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ARSENIC AND TIN IN FOODS

Reviews of Commonly Used Methods of Analysis
(Revised September 1978)

prepared by
William Horwitz, Ph.D.
Food and Drug Administration
Washington, D.C. 20204, U.S.A.

Published with the cooperation of
The World Health Organization
and
The United Nations Environment Programme

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PART I

REVIEW OF COMMONLY USED METHODS OF ANALYSIS FOR ARSENIC IN FOODS

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INTRODUCTION

Arsenicals have been known for centuries to be toxic to all forms of life. The trivalent form of arsenic is considerably more toxic than the pentavalent form. The use of arsenic trioxide as a poison prompted investigations into methods for its detection and determination in forensic situations. In fact, the detection of arsenic in trace amounts over a century ago can be considered as one of the earliest examples of trace analysis in food chemistry.

The biological role of arsenic has been the subject of considerable discussion and controversy for many decades. Evidence is now accumulating which indicates a role as an essential nutrient for arsenic in some animals (1).

Fifty years ago arsenicals were among the few available and effective pesticides, but their use has declined considerably since the introduction of the organic pesticides. Arsenicals are still used as contact weed killers, as insecticides on fruit, and as desiccants and defoliants on cotton. They are also used as direct additives to poultry and swine feed as growth promotants.

Arsenic compounds are a byproduct of the smelting industry, accompanying sulfur and phosphorus. The production of basic inorganic chemicals, particularly sulfuric acid, from the same mineral sources

resulted in a potential for arsenic contamination in processed foods utilizing these raw materials. Therefore a limit for arsenic, usually of the order of magnitude of 1 to 10 mg/kg and frequently 3 mg/kg, has been included in specifications for industrial and reagent chemicals, drugs, and food and color additives, as well as for food whose processing involved the use of common acids, alkalies, and salts. Modern production no longer utilizes high-arsenic sources of raw materials. Thus this potential source of arsenic in the diet has been eliminated.

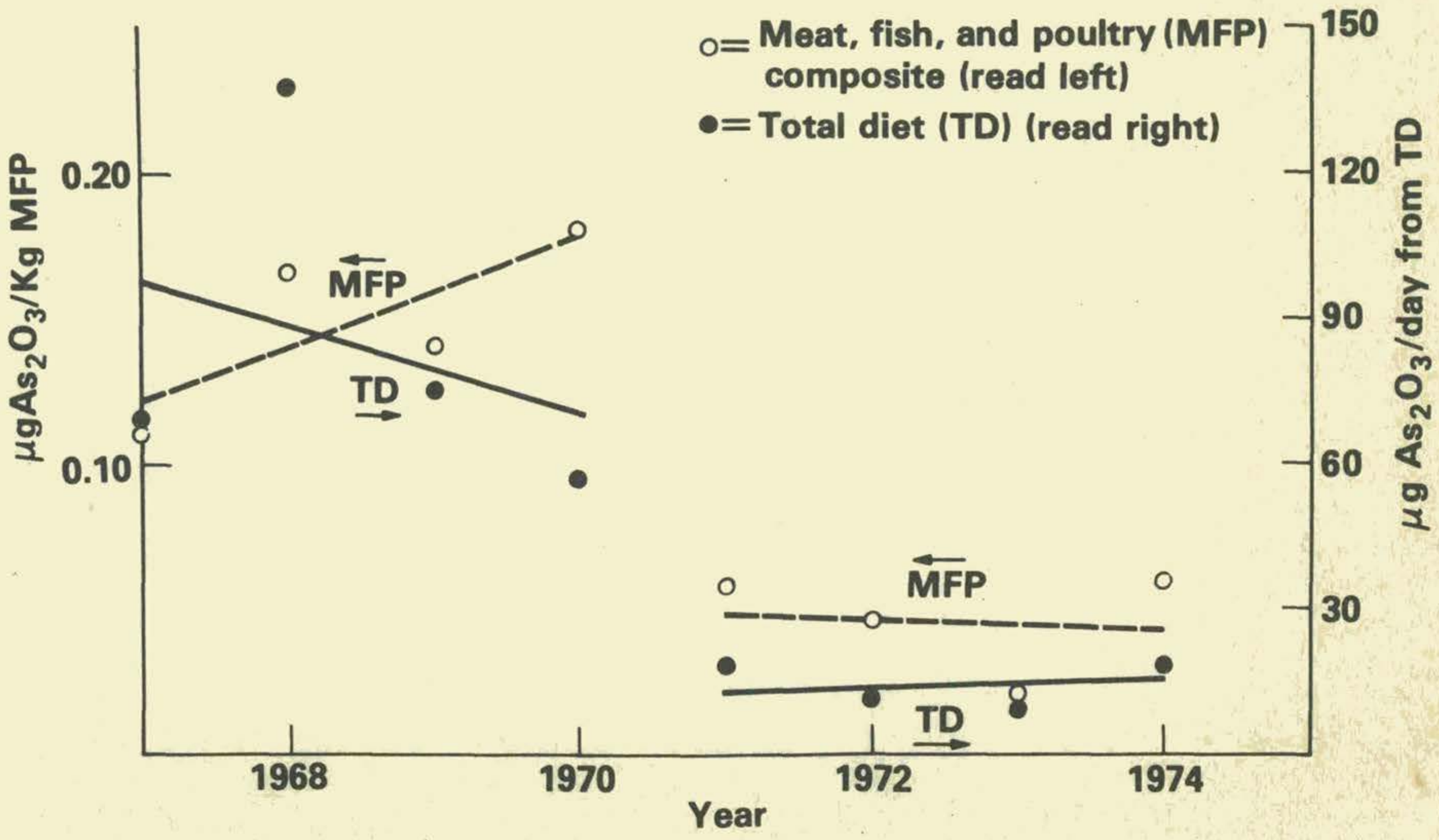
OCCURRENCE

Arsenic is a ubiquitous element; it is present in the environment (air, water, soil) and in living organisms. Schroeder and Balassa (2) estimated that man's daily intake of arsenic is 0.90 mg, 99% from food and 1% from water. In the light of later, more comprehensive surveys, this estimate is much too high. The Joint FAO/WHO Expert Committee on Food Additives (3) tentatively placed the maximum acceptable load of arsenic at 0.05 mg/kg body weight/day, which corresponds to 3.5 mg/day for a 70 kg man. Since daily food and water intake is roughly 3 kg, this maximum acceptable daily intake is equivalent to an arsenic content of 1 mg/kg for all food and water. The Committee estimated that the actual intake from normal diets by persons not exposed to special occupational hazards ranges from 0.007 to 0.06 mg/kg body weight. Subsequent estimates by improved methods of analysis for low levels of arsenic place the intake even below the lower end of the range.

The remarkable influence of methods of analysis on estimates of arsenic intake is shown by Jelinek and Corneliussen (4) in their summary of the arsenic content of the total diet composites in the United States during the reporting periods of 1967 through 1974. (All values are calculated to As_2O_3 although they may be designated as "arsenic.") The meat, fish, and poultry component of the total diet program, which constitutes only 9% of the 3 kg intake (including water), contains from 33 to 100% of the arsenic content of the diet. The average annual level of As_2O_3 in this diet component over the period 1967 to 1974 is shown in Figure 1 (open circles, dotted line, left scale). The average annual daily intake of arsenic from the total diet during the same period is also given (closed circles, solid line, right scale). A substantial discontinuity occurs between 1970 and 1971. At this time the program was consolidated in a single laboratory and the molybdenum blue method of analysis was changed to the silver diethyldithiocarbamate method with a resulting lowering of the blank and operation at a lower limit of reliable measurement. Thus much of the apparent decrease of the As_2O_3 content of the diet from an average of 80 μg/day during 1967-1970 to 15 μg/day during 1971-1974 may be an analytical artifact that may not reflect a drastic decrease in the arsenic intake during this period. An identical artifact is noted in the Canadian total diet program. The level of arsenic found during the first quarter of 1969, using a modified Gutzeit method, would contribute not more than 95 μg to the diet per day. Subsequently the method was changed to the silver

Annual Average Levels of Arsenic (as As_2O_3) in Meat, Fish, and Poultry Composite and Annual Average Daily Intake of Arsenic (as As_2O_3) in the Total Diet

(U.S. FDA Data)



diethyldithiocarbamate procedure of Hundley and Underwood (6) as used by FDA, but preceded by wet ashing (7). The maximum levels of arsenic in the total diets dropped to not more than 30 μg/person/day in 1970 (7), 30 in 1971 (8), and 35 in 1972-1973 (9). In 1977 the U. S. program further changed the method of analysis to the hydride evolution-atomic absorption procedure with a consequent reduction in the limit of reliable measurement from 0.1 to 0.02 mg/kg. Although the data has not yet been assembled or evaluated, there appears to be a further apparent reduction in the arsenic content as a result of this change.

In the U.S. total diet program, components of the meat, fish, and poultry group were analyzed individually. The finfish portion contained the highest levels of As_2O_3. Ninety five samples ranged from none detected to 19 mg/kg with a mean of 1.47; the next highest component was shrimp with a range of 0.3 to 1.5 mg/kg and a mean of 0.67 in 10 samples (4). These values for As_2O_3 are reported on an edible, cooked basis and therefore may not be comparable to most of the values reported in the literature. However, when allowance is made for the reporting basis, they are of the same magnitude as summarized by Zook et al. (10) and by Lunde (11). Zook et al. (10) also reported an average arsenic content of 2.6 mg/kg in the edible muscle of 121 samples from 11 species of fish.

Dairy products, which constitute about 26% of the U.S. Total Diet, make practically no contribution to the daily arsenic intake. Rice had

an unexpectedly high As_2O_3 content with a range from none detected to 0.4 mg/kg and a mean of 0.16 in 27 samples. Overall, the average daily intake and the levels in the various foods covered in the U.S. and Canada pose no hazard to the consumer.

Pentavalent organic arsenic compounds (derivatives of phenylarsonic acid) are deliberately added to poultry and swine feed as growth promotants. Regulatory restrictions have been established in many countries for residues of arsenic in food from various applications. For example, the U.S. Food and Drug Administration has established tolerances for residues of combined arsenic (calculated as As) in the uncooked (raw) edible tissues as follows: 2 mg/kg in byproducts of chickens and turkeys and in the liver and kidney of swine; and 0.5 mg/kg in the muscle of chickens, turkeys, and swine, in byproducts other than liver and kidney of swine, and in chicken and turkey eggs (12). These tolerances are easily met by withdrawing arsenic-containing feed at least five days before slaughter. A tolerance of 0.7 mg/kg (calculated as As_2O_3) exists for residues in cattle and horse meat as a result of dermal application of potassium arsenite for tick control, replacing the previous use of chlorinated hydrocarbons. A large amount of the arsenic fed to poultry and swine is excreted in the urine and feces. Animal wastes are being considered for recycling as animal feed (13). The addition of processed animal wastes to animal feeds will contribute to the arsenic content of the meat and poultry portion of the diet.

A tolerance of 3.5 mg/kg as arsenic trioxide (2.65 mg/kg as As) also exists in the United States for residues resulting from the application of calcium, copper, magnesium, and sodium arsenates to various

berries and vegetables. A tolerance of 7 mg/kg has been promulgated for residues of lead and the associated arsenic (2.5 mg/kg) from application of lead arsenate to various berries and fruits (14).

Both liver and applesauce, foods most likely to show the result of legal use of arsenic compounds, have mean values of less than 0.1 mg/kg in the U.S. Total Diet (4).

SCOPE OF REVIEW

The analytical problem to be dealt with in this review is the determination of arsenic at levels ordinarily ranging from 0.1 to 10 mg/kg in complex food substrates such as muscle (protein and fat), which also normally contain considerable amounts of the related element phosphorus and the problem agent chloride. Gorsuch (15) classifies arsenic as an element which causes analytical problems under most conditions, particularly its loss as volatile arsenic trichloride during wet ashing and its poor recoveries on direct ashing.

This review is not intended to be all encompassing. Rather it will cover the determination of arsenic in food and related substrates (animal feed, water). It will cover those techniques capable of application in the ordinary well-equipped laboratory engaged in monitoring and enforcement work. Classical procedures are included which can be applied successfully where modern instrumentation might not operate properly because of lack of stable power sources, unavailability of trained operators and electronics technicians, and inaccessability of reagents.

This review will not cover those techniques which require a large investment in capital and operators (e.g., neutron activation). Such techniques may be mentioned in connection with the establishment of reference values or for occasional utilization for quality control. The practicality of techniques should be considered not only in terms of resource requirements, but also in connection with the availability of samples for examination. Very rapid procedures for monitoring, for example, are not efficiently utilized unless sample collection operations maintain a smooth and abundant flow of samples.

This review will also concentrate on published, collaboratively studied, general purpose methods and collateral studies. Such methods are already known to perform satisfactorily in laboratories not associated with their development. They also have available unbiased data relevant to accuracy, precision, applicability, sensitivity, limit of detection, and practicability, as appropriate. Such data have already been accumulated in a number of representative laboratories on typical substrates analyzed as unknowns. Laboratories that cannot reliably analyze samples by these recommended methods will usually find it more profitable to examine their laboratory operations, particularly their standards, preparation of standard solutions, instrument calibration, and analyst training and capabilities, rather than attempt to "improve" the method.

Methods which have been developed for specific foods, such as sugars, wines, cereals, milk, etc., have not been considered because of

presumed limited applicability. In some cases, such methods may be superior for the limited application for which they were tested, since most of them were derived from the general purpose methods.

Reviews of broader scope, with emphasis on newer techniques, involving more sophisticated instrumentation, including other substrates and other elements, will be found in the following articles:

Crosby, N. T. (1977) Determination of metals in foods: A review. Analyst 102, 225-268. Best recent review of techniques applicable to metals of interest in food analysis. 346 references.

Talmi, Y. and Feldman, C. (1975) The determination of traces of arsenic: A review. Chap. 2 in Arsenical Pesticides, Woolson, E. A., ed. American Chemical Society, Washington, D.C. pp. 13-34. General review, including speciation. 167 references.

Talmi, Y. and Bostick, D. T. (1975) The determination of arsenic and arsenicals. J. Chromatographic Sci. 13, 231-237. Similar to previous review but with greater emphasis on chromatographic techniques. 52 references.

Lewis, R. G. (1977) Determination of arsenic and arsenicals in foods and other biological materials. Residue Reviews 68, 123-149. General review, including collection and storage methods and the most recent methods for sample preparation and analyses. 130 references.

The classical work on the subject is:

Monier-Williams, G. W. (1949) Trace Elements in Foods. John Wiley & Sons, Inc. New York. Chapter 5 Arsenic. Contains the history of the interest in arsenic as a food contaminant and reviews the earlier methods including the Gutzeit and molybdenum blue, up to about 1945. 129 references.

SAMPLING

There are no special requirements regarding the sampling of foods and biological materials for arsenic determination when the objective is trace metal content or adherence to specifications. When applied as a pesticide, an arsenical is likely to be deposited irregularly, and consequently as large a sample as possible should be taken for analysis. When applied to fruits, lead arsenate is concentrated at the calyx and stem ends. Arsenic, like phosphorus, is associated more with bone than with tissue. When introduced through use of processing chemicals, arsenic is more likely to be associated with the aqueous phase than with the insoluble solids. It is not usually associated with the lipid fractions.

Occasionally arsenic may contaminate a lot of bulk food through shipment in the holds of vessels or in railroad cars which previously contained ores, minerals, or bulk chemicals. In such cases, representative sampling serves only to dilute the contaminant. Sampling should be selective in such situations; attempts should be made to obtain portions most likely to be in contact with the contaminant. Rapid optical microscopic examination for the contaminant is likely to be helpful in locating the desired portions containing extraneous contamination.

METHODOLOGY

All of the methods for the determination of arsenic now in common use consist of three basic steps: (1) Elimination of organic matter; (2) isolation of the arsenic as a volatile halide or as arsine; and

(3) determination, originally as a stain on paper strips (Gutzeit method) and now by colorimetry or atomic absorption spectrometry.

Organic matter is eliminated by either wet or dry oxidation. Apparently both are equally satisfactory when properly conducted, provided the volatility of arsenic compounds is kept in mind. The choice is more a matter of convenience depending upon the nature and number of samples.

Arsenic must be separated from the digested matrix because of its similarity to the common related elements phosphorus and silicon. Antimony also reacts like arsenic but is rarely encountered in foods, feeds, and related materials. The volatility of arsenic halides or arsine compared with the corresponding or nonvolatile compounds of potentially interfering elements provides the needed separation.

In most methods to be discussed here, arsenic(V) is reduced to arsenic(III), which in turn is reduced to arsine (AsH_3) by the action of hydrogen liberated by zinc and hydrochloric acid or in more recent methods by sodium borohydride. The arsine is "scrubbed" with lead acetate to remove sulfides, a step which is unnecessary with atomic absorption methods. The arsine can be measured by three fundamentally different methods. The simplest and least precise consists of reacting the arsine with a mercuric salt impregnated on filter paper strips or disks and comparing the intensity and length of the stain with standards. Although characterized as "semi-quantitative," this method was the only one available for many decades and satisfactorily served to monitor arsenic

residues on fruits and accidental contamination in foods. Now the arsine is measured more precisely by various modifications of atomic absorption spectrometry. Finally, the arsine can be trapped by an oxidizing or solubilizing solution and the arsenic measured colorimetrically by forming molybdenum blue or by reacting with silver diethyldithiocarbamate. Table 1 and Figure 2 show the various steps involved in the determination of arsenic and the interrelationships of the various methods.

In many cases the methods have been studied as a complete unit so that the contribution each step makes to the total error is not easily isolated. Important sources of error in arsenic analysis are derived from environmental contamination and variability of blanks caused by heterogeneity of reagents. Handling samples and containers and performing chemical operations in near "cleanroom" facilities, and keeping vessels and containers covered whenever possible will minimize contamination. Dust and other particulate matter such as cigarette ash may contain arsenic. Only the highest quality, low-arsenic reagents should be used in trace analysis. Deionized water is also preferred for elemental analysis. Containers washed with phosphate detergent should be rinsed thoroughly with distilled or deionized water and, where appropriate, with dilute nitric acid. Phosphate detergents have been reported to contain appreciable quantities of arsenic (16, 17). In fact, Buttrill recommends that glassware not be washed routinely with soap or detergents (29). Ihnat and Miller (18) specify a reagent procedural blank with each set of five determinations to correct for arsenic introduced from the reagents and the environment.

TABLE 1. DETERMINATION OF ARSENIC

ELIMINATION OF ORGANIC MATTER

Wet oxidation (H_2SO_4, HNO_3, $HClO_4$)

Dry ashing (Mg salts, Na_2CO_3)

ISOLATION OF ARSENIC

Halide distillation ($AsCl_3$, $AsBr_3$)

Arsine (Zn + HCl, $NaBH_4$)

DETERMINATION

Gutzeit stain on Hg(II) impregnated paper

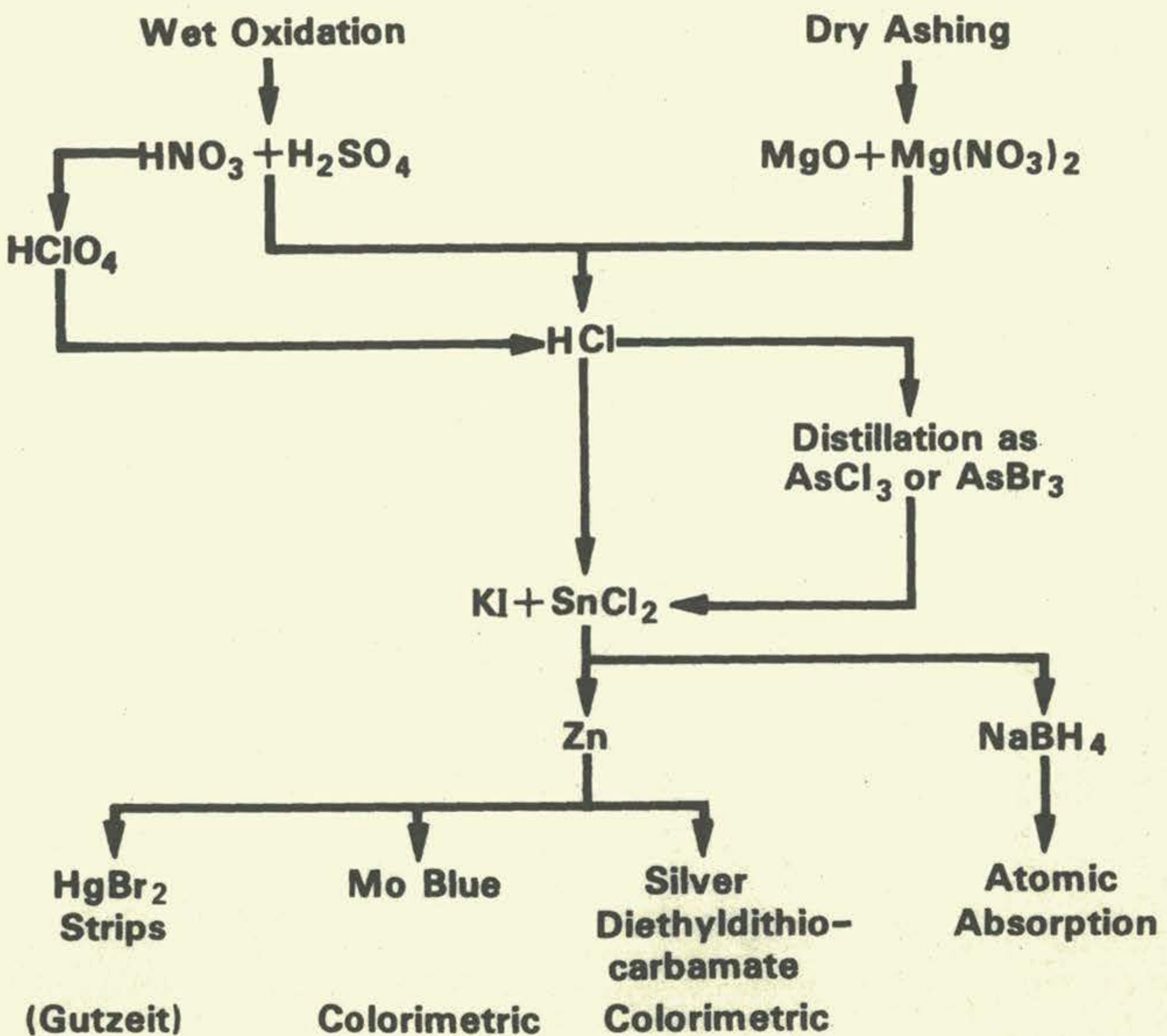
Colorimetric

Molybdenum blue

Silver diethyldithiocarbamate

Atomic absorption

Interrelationships of Methods of Analysis for Arsenic



ELIMINATION OF ORGANIC MATTER

Although there may be a few cases in which mild solubilization treatment may make arsenic available directly for determination (19), food analysis generally requires the elimination of organic matter, especially from bone and fatty tissue. This is usually accomplished by wet oxidation with nitric and sulfuric acids or by dry ashing in the presence of an alkaline fixative.

Gorsuch (20) believes that the ashing stage introduces more error into the arsenic determination than all the other effects. However, when the details of the digestion were specified (details not given yet) in a study which included isolating the digestion error and comparing it with the systematic and determinative errors of hydride generation techniques, Ihnat and Miller (21) found that the sample digestion contributed little variation to the final result. As Gorsuch mentioned (20, p 104), the nature of the organic material appears to have a bearing on the suitability of the ashing technique.

The greatest source of difficulty in the ashing of arsenic (20, p 27) is the presence of chloride, a normal component of many foods and an added component of many processed foods. Arsenic transformed into $AsCl_3$ is relatively volatile, and this fact is the basis of a well known analytical method for separation of arsenic from interfering materials. Ordinarily, predigestion with nitric acid will remove the chloride ion as nitrosyl chloride (bp -5.5^{o}) (20, p 27). Mechanical losses are

minimized by the use of long-neck flasks during digestion and the refluxing action produced. Sublimation is avoided during wet digestion by shielding the flask above the acid level from the heat source. Additional nitric acid should be added whenever the contents of the flask begin to darken and char.

The two major ashing techniques may be considered complementary to each other for the determination of arsenic in foods as shown by Table 2.

Some foods and plant products present special difficulties in the wet oxidation procedure. Hoffman and Gordon (22) reported low and erratic recoveries from fish subjected to the ordinary sulfuric and nitric acid digestion. These difficulties were overcome by completing the digestion with small volumes of perchloric acid, a fact also reported by Cassil (23). This suggestion is incorporated in the AOAC general method for arsenic. Tobacco, which contains ring nitrogen compounds, presented similar problems which were also solved by perchloric acid digestion. When perchloric acid is used, removal of nitrogen oxides by ammonium oxalate is unnecessary, since nitrosyl sulfuric acid is decomposed by boiling perchloric acid. Leblanc and Jackson (24) found similar difficulties in using wet oxidation with fish and NBS orchard leaves, but ashing with magnesium nitrate at 500° gave satisfactory values. Methylated arsenicals, which are present in marine organisms, are extremely resistant to oxidation, according to Uthe et al. (25); they found that dry ashing with magnesium oxide and nitrate gave incomplete recovery. Recoveries were improved an average of 6% by applying a

TABLE 2. COMPARISON OF THE ADVANTAGES AND DISADVANTAGES OF WET OXIDATION AND DRY ASHING OF FOOD FOR THE DETERMINATION OF ARSENIC

Characteristic	Wet Oxidation	Dry Ashing
Time	Less elapsed time More operator time	Long elapsed time Less supervision (overnight)
Temperature	Low (less volatilization and retention)	High (more volatilization and retention)
Reagent blank	Higher	Lower (fewer reagents)
Sample size	Large samples inconvenient	Large samples easily handled
Nature of sample	Generally less sensitive	Generally more sensitive
Number of samples	Best for few	Best for many

Adapted from Gorsuch (15, p 494)

wet oxidation with sulfuric and nitric acids, using vanadium pentoxide catalyst. They speculated that greater losses in the dry ashing procedure were related to higher fat contents in the product. Wichmann (26) had also reported that the magnesium nitrate dry ashing method failed with the organic arsenic present in shrimp.

Tam and Conacher (27), on the other hand, found that the "fish-arsenic" of sole and shrimp was quantitatively recovered by dry ashing with magnesium nitrate and oxide at 500° overnight and determining the arsenic by the silver diethyldithiocarbamate method. Recovery in this case was monitored by X-ray fluorescence, an absolute and non-destructive method for the arsenic determination. These authors also found the digestion with nitric, sulfuric, and perchloric acids satisfactory, but preferred the dry ashing procedure because of its simplicity.

Most methods for the determination of arsenic have been studied with the ashing step as an integral part of the procedure. The suitability of a method for a specific purpose can be evaluated as far as ashing is concerned in the light of the above discussion. In addition, the general section on "The Destruction of Organic Matter" of the Society for Analytical Chemistry's publication (28), which was developed as a result of series of committee reports that included experimentation and validation, provides a wide choice of wet decomposition methods. Recommendations are made on the basis of the nature and reactivity of the sample. Wet ashing procedures are suggested for the preparation of

samples for arsenic determination by the methods described in the arsenic section (28, p 23-30). Several dry decomposition methods are also given, of which only ashing at 420° maximum to a grey powder in the presence of 20% dry sodium carbonate appears to be the only procedure applicable to the determination of arsenic.

Buttrill (29) points out the necessity for suitable temperature control of the furnaces used for dry ashing. Even in the presence of fixatives, arsenic may be lost at over 600°. Furnaces require checking at many points to guard against "hot spots." Hamilton et al. (30) reported that although the thermocouple may indicate 450°, there could be a temperature gradient of 50° depending on the position in the furnace. At 900°, a difference of ±200° in some parts of the furnace is possible. Unreported and probably unsuspected differences from this source may be responsible for the discordant reports in the literature regarding complete recovery and significant losses of arsenic on dry ashing. Therefore, unless temperature calibration equipment is available and used, dry ashing should be avoided.

Very recently, Holak (81) applied a high pressure nitric acid digestion at 150° in a sealed Teflon bomb (82) to the preparation of a single solution which could be used for the determination of residue levels of lead, cadmium, copper, zinc, selenium, and arsenic in foods. This method of preparing a digested solution had previously been validated and approved by the AOAC for mercury in seafood (31, sections

25.106-25.107) (83). Arsenic added to strained chicken and to applesauce at 0.5 and 2.0 μg/kg, respectively, prior to digestion, was recovered by a hydride-atomic absorption spectrophotometric determinative step to the extent of 95-100% by seven collaborators, with a coefficient of variation of about 15%, with fewer than 10% outliers. The method is simple and requires little attention, but it does need an investment in a special piece of equipment for each sample digested simultaneously. This becomes very expensive for survey work. The Teflon liners, however, are relatively permanent, if not abused.

Occasionally the need for elimination of organic matter can be reduced considerably by taking advantage of the physical situation. Application of inorganic pesticides to fruit results in a deposition of the pesticide on the skin. The AOAC directions for fresh fruits (31, 25.008(a)) call for using only the peelings and the stem and blossom ends for the digestion, but calculating to a whole fruit basis. Hamelle et al. (32) have verified that most of the arsenic of apples is localized in the skin.

Fahey et al. (33), constructed a mechanical shaking machine which causes a tumbling action that permits the fruit to rub against itself and the walls of the jar, permitting removal of lead arsenate residues into an aqueous acid or alkaline medium. Less than 1% of the total arsenic was recovered in a second tumbling.

Conclusion.--Both wet oxidation and dry ashing are satisfactory for the elimination of organic matter prior to the determination of arsenic if suitable precautions are taken. Dry ashing should be used only with muffle furnaces which maintain a uniform temperature within the heated chamber. Wet oxidation is faster but dry ashing can handle many samples simultaneously. The sealed Teflon bomb technique appears to be satisfactory if only a moderate number of samples are to be analyzed simultaneously.

HALIDE SEPARATION

A common technique in macro analytical chemistry of arsenic is to separate it from accompanying interfering materials and the matrix by distillation as the volatile bromide or chloride. This method, in its micro modification, followed by the molybdenum blue colorimetric determination is currently favored by the Analytical Methods Committee (34). The method, which utilizes wet oxidation, is relatively fast for single samples but requires a special still. A halide distillation using the same Kjeldahl flask of the digestion and a bent air condenser trapped by water in an erlenmeyer, followed by a micro bromate titration, was at one time an AOAC method (35). It was deleted in 1965 because of the successful application of the colorimetric methods which used the simpler arsine distillations.

The main advantages of the halide distillation are speed for a limited number of samples and excellent separation from the interference

caused by "heavy metals" and by large amounts of phosphates and salts. However, the distillation requires constant attention; it should be conducted in a hood; and it is not amenable to multiple sample operation. It has no special superiority over the recommended methods in accuracy, precision, or sensitivity.

GUTZEIT METHOD

For over a half century, the Gutzeit method was the only well recognized method for the determination of trace quantities of arsenic. It is considered obsolete by modern standards because colorimetric methods can provide more reliable results in practically the same time. It is considered here because it is a true screening method which can be performed without special instruments. It separates those samples which are of no interest from those containing an amount of arsenic which should be examined by more reliable methods. An aliquot of the same digest prepared by wet oxidation or from the hydrochloric acid solution of the residue from dry ashing can be used for the additional examination.

In this method, relatively large samples (5-140 g) are oxidized with sulfuric and nitric acids; oxidizing conditions are continuously maintained with additional portions of nitric acid. The excess nitric acid is removed by boiling to fumes of sulfuric acid and the last traces are destroyed with ammonium oxalate. An aliquot of the digest containing about 20 μg As_2O_3 (15 μg As) is neutralized and then adjusted to a constant amount of hydrochloric or sulfuric acid. Arsenic(V) is com-

pletely reduced to arsenic(III) with potassium iodide and stannous chloride, and zinc is added to produce hydrogen, which in turn converts the arsenic to arsine (AsH_3). The arsine is scrubbed with lead acetate to remove sulfides and is then conducted over filter paper strips impregnated with mercuric bromide. The length and intensity of the yellowish to brownish stains produced are related to the arsenic content of the sample. The stains are compared to those produced by standard amounts of arsenic treated similarly.

The optimum amount of As_2O_3 handled at the determinative step is 20 µg. If the aliquot represents a 20 g sample, this concentration is equivalent to 1 mg/kg. The blank should not exceed 1 µg. Reagents extremely low in arsenic, especially the digesting acids, are commercially available. The least difference observable is about 0.5 µg and the customary range is up to 30 µg As_2O_3. Both the range and sensitivity can be adjusted by changing sample sizes and aliquots. An "ultramicro" version (36) is available to extend the range of quantitative measurement down to 0.04 µg when only small samples are obtainable, as in clinical work.

Care and experience are required in obtaining and examining the stains produced by the method. Maintaining uniform conditions among all the samples, controls, and standards during the generation of the hydrogen and arsine is essential.

During the past century a considerable literature has accumulated on attempts to standardize the liberation of arsine and the production and reading of the stains. Much of this effort is unproductive, since there are so many potential sources of error in this method that minimizing or controlling one cause of error still permits considerable variability from the remaining sources. The method should be utilized for its basic advantages as a simple, rapid, semi-quantitative method for the determination of arsenic. Many sample solutions, once they have been prepared, can be handled simultaneously. The bottleneck is in the sample preparation by wet oxidation. Much of the study of the generation and distillation of arsine is applicable to present methods since many of them still separate arsenic from the substrate by this procedure.

The AOAC version of the Gutzeit method last appeared in the 11th edition (1970) (37). This method resulted from a symposium conducted in 1930 to harmonize the various versions of the then official method (38). The Gutzeit method was not reprinted in the 12th edition (1975) since it was declared "surplus" by the AOAC. A surplus method is still considered technically sound for its intended purpose, but it is no longer in common use for various reasons. In this case, the reason for removal was the availability of superior colorimetric methods.

The Gutzeit method of the Nordic Committee on Food Analysis (39) is almost the same as the AOAC Gutzeit method and is more specific in various details, except for the unexplained addition of ferric ammonium

sulfate solution to the generator before addition of the zinc. Ferric ion is reported to be "injurious" in the determination (40).

The Society for Analytical Chemistry (SAC) (41) has also published a version of the Gutzeit method in which the arsine reacts with the mercuric salt on a circle of paper rather than on a strip. The British literature in general emphasizes the use of disks whereas the North American literature seems to favor the use of strips. The SAC method also utilizes an arsenic trichloride distillation after wet oxidation and before the further separation as arsine. This appears to be an unnecessarily complex method for semi-quantitative use and therefore is not considered further here. Moreover, there does not appear to be any data supporting the performance for this particular version of the Gutzeit method.

The conclusions from investigations of a number of factors influencing the results by the Gutzeit method are summarized below.

<u>Use of potassium iodide</u>.--Reduction of arsenic is incomplete in the case of refractory substances when potassium iodide is omitted. The function of the stannous chloride is primarily that of an accelerator, removing iodine as it is formed. It also activates the zinc. Low results were obtained with dyes and phosphates in its absence (42).

<u>Nature and amount of acids for generation of hydrogen</u>.--Although both sulfuric and hydrochloric acid are permitted, hydrochloric acid is preferred. Sulfuric acid from wet oxidation is neutralized before final

acidification to a specific volume because the amount remaining is unknown. Improper conditions result in formation of sulfur dioxide from sulfuric acid during the reduction of arsenic.

Time of evolution of hydrogen.--Although most of the arsine is evolved within the first 30 minutes, the reaction is allowed to continue for 1.5 hours in both the AOAC and Nordic methods.

Rate of evolution of hydrogen.--Too rapid evolution of hydrogen at the beginning of the determination results in an elongated stain which is difficult to measure. Too slow evolution results in very intense, short stains of poor sensitivity. Evolution should never be so rapid as to exhibit frothing. Ishida and Brown (43) were able to improve the recoveries of the Hoffman and Gordon colorimetric method (60) by 9% by controlling the formation of arsine by stepwise addition of the hydrochloric acid. Smoothing out the reaction improves the entrapment of the liberated arsine.

Temperature.--Heidenhain (44) pointed out the importance of controlling the temperature of both the generation apparatus and the absorption apparatus. The temperature affects the function of the two parts of the apparatus in opposite directions. Lower temperatures of the solution in the generator result in slower gas evolution, producing shorter and more intense colors than high temperatures; lower temperatures in the absorption apparatus produce long, drawn-out colors with less defined end points. More readable and uniform stains for comparison are produced by immersing the entire apparatus up to within 3 cm

of the top of the tubes holding the strips, using lead rings as weights, and maintaining a constant temperature from run to run. Standards must still be run with each batch for proper comparisons. A constant temperature between 20° and 25° is currently recommended (45).

Undigested ring nitrogen compounds.--All pyridine compounds retard the evolution of hydrogen and arsine (46). Such compounds might be encountered as a spray residue resulting from the use of tobacco or nicotine or in the examination of such materials as nicotinic acid for compliance with food additive specifications. The interference persists even after very thorough acid digestion. It may be eliminated by various procedures: digestion with perchloric acid (22); distillation of the arsenic as the bromide or chloride (34); or coprecipitation of the arsenic with magnesium ammonium phosphate as in the classical magnesium gravimetric determination, solution in hydrochloric acid, and application of the Gutzeit method to the solution (47).

Zinc.--Either 20-30 mesh (850-600 μm ISO sieve), uniform sticks, or pellets of zinc may be used in the Gutzeit method with equivalent results (48). The lengths of the stains for 20 μg of As_2O_3 were 29, 35, and 49 mm for stick, 20-, and 30-mesh zinc, respectively, but the coefficient of variation remained constant at 2.5%. Therefore, the same physical form, amount, and surface area of the zinc must be used throughout a set of determinations so that the evolution of the hydrogen is uniform. As Cassil points out (49), many of the conditions controlling the arsine evolution, such as temperature and concentration of acid and

salts, are usually kept constant in most laboratories, but the type of zinc used may vary greatly from bottle to bottle.

Heavy metals such as lead (from the scrubber) or mercury (from the strips) must not be permitted to contaminate the solutions in the generators since their presence will inactivate the zinc and reduce or stop the formation of arsine. Once contaminated, the zinc is unfit for further use. The zinc should be added as soon as convenient after addition of stannous chloride to avoid the formation of free iodine, which in turn may retard arsine formation. Stick zinc provides a practically constant rate of hydrogen evolution during the entire evolution period (50). The zinc contributes a substantial proportion of the arsenic of the blank. For this reason also, the same amount of zinc from the same lot should be used in a series of determinations. Mills (51) suggested melting pure stick zinc in porcelain and casting pellets 9 mm diameter and 12.5 mm long in a bullet or porcelain mold in order to obtain uniform pieces of zinc.

Paper strips.--Uniformity of paper and its impregnation is essential for reproducibility of the stains. By far the easiest procedure is to use a sheet of commercially precut strips (designated as "Hanford-Pratt" strips) for immersion. After immersion, the strips should be dried by waving them in the air, not by blotting. One point which might be overlooked is to make a pencil mark across the strips a few millimeters from the solid uncut portion of the batch of strips at both cut ends so that the marked ends (when cut in half) will always be placed uppermost

in the Gutzeit apparatus (45). The glass tubes holding the strips during the determination must be of the correct size with only 0.05-0.1 mm clearance at the sides, so that the strips are held straight and are not permitted to curl. Cassil (49) suggested performing the impregnation by evacuating the container with the immersed strips to 70 mm pressure and keeping them at this pressure for 1 hour to remove the air from the paper, and then drying in the usual manner. The mercuric bromide solution may be kept for at least 3 or 4 months if it is filtered just before each use (52). Sensitized strips may be kept for as long as 21 days if protected from the atmosphere, e.g., in a desiccator or between sheets of clean paper. However, the same batch of sensitized strips must be used in the entire series--blanks, controls, standards, and unknowns.

When the intensity and length of the stains on the two sides of the strips are unequal, an undesirable situation, the two values are averaged.

Repeatability and reproducibility.--Neller (45) has given a realistic appraisal of the results on replicate aliquots from the same solution by the same analyst. Approximately half of the determinations (i.e., probable error) would be expected to check a duplicate within 10% of the mean, or a coefficient of variation of 15% (relative standard deviation). Since, as a first approximation, the between-laboratory variability can be considered to be approximately twice the within-laboratory variability, the coefficient of variation of the Gutzeit method between laboratories would

be about 30%. Because of this relatively large variation, the Gutzeit method is considered to be only semi-quantitative.

In a five laboratory comparison of the British Pharmacopoeia Gutzeit method and the British Standards Institution silver diethyldithiocarbamate (AgDDC) method for the determination of approximately 0.3 mg arsenic/L of beer, the overall coefficient of variation was found to be about 22%, for both methods, and 11% and 8% within-laboratories, respectively (53). In this case, there was no difference if the determination was run directly on the beer or after wet oxidation. The value by the AgDDC method was approximately 10% lower than that by the Gutzeit method; the blank by the AgDDC method was also lower.

COLORIMETRIC DETERMINATIONS

MOLYBDENUM BLUE PROCEDURE

The molybdenum blue procedure has been one of the standard methods for the determination of phosphorus in biochemistry for over a half a century. A heteropoly blue compound is also formed by the condensation of arsenate and molybdate ions to form molybdiarsenic acid, which is reduced by hydrazine sulfate to the stable blue compound with an absorbance maximum at about 840 nm, depending upon the conditions of color development (54). The color is stable at least 24 hours (55). The hydrogen carrying the arsine is bubbled through an oxidizing solution of sodium hypobromite or dilute iodine. Ammonium molybdate in strong sulfuric acid and hydrazine sulfate are added and the mixture is allowed

to stand or is heated for color development. It is important to have enough acid present during color development to avoid reduction of the molybdate reagent itself (54).

Antimony does not interfere since it does not form a molybdenum blue complex. Phosphorus and silica, which are potential contaminants from detergents used in cleaning glassware, would interfere but they do not distill as the halide or with the arsine. Laboratory fumes, although not containing arsenic, are reported to increase the blank of the method (56).

The molybdenum blue calibration curve is linear over a broad range, at least up to 120 μg (57), so that it is unnecessary to make preliminary tests to determine if unknown concentrations are suitable. The method is also easily applicable to low levels of arsenic of the order of magnitude of 0.1 mg/kg (54). A ruggedness test (29) has shown that small variations in amounts of reagents have little or no effect on the values obtained.

The molybdenum blue method combined with magnesium nitrate ashing at 600° was applied to the determination of arsenic in eggs, a high fat food, and to livers of birds that had been fed arsenic compounds by Evans and Bandemer (58).

The Analytical Methods Committee of the Society for Analytical Chemistry (59) studied the molybdenum blue method for arsenic and provided a recommended procedure. Unfortunately this study was

made in conjunction with an over-elaborate isolation procedure (wet oxidation, extraction with diethylammonium diethyldithiocarbamate in chloroform and destruction of the organic matter thus introduced; distillation as trichloride if more than 1 mg of heavy metals or considerable insoluble material is present). Hydrazine sulfate was preferred over stannous chloride as a reductant for several reasons: it formed a stable complex immediately; the complex was less affected by variations in acid concentration; and the reagent was stable and contained no metal ions. The study was conducted with 0.65% arsenic (6500 mg/kg) so it is not known if the same factors would be operating at 0.01-0.0001 times this amount. The lower limit of operation of the method was 0.6-1.5 µg of arsenic, depending upon the reagents used. With purified reagents, this limit could be extended to 0.15-0.45 µg. The final collaborative study of this method showed a coefficient of variation of about 6% at the 5-10 µg arsenic levels and about 15% at the 0.5-2 µg level. Each laboratory added its own (known) amount of arsenic to an arsenic-free sucrose. Therefore these values cannot be considered as truly representative of analytical values which would be reported as true, unbiased unknowns.

Three studies form the basis of the AOAC-approved molybdenum blue methods. The first, conducted by Hoffman and Gordon (60) on apple digests containing the equivalent of 1 and 2 mg/kg in the original fruit, did not reveal any factors which would indicate an overall or conclusive superiority of the molybdenum blue method over the silver

diethyldithiocarbamate method. The values by the molybdenum blue method averaged about 3% lower than those by the silver diethyldithiocarbamate method and the reproducibility is about the same. The results by 6 laboratories out of 14 were not used, 4 because they did not use the correct mesh size of zinc, which caused a higher variability in the individual values with coarser zinc than those of analysts who used the correct size. A faulty spectrophotometer tube and failure to follow replication directions resulted in rejection of the other two laboratories.

The second study (29) utilized pork and chicken livers fortified with arsenic (from arsanilic acid) in three ranges: 0.25-0.75, 0.76-1.50; and 1.51-2.50, and unfortified. The ashing procedure of Evans and Bandemer (58) was combined with features of the Kingsley and Schaffert method (19). After more than one third of the data from the nine laboratories was rejected for chemical and statistical reasons, the remainder showed a coefficient of variation of about 14%. Since replicates were not conducted, it is not possible to determine the repeatability (within-laboratory) portion of error. The author of this report comments that five of the nine laboratories did not have muffle furnaces that could be controlled to a specified temperature. Extreme care is required in matters of cleanliness to eliminate contamination, in quality of reagents for low blanks, and in quality of equipment to avoid losses if valid values are to be obtained in residue analysis.

The third method (61), for arsenic in animal feed, utilizes a magnesium oxide-magnesium nitrate ashing at 550-600° for 2-4 hours, evolution of arsine, absorption in acid permanganate-mercuric chloride solution, and development of the molybdenum blue color. Eighteen collaborators analyzed three feeds containing 0, 62, and 100 ppm arsanilic acid (0, 21, and 35 ppm As). The results of only one collaborator were rejected as outliers, recovery was essentially 100%, the blank was about 4 ppm, and the overall coefficient of variation was about 10% at the two levels. These results are considerably different from those obtained on tissue residues one and two orders of magnitude lower. Subsequent work by Hoffman (62) to shorten the ash time to 1 hour by increasing the ashing temperature to 700° resulted in variation between laboratories of 15%, or 50% greater than that of the previous study using a maximum temperature of 600°. A simpler method of extracting with potassium bicarbonate and wet ashing the extract, although recovering all the arsenic, showed an even greater coefficient of variation of 21%.

SILVER DIETHYLDITHIOCARBAMATE PROCEDURE

The reaction of arsine with a pyridine solution of silver diethyldithiocarbamate (AgDDC) to produce a red color was introduced by Vasak and Sedivec (63) for the determination of arsenic as an alternative to the molybdenum blue procedure. The hydrogen carrying the arsine is bubbled through the reagent; the red color is then measured directly

at 540 nm, in the spectrophotometer. The calibration curve is linear over the range 1-20 μg of arsenic. Liederman et al. (64) found that only antimony reacted similarly to form a red color with a maximum absorbance at 510 nm. The antimony complex exhibits an absorbance of only 8% of that of arsenic at 540 nm, however. Even this potential interference could be eliminated by increasing the volume of stannous chloride solution used for the reduction of arsenic(V) to arsenic(III) (22).

The simplicity and convenience of this method led to a number of applications in food analysis. J. I. Morrison (65) combined this new method with the AOAC magnesium oxide-magnesium nitrate dry ash method at 550-600° to determine levels of about 10 mg of arsenic/kg in animal feeds. A similar ashing procedure previously used by Stone (66) with the molybdenum blue method was combined with the AgDDC colorimetric method for the determination of 0.1-1 mg arsenic/kg of animal tissues by J. L. Morrison and George (67). The average recovery of added arsenic to tissues was about 80%, which is compensated by the use of a determinative standard curve, produced by adding standards to the tissue and subjecting the spiked tissues to the entire determination, including ashing.

Hoffman and Gordon (60) conducted a collaborative study of the AgDDC method on apple digests containing 1 and 2 mg arsenic/kg original fruit. The results of eight collaborators, after rejection of outliers,

warranted adoption of the method as an alternative to the molybdenum blue colorimetric method.

The general applicability and usefulness of this method was demonstrated by Hundley and Underwood (6), who applied the AgDDC method to the U. S. Food and Drug Administration's total diet program of determining the arsenic content of the diet of a teenager. The method was applied routinely to the following 12 classes of food:

I	Dairy products	VII	Root vegetables
II	Meat, fish, and poultry	VIII	Garden fruits
III	Grain and cereal products	IX	Fruits
IV	Potatoes	X	Oils, fats, and shortenings
V	Leafy vegetables	XI	Sugars and adjuncts
VI	Legume vegetables	XII	Beverages

Recoveries of added arsenic in the 0.1-1.0 mg/kg range were 80-120%. The molybdenum blue method had too high a blank for routine application to the lower end of this arsenic range.

The Morrison method (65) and the molybdenum blue method were subjected to a collaborative study on three animal feeds containing respectively either no arsenic, 50 ppm arsenic supplied by 3-nitro-4-hydroxy-phenylarsonic acid, or 50 ppm arsenic from arsanilic acid (68). Sixteen collaborators analyzed each feed at least in duplicate on each of 2 days. All values were satisfactory. The AgDDC method gave slightly

lower results than the molybdenum blue method by about 4% and its coefficient of variation was slightly but not significantly higher, 9% vs. 7%.

The comparison of methods was again performed the following year (69) using a blank feed and blind triplicates of a feed containing 145 ppm arsanilic acid (50 ppm arsenic). Fifteen collaborators participated and no values were rejected as outliers. The molybdenum blue method was compared again with the AgDDC method and with the AgDDC method modified by conducting the arsine evolution from a solution cooled to 5°. The values for the blank feed by the three methods were 0.6, 0.2 and 0.2 ppm arsenic. Cooling the solution before evolution of the arsine improved the recovery slightly but also led to more variability both within and between laboratories. In this test, the higher recovery of the molybdenum blue method persisted, but the reproducibility was reversed. Therefore, the coefficient of variation of the two methods is about the same, 8%, at the 50 ppm level of arsenic in feeds. The AgDDC method in this case was not adopted by the reviewing AOAC subcommittee because the subcommittee saw no superiority of the method over the existing molybdenum blue method.

One of the objectionable aspects of this method is the necessity for the use of pyridine as a solvent for the silver diethyldithiocarbamate reagent. Woidich and Pfannhauser (70) replaced the pyridine with a 0.5% solution of hexamethylenetetramine and obtained reasonable values for arsenic in foods of marine origin. No comparisons with other

methods were given. Difficulties have been experienced in obtaining a consistently satisfactory silver diethyldithiocarbamate reagent (34).

Conclusion: Both the molybdenum blue and the silver diethyl dithiocarbamate (AgDDC) methods are satisfactory for the determination of 50 ppm arsenic in animal feeds, with coefficients of variation of about 8%. The AgDDC method, however, recovers about 4% less arsenic than the molybdenum blue method. Both methods are suitable for the determination of arsenic at contaminant levels of 0.1 to 10 mg/kg. The AgDDC method often shows a lower blank and therefore may be more suitable with smaller quantities of arsenic. The choice of method will also depend upon the laboratory facilities, operator preference, and the nature of the samples, since there is little basis for choice with reference to accuracy and precision. The molybdenum blue method has a greater range of applicability but the AgDDC method is faster and requires fewer reagents. The AgDDC method gives slightly lower values and is subject to variability from reagent quality. The odor of pyridine, used in the AgDDC method, is objectionable to some analysts. Although replacements for the absorbent have been suggested, their suitability has not been verified. The molybdenum blue method is subject to interference from phosphate (detergent) contamination and is not as suitable for the lower levels of arsenic.

The silver diethyldithiocarbamate method is also the recommended method for the determination of the extremely low levels of arsenic encountered in waters (71), ordinarily of the order of 1-10 µg/L, and in atmospheric particulate matter (72). The relative standard deviation

reported for 46 laboratories analyzing a synthetic water sample containing 40 μg of arsenic/L was about 14%.

It is important to note, however, that the collaborative studies of the colorimetric determination of arsenic at the level of 0.1 to 10 mg/kg in food have been perturbed by an unacceptable number of outlying determinations. The outliers are both very high and very low, and blanks may be high as well. Outliers also occur sporadically within a laboratory. Control determinations with standard reference materials or house standards or the use of quality control techniques such as independent reanalysis or standard additions must be practiced routinely with the determination of arsenic at residue levels. Analysts should be given practice samples and assigned time to gain experience and proficiency with the method.

A summary of the statistical parameters of the two colorimetric methods is given in Table 3. Recoveries are not listed, since the average values reported by the large number of laboratories involved are usually in the range of 90-100%. The values are satisfactory for levels of arsenic 14 mg/kg and above. The two studies utilizing the low levels of arsenic are satisfactory only after rejection of about 40% of the values. The within-laboratory coefficient of variation by both methods is very low for such a low level and the ratio of coefficients of variation of within-laboratories:between-laboratories is unusually low for the molybdenum blue method. This indicates that analysts can check themselves easily, but they cannot check the values of other laboratories as readily.

TABLE 3. SUMMARY OF STATISTICAL PARAMETERS OF COLLABORATIVELY STUDIED METHODS OF ANALYSIS FOR ARSENIC

Commodity and reference	Collaborators No. Used	Collaborators % Outliers Rejected	Arsenic mg/kg	Method	Coefficients of Variation: Within Laboratories	Coefficients of Variation: Between Laboratories	Ratio
				Wet ash			
Apples (60)	8	43	1-2	Mo Blue	2.1	5.7	0.37
(1963)				AgDDC	2.2	4.6	0.48
Livers (29)	4-6	37	0.3-2.4	Dry ash		14	
(1973)				Mo blue			
Animal feed (61)	17	1	21	Dry ash	5.6	9.8	
(1957)			35	Mo blue	5.7	10.0	0.57
Animal feed (62)	21	0*	14	Ash 700°	6.6	15	0.44
(1958)				Mo blue			
	20	0**	14	Wet ash of extract,	6.5	21	0.31
				Mo blue			
				Dry ash			
Animal feed (68)	16	0*	50	Mo blue	3.0	7.4	0.41
(1963)				AgDDC	4.5	9.0	0.50
Animal feed (69)				Dry ash			
(1964)	15	0	50	Mo blue	4.6	9.7	0.47
				AgDDC	3.9	7.6	0.51
				AgDDC cooled	6.3	10.4	0.61

* One collaborator with satisfactory results omitted because of failure to follow replication pattern
**Two collaborators with satisfactory results omitted because of failure to follow replication pattern

It should be noted that the method for phosphate in processed cheese of the International Organization for Standardization (73), which is also a molybdenum blue method, was not recommended for adoption by the AOAC because two of the seven collaborators were outliers (74).

The following collaboratively studied colorimetric methods for the determination of arsenic are recommended:

AOAC (1975) 25.006-25.013	General purpose method; wet oxidation, arsine evolution, and colorimetric determination by molybdenum blue or silver diethyldithiocarbamate
AOAC (1975) 25.014-25.019	For meat and poultry; Dry ashing with magnesium nitrate at 600°, arsine evolution, and colorimetric determination by molybdenum blue
AOAC (1975) 41.009-41.012	For tissue residues; Dry ashing with magnesium oxide/nitrate at 555°; arsine evolution; and colorimetric determination with silver diethyldithiocarbamate
AOAC (1975) 42.005-42.010	For feeds; dry ashing with magnesium oxide-magnesium nitrate at 550-600°; arsine evolution, and colorimetric determination with molybdenum blue.

ATOMIC ABSORPTION METHODS

The general availability of atomic absorption spectrophometers prompted an exploration into their use for the determination of arsenic. This element, however, has a relatively high limit of detection (mg/kg) in conventional atomic absorption spectrophotometry. Its direct determination suffers from sensitivity to acid and salt concentrations as well as interference from a number of other elements.

The general simplicity, high sensitivity, and low limits of detection of atomic absorption spectrometry prompted a search for means of taking advantage of these attributes for the determination of low levels of arsenic. Holak (75) utilized the transformation to arsine for this purpose, combining the AOAC method for producing arsine and hydrogen, as in the Gutzeit procedure, with collection of the arsine in a cold trap cooled with liquid nitrogen. The arsine was later volatilized and swept into the spectrophotometer. Simpler techniques followed, including trapping in silver nitrate solution, use of a collection reservoir, and high temperature decomposition of the arsine to arsenic atoms.

Current procedures favor generation of the arsine with sodium borohydride ($NaBH_4$). It has the advantages that it reacts faster than the zinc-hydrochloric acid system, has a low blank, and is capable of being automated. The technique is complex and the introduction of hydrogen together with the arsine produces background changes which

require compensation. None of the elements commonly present in food interferes in this method (76). A complete bibliography of procedures for liberating arsenic and selenium as their hydrides, AsH_3 and H_2Se, coupled with atomic absorption spectrophotometric detection techniques, is given by Ihnat and Miller (18). Optimizing the conditions and instrumentation appears to be complex, but once optimized, execution of the analyses is extremely simple (18).

A limited interlaboratory study was conducted by Fiorino et al. (77) on the hydride generator developed in the laboratories of the Food and Drug Administration. Four laboratories had a coefficient of variation of 5% on five fish samples containing 3-30 mg arsenic/kg dry weight (corresponding to 0.6-6 mg/kg wet weight).

A more extensive study of the hydride methods as developed and evaluated by the Food and Drug Administration and Agriculture Canada was initiated by Ihnat and Miller (21). Fourteen samples, which included National Bureau of Standards (NBS) and International Atomic Energy Agency (IAEA) reference standards, were examined by 24 laboratories. The participants were requested to follow a specific wet oxidation procedure but were permitted to use their own hydride generation equipment, ranging from commercial devices to semiautomated and fully automated custom-made instruments. All raw data including instrument charts and operating parameters were submitted, which permitted correction or verification

of apparently aberrant values or, as was frequently the case, calculation of individual results when averages were reported. The experimental design in all cases permitted resolution of the total error into "between-laboratories" and "within-digests;" in half the cases, it also permitted calculation of the "between-laboratories" and "between-digest" standard deviations. The overall variability as shown in Table 4 is so large that in many cases the digestion variance contributed little to the total error. It was concluded that a laboratory-dependent systematic error exists in the method and also that imprecision exists in the determination step, most likely arising from the hydride generation-transport step rather than in the atomic absorption measurement step.

In general, an acceptable method should not have a coefficient of variation greater than 20% at the mg/kg level. Only 2 of the 13 different samples (4 known and 2 blind pairs; 19 total samples) were acceptable in this respect, and one of these samples (NBS orchard leaves) was undoubtedly recognized by many of the collaborators from its distinctive odor. However, when it is realized that these values were calculated after rejection of outlying laboratories and determinations, an examination of the outlier pattern is in order. Of the 24 participants, only 6 (25%) produced usable values for all samples analyzed. Another 6 (25%) produced values from which only one value had to be excluded. All values from four laboratories (17%) had to be excluded.

TABLE 4. Summary and Statistical Analysis of the Cooperative Results for Arsenic in Foods by the Hydride-atomic Absorption Spectrophotometric method as reported by Ihnat and Miller (21) (Numbers in parentheses, except for column 1, are the number of results entered into the associated number).

Sample (Code No.)	Concn found µg/g (as is)	Conc found µg/g (dry basis)	Selected Ref. Value µg/g (dry basis)	Coefficients of Variation		
				Within	Total	Ratio
Skim milk powder (11)	0.00012 (14)				22500	
IAEA Flour (10)	0.0312 (14)	0.035 (14)	0.024 (3)		190	
Flour (12)	0.0314 (15)				210	
NBS Bovine liver (8)	0.0433 (17)	0.046 (17)	0.055	62.4	86.4	0.72
Kale (7/14)	0.0931 (17)	0.101 (17)	0.141 (7)	27.9	39.7	0.70
NBS Spinach (9)	0.114 (16)	0.120 (16)	0.150	17.8	43.7	0.41
Apple (5A)	0.918 (10)				26.9	
Apple (5B)	1.45 (3)				48.0	
Swordfish (4)	2.38 (19)				47.9	
NBS Tuna (2)	3.06 (19)	3.160 (19)	3.900 (4)	11.0	22.4	0.49
NBS Orchard Leaves (1)	9.27 (16)	9.940 (16)	10.000	8.9	9.1	0.98*
IAEA oyster (3/13)	11.4 (18)			8.0	13.9	0.58
Flounder (6)	13.1 (15)				32.9	

*Very unusual ratio which probably indicates that the sample was recognized by most collaborators

With the remaining 8 laboratories, the data from two to nine samples per laboratory had to be excluded from consideration. It is promised that the data will now be examined to discover common factors which led to outlying or excessively variable results.

This study provides some sober reflections on laboratory (analyst) performance. Laboratories who use their own version of the hydride-atomic absorption method should not deceive themselves into thinking that they can perform better than the group of outstanding laboratories whose cooperation was acknowledged by Ihnat and Miller. More sobering yet is the poor performance of other techniques used by reference laboratories in establishing the "best values." Very few values by the following techniques were usable: Neutron activation analysis with radiochemical separation after radiation; isotope dilution spark source mass spectrometry; silver diethyldithiocarbamate colorimetry; and graphite furnace atomic absorption spectrophotometry.

Very recently, the situation has shown some indication of improvement. Holak (81), using a specific, simple hydride generator in conjunction with high pressure nitric acid wet oxidation in a sealed Teflon bomb with a final magnesium nitrate dry oxidation at 450°, demonstrated that the hydride atomic absorption method could produce acceptable results in a collaborative study. Seven laboratories analyzed two samples, a strained chicken and a strained applesauce containing 0.5 and 2.0 mg added arsenic/kg, respectively, and the

two corresponding blank samples. The reproducibility coefficient of variation was about 14%, which is about the expected variability at the mg/kg level. The repeatability coefficient of variation is about 12%. The ratio of the two coefficients of variation is about 0.8. This is rather high, indicating that most of the variability is from the analysts, and not from between laboratories. Only one outlier appeared in the 28 determinations (4%), although two blank values were too high in the matching 28 blank determinations. High blanks are usually caused by contamination.

Conclusion: The limited collaborative studies by Fiorino et al. (77) and by Holak (81) indicate that the hydride atomic absorption methods recommended by these investigators, if followed explicitly, can produce reliable results. However, personal versions of this method, if not monitored by reference samples and quality control techniques, can produce a high proportion of outliers, with consequent misleading results, as shown by Ihnat and Miller (21).

OTHER METHODS

The only other method which may have a potential usefulness for routine surveillance and compliance operations in environmental and regulatory laboratories involves polarography in its various aspects. But polarography is not a popular technique in the general laboratory, although it is becoming more prevalent. It has its strong advocates but many analysts have not taken the trouble to become informed of the

simplicity and other advantages of this technique. A review of the polarography of arsenic has been prepared by Arnold and Johnson (78). Holak (79) has explored some analytical applications. The main advantage of this technique is that it is not subject to interference from antimony. Of the common toxic elements, lead and tin interfere. Polarography is very sensitive to the nature and concentration of the solutes of the medium, and therefore the arsenic must be separated by one of the conventional distillation procedures. As long as this step is necessary, most analysts prefer to use a more familiar technique. Anodic stripping voltammetry (80) permits extension into the nanogram range. No collaborative studies have yet been performed with these techniques for the determination of arsenic.

SUMMARY AND CONCLUSIONS

Total diet studies show that current concern about arsenic in food can be confined to fish and other seafood. If the feeding of poultry and swine wastes containing excreted arsenicals becomes widespread, additional monitoring of meat may become necessary. The total diet programs now conducted by many countries require suitable methods for the determination of arsenic at levels lower than 0.1 mg/kg in order to accurately estimate the total daily arsenic intake. Most foods contain arsenic at levels lower than the current limit of reliable measurement, approximately 0.1 mg/kg. Present dietary intake calculations are inaccurate and biased toward the low side because of the uncertainties involved in assigning zero or finite values to analytical data below the limit of reliable measurement.

The only method which can receive an unqualified recommendation is the AOAC method for the determination of arsenic in animal feeds at levels of 15 mg/kg and above. It consists of ignition at not over 600° with a magnesium fixative, separation by arsine evolution, and determination by the colorimetric molybdenum blue method. Recoveries are greater than 90%, and the coefficient of variation between laboratories is 10-15%. The method is very dependable, generating few outliers.

The AOAC methods for residues in general, for tissues, and for meat, involving wet and dry ashing, arsine evolution, and colorimetric determination by molybdenum blue or silver diethyldithiocarbamate, can be recommended only with the qualification that performance must be continuously and closely monitored by quality control techniques because of their erratic behavior both within and between laboratories. A similar qualification must be made for the sealed bomb, nitric acid digestion, magnesium oxide ashing, and hydride atomic absorption determination method now being considered for adoption by the AOAC, but with emphasis on the within laboratory (analyst) variability. Personal versions of this method cannot be recommended at this time. A list of standard reference materials for this purpose, available from the National Bureau of Standards, is given in Table 5. Collaborative studies show that 40% of the colorimetric values may be expected to be rejected. The remaining values, however, show a reasonable overall coefficient of variation of between 5 and 15% at levels from 0.5-2 mg/kg.

TABLE 5. REFERENCE MATERIALS USEFUL FOR CONTROL OF ARSENIC ANALYSES

NBS SRM No.	Name	Value, µg/g ±2 s.d.
		Certified
1570	Spinach	0.15±0.05
1571	Orchard Leaves	10 ±2
1573	Tomato Leaves	0.27±0.05
1575	Pine Needles	0.21±0.04
1567	Wheat Flour	0.006*
1568	Rice Flour	0.41±0.05
1577	Bovine Liver	0.055±0.005
Research Material 50	Albacore Tuna	3.3±0.4

Available from: National Bureau of Standards, Office of Standard Reference Materials, Washington, DC 20234

Abbreviations: NBS = National Bureau of Standards
SRM = Standard Reference Materials
s.d.= Standard Deviation

*Noncertified informational values

Information as of February 1978

Since the erratic results for arsenic appear to be independent of the method, the cause should be sought in such factors as analyst training and performance, contamination, standards, and instrument calibration, including muffle furnace temperatures.

As stated at the onset, arsenic is a very difficult element to determine with even a tolerable degree of reliability at the mg/kg level and below. Application of new, modern, sophisticated techniques does not appear to be able to improve this situation. Perhaps training and utilization of competent, experience chemists is more critical than instrumentation.

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PART II

REVIEW OF COMMONLY USED METHODS OF ANALYSIS FOR TIN IN FOODS

prepared by

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INTRODUCTION

Tin is known throughout the world as the protective surface of "tin cans." Exposure to tin is decreasing by the replacement of tin-coated metal cans by other types of protective containers such as glass, paper, foils, and plastics. A concentration of 250 mg of inorganic tin/kg of food is generally considered a permissible limit in canned food (1). This specification is included in many of the international Codex standards for canned fruits, vegetables, and juices. Above this level, consumers begin to find the food objectionable, characterizing it as "tinny" or "metallic." The presence of tin has been considered as self limiting before the concentration approaches a toxic level in food. However, as a result of several incidents of apparent tin poisoning in Sweden (2), involving corrosion of tin containers, analysis of canned peaches, green beans, and tomato paste, some of which were involved in the outbreaks, showed tin levels above and below 250 mg/kg without a noticeable characteristic taste in the products.

Inorganic tin salts are poorly absorbed and are excreted mainly in the feces. These compounds have a low toxicity (1). Cyclic organic tin compounds are on the average moderately toxic and the alkyl tin compounds are highly toxic (3).

The presence of tin residues is regulated in many countries. For example, in the United States, various salts of tin are permitted as food additives. Stannous chloride is considered generally recognized as safe (GRAS) as a preservative at levels not to exceed 15 mg (as tin)/kg. Stannous chloride may be added to asparagus packed in glass for color retention up to a maximum of 20 mg (as tin)/kg. Tin compounds are also permitted as indirect additives in various food packaging materials, such as adhesives, coatings, paper, rubber, and plastics. Organic tin compounds are used as insecticides and fungicides. Some examples of pesticide tolerances include 60 mg/kg (the highest) of tricyclohexyltin hydroxide on almond hulls. A tolerance of 0.2 mg/kg of the same compound has been established for meat in the U.S. to cover the legal usage of tin compounds in feeds. The lowest tolerance is 0.05 mg/kg for triphenyltin hydroxide in a number of crops and for tricyclohexyltin hydroxide in milk fat.

Tin is not one of the elements which is monitored by the "total diet" programs of various countries. Surveys of tin content of foods have usually been confined to canned foods having a tin surface, and usually for the technological purpose of correlating protective action or corrosion of surfaces with various production factors or storage conditions. The values for tin usually found in canned foods are from less than 10 to several hundred mg/kg. Zook et al. (4) determined the tin content of a series of wheats, flours, and prepared products. The

tin concentration ranged from 4 to 32 mg/kg (dry weight basis). The daily intake of tin by adult man is estimated as 3.5 mg (1), which, distributed over a 3 kg daily intake of food and water, corresponds to a concentration of 1 mg of tin/kg of the dietary intake.

SCOPE OF REVIEW

The analytical problem to be dealt with in this review is the determination of tin at two distinctly different levels:

(1) Residue levels, 1 mg of tin/kg and below, resulting from the legal application of organotin compounds to crops as a pesticide, and their appearance in animal food products as a result of ingestion of treated or contaminated feeds, and as migrants from food contact surfaces.

(2) Technological or contaminant levels, 10-1000 mg of tin/kg, resulting from the deliberate addition of tin to foods as a flavor or color preservative, or as an inadvertent solubilized component of foods packed in tin-lined cans. The higher levels of tin may result from surface defects which permit corrosion or from prolonged storage under unfavorable conditions. The possible presence from abrasion of elemental tin in solid foods packed in tin-lined containers should not be overlooked.

This review is not intended to be all encompassing. Rather it will cover the determination of tin in food and related substrates. It will cover those techniques capable of application in the ordinary well equipped

laboratory engaged in monitoring and enforcement work. Classical procedures are included since they are applicable directly to the higher contaminant levels. The numerous spectrophotometric determinations for tin are easily adapted as visual colorimetric procedures, using standards developed simultaneously for comparison. These manual procedures can be applied successfully under conditions where modern instrumentation might not operate properly because of lack of stable power sources, unavailability of trained operators and electronic technicians, and inaccessability of reagents. This review will not cover those techniques which require a large investment in capital and operators, such as neutron activation. The practicality of techniques may also be considered not only in terms of resource requirements, but also in connection with the availability of samples for examination. Very rapid procedures for monitoring, for example, are not efficiently utilized when sample collection operations do not maintain a smooth and ample flow of samples to the laboratory.

This review sought published, collaboratively studied, general purpose methods and collateral studies. Such methods are already known to perform satisfactorily in laboratories not concerned with their development. Associated with collaboratively studied methods are unbiased data relevant to accuracy, precision, applicability, sensitivity, limit of detection, and practicability, as appropriate. Such data have been accumulated by a number of representative laboratories on typical substrates over the applicable range analyzed as unknowns. Laboratories that cannot reliably analyze samples by these recommended methods will

usually find it more profitable to examine their laboratory operations, particularly their standards, preparation of standard solutions, instrument calibration, and analyst training and capabilities, rather than attempt to "improve" the method.

Methods which have been developed for specific foods, such as sugars, wines, milk, etc., except for canned foods as a class, have not been considered extensively because of presumed limited applicability. In some cases, such methods may be superior for the limited application for which they were tested. However, a general purpose laboratory has little control over the nature of the samples it is called upon to analyze, and it must rely upon general purpose methods.

There are very few general reviews of the determination of tin in foods. The following articles will be found useful:

Crosby, N. T. (1977) Determination of metals in foods: A review. Analyst 102, 225-268. Only recent review of all techniques applicable to metals of interest in food analysis, including tin. 346 references.

Monier-Williams, G. W. (1949) Trace Elements in Foods. John Wiley & Sons, Inc. New York. Chapter 4 Tin. The classical work on the subject covering methods up to about 1945. 71 references.

SAMPLING

There are no special requirements regarding the sampling of foods and biological materials with reference to tin. The usual inorganic salts are water-soluble; there is little organized information on the solubility of the common organic salts and compounds of tin used in agricultural and technological applications.

If the purpose of the analysis is to determine the tin content for composition or as a pesticide or food additive, the customary sampling plans and technical sampling procedures are applicable. If the purpose is to solve problems dealing with the protective action of the tin coating in canned foods, probably special selective sampling procedures are required which obtain the desired samples as a function of processing, storage, or exposure.

Tin-plated equipment for sampling and tin-plated containers for storage must be avoided.

PREPARATION OF SAMPLE

In most analyses of canned foods, the contents of the entire can are homogenized. If the distribution of tin between the solid and liquid portions of canned foods is desired, the two portions can be separated by draining as for drained weight determinations and each portion analyzed separately.

The equipment used for the preparation of samples for tin analyses requires special attention. Various food mills and grinders contributed from 0 to 1.4 mg of tin/kg to the analytical sample (5). Tin-plated steel, a material often used for the construction of food grinders, must be specifically avoided. Stainless steel, cleaned with 6N hydrochloric acid, if necessary, was found by Corbin (5) to be superior to nickel and aluminum. Blending and regrinding coarsely ground samples is a worthwhile step since it has reduced the differences of duplicates from 12 to 1.5% on similar samples.

Biston and Verstraeten (6) prepared samples of canned vegetables by direct extraction with cold 10N hydrochloric acid. The tin was then directly determined by oscillographic polarography without removal of oxygen. The results obtained on canned beans, carrots, celery, and potatoes were in good agreement with those obtained spectrophotometrically with dithiol or phenylfluorone. However, it is not clear if the spectrophotometric results were obtained on the same hydrochloric acid extract or on a separate wet oxidized solution. Which prepared solution was used makes considerable difference in interpreting the suitability of the hydrochloric acid extraction method for the determination of tin in canned foods.

The Health Protection Branch of Canada (48) is exploring the use of boiling hydrochloric acid as a method for preparing samples of canned food for tin analysis by atomic absorption spectrometry, as described by Simpson and Blay (49). Although the preliminary results show low and

variable recoveries at levels below 100 mg/kg, the procedure appears to be adequate to monitor a 250 mg/kg regulation level.

METHODOLOGY

The relatively low toxicity of inorganic tin salts has resulted in little attention being paid to the perfection of analytical methods for the tin determination. The only extensive collaborative study on methods of analysis for tin was published in 1915.

The higher levels of tin usually are determined by wet oxidation with nitric and sulfuric acid followed by a spectrophotometric determination by one of the numerous colorimetric reagents which are available for the tin assay. Residue levels are determined similarly but they require a separation from potentially interfering elements. Use of the atomic absorption determinative step is desired by laboratories having this equipment.

Doubts have been raised regarding the suitability of both the wet and dry methods of oxidizing organic matter for the tin determination. These doubts have not been resolved. A few comparative studies have been performed within a single laboratory to assess the relative merits of the various methods. Only the gravimetric method (precipitation as the sulfide and ignition to the oxide) and the titrimetric method (precipitation as the sulfide, solution of the sulfide, and titration of the

tin(II) with iodine) have been subjected to an interlaboratory collaborative study under the same conditions so that their relative reliability can be compared. No true collaborative study of any of the colorimetric procedures has been located.

Table 1 lists the analytical operations and the various methods available for general laboratory use for the determination of tin in food. There are several important sources of possible tin contamination in the laboratory that must be avoided. Distilled water lines, particularly in older laboratories, were constructed from "block tin." Such water should be further treated by deionization. Deionized water generally should be used for elemental analysis. Plastics used to conduct distilled water may contain organotin compounds as stabilizers. Solder, which contains up to 50% tin, is often used to join metals and is an essential component of electrical connections. Reagents, if packed in tin-lined containers, may pick up tin by abrasion. Such a source might not be suspected if bulk reagents shipped in tin-lined containers are later repackaged into glass or plastic. Conducting blanks through the entire procedure is essential to control these types of contamination.

ELIMINATION OF ORGANIC MATTER

Most methods now rely upon wet oxidation for destruction of organic matter because dry ashing was reported to leave the tin as insoluble oxides. Furthermore, the solution from wet oxidation can be used directly for the determination of numerous other elements. Wet oxidation using

TABLE 1. DETERMINATION OF TIN

ELIMINATION OF ORGANIC MATTER

Wet oxidation (H_2SO_4, HNO_3)

Dry ashing

ISOLATION OF TIN

Precipitation as sulfide

Extraction as tin iodide into hexane

Distillation as stannic bromide

Coprecipitation with iron (III) or hydrous manganese dioxide

DETERMINATION

Gravimetric

Titrimetric

Colorimetric

Catechol Violet

Quercetin

Dithiol

Phenylfluorone

Polarography

Atomic absorption

nitric acid was initially avoided because it was thought that the presence of nitric acid led to the formation of insoluble metastannic acid (H_2SnO_3). Apparently no specific studies have been directed to this point, although most analytical investigations incorporate the usual recovery procedures of adding known amounts of tin. Gorsuch (7) raises doubts regarding the use of perchloric acid, which may be reduced to chloride ion during oxidation of some types of organic material at high temperatures. Chloride ion may then cause losses of tin as the relatively volatile stannic chloride.

The report of the Analytical Methods Committee on the determination of small amounts of tin (8) recommends the use of any of the following combinations: nitric and sulfuric acids; nitric, perchloric, and sulfuric acids; or 50% hydrogen peroxide and sulfuric acid. The report indicates that reproducible results were obtained only when completely colorless digests were produced. The Association of Official Analytical Chemists (AOAC) method (9) digests the sample with sulfuric and nitric acids as for arsenic determinations but indicates that digestions can be greatly facilitated by additions of small portions of perchloric acid. The Nordic Committee on Food Analysis (10) uses only nitric and sulfuric acids. Corbin (5) emphasizes that the amount of nitric acid present must at least equal the amount of water present in the sample or organotin compounds will be lost.

Engberg (11) found that wet oxidation with sulfuric acid and hydrogen peroxide is usually satisfactory with much canned goods, fruit juices, and other easily digested samples, but nitric acid must be used when carotenoids or tartrazine is present and the quercetin color reaction is used, since the absorption maximum of the complex is very close to that of these food colors.

Schroeder et al. (12), in their survey of sources and exposure of man to tin, used ashing at 450° for foods, tissues, and vegetation. Recoveries are stated to be satisfactory but details are omitted.

Zook et al. (4) used dry ashing for the elimination of organic matter in a study of the metal content of wheat products. Fifty-five gram samples were ashed overnight at 480°. The residue was treated with 6N hydrochloric acid, dried, and re-ashed overnight. This procedure was repeated until no carbon remained. The residue was extracted with dilute hydrochloric acid. No mention is made of any difficulties from insoluble tin salts or from volatility of tin(IV) chloride. Recoveries of tin added before ashing averaged 90% with a standard deviation of 10% at levels of 0.05-1.0 mg tin/kg. The values by the atomic absorption method used agreed with the values obtained by a colorimetric method, but it is not clear whether or not the same ash solution was used for the comparison. Tin levels of wheat and its products as reported by Schroeder et al. (11) (average 0.7 mg/kg for 4 samples) differ markedly from those reported by Zook et al. (4) (average 11 mg/kg for 14 samples).

<u>Conclusion</u>: Since there is no definitive study comparing the various ashing procedures for tin, the flexible recommendations of the Analytical Methods Committee may be accepted as suitable for the preparation of food samples for the tin determination. This involves the use of sulfuric and nitric acids, supplemented by perchloric acid or 50% hydrogen peroxide, and digesting to a completely colorless solution. It should be pointed out, however, that Gorsuch (13) classifies tin as an element which causes problems under most ashing conditions.

ISOLATION OF TIN

When dealing with residue levels of tin in food, it is necessary to isolate the tin from interfering metals or from the large amounts of salts and acid introduced by wet oxidation. Three methods are in common use for this purpose: (1) The oldest is the sulfide precipitation from acid solution; (2) tin(IV) bromide distills readily at relatively low temperatures; and (3) tin(IV) iodide can be extracted into hexane. Coprecipitation with iron(III) and manganese are mentioned in the analytical literature but have not been applied to food to any appreciable extent.

The sulfide separation precipitates a large number of other elements together with the tin so that it is useful primarily to eliminate salts and acids. Unless the matrix is free from other "heavy metals," the determinative step must be selective with respect to tin. This classical separation is used in both of the AOAC methods, gravimetric and titrimetric, for the determination of tin in foods. The separation

is long and tedious by modern standards and calls for the use of skills and experience which are probably little practiced today.

Law (14) separated tin(IV) as the bromide from a sulfuric and hydrobromic acid solution at 220° in a current of carbon dioxide over a one hour period. An advantage of this technique is that the sample need only be charred prior to distillation. Of the elements likely to be present, only arsenic and boron distill under these conditions. Relatively large amounts of arsenic are required to interfere with the dithiol colorimetric determination and boron does not react with this reagent. It is difficult to estimate the reliability of Law's procedure since the results are confounded with the unreliability of the gravimetric determination at milligram weighing levels which was used for comparison and by the necessity for use of visual color comparisons in the dithiol reaction employed in the determinative step.

Corbin (5) utilized the distillation procedure after oxidation with sulfuric, nitric, and perchloric acids for determining organotin residues below 1 mg/kg. He simplified the distillation apparatus by using the digestion flask as the distilling flask and by eliminating the use of carbon dioxide carrier. The distillation was conducted from a mixture of hydrochloric and hydrobromic acid to fumes of sulfur trioxide, which required about 0.5 hour. Arsenic and antimony would be expected to distill under these conditions.

As an alternative to the distillation procedure, Corbin (5) also recommended a hexane extraction of stannic iodide from the wet oxidation solution after addition of potassium iodide. In both cases, the residue from the distillate or the extract is again oxidized with nitric acid and sulfuric or perchloric acid before a colorimetric determination with dithiol. The difference in results between the two methods of separation of tin--distillation and extraction--was found to be negligible. Gorenc et al. (15) characterize the extraction of stannic iodide into toluene as the best medium for the separation of tin if the concentrations of potassium iodide and sulfuric acid are properly adjusted.

TITRIMETRIC AND GRAVIMETRIC METHODS

The only approved methods of analysis for tin which are based upon collaborative studies are those of the Association of Official Analytical Chemists (AOAC). These methods are a gravimetric (16) and a titrimetric (17) procedure after oxidation with sulfuric and nitric acids. In the gravimetric method the tin is precipitated as sulfide from slightly acid solution, washed, and ignited to stannic oxide (79% tin). Using a 100 g sample, 200 mg tin/kg food will yield about 25 mg of weighed stannic oxide. The overall coefficient of variation of the five collaborators analyzing three samples at the 200-300 mg/kg level was about 15%; the repeatability (within-laboratory) coefficient of variation was about 3%. The recoveries were about 8% low. The method is lengthy and tedious, particularly the ashing of 100 g of sample, and the results are no

better than the faster colorimetric procedures available today. It also demands manipulative skills which are probably beyond the capabilities of many recently trained analysts. If approval were to be withdrawn by the AOAC, the action would probably go unnoticed.

The AOAC titrimetric method follows the gravimetric method through the precipitation as sulfide. The sulfide is dissolved by the action of chlorine produced with potassium chlorate and hydrochloric acid. While an inert atmosphere of carbon dioxide is maintained at all times, aluminum is added to transform all the tin into the stannous form and the solution is titrated with 0.01N iodine solution, or an excess is added and the excess is back-titrated with sodium thiosulfate solution. Using a 100 g sample, 200 mg tin/kg food will provide a respectable titration of about 20 ml of 0.01N iodine. The overall coefficient of variation of six collaborators analyzing the same three samples used for the trial of the gravimetric method was about 7%, about half that of the gravimetric method at the 200-300 mg/kg level; the repeatability (within-laboratory) coefficient of variation was 3.4%. Furthermore, the results closely approximated the amounts of tin added to the two known samples. This method was also considered best by most of the laboratories. The method would still be considered very lengthy and tedious because of the need to oxidize 100 g of sample and to precipitate, wash, and dissolve the tin sulfide before the determinative step. A major source of error is from air oxidation. Even the titrating solutions and reagents should be prepared from boiled water.

The titrimetric method of Gulbrand and Mathieson (18) was one of the three methods intercompared by Woidich and Pfannhauser (19). A 40 g sample was wet oxidized and the nitric acid completely eliminated. The digested solution was diluted to 5N with hydrochloric acid, the tin reduced with aluminum, an excess of 0.005N iodine added, and the excess back-titrated under carbon dioxide with 0.005N thiosulfate. This method is stated to be virtually interference-free and satisfactory down to about 50 mg tin/kg. Oxygen from the atmosphere must be avoided, since it too will oxidize tin(II). This range will handle all contamination problems with tin except those dealing with residue tolerances. However, this titrimetric method appears to give consistently low values as compared to the other two methods examined (quercetin colorimetric and atomic absorption spectrometry) and there is no basis in the Woidich and Pfannhauser study for determining which method is the more accurate. The long time required for the preparation of a 40 g sample makes the titrimetric method very lengthy. No collaborative study has been performed on this simpler version of the titrimetric method.

COLORIMETRIC METHODS

Spectrophotometric methods for the determination of tin have been generally preferred because of their relative simplicity. The high sensitivity of some of the reagents permits interfering elements and salts and acids to be diluted out when applied to canned foods. If preceded by distillation or extraction, the reaction can be made almost specific.

Some of the reaction products are colloidal in nature. Such systems are inherently difficult to develop reproducibly. There appear to be problems in obtaining pure and reproducible color reagents.

Table 2 provides a comparison of the molar absorptivities (absorbance/cell thickness (cm) X concentration (moles/liter)) of the tin-color complex of the common reactions used in food analysis. There is obviously a considerable discrepancy in the phenylfluorone absorptivity as calculated by Dagnall et al. (20) and as mentioned by Engberg (11). Gorenc et al. (15) quote a value near that of Engberg and provide the important information as to the effect of pH on absorbance.

DITHIOL METHOD

Clark (25, 26) introduced the use of dithiol (4-methyl-1,2-dimercaptobenzene, toluene-3,4-dithiol) as a fairly specific reagent for tin. Thioglycolic acid reduces the tin to the stannous form and the addition of agar-agar avoids the precipitation of the red colloidal complex. Kenyon and Ovenston (24, 27) found that sodium lauryl sulfate is superior to agar and that its use permitted the application of the spectrophotometer to the measurement of the absorption. Copper, if present at a concentration of more than 0.1 that of the tin, must be removed (28). Dickinson and Holt (28) recommend the use of diethylammonium diethyldithiocarbamate for this purpose rather than sodium diethyldithiocarbamate to permit the efficient separation without the loss of tin.

TABLE 2. MOLAR ABSORPTIVITIES OF COLORED COMPLEXES USED IN THE DETERMINATION OF TIN

Reagent	Wavelength nm	pH	Molar Absorptivity	$\frac{\Delta A}{\Delta pH}$	Reference
Catechol violet with CAB	662		95,600		Dagnall et al. (20)
Catechol violet with DBAC	665		71,200		Thomas and Tann (21)
Catechol violet	555		65,000		Ross and White (22)
	552		68,500		Newman and Jones (23)
	550	4	53,500	0.005	Gorenc et al. (15)
Quercetin	437		20,600		Engberg (11)
	450	3.5	35,500	0.01	Gorenc et al. (15)
Dithiol	533		7,100		Ovenston and Kenyon (24)
Phenylfluorone	510		4,000		Dagnall et al. (20)
			60,000		Engberg (11)
		1	80,000	0.12	Gorenc et al. (15)

CAB = Cetyltrimethylammonium bromide

DBAC = Benzyldimethyl-n-hexadecylammonium chloride

$\frac{\Delta A}{\Delta pH}$ = Change of absorbance relative to pH

The working range of the dithiol method as applied by Corbin (5) to the determination of organotin residues is from about 0.05 to 8 mg/kg, using a 100 g sample and a 10 cm optical cell. Antimony seriously interferes in the dithiol method, but this element is not ordinarily encountered in food. If present, the hexane extraction of the tin(IV) iodide tolerates as much as 2 mg of antimony (equivalent to 20 mg/kg). Arsenic at levels of 0.15 mg (equivalent to 1.5 mg/kg) does not cause an error in excess of 1 µg of tin. Other elements likely to be present in food which do not interfere in the dithiol method in ordinary amounts are calcium, magnesium, sodium, potassium, phosphorus, and iron.

The dithiol method is recommended by the Analytical Methods Committee for amounts of tin from 30 to 150 µg (29). The sample containing 30-150 µg of tin is digested with nitric and sulfuric acids, with nitric, sulfuric, and perchloric acids, or with 50% hydrogen peroxide and sulfuric acid. Any copper present is removed, after dilution, with dithizone. The tin reacts with zinc dithiol in the presence of thioglycolic acid, with sodium lauryl sulfate as a dispersing agent. All of the nitric acid present during digestion must be removed and the sulfuric acid concentration in the final aliquot must be carefully controlled. Only a very limited amount of data support the recommendation. Three known levels of tin were added to a single sample of dried carrots in three laboratories with satisfactory results.

Woidich and Pfannhauser (19) did not consider the dithiol method as one of the most useful methods for the determination of tin in foods because of the necessity for the removal of copper, which makes for a cumbersome procedure.

Schroeder et al. (12) reported difficulties with a new batch of reagent which resulted in their abandoning this method in favor of the phenylfluorone method.

According to Sandell (30), no color is produced at tin concentrations less than 0.25 μg/ml. Therefore a small amount of tin must be added to the blank solution to bring the final concentration to about 3 μg tin/ml. Without the addition, the sample values will be too high and the apparent blank will be zero.

PHENYLFLUORONE METHOD

The phenylfluorone (2,6,7-trihydroxy-9-phenylisoxanthene-3-one, 2,3,7-trihydroxy-9-phenyl-6-fluorone) method has been accepted by the International Organization for Standardization (ISO) for the determination of tin in fruit and vegetable products (31). A 10 g sample is oxidized with sulfuric and nitric acids, the solution is buffered to pH 1.0-1.2 with acetate, iron is complexed with ascorbic acid, and an orange complex is formed with the reagent which is stabilized with polyvinyl alcohol. The lowest point on the calibration curve is 10 μg of tin, equivalent to a concentration of 10 mg/kg sample, after aliquoting. The method is stated to be applicable in the presence of up to 1.25 g of copper, 0.6 g

of lead, 0.6 g zinc, and 40 g of phosphorus per kg. As customary with ISO final documents, no supporting data are provided to justify its reliability and practicability. The repeatability of the method is given as follows: "the difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed 5% of the mean value." Apparently the final version of the method had never been tried by the 20 countries which approved this standard, since errors in the text were brought to the attention of the Codex Committee on Methods of Analysis and Sampling (32). The statement in the ISO standard that the method had been approved by the AOAC is also in error.

The phenylfluorone method of Luke (33) was applied to the determination of tin in shrimp by Thompson and McClellan (34). A 10 g sample, preferably containing 100-400 μg of tin, was oxidized with sulfuric and nitric acids. Many interfering elements are removed by extraction with diethylammonium diethyldithiocarbamate solution. The tin is reduced to the stannous form and re-extracted into carbamate solution. After evaporation of the solvent, the residue is again oxidized with sulfuric, nitric, and perchloric acids and the color is developed with phenylfluorone and read at 510 nm. The sensitivity of the calibration curve is about 0.02 absorbance units/μg tin over the straight line portion 0 to 40 μg. The calibration curve varies to some extent with the age of the phenylfluorone and carbamate solutions. The calibration curve is prepared beginning with the first carbamate extraction since there is some loss of tin during the

carbamate extraction. The pH must be closely controlled at 1.8 during color development and stabilization. Phenylfluorone is very sensitive to traces of many metals, so all solutions and glassware must be protected from contamination. The double extraction and the necessity for conducting several standards along with a series of samples makes for a lengthy and time consuming procedure.

The phenylfluorone procedure of Gorenc et al. (15) appears attractive in view of its relative simplicity, despite a double extraction, and claimed almost absolute specificity through an iodide extraction and pH control, and a suitable limit of reliable measurement of about 1 mg/kg, utilizing samples of the order of magnitude of 1 g. The recovery and reproducibility data, however, are very limited.

PYROCATECHOL VIOLET METHOD

Ross and White (22) investigated pyrocatechol violet (catechol violet, 3,3',4'-trihydroxyfuchsone-2"-sulfonic acid) as a colorimetric reagent for tin(IV) and recommended the method as one that is at least as sensitive as and less subject to error than other methods then currently in use. The reagent has a low absorbance at the maximum for the colored tin(IV) complex at 555 nm. The calibration curve is linear in the range 0.24-1.6 μg/ml, but not below. Tin(II) does not react, but is readily oxidized under the color development conditions. The reagent is an acid-base indicator and therefore the pH must be controlled carefully

to pH 2.5 by the use of a potassium acid phthalate buffer. Acids and salts that might be present from wet oxidation suppress the color formation. Acids, if present in amounts greater than 4 mmoles, should be removed by evaporation to dryness. Of the common elements which might be present in food, only iron(III) produces serious interference at the pH of the reaction. Sulfates and nitrates do not interfere if chloride is present and the maximum ionic strength is not exceeded.

The Analytical Methods Committee selected the catechol violet method for the determination of small amounts of tin (8), based upon the work of Newman and Jones (23). Tin must be separated from many other metals, particularly iron and aluminum, and those which might be introduced through contact with processing metals, such as titanium, antimony, bismuth, and molybdenum. The separation recommended is the extraction of tin(IV) iodide into toluene from solutions of sulfuric acid greater than 8N in order to take advantage of the presence of sulfuric acid from wet oxidation.

The calibration curve is linear from 0 to 1.2 μg of tin/ml (30 μg maximum in the aliquot taken gives an absorbance of 0.68). Since the reagent may be variable in quality, a new calibration curve must be prepared with each new batch of catechol violet. Although straightforward, the method has a number of steps, including an extraction in a separatory funnel, and an adjustment using the pH meter. Except for

a 30-minute standing period before the color is read, the method requires constant attention from the operator.

A limited collaborative study (8) was conducted on a single sample of orange squash containing about 2 mg of tin/kg to which each of 4 collaborators (1 collaborator used two different batches of reagent, which are considered independent) added known amounts of tin equivalent to 1, 2, and 10 mg of tin/kg. An analysis of variance of these data performed for this review shows a repeatability coefficient of variation of 4.4% (essentially the variability of duplicates within a laboratory) and a reproducibility coefficient of variation of 8% (the variability between laboratories or total variability). This is not a true collaborative study since the collaborators were aware of the concentration of the added analyte.

Adcock and Hope (35) determined amounts of tin of the order of 0.75 μg such as would be encountered by migration of organotin stabilizers from poly(vinyl chloride) containers into vinegar, orange drink, and cooking oil. The catechol violet complex formed at pH 3.8 in acetate buffer is retained on a column of asbestos or cellulose powder while the excess color reagent passes through. The complex is eluted by an anionic or nonionic surface active agent and measured directly. Complex formation at these levels requires 3 hours. The organotin compound is removed from aqueous liquids by continuous extraction with

chlorinated solvents or, for a cleaner extract, by petroleum ether. Silica gel removes the stabilizers from cooking oils. Aluminum and iron as well as inorganic tin salts are separated from the mercaptoacetate tin stabilizer by paper chromatography with chloroform, which leaves the inorganic material behind.

The catechol violet method was applied by Thomas and Tann (21) to the determination of very small levels of tin such as found from the application of triphenyltin compounds as a fungicide to potatoes. The U. S. tolerance for triphenyltin hydroxide on potatoes is 0.05 mg/kg (36). A sample of 250 g of potatoes is macerated with acetone to remove water and the triphenyltin compounds are extracted with dichloromethane. Coextracted inorganic tin is removed as the EDTA complex at pH 7-8. Most of the solvent is removed under reduced pressure and the tin is transferred to sulfuric acid, converted to inorganic tin, and reextracted as the iodide into carbon tetrachloride. The tin was transferred to hydrogen peroxide and the solution oxidized with nitric and sulfuric acids. The tin was determined with catechol violet in the presence of a quaternary ammonium compound (benzyldimethyl-_n_-hexadecyl ammonium chloride). The limit of detection by this method is given as 0.5 μg of tin. The average value found in 44 samples of potatoes corresponds to less than 0.5 μg of tin after a blank of 0.75 μg is subtracted. These values are too close to the blank to provide meaningful information as to the apparent presence of the organotin compound. Few other data are given

as to the performance of the method; recoveries were run at an order of magnitude higher than the actual samples.

Corbin (37) studied the characteristics of the catechol violet method as applied to biological samples at residue levels (less than 1 mg tin/kg). Tin is extracted from the wet oxidized sample into hexane as the iodide to remove much of the sulfuric acid and insoluble material (often calcium sulfate). Standard tin solution containing 0.5 μg is added to the solution to bring the absorbance reading into a more convenient region. The calibration curve in his system was not linear below 0.5 μg (0.01 mg/kg). The sensitivity (10 cm cell) was 0.17 absorbance unit/μg of tin. The most critical factor in the determination was the color reagent concentration, which is easily controlled by pipetting. A high tolerance for foreign elements (particularly phosphate, calcium, and iron) is claimed with an applicability to levels as low as 0.01 mg tin/kg, using 5-10 g samples.

QUERCETIN METHOD

Kirk and Pocklington (38) applied the quercetin (3,5,7,3',4'-pentahydroxyflavone) reaction with tin(IV) to its determination of 10-500 mg/kg in canned food. The bright yellow-orange complex (maximum at 437 nm) requires at least 50% alcohol to remain in solution. The color of the blank, from the reagent itself, is appreciable (an absorbance of 0.4 in a 4 cm cell) and this solution is used in the reference cell.

Because the acidity of color development must be controlled carefully, the digest from wet oxidation must be neutralized with sodium carbonate before addition of a constant amount of hydrochloric acid. The range of applicability is very large, 10 to 500 mg tin/kg (0 to 500 μg). The size of the aliquot used for color development must be limited to avoid salt precipitation in the alcoholic medium. When more than 1 mg of tin is present in the digested sample, low results were obtained, possibly by conversion of the tin, under the influence of nitric acid, to metastannic acid. When more than 2 mg of tin are present, opalescence is often apparent and a smaller sample should be used for a repeat digestion. Up to 1% of iron is masked by the use of thiourea; sulfates and nitrates from the wet oxidation do not affect the reaction. Phosphates at levels of 0.5% supresses the color to some extent, which is overcome by taking a smaller sample. Six samples can be completed in 3.5 hours if silica flasks are used and the results are comparable to those obtained by the dithiol method.

Engberg (11) simultaneously studied the quercetin method and an atomic absorption method for the determination of tin in food. She found the quercetin method convenient for canned food utilizing a range of 0.6-6 μg/ml or 30-300 μg in 50 ml final solution. The tin(IV) iodide extraction into toluene was used to obtain improved specificity. By using longer cells or scale expansion, or both, the method is also suitable for organotin residues, since the calibration curve is linear down to at least 0.02 μg/ml or 1 μg in 50 ml final solution. Engberg

also measured the various factors influencing the precision of the method. The final steps--instrumental measurement and holding the solutions for 1 hour before measurement--contributed little variability. The error introduced from adding the color reagent and mixing was appreciable. Therefore the addition of the colored quercetin solution by pipet must be performed reproducibly. The extraction and oxidation steps did not contribute appreciably to the variability although there was evidence that the acid-alkaline-acid sequence after the extraction contributed a decrease in absorbance and a lowering of the calibration curve. This source of variability coupled with a considerable day-to-day variability in the complete procedure is interpreted as being caused by a tendency of tin to hydrolyze or polymerize during the determination. Therefore the procedure must be carried out as rapidly as possible, which limits its application with an extensive series of samples. The spectrophotometric measurement must also be conducted without delay since the absorbance is temperature dependent and there may be some liberation of free iodine under the influence of the light beam. It is estimated that the practical limit of reliable measurement is about 1 μg of tin in the final aliquot with a linear working range of 1-400 μg in that aliquot.

The quercetin method was compared with the atomic absorption method using the aliquots from the same wet oxidized samples of baby food with standard additions of tin. The results by the two methods were not significantly different. It was concluded that the quercetin method is

preferred for very low concentrations of tin such as those found in residue examinations while both methods are equally suitable for the determination of tin in canned food.

Woidich and Pfannhauser (19) in their intercomparison of the quercetin, atomic absorption, and iodine titration methods found the quercetin method suitable for tin levels above 20 mg/kg. Accuracy decreased below this level and phosphate decreased the absorbance. In general, the quercetin method gave values about 7.5% lower than the atomic absorption method studied in the range of 15 to 225 mg of tin/kg. Since this comparison was performed on apparently commercial samples, it is not possible to determine which results are more accurate.

Conclusion. There are at least three sensitive reagents which are suitable for the spectrophotometric determination of tin at contaminant levels. These sensitive reagents, however, are colored themselves, thus limiting their applicability at lower residue levels. The disadvantage of a lower sensitivity of the dithiol reagent is offset by a colorless reagent blank but a minimum amount of tin must be present before a color is produced. None of the reagents are specific, but with the separation techniques available, the final determinative step can be made specific even at residue levels. When separation is required, the methods become lengthy. The unreacted reagent is often an indicator, so the colored complex, which may be colloidal, must be developed in highly buffered

solutions which may contain high salt concentrations from the neutralized wet oxidation solution. If the color can be developed in acid solution, it may be affected by a variable, unknown amount of residual acid.

No study has been performed to compare the various procedures on the same samples to determine the applicability and limitations of the various reagents for determining tin at the contaminant, technological, and residue levels of tin. Nor has a properly designed collaborative study been performed which would permit the recommendation of a colorimetric method for any purpose on the basis of unbiased, comparative data.

ATOMIC ABSORPTION METHODS

Engberg (11) studied the performance of the atomic absorption method in comparison with the quercetin colorimetric procedure after wet oxidation. The lowest possible detection limit is obtained with the hydrogen-air flame, with a linear response of 0.2-100 µg of tin/ml, corresponding to 4-2000 mg/kg in the final aliquot; the corresponding values for the air-acetylene flame are 2-360 µg/ml and 40-8000 mg/kg, respectively. These ranges make the aromic absorption method more suitable for the determination of tin in canned food than for organotin residues.

For use of the atomic absorption method, the tin in the oxidized solution is extracted into toluene as the iodide and reextracted into

potassium hydroxide (rather than sodium hydroxide) to form the stannate. The solution is reacidified with hydrochloric acid for the final measurement to gain the enhancement caused by potassium and to avoid suppression caused by sulfate. The removal of iron interference is not necessary. A deuterium background corrector is necessary, however, particularly near the detection limit, since its use lowers the standard deviation of a single measurement by a factor of 4-5. A concentration of about 1.5 μg /ml final solution corresponds to 1% absorption. Using the relative magnitude of the standard deviations as the basis for estimation, the atomic absorption method has a detection limit about four times higher than the quercetin method. The relative precisions are comparable, however, in the range useful for canned food.

Woidich and Pfannhauser (19) handled the interference problem in atomic absorption by extracting the tin from 3N hydrochloric acid solution with three portions of methylisobutylketone after wet oxidation. The method was usable over the range of 5 to 1000 mg of tin/kg. It is especially suitable at the lower levels where the titrimetric and quercetin methods were not usable. In general, this method gave values for tin about 8% higher than the quercetin method and was the method of choice of these workers. However, since the comparison of methods was performed on apparently commercial samples, it is not possible to determine which results are more correct.

Meranger (39) analyzed a number of fruit juices and carbonated beverages for nine metals, including tin, by centrifuging out large particles and removing carbon dioxide, when necessary, and using the method of additions (40). Sugar reduces the sensitivity of atomic absorption methods and is compensated for by the method of additions. Centrifuging may result in loss of particulate and adsorbed or occluded tin, particularly since acidification to solubilize tin was absent. No reliability data are reported.

Meranger (41) also described a rapid screening method to determine the presence of di-(n-octyl) tin stabilizers in alcoholic beverages using a heated graphite atomizer coupled to an atomic absorption spectrophotometer. Benzene selectively extracts the tin stabilizers in the presence of inorganic tin from the plastic containers or from the alcoholic beverages stored in them. Although up to 1.7 mg of tin/g plastic was found in the container by X-ray fluorescence spectrometry in five types of plastic containers, no di-(n-octyl) tin was found in 22 samples of various alcoholic beverages stored therein. The method used would have detected 0.04 mg tin/kg. Recovery of 0.3-0.6 mg organic tin/kg added to the alcoholic beverage was 85-118%, while inorganic tin added at the same levels was not extracted.

Although the detectability of the graphite furnace technique for tin is excellent compared to other methods for tin, it is still rather poor compared to methods for elements such as cadmium or zinc, where the ratio of detectability of element to that for tin is about 5000:1 (42).

Tin forms a hydride (SnH_4) with sodium borohydride ($NaBH_4$) which can be determined by atomic absorption spectrophotometry (43). The hydrochloric acid concentration during the liberation must be less than 0.5N. The repeatability of the determinative step was the poorest of the group of seven elements tested (As, Bi, Ge, Sb, Se, Sn, Te); the coefficient of variation was about twice that for arsenic, which has one of the best repeatabilities. The determination of arsenic by the hydride method can not yet be recommended for general laboratory use, however (44). An unsuspected source of high blanks in this method may arise from storage of the sodium borohydride in tin-lined containers at some stage of its distribution.

An atomic absorption method for the determination of tin is currently undergoing collaborative trials under the auspices of the AOAC (45).

POLAROGRAPHY

Very small amounts of tin, of the order of magnitude of 0.1 ppm, can cause an intolerable amount of haze in beer. Colorimetric methods have not been satisfactory at this low level and are not sufficiently specific. Rooney (46) found polarography in 5N HCl was satisfactory for small amounts of tin in beer. The wet oxidation and the necessity for fuming off all acids in order to provide a satisfactory electrolyte results in a lengthy procedure. The only likely interference is lead, whose peak is readily differentiated from that of tin.

The sample is digested with sulfuric, nitric and perchloric acids, and all the acids are removed by fuming to dryness. The residue is dissolved in hydrochloric acid and an aliquot is polarographed. The useful tested range of the method is 0.02-0.5 μg/ml. Blank values from bottled beer were less than 0.01 mg/kg.

Polarography was applied to the determination of tin in canned food by Markland and Shenton (47). A 5 g sample was oxidized by nitric and sulfuric acids. All residual nitric acid was removed by two treatments with ammonium oxalate solution. After addition of supporting electrolyte and gelatin, the solution was polaragraphed directly. The lead wave is indistinguishable from tin but was ignored on the basis that modern cans do not contain sufficient lead to interfere. Added tin (0.1-1.25 mg) was recovered to a satisfactory extent and the results on three samples at the 100-500 mg tin/kg level were comparable to those obtained by the titrimetric method.

Polarography in its various aspects may have a potential usefulness for routine surveillance and compliance operations in environmental and regulatory laboratories. But polarography is not a popular technique in the general laboratory, although it is becoming more prevalent. It has its strong advocates but the majority of analysts have not taken the trouble to become informed of the simplicity and advantages of this technique. Polarography is sensitive to the nature and concentration of the solutes in the medium; therefore tin must be separated by one of the

conventional separation techniques from the reagents of the wet oxidation customarily used. Once this step is necessary, most analysts will prefer to utilize a direct colorimetric procedure. No collaborative studies have yet utilized this instrument.

SUMMARY AND CONCLUSIONS

Inorganic tin, the form present predominantly as a contaminant in food at levels of several hundred mg/kg, is relatively nontoxic. Therefore little attention has been given to its determination in food. Organic tin salts are more toxic. They have minor uses as pesticides and as a stabilizer in plastics where tin may be present as an indirect additive at levels of 1 mg/kg and below.

Only two methods of analysis for contaminant levels of tin have been collaboratively studied. Although the titrimetric method has an acceptable interlaboratory relative standard deviation of about 7%, it requires wet oxidation of a 100 g sample, a sulfide precipitation, solution with chlorine, and iodine titration in an inert atmosphere. It is therefore too lengthy for practical laboratory operations. The gravimetric method of ignition of the sulfide to the oxide is less precise by a factor of two.

A number of reagents have the potential of providing sensitive spectrophotometric methods for tin. They are nonspecific, requiring separation of the tin by distillation, precipitation, or extraction; or

the method is sensitive to the presence of the acids and salts from wet oxidation, when applied to concentrations sufficiently high to permit dilution of interfering elements. Most of these methods also measure the color in the presence of an excess of the colored reagent.

The suitability of both direct ashing and wet oxidation for destruction of organic matter has not been settled satisfactorily, nor has the chemical nature of the tin in foods been investigated. If tin is not tightly held to the protein or to other polar constituents, it might be made available quantitatively by the simultaneous application of hydrochloric acid in a high speed blender, and extracted in a form for direct application to atomic absorption spectrophotometry or polarography.

At present no method of analysis can be recommended for the determination of tin in foods at either the contaminant or residue levels. No reference standards exist that permit assurance that analyses are being performed in a reliable manner.

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