Methodological Evaluation of Method for Dietary Heavy Metal Intake

J. Żukowska and M. Biziuk

ABSTRACT: Exposure to environmental pollutants is an important problem of environmental toxicology. Heavy metals are regarded as toxic to living organisms because of their tendency to accumulate in selected tissues. Moreover, their presence is a causative agent of various sorts of disorders, including neuro-, nephro-, carcino-, terato-, and immunological. Exposures of human to environmental chemicals can occur simultaneously from various sources. One exposure route is ingestion of hazardous chemicals through contaminated food and beverages. Considering the above-mentioned menace, efforts should be focused on the estimation of dietary intakes of potential toxic agents by consumers. Dietary exposure assessment to nonnutrients is usually performed by combining 2 sets of data—the concentration of elemental contaminants in various food products and the consumption data of these food items. A variety of approaches exist for evaluating exposure to food chemicals, and the method chosen is influenced, among others, by the intended goal, the availability of data, cost, and time frame. Moreover, it is also important to note how accurate and detailed the information concerning toxic elements intake needs to be. There are a number of sources of food consumption data currently used in exposure assessments, which range from 1 d to habitual intake. Frequently, the heavy metals for which dietary exposure is of interest are present in trace and ultra-trace quantities. Hence, an analytical technique with sufficient sensitivity is required for the accurate determination of these chemicals in food samples. It is important to remember that the accuracy of quantitative analysis is strongly dependent on the sampling and preparation steps.

Keywords: dietary exposure, food analysis, food contamination, heavy metals, risk assessment

Introduction

Contamination of food products by heavy metals is becoming an unavoidable problem these days. Air, soil, and water pollution are contributing to the presence of harmful elements, such as cadmium, lead, mercury, and arsenic in foodstuff. The occurrence of heavy metals-enriched ecosystem components, firstly, arise from rapid industrial growth, advances in agricultural chemicalization, or the urban activities of human beings. These agents have led to metal dispersion in the environment and, consequently, impaired health of the population by the ingestion of victuals contaminated by harmful elements.

One of the most glaring examples of metal poisoning by industrial water pollution is Minamata disease. This illness' occurrences, which took place at Minamata City (1956) and then at Niigata City (1965) in Japan, were attributed to the intake of fish and zoobenthos contaminated with methyl mercury (Kudo and others 1998). The above-mentioned organic form of mercury is the most prevalent and particularly toxic compound, which is bioaccumulated and passed up the food chain.

As mentioned previously, metal contamination can be influenced by factors ranging from environmental conditions during growth to the processing, handling, and storage of food products (Morgan 1999). Contact between food and the coat metal surface of packing containers or the processing equipment is a significant source of toxic contamination in food. Thus, the presence of these elements in crops originating, for example, directly from harvest, can undergo a change owing to the effects of food processing. A number of papers presented data about the influence of some processing factors on the content of metals in foodstuffs (Kroyer 1995; Atta and others 1997; Watzke 1998; Ersoy and others 2006).

The toxicity of heavy metals for humans is mainly caused by their persistence in the environment and hinged strongly upon the chemical form in which they are ingested. In this regard, inorganic arsenic (As^{3+}) and organic forms of mercury (methyl- and dimethyl mercury) belong to the most toxic forms of discussed metals. Nonetheless, all other forms of mercury are toxic (Gochfeld 2003). As distinct from acutely toxic trivalent arsenic trioxide, elemental as well as organic arsenic compounds (with the exception of methylarsonic acid [MMA] and dimethylarsinic acid [DMA]) are considered to be virtually nontoxic. According to a report published by the U.S. EPA, alkyl-lead compounds (especially tetraethyllead [TEL] and tetramethyllead [TML]) have a higher toxicity than inorganic forms of lead (USEPA 1999). The lipophilic nature of alkyllead and its ability to permeate biological membranes render these compounds readily bioavailable. Unlike particularly toxic forms of described metals, all inorganic forms of cadmium have the same toxic endpoints; however, the toxicity depends upon whether it is ingested or inhaled. Cadmium is not as toxic via oral routes as through inhalation (Robson 2003).

There are a number of different factors that can influence a metals' toxicity (Table 1), but generally the poisonous effect of heavy metals is a function of a concentration which is attained in a target organ in the human body. Thus, similar effects will occur following long- and short-term exposure to low and high cadmium levels, respectively (Wang 1984). Consequently, both acute and chronic systemic toxic effects may be induced.

Among possible target organs of heavy metals, soft tissues such as the kidney and liver and the central nervous system appear to be especially sensitive (Apostoli 2002).

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Considering the above-mentioned problem, efforts should be focused on the evaluation of dietary intakes of heavy metals by consumers in order to assess risk.

Whenever feasible, monitoring data from dietary intake studies should be compared with the acceptable or tolerable daily intake (ADI/TDI) recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The ADI refers to substances that can be ingested daily without substantial health risks, whereas TDI is used to emphasize the importance of limiting the daily intake of food contamination over a period of lifetime without appreciable health risk. Regulation for food contaminants with cumulative properties is distinct from ADI/TDI guidelines. In the case of several heavy metals, such as As, Pb, Cd, Hg, which are able to accumulate within the body, the tolerable intakes are expressed as provisional tolerable weekly intake (PTWI). The PTWI from the JECFA represents permissible weekly human exposure to contaminants that can cause adverse effects on health. Table 2 is focused on the characteristics of detrimental elements, commonly monitored in food.

Risk assessment

Food contains a wide range of chemicals, both essential because of fundamental importance to human health (such as mineral components and vitamins) and deleterious substances that can contribute to the appearance of pathological effects. Among the undesired compounds that are present in food, 2 types of contaminants are contained:

Table 1 — Factors influencing metal toxicity (Nordberg and others 1978).

Factors
• physical and chemical properties (among others chemical forms of element) • element interaction • formation of compounds or complexes among metal and other metalloids • interchange of metal bounds to proteins • sources and sinks, environmental transport, and transformation • influence of concentration and other exposure variables (for example: time, route, pattern of exposure, bioavailability) • nutritional status • taking drugs such as alcohol and nicotine

Table 2 — Properties and sources of heavy metals exposure (Oh and others 1981; Ribeyre and others 1995; Badiello and others 1996; Goyer 1997; Aydin 2001; Grosicki and Kowalski 2002; Gochfeld 2003; Satarug and others 2003; Eustace and others 2004; Raymond and Ralston 2004; Nordberg and others 2005; Miyazaki and others 2005; Duker and others 2005).

Element	Sources	Interacting elements	Chronic effects	PTWI[a] [μg/kg b.w. /week]
As	• volcanic action • mining • smelting of non ferrous metals • burning of fossil fuels • smoking • wood preserving, pesticide production, and application • coal/oil production and processing	Cd, Se, Zn	• dermal lesions • peripheral • neuropathy • skin cancer • peripheral vascular disease	15 (inorganic As)
Cd	• consumption of tobacco products (a cigarette box contains 2 to 4 μg Cd), • phosphate fertilizer sewage sludge • plated/galvanized equipment • enamels and glazes • industrial emissions	Zn, Cu, Ag, Hg, Se, Fe, As, Mg	• lung cancer • pulmonary adenocarcinomas • prostatic proliferative lesions • bone fracture • kidney dysfunction • hypertension	7
Hg	• coal-fired power station • chlor–alkali works • fossil fuel burning • power plants • manufacturing industries • crematories • various biocidal coatings • dental amalgams	Se, Ag	• Minamata disease • neurotoxic effects • renal, pulmonary, reproductive, and cardiovascular toxicity • gingivitis, insomnia, memory loss, anorexia, paresthesias	5 (total Hg)
Pb	• exhaust fumes from cars • industrial gases and liquid effluents • the production and disposal of storage batteries • some phosphate fertilizers and pesticides	Fe, Zn, Cu, Se, Ca	• plumbism • anemia • nephropathy • gastrointestinal colic • central nervous system symptoms	25

[a]PTWI = provisional tolerable weekly intake.

1. natural chemicals, unintentionally present in foodstuff
2. intentionally added, to modify food properties (for example, food additives and pesticides; Nasreddine and Parent-Massin 2002)

Considering that food is a particularly important source of the overall metals exposure, undertaking a risk assessment appears to be justified.

Risk assessment, as a part of risk analysis, is defined as the process of evaluating the possibility of adverse health effects that may occur as a consequence of exposure to a hazard. This scientifically based process consists of hazard identification, hazard characterization, exposure assessment, and risk characterization (Figure 1).

At the beginning of the risk assessment process, the sources and types of potential hazard should be considered and the ensuing appropriate monitoring ought to be established. For this purpose, various factors, such as:

- the threshold amount of toxic contaminants (PTWI) that results in no adverse health effects,
- the relationship between the exposure level and frequency of adverse health effects (dose–response assessment),
- the severity of the health effects, by considering multiple biological endpoints (for example, morbidity, fatalities),

which have an effect on the health threat, should be taken into consideration.

Finally, it is important to emphasize the qualitative description and quantitative evaluation of the intake of potential toxic agents via food.

Intake measurement

A quantitative evaluation of exposure can be realized using:

- direct assessment, including the following approaches: "point–of–contact measurement" (duplicate diet), and "reconstruction" (biomarkers),
- indirect assessment, including "scenario evaluation" (Kroes and others 2002).

Both of the above-mentioned approaches are mutually distinct. The indirect assessment is a modeling approach, whereas in the case of direct assessment, the measurement of exposure is done directly using personal monitoring. Each approach relies on different kinds of information and has different benefits and weaknesses. These distinctions have been summarized in Table 3.

Different approaches, both direct and indirect, for an estimation of the dietary intake of food contaminants recommended by the World Health Organization (WHO) are available (Figure 2).

Food contaminant monitoring and exposure estimation is a complex process and no single approach is suited to all circumstances. The final choice of method to estimate heavy metal intake depends on the intended goal and the availability of data, and other resources, such as cost, time frame, the concentration of the chemicals in the food, and so on. Moreover, it is also important to note how detailed and precise the information concerning toxic elements intake needs to be. The multiplicity of sources of data related to food consumption enables the selection of an appropriate method.

Direct method

The duplicate diet (DD) study is a particularly useful methodology (Thomas and others 1997; Leblanc and others 2000) because it provides the most accurate information on the ingested dose of food contaminants. The direct approach pertains to the analysis of all food and beverages prepared for consumption. Thus, it reflects the dietary habits of meal preparation of the surveyed population. It is especially important, due to the fact described previously, that toxic elements are sensitive to processing effects. Peeling, washing, cooking, frying, and other culinary activities can have a significant influence on the content of heavy metals in foodstuffs (Watzke 1998; Ersoy and others 2006). However, as mentioned previously, direct methods are burdened by difficulties connected with finding willing consumers who will conscientiously supply their pattern of food consumption. A number of papers suggest that the above-described human behavioral differences as well as socioeconomic factors could be potentially contributing agents arising to errors, in the most significant sense (Peterson 1995).

Indirect methods

In the case of indirect measurements, the analysis of individual food items from the so-called total diet study (TDS) is the most common approach. The total diet study, also known as a market basket study (MBS), is based on the estimation of the dietary

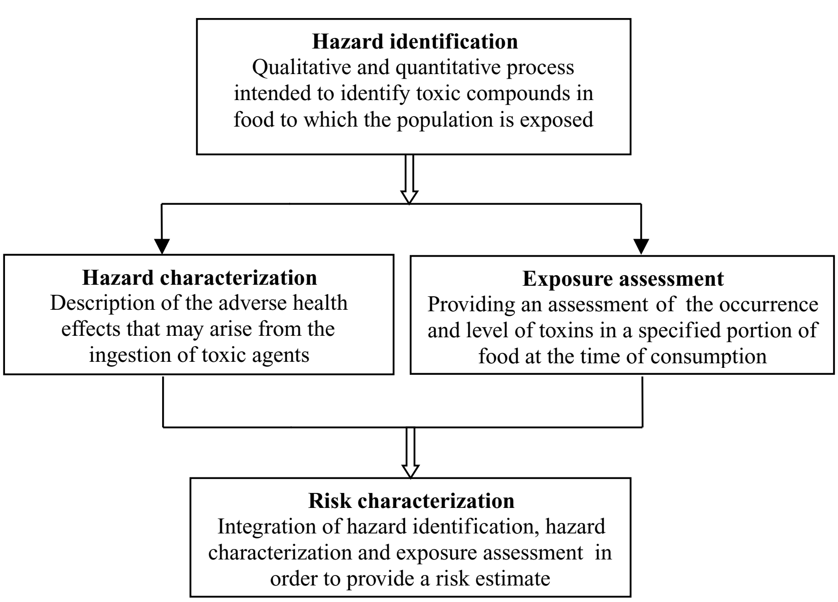

Figure 1 — Components of risk assessment.

intake of specific age-sex groups in the population. TDS surveys involve purchasing samples of food items representing products most commonly consumed in a defined population, preparing the foods in accordance with standard household procedures, combining the foods into food composites or aggregates, and analyzing each food group to measure the levels of selected food contaminants. The combination of the toxic concentrations obtained in the food products analyzed and the information on consumption permit the estimation of dietary exposure in population groups. The main asset of the total diet surveys is the capacity to monitor trends of contaminant exposure without burdening the participants of the study. As mentioned previously, the TDS approach covers only a limited number of foods (representative of diets) in a studied population. Therefore, the important foods for contaminants with an unusual distribution (such as mercury) may not be included within the foods tested. On the other hand, the levels of these compounds in the foods investigated may not be representative. Finally, the assessment of heavy metals intake may be either under- or overestimated. Furthermore, total diet study provides information that is understandable for use by government surveillance programs.

Both food supply data and household surveys provide only rough estimates of foods available. However, they cannot be used to determine the intakes of individuals. The main advantage of these approaches is no requirements for costly stages connected with burdensome foodstuffs sampling and their subsequent analysis. Consequently, it contributes to a lack of knowledge to the point of actual exposure levels.

Food supply studies (also known as food balance sheets [FBS]) have been developed because of the need for assessing the amount of food and nutrients available for human consumption. FBS data depict a comprehensive picture of the pattern of a country's food annually consumed by the population. Information on foods quantities is collected based on a calculation of the annual amount of foodstuffs produced in a country, changes in stocks and imports and exports transactions during a specified period. Such measurements are valuable due to the realizability of assessing the overall deficiencies or surpluses in the food supply in the country. However, food balance data do not indicate any differences between food consumption levels between different regions, different occupations, or at different income levels.

Such information can be obtained by household surveys (HS) that deliver detailed data on the distribution of food consumption among different population groups. For assessing food availability in a household, budget surveys and consumption studies can be used. Budget surveys provide information about meal expenditure, whereas in the consumption survey, the quantities of food and beverages brought into the household are recorded. In general, the application of the HS method enables the obtainment of data on food consumed in the household as well as away from home.

Among indirect approaches to assess exposure to deleterious substances, individual dietary surveys can be used. In accordance with the previously enumerated items, 4 basic methods may be applied for describing the diet of individual people.

Recall methods as well as food diaries are widely used in consumption studies for the sake of administration simplicity and low cost. However, using the recall approach requires highly trained interviewers. They are engaged in asking and probing the respondents to identify and quantify all food and drink consumed during the past 24 h. The procedures for getting 24-h recalls can vary greatly. In general, the interview is conducted personally or by telephone with the subjects, but in particular situations the respondents can be asked to complete the recall on paper or a computer without the assistance of an interviewer.

The food diaries approach is based on the description of types and amounts of all the food items and beverages consumed during a prescribed time period, usually 3 to 4 d (Pekkarinen 1970) or up to 10 d (Massey 1997). Moreover, the participants can be asked to measure and weigh each food item before consumption. In some cases, food consumption may be assessed on the basis of standard portion size. It is obvious that the former manner is more accurate but simultaneously more costly.

In the context of these surveys, indicating whether or not the day had consisted of typical eating habits is significantly important. The number of days needed for the providing of accurate dietary information depends on both day-to-day variation and seasonal variation in food consumption. To characterize usual metal intake, collection of data on dietary ingestion over several days in each season of the year is deemed advisable (Kroes and others 2002).

To assess usual food consumption over a relatively long time, both the food frequency method and dietary history can be used.

Table 3—Comparison of direct and indirect approaches for intake measurement (Duan and Mage 1997; Klepeis 1999; Pennington 2004).

Type of approach	General information	Advantage	Disadvantage
Direct	Incorporation of a large number of personal exposure monitoring and biological markers of exposure	• accurate method • simplicity	• costly and time consuming • probability that participants may not provide equal portions of foods, leading to underestimation of intake • does not allow for identification of the contribution of individual foods to total dietary exposure
Indirect	Prediction of personal exposure using exposure factor information, modeling, and questionnaires	• rapid and inexpensive method • useful for determining the sensitivity of the exposure level to quantifiable parameters	• need for systematic validation • necessity of comparison of results with an independent set of directly measured exposure levels • the data-intensive nature of the approach • vulnerable to systematic measurement errors

A food frequency questionnaire (FFQ) method is utilized as a tool in assessing the frequency of individual foods or food group's intake over extended periods of time (weeks, months, or years). The underlying principle of this approach is the possibility of a long-term evaluation of a diet, which may provide more valuable information for estimating average exposure to chemicals than short-term methods. An FFQ consists of a structured list of foods and a frequency of its consumption by respondents. Moreover, the questionnaire may include questions regarding the amount of food consumed, usual food preparation methods, use of dietary supplements, and so forth. In assessing exposure to deleterious substances, the FFQ enables the obtainment of useful information on the consumption of particular types of foodstuffs containing a high content of toxicants. Consequently, FFQ approaches are increasingly used to measure dietary intakes in epidemiologic studies of chronic disease (Tucker 2007).

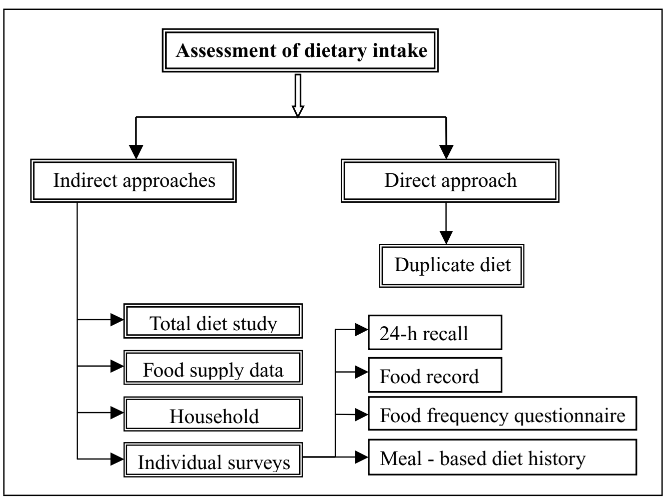

Figure 2—Dietary intake assessment methods.

Dietary history methods are designed to assess an ordinary individual's total food consumption and meal pattern. The main purpose of this approach is to obtain a picture of dietary consumption habits, which seems to be more related to slowly developing diseases than intake over a short time, which does not reflect habitual nutrition. However, Hoffman and others (2002) have reported that a repetition of short-term measurements (24-h recalls), performed on 2 nonconsecutive sampling days, can be used to describe the habitual dietary intake distribution in food consumption surveys.

The interviewer's task is to elicit detailed information on the types of foods and beverages commonly eaten over an extended time period, which is often a "typical week." Moreover, dietary survey questions may apply to recall the meals eaten during the previous 24 h. In some cases, participants are also asked to report a 3-d estimated record (Committee on Diet and Health 1989).

Distinct from FFQs, data of dietary history possess limitations in estimating dietary exposure of the population. Dwyer and others (1987) have demonstrated that respondents participating in a study may both fail to report foods actually eaten and recall foods that were never eaten. Consequently, information about kinds and amounts of food consumed can be inaccurate.

Each of the described methods used for dietary heavy metal uptake has some advantages and limitations (Table 4), some methods being better than others for a specific goal. The final choice of the approach depends to a high degree also on the population size study. Research comprising a relatively small number of participants may use resources of intensive methods (such as a duplicate diet) for measurement of metal intake. Employment of the mentioned approaches is useful also in the case of dietary assessment in collective nutrition in different sectors, that is, hospitals, old people's home, and so forth. Nevertheless, it should be remembered that in this field of surveys, dietary exposure to heavy metals is representative only for a specific population subgroup. For comprehensive national nutrition surveys, the efficiency of dietary methods in terms of time and cost was shown to affect their choice

Table 4—Advantages and disadvantages of individual food consumption approaches (Petersen and others 1994; Martin and others 2002; Shahar and others 2003; Forshee and Tech 2004; Rutishauser 2005; Tucker 2007).

Type of approach	Advantages	Disadvantages
Recall method	• provides detailed quantitative assessment of intake • the data can be used to estimate acute or chronic exposure • possibility of calculating average and distribution of exposure • estimates can be produced for specific population groups	• under reporting of food intake or "restricted" eating • difficulty in estimating portion sizes • in case of 24-h recall—depicting only a single day
Food record	• lack of limitations by a precoded food list • average of multiple days can provide information about diverse dietary practices	• inaccuracy for estimating an individual's usual relative or absolute intake • requires multiple contact with participants • likelihood of poor compliance
Food frequency questionnaire	• accuracy for estimating average exposure to chemicals • easy for subjects to complete • feasible for prospective studies involving tens of thousands of subjects • inexpensive • relatively low burden for participants	• specific • time consuming, • limitation of flexibility by the precoded food list • losing information on cooking method • semiquantitative • underestimate of intake
Dietary history	• can provide an estimate of habitual intake for individuals	• time consuming • susceptible to recall bias, and encourages psychological tendencies to report what is socially acceptable • labor-intensive

(Pennington 1998). Among the methods applied for large-scale studies, the most popular approach was the total diet study (Berry and Johnson 1997).

Dietary sources of heavy metals

Data from the literature on the dietary metal intake in various countries show great variation (Marzec and Bulinski 1990; Johansen and others 2000; Schrey and others 2000; Erzen and others 2002). It is obvious that food choice, like any complex human behavior, is influenced by many interrelated factors, including various physiological, social, and cultural factors. Nutritional customs can vary locally, regionally, and nationally.

Johansen and others (2004) evaluated the dietary intake of chemical contaminants in Greenland. It has been reported that inhabitants of Greenland are more exposed to contaminants from their diet than people in Europe and North America. It was also recognized that seal blubber, seal muscle, seal kidney, and whale blubber are the dominant contributors of contaminants in this diet. The study suggests that avoiding or limiting the consumption of specific diet items will contribute to a reduction of the contaminants' intake.

Data assembled by Peterson (1995) pointed that the major sources contributing to dietary cadmium intake, in almost all countries, are potatoes and cereals. In the case of Sweden, the consumption of hand-peeled shrimps with the viscera was related to high intakes of cadmium. The results of the study presented by Muñoz and others (2005) showed that fish and shellfish consumption has the greatest contribution in total cadmium intake.

Currently, the most common route of exposure to lead, in countries where leaded gasoline has been banned, is through food (CDC 1999), whereas the amounts of Pb in foodstuffs originating from plants are found to be higher than those from animals (Krížová and others 2004; Muñoz and others 2005). Unlike lead, the mercury content in food originates from animals—fish and shellfish are the main sources of this toxicant in human beings (Llobet and others 2003; Muñoz and others 2005). Available data on the content of arsenic in food indicate that, similar to Hg, fish and seafood tend to concentrate environmental arsenic (Robberecht and others 2002; Llobet and others 2003; Muñoz and others 2005). Nevertheless, the great majority of seafood derived arsenic compounds occur in less toxic organic forms.

In addition, the consumption distinctions between different consumer groups (for instance, between vegans and vegetarians; infants and adults, and so forth) have been observed. Data on the diversification of metal intake in different population groups have been reported in a number of countries (Stanek and others 1998; Wilhelm and others 2002; Llobet and others 2003). Data demonstrated by the Natl. Food Agency of Denmark (Levnedsmiddelstyrelsen 1990) showed that the daily intake of cadmium for men is higher than for women.

Analytical procedure in food analysis

Frequently, the heavy metals for which dietary exposure is of interest are present in trace and ultra-trace quantities. Hence, an analytical technique with sufficient sensitivity is required for the accurate determination of these chemicals in food samples. The major techniques employed for heavy metal analysis are flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), cold vapor atomic absorption spectrometry (CVAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) (Table 5). Besides those mentioned previously, many other techniques such as differential pulse cathode stripping voltamperometry (DPCSV) have also been shown as an excellent tools for the trace and ultra-trace analysis (Inam and Somer 2000; Szefer and Nriagu 2007). The final choice of the method often depends on several factors, including detection capability, desired speed, accuracy, and sensitivity of assay, simplicity of use, and cost of analysis. Thus, although ICP-MS offers the advantages over, for example, FAAS, of lower detection limits and possibility of simultaneous multi-element analysis, in case of determination only one or few elements, the less expensive method, that is, flame atomic absorption spectrometry, will be more suitable.

Neutron activation analysis (NAA) is a less frequently used technique in the determination of metals in food samples because of the necessity of accessing the reactor. Furthermore, NAA cannot be used to determine some elements, for example, Pb. Despite the mentioned negative features, neutron activation analysis possesses a number of specific advantages and possibilities. Among these are lack of necessity of chemical destruction (in the case of instrumental neutron activation analysis [INAA]), appropriate accuracy, possibility of simultaneous quantification of many elements both in very small samples (a few milligrams) and very large samples (up to several kilograms).

As distinct from INAA, in the spectroscopic methods, like in most determinative techniques for elements, food samples must be prepared for solubilization by an appropriate dissolution method.

Table 5—Studies examining the heavy metal contents of different food samples.

Analyte	Sample type	Country	Analytical technique	Literature
Pb, Cd	Duplicate diet	China, Japan	GFAAS, ICP-MS	(Zhang and others 1997)
Pb	Vinegar	California	GFAAS, ICP-MS	(Ndung'u and others 2004)
Cd	Foodstuffs and drinks	Canary Island, Spain	GFAAS	(Rubio and others 2006)
Cd	Duplicate diet	Germany	GFAAS	(Wilhelm and others 2002)
As	Duplicate diet	Belgium	GFAAS	(Robberecht and others 2002)
As, Cd, Hg, Pb	Total diet study	Spain	ICP-MS, HGAAS, CVAAS	(Llobet and others 2003)
As, Cd, Pb, Hg	Foodstuffs	Korea	ICP-AES, CVAAS	(Lee and others 2006)
Cd	Duplicate diet	Belgium	GFAAS	(Van Cauwenbergh and others 2002)
Among others: Cd, Hg, Pb, As, Se, Ca	Market basket study	The Netherlands	FAAS, CVAAS	(Van Dokkum and others 1989)
Fe, Mn, Zn, Cu, Pb, Cr, Ni, K, Ca, Mg	Cheese	Turkey	FAAS, GFAAS	(Mendil 2006)
As, Se, Hg	Duplicate diet	Germany	HGAAS, CVAAS	(Wilhelm and others 2003)
Cd, Pb, Hg, As	Total diet study	Santiago	HGAAS, GFAAS, CVAAS	Muñoz and others 2005)
27 elements including As, Cd, Pb	Foodstuff	Jamaica	NAA, AAS, FIA	(Howe and others 2005)

Both sampling and preparation are usually the most important steps of any analytical procedure (Namieśnik 2001, 2002). Errors committed at these stages influence the quality of the final results and cannot be corrected during further analysis. Directions for proper sample collection have been widely described in many studies (Horwitz 1990; Keith 1991; Markert 1995; Melcher and others 1996; Stoeppler 1997).

There is a plethora of sampling tools, which depend greatly on the samples to be analyzed and expected concentrations of analytes to be determined in food. Considering the character of the targeted compounds, the collection of food samples for heavy metal determination has to be carried out by applying carefully selected and cleaned vessels. A suitable choice of vessels is important to minimize the potential risks of contamination, especially in most cases, where the investigated analytes are present in very low concentration levels in food samples. The basic requirement is maintaining the representative character of the collected food sample to be analyzed. The following factors may affect the contents of the analytes in collected food sample:

- contamination derived from handling of foodstuffs,
- adsorption of metal ions on the vessels wall and instruments used for analysis,
- desorption of harmful chemicals to be analyzed from contaminated walls of containers as well as from the tools and devices used during laboratory analysis,
- contamination of the sample with compounds from reagents used during different steps of the analytical procedure.

To counteract chemical composition changes in samples, several basic principles should be followed (Boutron 1990):

- use of a clean laboratory, together with a very careful choice of labware, ultra-pure water, and reagents of high purity,
- all standard solutions should be acidified and storage in a refrigerator or freezer,
- prudent choice of laboratory materials used during the various stages of the analytical procedure,
- cleaning of all the containers and the labware using acid cleaning baths,
- storage of food samples for a short time in a refrigerator, and for longer periods in a freezer.

One of the most critical steps in the analytical process that can affect heavy metal content in food samples is sample preparation. In most cases, treatment stage includes several operations, such as drying (most often by freezing), homogenization, grinding, subsampling, digestion, and dissolution. Each of these stages may be a potential source of contamination; thus a great attention should be paid to prevent the original chemical constitution of the sample.

The sources of major errors are most often related to the materials from which the vessels and labware are made as well the purity of reagents used during sample treatment.

Buldini and others (2002) have reported the average content of some elements in the materials frequently used for storage vessels and other equipments. According to these data, laboratory tools made from isostatic molded polytetrafluoroethylene (PTFE) and ultra-pure quartz appear to be best suited for metal determinations. The least proper seems to be a pyrex glass, which, compared to PTFE and quartz, contains a considerably higher level of metals. For example, the content of As in ultra-pure quartz averages about 0.1 μg/kg whereas in pyrex glass ranges from 500 to 20000 μg As/kg.

Because of heterogeneous food samples collected for analysis, many operations have to be carried out to obtain a more representative subsample. Dependent on the type of samples, analytical portions of foods are drying either before the homogenization process or after this step. Removing water from the food sample allows the obtainment of a material that is easier comminuted by suitable equipment. Unfortunately the drying process can contribute to removal of more compounds than just water from of the fresh food sample and consequently alter the initial chemical composition of the sample. Homogenization apparatus may also be a source of contamination. The impact of comminution on changing the chemical composition of the original sample is often underestimated. The most known reasons are the abrasion of the surface of the instrument and application of unsuitable equipment's material (Cubadda and others 2001). Among others, plastic and equipment with titanium blades instead of stainless steel are the most appropriate materials for homogenization apparatus, which has a contact with the food sample.

Most commonly used techniques to quantitative analysis of heavy metal in food samples require the complete digestion of the material. It is evident that there is a range of methods that may be used to destroy the composite organic matrix. Generally, sample matrix decomposition can be carried out by the dry ashing or wet digestion procedures. Comparison of methods for mineralization of food samples is presented in Table 6.

Regardless of the type of mineralization, all manner of different decomposition methods can contribute to losses of some elements, for example, by volatilization.

The problem of volatilization of some elements of interest, often encountered during dry ashing (these principally concern arsenic and mercury [Hoenig and others 1998; Vassileva and others 2001]), may be avoided by addition of ashing aids. Typically a variety of ashing aids, for example, magnesium nitrate ($Mg(NO_3)_2$ and magnesium oxide (MgO), are used. Moreover, data reported in the literature show that the attainment of ashing temperature by a slow gradient allows avoiding losses of analytes. Another parameter, such as ashing vessels, has to be also taken into account. The most commonly used vessel materials for dry ashing are quartz, porcelain, and platinum, which resist all acids.

Decomposition of the sample strongly depends on the type of analytes to be determined. Hence, an analysis of particularly volatile elements force necessitates the use of procedures based on wet digestion methods. The oxidizing power of a wet digestion method is based on the use of a wide range of chemical reagents (acids and oxidants) of which the most commonly used are nitric, sulfuric, and perchloric acids, and hydrogen peroxide

Table 6—Comparison of the mineralization methods for food analysis (Jorhem 1995; de Paz and others 1997; Parmigiani and Midio 1997; Hoenig 2001; Capar and Szefer 2003).

Decomposition method	Simplicity	Laborious	Cost	Digestion time	Reagent volumes and handling	Temperature attained	Losses of elements on the mineralization vessel walls	Risk of volatilization losses	Sample amounts
Dry ashing	Yes	Low	Low	Long	Low	High	Frequently	Substantial	Large
Wet digestion	No	High	High	Relatively short	High	Low	Less frequently	Lower	Small

(Gorsuch 1970; Skurikhin 1993), and their combination. Moreover, the large number of different types of dissolution apparatus to mineralization using acid digestion causes this approach to be increasingly applied in sample treatment.

Conclusions

Among other routes, food is one of the main sources of consumer exposure to heavy metals. Since increased dietary metals intake may contribute to the development of various disorders, there is a necessity for monitoring of these substances in the human diet. To estimate the dietary intake of food contaminants, the different approaches can be utilized. The exposure estimation is a complex process and no single approach is suited to all circumstances. The final choice of method to estimate heavy metal intake depends, among other things, on the intended goal, the availability of data, the nature of the chemicals, and so forth. In addition, it is important to note how detailed and precise the information concerning toxic elements intake needs to be. Considering that heavy metals are present in low concentrations in food samples, sensitive analytical techniques are required to measure their concentrations with appropriate accuracy. In most inorganic laboratories, spectrophotometric techniques are usually employed for the quantification of metals. Nevertheless, the application of many other techniques have also been shown as an excellent tools for the trace and ultra-trace analysis. It is important to remember that sampling and preparation steps are the most critical parts of any analytical procedure because they are responsible for the largest source of errors. Therefore, a great attention should be paid to prevent the original chemical constitution of the sample by the suitable choice of, among others, vessels using ultra-pure water and reagents of high purity.

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