous $KMnO_4$ oxidation. The high degrees of variation between replicate readings was thought to result from the varying degrees of incomplete reduction due to the presence of residual chlorine. Further experimentation with

Table 13. Total Mercury Content of Foods: Sugar, Salt and Bread

Food Item	Mercury Concentration (ppm) Range		Mean
Sugar (White) (i)	0.01	(6)'	0.01 ± 0.005
Sugar (ii)	0.01	(6)	0.01 ± < 0.005
Salt (non-iodized)	0.03 -0.09	(6)	0.06 ± 0.02
Bread (White)	≤ 0.005-0.01	(6)	0.005 ± < 0.005

'Figures in parentheses refer to number of determinations.

Table 14. Summary of Base-Line Levels of Mercury in Major Food Groups--Comparison with Reported Values

Food Class	Mercury Concentration (ppm)		Reported Mercury Conc.[a] (ppm) ± 30%	
			Minimum	Maximum
Red Meat (Pork and Beef)	(18)[1]	0.01	0.010	0.05
White Meat (Chicken)	(6)	0.024		
Eggs	(6)	0.04		
Fish	(17)	0.17		
Milk	(21)	0.01	0.002	0.02
Liver (Pork and Beef)	(18)	0.02		
Cereals	(31)	0.02	0.02	0.05
Legumes	(12)	0.03	0.002	0.010
Fruits and Vegetables	(39)	0.01		

[a] Mercury levels (by food class) reported by Corneleiussen (1969) for major cities in the U.S.

[1] Figures in parentheses refer to the total number of samples analyzed.

technique is required to completely eliminate all traces of chlorine before the final reduction step, to verify the results of this study.

Levels of mercury obtained for sugar and bread are consistent with those reported by other workers (Lee et al., 1972).

Methodology

Comparison of Mercury Recoveries from Digestion Procedures I, II and III

The results of comparative recoveries of mercury from fish and egg with digestion procedures I, II, and III are shown in Table 15. Already monitored reference samples of fish (of known mercury content) were subjected to the three digestion procedures. In digestion I and II samples were analyzed at intervals of 1 hour and 12 hours following digestion.

The results of Table 15 indicate comparable recoveries of mercury from fish, with digestions I and II. Concentrations of mercury in both cases showed excellent agreement with the monitored value. Mercury concentrations in fish and egg samples, obtained with digestion III, were lower by over 50 percent than those from digestions I and II and for this reason digestion III was considered inadequate. The lower results are probably attributable not to losses of mercury in digestion but to incomplete release of organically bound mercury in the foods examined when HNO_3 (35%) was used alone.

Table 15. Levels of Mercury in Fish and Eggs Determined by Three Digestion Procedures

Method	Digestion		Mercury Concentration (ppm) [a]	
	Acids	$KMnO_4$ Oxid.	Fish[b]	Eggs
Dig. I	H_2SO_4	1 hour from Dig.	0.05	-
	(Sp.Gr. 1.84)	12 hours from Dig.	0.26	0.035
Dig. III	HNO_3	1 hour from Dig.	0.15	-
	(35% w/v)	12 hours from Dig.	0.15	0.01
Dig. II	HNO_3:H_2SO_4	1 hour from Dig.	0.27	-
	v/v 2:1	12 hours from Dig.	0.285	-
	HNO_3	-	0.06	
	(35%)			

(a) Mean of 4 to 6 replicates.

(b) Samples of fish monitored by the M.S.U. Institute of Water Research Lab., by Atomic absorption spectrophotometry and H_2SO_4:$KMnO_4$ digestion, to contain 0.24 ppm mercury.

A difference in mercury concentrations between samples of fish, at 1 hour and 12 hour intervals from digestion, was observed with digestion I. Final oxidation with $KMnO_4$ was carried out on the steam bath instead of by direct heating on a hot-plate as in the Barret (1956) and Dow (1970) procedures and these oxidation conditions were probably not vigorous enough to complete the oxidation. Oxidation was apparently completed in the 12 hour standing period as suggested by the higher mercury concentration at the end of this interval. Overnight standing of the digests from digestion I before final analysis was therefore adopted as a routine practice.

Recovery Results

The recovery data for digestion procedures I and II using methylmercuric chloride are shown in Tables 16 and 17. Table 16 shows the recovery data obtained with digestion I using 1 gm. samples of chicken meat spiked with methylmercury standards in the range 0.01 to 0.2 µg. mercury. An average recovery of 83 percent was obtained in this range. Recoveries of mercury from methylmercuric chloride spiked samples of barley, using digestion II are shown in Table 16. Average recoveries by this method were 87 percent. Recovery of mercury from mercuric chloride spiked samples of food are shown in Table 18. An average recovery of 97.6 percent of mercury added to milk, barley, potatoes, beans and chicken meat was obtained.

Table 16. Recovery of Mercury Added As Methylmercuric Chloride to 1 gram Samples of Chicken Meat (Leg Meat) by H_2SO_4:$KMnO_4$ Digestion--Digestion I

µg. Hg. Added	Net µg. Hg Estimated	µg. Hg Recovered		Percent Recovered		Mean % Recovered
		Maximum[1]	Minimum[2]	Maximum	Minimum	
0.01	0.035	0.01	0.005	100	50	
	0.030	0.005	0.00	50	-	63
	0.035	0.01	0.005	100	50	
	0.035	0.01	0.005	100	50	
0.03	0.055	0.03	0.025	100	83	
	0.060	0.035	0.030	116	100	91
	0.055	0.030	0.025	100	83	
	0.050	0.025	0.020	83	66	
0.05	0.07	0.045	0.040	95	80	
	0.08	0.055	0.050	110	100	98
	0.08	0.055	0.050	110	100	
	0.075	0.050	0.045	100	90	
0.10	0.110	0.085	0.070	85	70	
	0.100	0.075	0.070	75	70	79
	0.110	0.085	0.075	85	75	
	0.115	0.090	0.085	90	85	
0.20	0.20	0.175	0.170	88	85	
	0.220	0.195	0.190	98	95	84
	0.190	0.165	0.160	83	80	
	0.180	0.155	0.150	78	75	
				Average Recovery		83%

[1]Net-minimum blank reading--.025 µg.

[2]Net-maximum blank reading--.030 µg.

Blank includes a reagent blank and mercury content of the chicken meat.

Table 17. Recovery of Mercury Added As Methylmercuric Chloride to 1 Gram Samples of Barley--(HNO_3:H_2SO_4) Digestion II

µg. Hg Added	Net µg. Hg Estimated	µg. Hg Recovered Maximum[1]	µg. Hg Recovered Minimum[2]	Percent Recovered Maximum	Percent Recovered Minimum	Mean % Recovered
0.01	0.045	0.01	0.005	100	50	75
	0.045	0.01	0.005	100	50	
0.03	0.06	0.025	0.020	83	66	83
	0.065	0.030	0.025	100	83	
0.05	0.08	0.045	0.040	90	80	90
	0.085	0.050	0.045	100	90	
0.10	0.135	0.100	0.095	100	95	92
	0.125	0.090	0.085	90	85	
0.20	0.230	0.195	0.190	97	95	95
	0.225	0.190	0.185	95	93	
				Average Recovery		87%

[1]Net µg. Hg--minimum blank reading-0.035 µg.

[2]Net µg. Hg--maximum blank reading-0.040 µg.

Blank includes a reagent blank and mercury content of the barley.

Table 18. Recovery of Mercury Added As Mercuric Chloride to 1 Gram Samples of Food

Food Item	Digestion	µg. Hg Added	Percent Hg Recovered	Mean % Recovery
Milk Spray Dried	H_2SO_4 (Dig. I)	0.03	107 106	106.5
Barley	HNO_3:H_2SO_4 (Dig. II)	0.04	80 105	92.5
Potatoes	HNO_3:H_2SO_4 (Dig. II)	0.05	111 94	102.5
Beans, Navy	HNO_3:H_2SO_4 (Dig. II)	0.01	88 92	90
Chicken Meat	H_2SO_4 (Dig. I)	0.20	95 98	96.5
		Average Recovery		97.6% ± 8.6

The variation in replicate recovery values at the 0.01 µg. level need to be interpreted in terms of limitations of instrument readings, on account of which a difference of scale reading of 0.005 µg. results in a $\pm$ 50 percent difference in recovery.

Results of interlaboratory comparison of the mercury content of fish samples is represented in Table 19. Excellent agreement between the values obtained (using atomic absorption spectophotometry and H_2SO_4:$KMnO_4$ digestion) reflects the accuracy of the analytical techniques and the degree of instrument reliability.

Table 19. Interlaboratory[a] Comparison of Mercury Concentration of Fish (ppm)

Fish	Digestion	Mercury Concentration (ppm) Determined Range	Determined Mean[1]	Monitored[b] Mean
Sunfish, Pumpkin Seed	Dig. I	0.11-0.14	(6) 0.12 ± 0.00	0.11
Sunfish, Pumpkin Seed	Dig. II	0.14-0.16	(4) 0.15 ± 0.01	--
Yellow Perch	Dig. I	0.12-0.18	(5) 0.15 ± 0.00	0.16
Largemouth Bass	Dig. I	0.22-0.28	(6) 0.24 ± 0.025	0.24

[a] Institute of Water Research Laboratory, M.S.U.

[1] Figures in parentheses refer to number of replicates.

[b] Samples monitored at Water Research Lab. by Atomic absorption Spectrophotometry using H_2SO_4:$KMnO_4$ digestion.

GENERAL DISCUSSION

The primary objective of the study was the determination of trace mercury background levels in foods of Michigan origin, in which there was no direct exposure to mercury contamination. The results indicate that practically all the foods examined contained traces of mercury and for the most part, these levels were barely above detection limits. The results of this study are consistent with those of surveys carried out in the U.S. (Corneliussen, 1969) and more recently in the U.K. (Ministry of Agriculture, Fisheries and Food, U.K., 1971), indicating no overt contamination of foods above the base levels ranging from 0.01 to 0.03 ppm. The levels detected probably represent a background level of naturally occurring mercury derived from soil, ground water or atmospheric sources.

However, it is difficult to differentiate between mercury of social and industrial origin, from the naturally occurring geologically related concentrations of mercury in nature. The wide variations in values of background mercury levels in soils, reported by different workers illustrates this. Martin (1963) reported natural background levels of some English soils to be in the range of 10-60 ppb while Anderson (1967) reported values of 20-920 ppb in Swedish soils.

Kimura and Miller (1962) found levels of 116 ppm for soils from Washington, in the U.S. Relatively high mercury concentrations were reported in soil samples taken from Central Michigan (U.S. Dept. of Interior, 1971), in the range 200-1,500 ppb as compared to a 71 ppb national average.

The major atmospheric sources of mercury contamination arise from the burning of fossil fuels such as shale oil and coal. Mercury from such sources could be transported considerable distances by the wind and be deposited on vegetation situated windward from such sources. Atmospheric concentrations of mercury further add to soil and ground water levels by being washed down in rain water. Erikson (1967) reported concentrations of mercury in rain water ranging from 0.000-0.20 ppb. Background levels in ground water have been reported by Dall'aglio (1968) in Italy ranging from 0.01-0.05 ppb, while Hinkle and Learned (1969) reported higher ranges of 0.2-0.7 ppb in the U.S. While soil and ground water levels of mercury contribute in greatest measure to the background levels of systemic mercury in foods, mercury from atmospheric sources cannot be entirely discounted particularly in relation to physical contamination of exterior surfaces and entrappment. Additionally, many parameters have been found to influence the movement of mercury and metal ions in the environment, including pH, temperature, presence of biological agents, etc. Variations among these and other unknown parameters can increase the mercury available to a

system many times beyond the normal background level (D'itri, 1972).

The foregoing evidence offers a basis for the presence of a background level of mercury in most common foods. It is important therefore to set and evaluate tolerance levels of mercury in relation to background levels inasmuch as it is important to continuously monitor foods and food sources to ensure that the background levels are well within safety standards.

The overall mean of mercury concentrations in animal products was higher than that in plant products. The generally higher background level of mercury in animal products is probably a result of bioaccumulations within the food chain. A recent survey of mercury levels in foods (Ministry of Agr. Fisheries & Food, 1971) carried out in Britain indicates that mercury in animal feeds was higher in comparison to most human foods specially in fish meals and some grains. It is conceivable that animals could concentrate the mercury derived from such feed sources.

Dried plant products, viz cereals and legumes had higher levels in comparison to fruits and vegetables. This latter difference could be a reflection of the differing water contents of these two categories of food or could be related to the higher protein content of the cereals and legumes as compared to fruits and vegetables.

Though different species of animal and plant materials were analyzed there was no substantial difference in levels

between the different species except in the case of fish.

The results of the present study also confirm the findings of Pillay et al. (1971) in relation to mercury losses in vacuum drying and freeze drying of samples as a preliminary step to digestion. Neither of these treatments is therefore desirable for the concentration of samples prior to digestion. However, the losses of mercury in freeze-drying may find application in the preparation of mercury-free fish-meals or fish protein concentrates from mercury contaminated fish.

Methodology

While the main objective of this study was the evaluation of mercury contents of foods, it was necessary to develop a suitable analytical method for determining sub-microgram quantities of mercury in a wide variety of organic matrices. A rapid, simple and adequately sensitive analytical procedure capable of being applied to the routine monitoring of a wide variety of matrices was sought.

The basic demands on the analytical method were

1. Use of a small sample size
2. Limited digestion time
3. Moderate digestion conditions
4. Use of simple equipment, permitting several samples to be processed concurrently with little attention.

Choice of sample size was dictated by the need to minimize reagent volumes and digestion time, while permitting

the use of small 100 ml. capacity digestion flasks, without loss of material in the initial reaction with the digesting acids. After preliminary experimentation a sample size of 1 to 3 gm. was found optimum both in terms of digestion time and of giving results within the limits of sensitivity of the method.

Digestion

The H_2SO_4 digestion was found applicable to most animal products except beef liver, pork liver and cheese. While liver samples tended to foam in the final reduction-aeration step either from incomplete breakdown and/or from the formation of surface active compounds, cheese samples gave too high a residue of undigested fat in acid digestion. Though the latter limitation was observed also with eggs, both digestions II and III yielded much lower recoveries of mercury from eggs. The mixed acid digestion (Digestion II) was however used for the analysis of both liver and cheese.

Due to the high content of carbohydrate in plant materials, Digestion I generally resulted in charring and carbonization. Mixed acid digestion was therefore used on all plant materials. Dried plant materials, notably cereals and legumes, reacted violently in the early stages of digestion when concentrated HNO_3 (Sp. Gr. 1.47) was used. Laug and Nelson (1942) pointed out that a violent reaction and excessive foaming in the early stages of the reaction may lead to losses of mercury. Abbott and Johnson (1957) showed that mercury may be carried

away though the digestion mixture by the carbon dioxide which was evolved, but Gorsuch (1959) working with different experimental conditions found no such losses. Nevertheless in all cases, 35 percent HNO_3 in the mixed acid digestion was found to give a smoother reaction with minimal foaming and was used in place of concentrated HNO_3. Digestion was started with HNO_3 to a point of partial dissolution before addition of H_2SO_4 to moderate the initial reaction that otherwise occurred on addition of the mixed acids together. Pickard and Martin (1960) used the same expedient in the digestion of tomatoes and coffee beans. Cooling of the digest in ice during the addition of the H_2SO_4 followed by gradual heating eliminated the sudden and violent evolution of nitrous fumes which otherwise occurs.

The use of selenium as a fixative to prevent losses of volatile mercury has been reported by a number of workers (Kunze, 1948; Abbott and Johnson, 1957), with analyses involving dithizone complexing and colorimetric estimation of mercury. Limited experimentation with selenium suggest its use is not applicable when analysis is accomplished by the flameless atomic absorption technique, because of spurious high signals either from absorption by selenium or some volatile compound of selenium at 253.7nm.

Oxidation

The oxidising agents normally used in the wet digestion procedure are $HclO_4$, H_2O_2 and $KMnO_4$. Gorsuch (1959) first

found that mixed HNO_3:H_2SO_4:$HClO_4$ acid could be used in determining traces of mercury in foods. The method was also proposed by the Analytical Methods Committee of the Society for Analytical Chemists (1960) but was not recommended for trace residue levels due to losses arising from the formation of volatile chloro compounds.

Polley and Miller (1955) used H_2O_2 in microdetermination of mercury in soils and biological materials. The method was investigated by Kudsk (1964) who reported low recoveries as did Campbell and Head (1955). In this study, poor recoveries of mercury added as mercuric chloride in the 0.05-0.15 µg. range, were obtained when H_2O_2 oxidation was tried in preliminary experimentation with wheat.

Equipment

A number of authors have stressed the necessity of using special digestion vessels with complicated condenser and recovery systems in order to avoid losses of mercury. Kudsk (1964) recommended that a Leibig condenser was completely adequate when used in conjunction with long-necked digestion flasks. Lee and Laufman (1971) used capped centrifuge tubes for digestion of 1 gm. samples of paper pulp with aqua regia, while Hoover et al. (1970) used 125 ml. Erlenmeyer flasks and heated directly on a hot-plate with no attached recovery systems. They were able to obtain recoveries of 95-100 percent of added mercury. The recoveries obtained in this study under different digestion conditions indicated

that the use of long-necked volumetric flasks with moderate digestion temperatures removes the need for elaborate recovery equipment, thereby permitting the analysis of a large number of samples at one time.

Recovery

There is only limited knowledge of the possible variation in chemical forms of mercury present in various foods. Recovery data using methylmercury spiked samples of food served as an indication of the extent of methylmercury breakdown as well as losses of mercury in digestion. The recovery data from the mercuric chloride spiked samples served as an indication of the extent of mercury losses in digestion. Organic and inorganic compounds of mercury were used in recovery studies to obtain an estimate of the extent of mercury release from organically bound forms and the extent of losses from volatilization during the digestion process. However, the use of one or the other chemical form of mercury in recovery studies does not necessarily indicate the extent of breakdown or release of mercury from the forms actually present in the sample. The differences in recoveries of mercury from eggs and fish under identical digestion conditions serves to illustrate this. Recovery data have to be applied and interpreted with a recognition of this limitation.

Precision and Sensitivity

Good agreement with results of total mercury evaluation on reference samples of fish, in interlaboratory comparative

studies served to confirm and validate the accuracy of the analytical and instrumental procedures. The variation in the standard deviation of the replicate analyses reflect only partly the sampling and experimental errors. Since all readings and calculations based thereon were approximated to the closest 0.005 µg., the standard deviation also reflects the limitations of instrument calibration. For this reason it was not attempted to differentiate results below 0.005 µg. with any degree of certainty. Maximum deviation of $\pm$ 50 percent was observed in replicate analyses at the 0.01 µg. level. However, this was thought to be acceptable in view of the low levels of determination and for the limits of precision sought in this study.

SUMMARY AND CONCLUSIONS

The results of this limited survey of Michigan foods indicate that practically all the foods sampled contained detectable traces of mercury and that a background level of a very low order ranging from 0.01 to 0.03 ppm is commonly present. The only significant levels of mercury observed occurred in fish, though these levels were within the F.D.A. tolerance limit of 0.5 ppm. From the data collected, levels of mercury in fresh fruits and vegetables were consistently $\leq$ 0.01 ppm. In comparison levels in cereals and legumes were in the range 0.01 to 0.03 (mean value 0.02) ppm and in the animal products levels were in the range 0.01 to 0.17 ppm with a mean of 0.03 ppm. From the samples analyzed it appears that levels in fruits and vegetables tend to be very low and at or below the lower limits of detection, while those in cereals and legumes are higher and were comparable to those found in animal products. Whether these differences are statistically significant has to be established by more large-scale and statistically valid sampling. While the levels of mercury in foods observed were within the 0.05 ppm guideline (WHO, 1963) for mercury in foods, they suggest that a zero tolerance would be unrealistic. The results of this study should permit some estimates

of the average daily intake of mercury in combination with data on the approximate composition of diets based on the food items examined.

Losses of mercury were noted in the vacuum drying and lyophilization of egg samples preparatory to analysis, precluding the use of these procedures for preliminary concentration or pre-processing of samples to limit bulk or reduce moisture content.

Quantitative monitoring of mercury in foods is handicapped largely by lack of suitable methods of analysis involving a minimum of sample preparation without destruction of sample and that would be adaptable to speedy handling of a large number of samples.

Modifications in methodology were adopted towards these ends. The main features of the methodology used were

1. The use of moderate and controlled digestion temperatures throughout the digestion.
2. The use of conditions minimizing the rate of initial reaction of sample and digesting acids.
3. Use of simple digestion equipment enabling a large number of samples to be handled concurrently.
4. Use of 35 percent HNO_3 in place of concentrated HNO_3 in the mixed acid digestion.
5. Use of one reaction vessel throughout the digestion and subsequent analysis up to the point of reduction-aeration in the aeration flasks, thus minimizing manipulative losses and contamination possibilities.

6. Maintaining the digest under oxidized conditions in excess of $KMnO_4$ up to the final reduction step thus minimizing losses of mercury (at very low dilutions) in the standing period between digestion and reduction-aeration (Uthe et al., 1970)

Results of recoveries of added mercury with the digestion techniques used, on a range of food materials indicates that the methods have merit for determining trace mercury levels in foods.

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