Food Chain Analysis for Lead and Mercury

Protocol Document

PURE EARTH

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Contents

Acknowledgements

This document was written by Alfonso Rodriguez and Emily Nash of Pure Earth.

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Disclaimer

This protocol is designed for use in specific projects and may not be universally applicable. It should be adapted or modified only with the guidance of relevant experts to ensure it meets the unique needs of each project. The creators of this protocol assume no responsibility for its misuse or for any outcomes resulting from its application beyond its intended scope.

1. Background Information

Diet has the potential to be a significant pathway of human exposure to lead and mercury. Therefore, quantification of the amount of these toxic metals entering the body through food is important to determine drivers of exposure and potential interventions.

As shown in Figure 1, lead may enter the food system through contaminated soil from industrial activities (including smelting, recycling, mining, or coal combustion), pesticides and fertilizers, paint chips, or residual contamination from leaded gasoline. Lead can be taken up by vegetable crops or plants. Lead in vegetation can be transferred to livestock, which has the potential to impact meat and dairy. The use of lead soldering for cans used to store food continues to be an issue in certain regions. In other cases, such as certain spices, lead may be deliberately added as an adulterant. Other potential sources of lead include aluminum, ceramic, and brass cookware and traditional cosmetics, such as eyeliners.

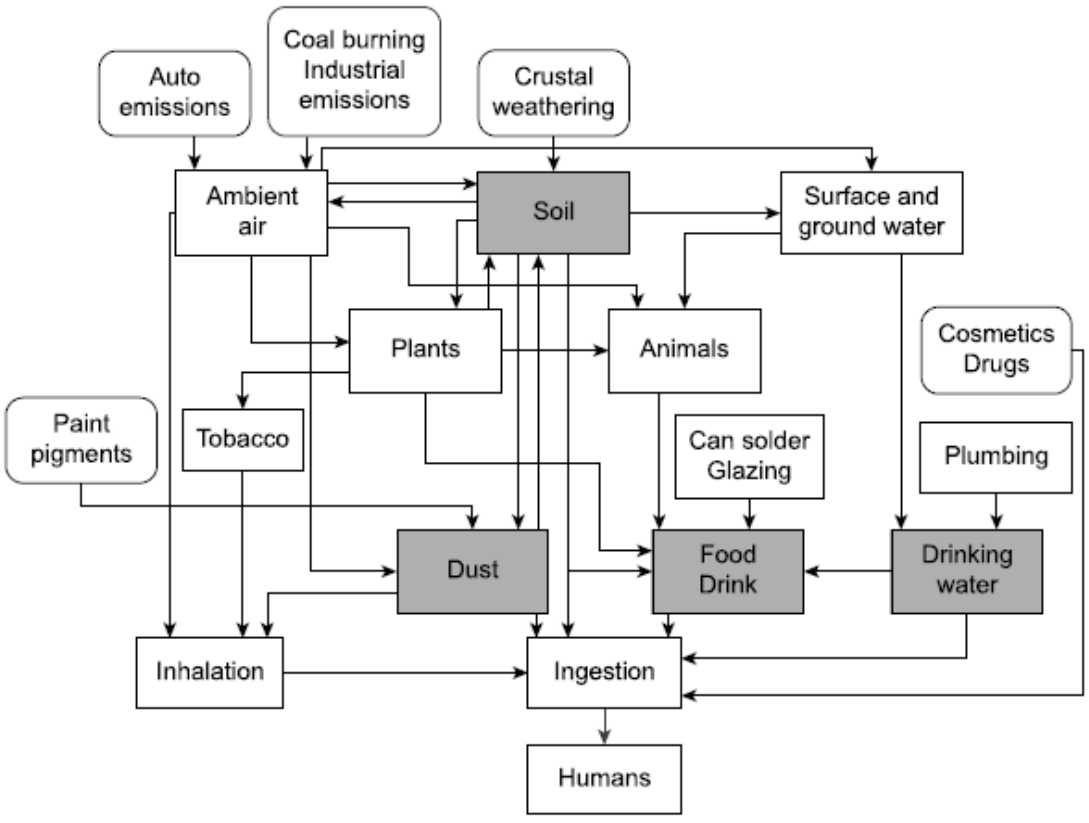

Figure 1. Sources and routes of lead exposure in general population (Source: Bergdahl & Skerfving, 2021)

Mercury enters the environment through both natural processes and industrial activities and can be transported in the atmosphere. Elemental mercury may be converted to an organic form, methylmercury. Methylmercury is highly toxic and is the form of greatest concern for human health through the dietary pathway. Methylmercury bioaccumulates in aquatic and marine organisms and has also been documented in terrestrial ecosystems. While seafood is often of most concern for methylmercury exposure, other food types have also been found to be associated with biomarkers of mercury exposure, including rice and vegetables (Wells et al., 2020).

Additionally, both lead and mercury can pass from a mother to child through the placenta and breast milk, which may be important components when considering exposure scenarios for children.

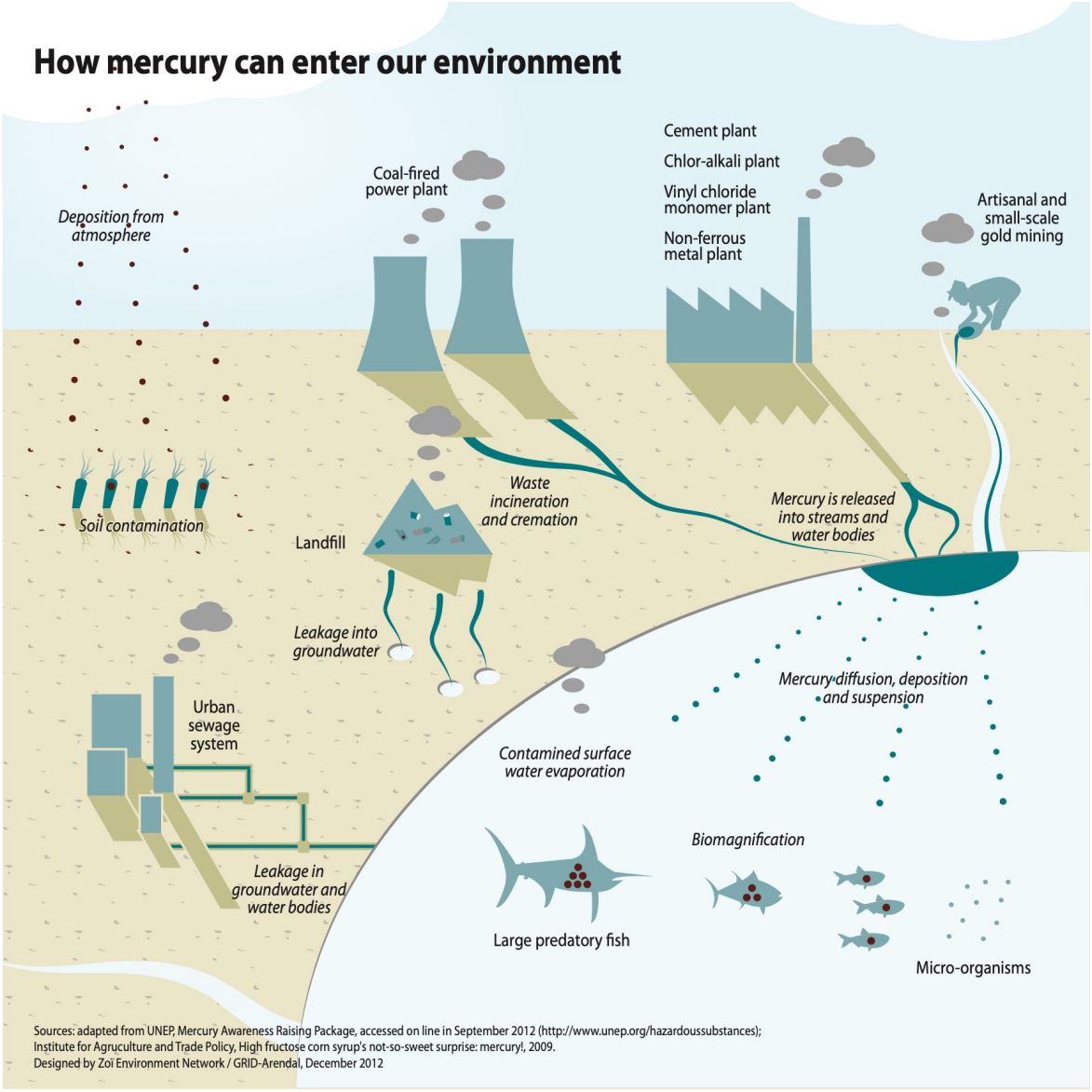

Figure 2 How mercury can enter our environment (Source: GRID-Arendal, 2012)

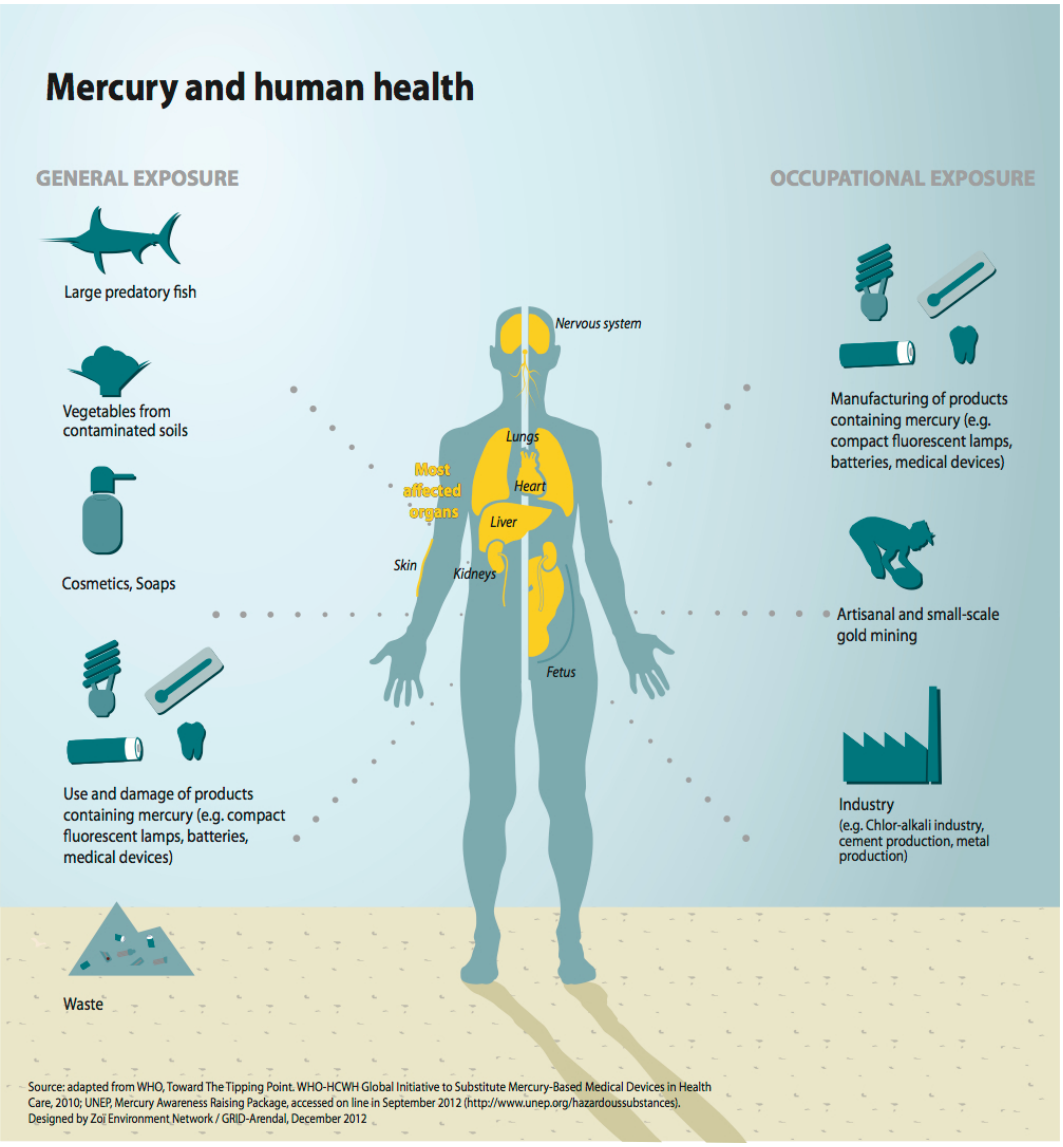

Figure 3 Mercury and human health (Source: GRID-Arendal, 2013)

NOTE: The techniques described in this protocol quantify total lead and total mercury. Additional analyses would be needed to determine concentrations of specific lead or mercury species. If budget and laboratory capacity allow, consider conducting these additional laboratory analyses. Metal speciation would provide more detailed information on bioavailability and how these metals enter food systems.

NOTE: Sampling individual ingredients or raw ingredients will not determine if additional lead is introduced into the diet from sources like aluminum, brass, or ceramic cookware. Additional studies (e.g., cookware leaching tests) would be required to assess the impact from these sources. For mercury analysis, the process of cooking food items such as fish may change the amount of bio-accessible mercury (Costa et al., 2022).

2. Design Considerations

The two main types of data required for assessing dietary intake of lead or mercury are:

1) food consumption data (i.e., the amount and frequency of a specific food item or category consumed by an individual), and
2) the concentration of lead or mercury in these food items.

The following are important considerations for the study design:

- The resources available for primary research (i.e., collecting metal concentration and food consumption data in the field), versus using existing data at the national or sub-national levels. Food consumption data may already exist from nutrition studies.
- Is the objective to estimate total daily lead or mercury consumption (in which case all major food groups should be sampled)? Or is the objective to identify whether specific food groups contain concentrations of concern?
- Will priority food groups be selected based on consumption levels from an existing or new dataset? Or will they be selected based on those previously found to contain toxic metals at concentrations of concern? Try to define categories consistent with available data.

The appropriate sample size depends upon study objectives, geographic scope, and budget. Sample size may also depend on whether data are available on for contamination levels to guide priorities. It is important to document the justification for the choice of study design.

NOTE: The study design must account for how samples are selected – from selecting regions, to towns, to markets, to individual food items within those markets.

Exploratory studies that aim to produce a broad picture of toxic metal contamination in food should be designed to generate a representative sample of the target geographic area. Another objective may be to compare the concentrations of toxic metals in food from higher risk areas (such as those with environmental contamination or documented adulteration), against areas without these risk factors. Human-related sources of mercury contamination in the environment include mining and gold extraction, industrial uses (such as chlor-alkali plants), and coal combustion (Driscoll et al., 2013). Hotspots of lead contamination may result from industrial activities such as lead smelting, lead-acid battery recycling and manufacturing, and mining.

Consider the following design options:

Multi-stage cluster sampling:

- The entire geographic area is divided into groups or clusters (such as administrative regions or provinces), and then a subset of those clusters is randomly selected.
- These areas can then be divided further into smaller units (municipalities), and even smaller units (households, markets), which are also randomly selected.
- All items within the final unit are then sampled.
- This design can allow for random sampling within a large geographic area and can save money and time by focusing by sampling in fewer areas. However, this approach can lack precision.
- Cluster sampling can be combined with stratified sampling. If there is a particular characteristic of interest (rural versus urban districts, for example), the clusters can be divided by this characteristic, and a certain number from each stratum can be randomly selected.
- Cluster sampling can also be designed to be proportional to the population, with areas of higher population more likely to be selected for sampling.

Judgmental sampling:

- If resources are limited, and the objective is not to achieve a representative sample, samples can be selected based on prior information.
- For example, this approach could be used to confirm the presence of an issue of food contamination in a particular region with known risk factors.

Some guides to support the size samples are:

- www.nao.org.uk/wp-content/uploads/2001/06/SamplingGuide.pdf
- https://www.ndi.org/sites/default/files/samplesizecalculation.pdf

3. Data Required

Two primary datasets are required to assess dietary intake of toxic metals:

- The amount of lead and/or mercury in a specific food item, and
- The amount and frequency of that specific food item that is consumed by a specified population group.

3.1. Dietary intake

Dietary intake values for major food categories should be included in the analysis. This information can either be obtained through surveys of study participants or from existing literature. Dietary intake data should be specified:

- By age
- By region, if possible

Also consider that among some populations, there may be significant changes in diet based on the season. If the study objectives include aligning dietary intake with blood lead levels, the data should match the same time period.

Country- or region-specific data must be used because dietary norms vary greatly across cultures and geographies. The UN Food and Agriculture Organization and World Health Organization (FAO/WHO), with Tufts University and the European Food Safety Authority (EFSA), administer the Global Dietary Database, which provides estimates of individual food and nutrient intakes worldwide by country, year, age across the lifespan, sex, education level, urban or rural residence, and pregnancy/nursing status. The data can be downloaded here: https://www.globaldietarydatabase.org/

As an example of existing dietary intake data at the national level, the U.S. Environmental Protection Agency (EPA) publishes an Exposure Factors Handbook, which provides the mean and 95th percentile amounts consumed (in g/kg-day) per capita by age group, for the following food groups:

- Fruits and vegetables
- Fish and shellfish
- Meats, dairy products and fats
- Grain products
- Human milk

This dietary intake data allows calculation of the total amount of lead or mercury consumed daily. This can be compared to a standard value such as the U.S. Food and Drug Administration (FDA) interim reference level (IRL) for lead of 2.2 µg/day for children and 8.8 µg/day for females of childbearing age (US Food & Drug Administration, 2023). Having the dietary intake data also allows the use of the U.S. EPA's All Ages Lead Model to estimate how dietary lead impacts blood lead levels.

For mercury, there are different reference levels depending on the species of mercury in the food item. As previously noted, the analyses described in this protocol will generate results for total mercury. If there are available funds and sufficient laboratory capacity, additional analyses can be performed to determine the concentrations of individual mercury species.

In the absence of these resources, assumptions can be made about the likely composition of mercury species based on the food category. The FAO/WHO has set a provisional tolerable weekly intake (PTWI) of methylmercury of 1.6 µg/kg bodyweight (Joint FAO/WHO Expert Committee on Food Additives, 2007). This standard should be applied for fish and shellfish. In other foods, the mercury is presumed to be present as

inorganic mercury (European Food Safety Authority, 2012). For foods other than fish and shellfish, a PTWI of 4 µg/kg bodyweight per week for inorganic mercury can be applied (Joint FAO/WHO Expert Committee on Food Additives, 2011).

AIR	WATER	DIETARY EXPOSURE	TOTAL MERCURY
Annual average: 1 µg/m^3 **Long-term elemental mercury vapour**: 0.2 µg/m^3	**Inorganic mercury**: 6 µg/L	**Methylmercury from fish in pregnant women and children under 17 years**: 1.6 µg/kg body weight per week	**Tolerable intake**: 2µg/kg body weight per day

µg, microgram; m^3, cubic metre; L, litre; kg, kilogram.

3.2. Toxic metal concentrations in food items

To assess how much lead or mercury is being consumed through diet, representative food items must be analyzed for the amount of these contaminants they contain.

Lead and mercury concentrations in food, and the accompanying regulatory limits, are typically too low for analysis by XRF. Therefore, toxic metal concentrations should be assessed in a laboratory (see Analytical Methods).

The desired unit for lead or mercury concentration in food items is ppb (equivalent to µg/kg) or ppm (equivalent to mg/kg or µg/g).

Specific information on each food items should be recorded (see Data Management). This should include the food type category (e.g., vegetable, fruit, dairy, poultry, etc.), the food item (e.g. tomato or specific fish species), where it was purchased (geographic coordinates, town, market, etc.). Samples of the selected food must be representative of what is being consumed. These samples are then submitted for laboratory testing (e.g., the interior but not the peel of a banana would be submitted), as detailed below. Depending on the focus and objectives of the study, additional details may be needed, such as the length and weight of whole fish and which part was sampled, and/or brand names.

4. Sample handling and processing

All sample handling, transport, storage and processing considerations should be made in coordination with the receiving laboratory.

Typically, the amount of food that will be used during the analysis (the analytical portion) will be very small (0.5 - 5 grams).

Below are general guidelines for transporting samples (US Food & Drug Administration, 2018):

- Ensure all food items are separately bagged and labeled with a unique identifier.
- Keep frozen foods frozen.
- Freeze all meats and seafood items.
- Refrigerate perishables (dairy, vegetables).

Only the edible portion of food should be sampled, so some items may need to be processed at the lab before analysis. Examples include:

- Remove peels, rinds, and end pieces of produce if they are not typically consumed
- Rinse produce with deionized water, drain and dry
- Remove bones and inedible components of meat, poultry and seafood

Because sample portions are very small, food items should be homogenized when possible. This ensures the small amount of material analyzed will be representative of the item as a whole. Homogenization can be done with household appliances, such as blenders or food processors, or more specialized laboratory equipment. However, ensure that the equipment used does not inadvertently contaminate the sample with lead, mercury or other toxic metals during the homogenization process. For more specific guidelines for certain food types, please refer to Section 2.2 of the US FDA Elemental Analysis Manual for Food and Related Products (US Food & Drug Administration, 2021).

NOTE: Concentrations of toxic metals in food may be expressed as being by “wet” or “fresh” weight, or by “dry” (dehydrated) weight. Confirm which metric the applicable regulations use. Ensure consistency when carrying out the analysis. There are conversion factors that can be used if one has to transform between wet and dry weight using the moisture content of the food as determined by the laboratory; if a conversion factor is used, be sure to document it and cite the data source for the conversion factor.

5. Analytical Methods

5.1. Laboratory – ICP-MS

Inductively coupled plasma-mass spectrometry (ICP-MS) is the preferred analytical method for toxic metals in food. The following method allows for the quantification of total acid-extractable concentration of lead, arsenic, cadmium, chromium, copper, lead, manganese, mercury, molybdenum, nickel, selenium, thallium and zinc, in food items.

As shown in Figure 2, the food sample first undergoes microwave assisted acid digestion, before being analyzed via ICP-MS. The following flow chart captures the procedure. Details on the ICP-MS and digestion protocol can be found in Section 4.7 of the US FDA Elemental Analysis Manual for Food and Related Products (US Food & Drug Administration, 2021):

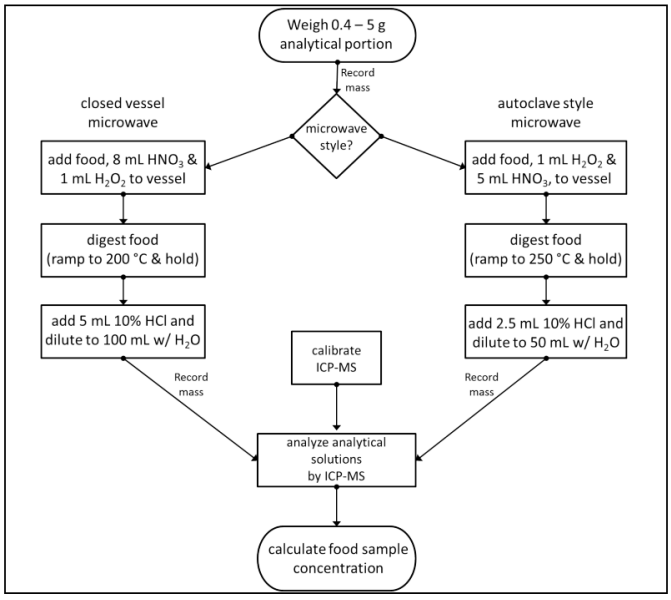

Figure 4. Procedure flow chart (US Food & Drug Administration, 2021).

5.2. Laboratory – GF AAS

When ICP-MS is not available or is prohibitively expensive, graphite furnace atomic absorption spectrometry (GF AAS) can be used for lead. As with ICP-MS, food samples require specific sample processing steps prior to analysis, which must be adapted based on the food type because of their complexity (for example, different

concentrations of organic compounds like proteins, fats, and sugars). Furthermore, different chemical modifiers may be needed during the analysis. Therefore, laboratory staff must be highly trained and knowledgeable in handling food samples. A European standardized method for determination of lead, cadmium, chromium and molybdenum in foodstuffs via GF AAS is available: EN 140836:2003 (EN 14083:2003).

5.3. Laboratory – CV AAS

Cold vapor atomic absorption spectrometry (CV AAS) may be a lower cost alternative to ICP-MS for mercury content analysis. As with the above-mentioned laboratory techniques, food samples must first be digested prior to CV AAS (Chirita et al., 2023). A European standardized method for determination of mercury in foodstuffs via CV AAS is available: EN 13806:2002.

5.4. Direct Mercury Analysis

An alternative to traditional laboratory analysis for mercury is direct mercury analysis (DMA). This process does not require sample preparation and can be faster, cheaper, and less labor intensive. DMA can be used for solid or liquid matrices. DMA quantifies total mercury content. Within the analyzer, the sample undergoes thermal decomposition (combustion); the mercury is concentrated using gold amalgamation, and quantified via atomic absorption spectrometry.

The device must be externally calibrated with certified reference materials (see Laboratory Quality Control) or aqueous standards.

Refer to US EPA Method 7473 for more details and important safety considerations: www.epa.gov/sites/default/files/2015-07/documents/epa-7473.pdf

6. Laboratory Quality Control

Ensure the laboratory is qualified to assess toxic metals in food specifically and has relevant experience. Ensure the laboratory will use the specified sample processing (homogenization, microwave digestion) and analytical methods (ICP-MS or appropriate alternative).

All analyses should be conducted with the highest purity materials, including ultra-pure water with a resistivity > 18 ohm meters and ultra-high purity grade reagents.

To ensure valid results, consider splitting a subset of samples for analysis by two labs (at least 10% of samples) at the beginning of the analysis. Confirm that the results align before analyzing the full set of samples.

Certified reference materials are available for different food matrices, which contain a known concentration of specific elements. This ensures that a laboratory is producing reliable results. Consider whether the project's budget and timeline allow for the provision of such reference materials.

A list of available CRMs for food matrices and their prices can be found here: https://www.metas.ch/dam/metas/de/data/dienstleistungen/referenzmaterialien-elemente-d.pdf.download.pdf/referenzmaterialien-elemente-d.pdf

7. Data Management

Consider an electronic data management platform such as ODK. Also retain a physical backup of datasheets.

The following variables should be included in the data collection format:

- Date of collection of sample
- Location – Country, District, Town
- Geographic coordinates
- Sampling location type – Market, Household, Other
- Sampling location details – such as:
    - Market name and type
    - If food sample is linked with a blood lead or home-based assessment, include participant ID
- Food type category – Vegetable, Fruit, Meat, Seafood, Dairy, etc.
- Food item – specific details of food item, including species where applicable
- Sample ID – unique identifier for each sample
- Sample photo

Information to be collected at laboratory:

- Sample weight (in grams) – this should be the wet or fresh weight unless otherwise specified by the regulations or project objectives
- Lead concentration in ppm (g/kg, µg/g) or ppb (µg/kg)

8. References

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