1.04 Sample Homogenization

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1.04.1 Requirements for Sample Homogenization in Environmental and Food Analysis

Environmental analytical data are of major importance and serve (1) to investigate how human activities influence the environment; (2) to develop, calibrate, and validate environmental models; (3) to assess whether there is a potential risk for the ecosystems; and (4) to comply with current directives.[1] On the other hand, food samples need to be controlled for pesticides and other contaminants to ensure human safety. Given the importance of analytical data, it is of utmost importance to provide accurate and trustful results and documents with relevant aspects of the sampling, sample preparation, and analytical protocol, following strict criteria of quality control and quality assurance in order to guarantee the quality of the analytical results, meanings that a given value lies around a true value within a known uncertainty.[2,3]

Trace analysis in environmental and food samples is complex, requiring skills and experience during sampling and analytical procedures. Considering that (1) any environmental/food entity is heterogeneous and that analytes are randomly distributed and/or have a large geographical or temporal variability; (2) analytical results depend on the type of sample and on the sample preparation techniques, which highly influence the precision and accuracy of the chemical measurement, and (3) subsampling of a heterogeneous material and loss or contamination during sample preparation leads to a high bias in the analytical measurement thus generating inaccurate data that do not represent the original sample, several aspects should be taken into consideration:

- Minimum analytical requirements imply the determination of organic contaminants at the ppt or sub-ppb level, and thus, the amount of sample handled should be such to detect trace concentrations in environmental and food samples
- Sampling and sample preparation procedures should 'smooth' the natural environmental variability
- Sample size should be carefully chosen (and reported) to minimize environmental lack of homogeneity (the larger the sample size, the lower the sample-to-sample variability)

- Sample preparation techniques (handling, filtering of water, sieving of soils/sediments, drying, homogenizing, etc.) should not influence the subsequent analytical result
- Subsamples should be representative and have the same chemical composition as the original sample, collected from any environmental or food entity
- Analysis of subsamples should be repetitive
- All steps regarding sample preparation should be described and validated to ensure that the results are of acceptable quality

In view of this, to obtain trace concentrations on a bulk sample and to make results from different subsamples equivalent and repetitive, homogenization of the bulk sample is mandatory. Homogenization is the process of reducing and mixing the original sample to enable the taking of representative and repetitive test proportion.[4] It comprises (1) a comminuting step, which refers to the grinding of solid samples into a powder consisting of small particles by applying mechanical forces that cause an increase in the particle surface area; and (2) homogenization as such, which is the random distribution of the substance to be measured within the sample. Homogenization is the step performed after 'sampling' and before 'sample preparation' (**Figure 1**).

Current sampling procedures include the collection of large sample sizes, which are necessary to achieve a high representation of the environment. Bulk samples have to be reduced to a small amount that is, analytically speaking, amenable. In many environmental and food chemistry laboratories, the quality of sampling and sample preparation, as opposed to the quality of chemical analysis, is often an overlooked step in the analytical process. This is because sample homogenization is a time-consuming and tedious step in the whole analytical process.[5] However, at this point it is necessary to indicate that the complex structure and composition of environmental and food matrices makes homogenization techniques compulsory prior to sample preparation and chromatographic analysis. Variable structure, texture, viscosity, and hygroscopic or hydrophobic nature of the environmental sample together with the complexity of chemical trace analysis of organic compounds contribute to the necessity of homogenizing the sample. However, the likelihood of error depends on the concentration range in which the substance to determine occurs in the sample to a larger extent than the physicochemical properties of the substance or sample.[6,7] Lichon[4] reports that the total error components, quantified as relative standard deviation (RSD), for an inhomogeneous matrix are

$$\mathrm{RSD}_{\mathrm{total}} = \mathrm{RSD}^2_{\mathrm{sampling}} + \mathrm{RSD}^2_{\mathrm{homogenization}} + \mathrm{RSD}^2_{\mathrm{sample\ preparation}} + \mathrm{RSD}^2_{\mathrm{determination}}$$

where sampling is the highest contributing factor, followed by homogenization, sample preparation, and finally analytical determination. Therefore, it is futile to attempt to reduce sample preparation and analytical errors, as the total error will be disproportionately dictated by the squared dominant factors (sampling and homogenization).[4]

Homogenization techniques and requirements imply (1) reduction of particle size, (2) mixing, and (3) verification. Reduction of particle size can be performed by cutting, shattering, and shearing. Mixing can be performed in very different ways and depends on the matrix. Contrarily to what might be expected, this step is complex due to some samples having shape and density differences, electrostatic and surface tension charging, and encapsulated structures that are difficult to disrupt. Therefore, mixing is crucial to obtain a fully homogeneous material. Within these steps, often two or more techniques are sequentially used to guarantee final homogeneity. The final step is verification, which can be performed in very different ways. Perhaps the most common technique is to run the analysis for the target compounds, but if this analysis is complex or costly it is prudent to run a simple and cheap analysis that confirms the homogeneity of the sample.

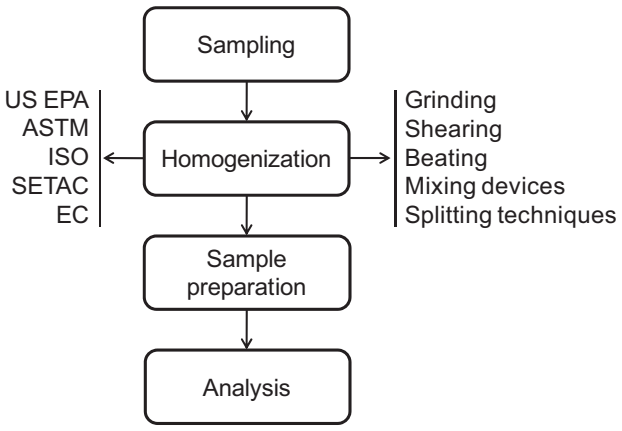

Figure 1 The analytical steps used in the analysis of contaminants in environmental and food samples. Homogenization techniques are indicated as well as the regulatory agencies that contemplate homogenization in the analytical protocol. US EPA, United States Environmental Protection Agency; ASTM, American Society for Testing Materials; ISO, International Organization for Standardization; SETAC, the Society of Environmental Toxicology and Chemistry – Europe; EC, Environment Canada.

1.04.2 Homogenization Theory

In mathematics and physics, homogenization is the study of partial differential equations with rapidly oscillating coefficients, which govern the physics of inhomogeneous or heterogeneous materials. Although all matter is inherently inhomogeneous, at some scale it can be treated as homogeneous. A good example is the continuum concept, which is used in continuum mechanics (fluids, solids, etc., all with their material properties). Differential equations govern the physics of the homogenization theory. However, this chapter is intended to give an overview of homogenization techniques and uses and is not directed to provide specific knowledge on the theory of homogenization. However, it is worthwhile indicating a few references regarding homogenization theory and its application in general in the field of new materials.

Chechkin et al.[8] describes several aspects of methods of homogenization theory, and discuss modern subjects and techniques developed in the last decade. Special attention is paid to averaging of random parabolic equations with lower order terms, to homogenization of singular structures and measures, and to problems with rapidly alternating boundary conditions. Cioranescu and Donato[9] provided a rigorous introduction to the theory of homogenization, with special emphasis on describing the behavior of composite materials. Typically these composite materials, while appearing homogeneous on a macroscopic scale, contain two or more constituents on a microscopic level. This study provides means based on homogenization techniques to derive the macroscopic properties of composite materials based on their microscopic configurations. Kozlov et al.[10] report asymptotic homogenization issues but also the more general homogenization of linear operators in the theory of partial differential equations. Oleinik et al.[11] deal with homogenization problems in elasticity as well as some mathematical problems related to composite and perforated elastic materials. Application of homogenization theory to environmental and food samples is seldom reported.

1.04.3 Standardized Methods for Sample Homogenization

In recent years, standardized methods in sampling procedures have been gradually implemented in many laboratories. Protocols include those recommended by the United States Environmental Protection Agency (US EPA), the American Society for Testing Materials (ASTM), the International Organization for Standardization (ISO), the Society of Environmental Toxicology and Chemistry – Europe (SETAC), and Environment Canada (EC). The Web pages of these agencies are given in the Relevant Websites section.

The US EPA is an agency of the federal government of the United States in charge of protecting human health and the environment, by writing and enforcing regulations based on laws passed by Congress. The US EPA describes specific procedures for sediment homogenization (EPA/600/R-94/025 and EPA/620/R-95/008), and for the homogenization of both soil samples (EPA/600/X-90/043) and waste samples (EPA530-D-02-002). EPA/600/R-94/025 (US EPA, 1994) reports that although sediment samples tend to settle during shipment, water above the sediment should not be discarded, but should be mixed back into the sediment during homogenization. To remove predatory organisms, large debris, or organisms taxonomically similar to the test species, this guide recommends press sieving sediment samples through a 1- or 2-mm stainless steel mesh screen. However, it may be not necessary to press-sieve sediments if previous experience has demonstrated the absence of potential interferences, including predatory or competitive organisms or large debris, or if large debris or predators can be removed with forceps or other suitable tools. If sediments must be sieved, it may be desirable to perform select analyses (e.g., pore-water metals or dissolved organic carbon, total organic carbon, acid-volatile sulfide) on samples before and after sieving to document the influence of sieving on sediment chemistry. This guide also recommends mixing sediments collected from multiple field samples by using stirring or a rolling mill, a feed mixer, or other suitable apparatus. It is preferable to homogenize sediments by gentle hand mixing. Nevertheless, a large number of sediments may demand the use of a mechanical aid, despite being potentially disruptive. In this case, homogenization can be accomplished using a modified 30-cm bench-top drill press or a variable-speed handheld drill outfitted with a stainless steel auger. These procedures could also be used to mix test sediment with control sediment in dilution experiments.

EPA/620/R-95/008[12] describes the homogenization procedures used to determine the silt-clay content, water content, grain-size distribution, and total organic carbon concentration of sediments collected for EMAP (Environmental Monitoring and Assessment Program). In the determination of both the silt-clay content and the sediment grain-size distribution, homogenization is accomplished by stirring the sediment with a spatula with a small amount of deionized water for at least 3 min. When determining both the percentage of water content and the total organic carbon, homogenization is accomplished by stirring sediments with a small metal spatula for at least 3 min. In the case of the water content, it is important not to add water to the beaker during the homogenization process.

EPA/600/X-90/043[13] presents both advantages and disadvantages of several methods used to obtain homogeneous soil samples. It is recommended to homogenize bulk soil samples in a closed recipient in order to reduce the loss of fines from dusting. However, it is also concluded that random sampling after grinding and sieving is the most efficient homogenization method, although these techniques have greater replicate variabilities than the other homogenization techniques.

EPA530-D-02-002[14] recommends carrying out homogenization of waste samples prior to analysis, since the benefits of homogenization may be temporary because gravity-induced segregation can occur during shipment, storage, and handling of samples. The preferred homogenization techniques used in the laboratory are riffling, fractional shoveling, and mechanical mixing (which can be also used in the field), cone and quartering, magnetic stirrers (e.g., to homogenize the contents of an open beaker), and V-blenders. Some of these techniques for homogenization, such as riffling and fractional shoveling, can also be used for

splitting and subsampling. The use of sheet mixing (also called mixing square) and vibratory spatulas is not recommended because these homogenization techniques tend to segregate particles of different density and size.

The US EPA has offices in the different regions of United States. Each EPA Regional Office coordinates tribal programs within their respective regions. The US EPA's Region 4, Science and Ecosystem Support Division (SESD), published a document describing general and specific procedures, methods, and considerations to be used and observed when collecting soil samples for field screening or laboratory analysis. This guide, SESDPROC-300-R1,[15] describes the most common method for homogenizing soil samples, which is the quartering procedure.

ASTM is a globally recognized leader in the development and delivery of international voluntary consensus standards. Today some 12 000 ASTM standards are used around the world to improve product quality, enhance safety, facilitate market access and trade, and build consumer confidence. ASTM members deliver the test methods, specifications, guides, and practices that support industries and governments worldwide.

ASTM describes specific protocols for the homogenization of both waste samples (ASTM D6323 – 98(2003) and ASTM D6051 – 96(2006)) and soil samples (ASTM E1726 – 01(2009)). Summaries of these documents are available online, but a fee must be paid for the whole document.

ASTM D6323 – 98(2003)[16] covers common techniques for laboratory subsampling of media related to waste management activities. This guide includes several sample homogenization techniques, including mixing and grinding, as well as information on how to obtain a specimen or split laboratory samples. The limitations and advantages of sample preparation options are also presented in detail.

ASTM D6051 – 96(2006)[17] discusses the advantages and appropriate use of composite sampling, field procedures, and techniques to mix the composite sample and procedures to collect an unbiased and precise subsample(s) from a larger sample. It discusses the advantages and limitations of using composite samples in designing sampling plans for characterization of wastes (mainly solid) and potentially contaminated media. This guide assumes that an appropriate sampling device is selected to collect an unbiased sample.

ASTM E1726 – 01(2009)[18] covers drying, homogenization, and acid digestion of soil samples and associated quality control samples using a hot-plate type method for the determination of lead utilizing laboratory atomic spectrometry analysis techniques such as inductively coupled plasma atomic emission spectrometry (ICP-AES), flame atomic absorption spectrometry (FAAS), and graphite furnace atomic absorption spectrometry (GFAAS).

The ISO/IEC Directives[19] define the basic procedures to be followed in the development of International Standards and other publications. The so-called ISO/IEC 17025, General requirements for the competence of calibration and testing of laboratories, includes (1) sampling, (2) method validation, and (3) traceability and measurement uncertainty. Within the sampling procedures, the ISO protocols indicate that laboratories are responsible for subsampling and homogenizing the sample to ensure that a representative test portion is used for analysis. The ISO procedures do not indicate the homogenization techniques to be used for each type of sample, but require that each laboratory should have documented procedures for subsampling and/or homogenization. Specific consideration for multiphase and label samples, aseptic handling, cross-contamination, and other issues to reduce the known errors associated with sample heterogeneity should be considered.

EC is the department of the Government of Canada with responsibility for coordinating environmental policies and programs as well as preserving and enhancing the natural environment and renewable resources. Since 1990, 21 standardized toxicity test methods and six supporting guidance documents have been published and distributed to a national and international audience. Among others, EPS1/RM/29[20] describes methods recommended by EC for the selection of sampling stations within a study site and the collection, handling, storage, transportation, and manipulation of samples of whole sediments from marine, estuarine, and freshwater environments, for the purposes of physicochemical characterization and/or biological assessment using whole sediments, pore waters, or sediment elutriates. This guide reports that mixing by hand or mechanical mixing may be used to achieve homogeneity of color, texture, and moisture. However, the efficacy of the method must be demonstrated, a priori, and the mixing time standardized and minimized to ensure consistency and to minimize changes to the size distribution of sediment particles, respectively. It is also said that mixing of sediments can take place in the sample/storage container, or the sample can be transferred to a clean mixing container. The recommended techniques for partitioning the sediment for distribution among test containers are coning or caking and quartering. Thus, if a sediment splitter is used, its efficacy must be demonstrated and documented and it must be made of a proper inert material.

Finally, SETAC is a nonprofit worldwide professional society directed to support the development of principles and practices for protection, enhancement, and management of sustainable environmental quality and ecosystem integrity. One of the scopes of SETAC comprises environmental aspects of quantity and quality of sediments, both as deposits and as suspended matter in freshwater, estuarine, and marine environments to conduct effective environmental risk assessment and management of sediment, including issues such as transport, fate, exposure, effect, impact analysis, guideline values and frameworks (regulations), and management strategies. SETAC provides a guide, which includes the handling of sediments from sampling to analysis, mainly in the field of ecotoxicological studies.

1.04.4 Validation Procedures

As in any other analytical step, validation is needed to guarantee the performance of any analytical step. In this case, validation refers to the quality control activities aimed to guarantee that a sample is homogeneous so that subsamples used in the batches are

identical, meaning that they belong statistically to the same population. In practice, each laboratory performs the validation step according to its experience and possibilities. Some laboratories adopt the method of preparation laboratory homogenization duplicate sample. This sample is used to estimate the homogenization error of the overall measurement uncertainty, and can be used as an independent check on the intrabatch precision. In other cases, the laboratory bulk sample is split and is analyzed in duplicate within one batch. The standard deviation and uncertainty are calculated in order to define whether the subsamples are homogeneous.

1.04.5 Homogenization Techniques

In the pre-World War II era samples were prepared by chopping and dicing, and it was not until 1940 that real homogenizers were implemented in the laboratories' praxis. Nowadays a large variety of devices are used for size reduction and homogenization of environmental, food, or other types of samples, no matter its origin. There is not a perfect system but each one has some limitations: (1) some of them generate heat that may alter or decompose the sample; (2) others may produce loss of volatile compounds[21,22]; (3) some can only accommodate small amounts of bulk sample; (4) depending on the construction material of the homogenizer, they may contaminate the sample.[23]

In this section the most common homogenization techniques used in environmental and food chemical analysis are described and discussed, in terms of use, efficiency, and application. Mechanical/physical methods for disrupting samples include grinding, shearing, beating, and shocking (Figure 2).

Prior to describing each method, some common aspects need to be discussed. When selecting the homogenization technique for environmental or food analysis, it is recommended to consider the final objective and desired characteristics of the final homogenate. Some general considerations to keep in mind are:

- *Material of the homogenization device*. Vessels in contact with the sample have to meet the standards of purity for trace analysis studies. Depending on the type of analytes and expected concentrations, this is an important factor to be considered to avoid sample contamination. Glass homogenizers used for shearing are the best option to eliminate any contamination source, but many lipophylic compounds might be adsorbed on the walls, producing a net loss of the contaminant load. Polytetrafluoroethylene (PTFE or Teflon) homogenizers have been widely accepted for their roughness and easy use, but might be a source of contamination if perfluorinated compounds have to be analyzed. On the other hand, plastic homogenizers under sterile conditions are used for homogenizing biological samples but can be a source of phthalates to the sample, which may interfere with chemical analysis. Finally, stainless steel homogenizers are widely used for their compatibility with the analysis of organic compounds, although they cannot be used when analyzing metals.

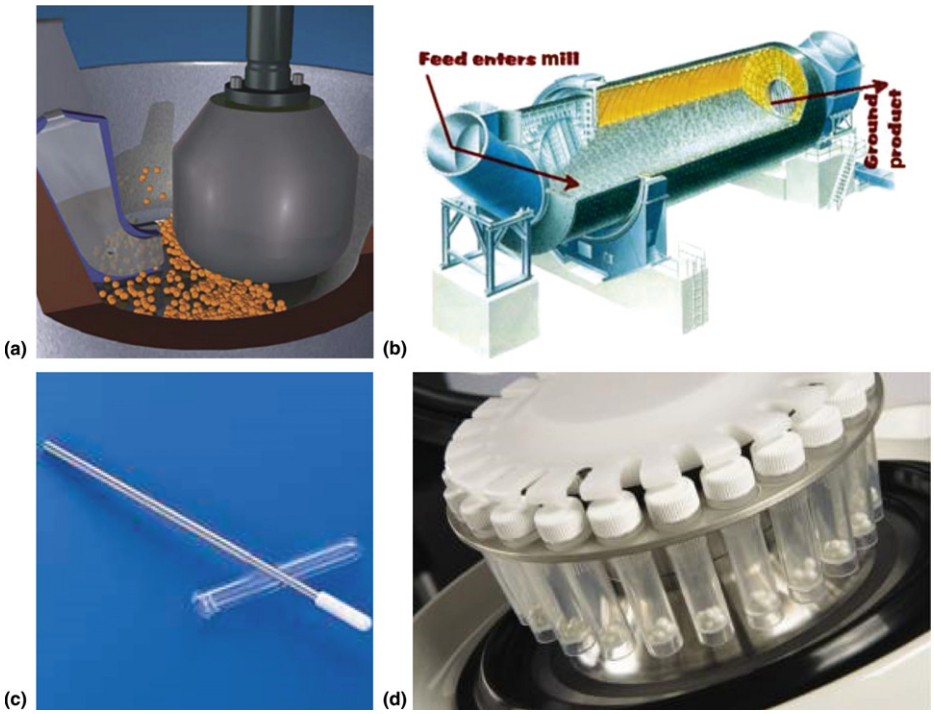

Figure 2 Different devices used for sample homogenization: (a) a mortar grinder; (b) a planetary ball mill; (c) a Potter–Elvehjem homogenizer; and (d) a bead beating.

- *Cleaning requirements*. As indicated earlier, target analytes in environmental and food matrices must be detected at the low ng/l or μg/l range. This means high cleaning requirements to avoid any external source of contamination or contamination between samples. Therefore, the homogenizers should be easy to clean and the cleansing technique should be effective enough to avoid memory carryover. In general, cleaning is performed with water, detergents, acids in some cases, and a set of solvents. Thus, the material in contact with the sample should be acid, solvent, and detergent resistant. Finally, independent of the homogenization technique, laboratory conditions (atmosphere, working areas, and hoods if necessary) should be such as to avoid any external source of contamination that can occur during sample homogenization, reduction, and transfer of the sample to the final container.
- *Quantification of organic compounds*. In environmental analytical chemistry, homogenization should be performed so that it enables the quantification of target analytes within a given sample. This means that the homogenization technique should liberate the analytes from the matrix, e.g., adsorbed to adipose tissue or to organic or colloidal matter in the case of soils and sediments.
- *Physicochemical properties of the environmental sample*. Particle size/hardness and texture of the material can affect the homogenization procedure and the obtained results.
- *Fineness of the homogenized material*. Different size homogenates may be obtained using different techniques, and their suitability depends on the final objectives of each study, e.g., quantification of target compounds to be measured, rupture of the matrix, and application of specific extraction procedure. Generally speaking there are 3 types of comminution: (1) coarse grinding down to a particle size of about 5 mm, (2) fine grinding down to 63 μm, and (3) extra-fine grinding down to a particle size <63 μm.[24]
- *Capacity*. The type of sample to be processed has a great influence on the quantity of sample that has to be gathered to obtain optimum representativeness. This means that the homogenizer should hold this collected amount. Considering that in many instances the extraction of 1 g of sample (e.g., sediments, sludge) is sufficient to detect target analytes, analysts have to calculate the amount sampled and homogenized so that this 1 g of sample is representative of the bulk sample. Furthermore, the total homogenate should be enough to duplicate or triplicate analysis and for storage. Therefore, homogenizers should be selected according to the volume to be processed, which can vary from a few grams (e.g., fish tissue, eggs, mothers' milk) to kilograms (sediments and soils, whole organs, tissues, food, etc.).
- *Throughput*. Haste of analysis is one of the main objectives in modern environmental laboratories, so as to reduce personnel costs and increase productivity. With the need of high-throughput screening strategies, sample homogenization often becomes a bottleneck. Many routine homogenization techniques rely in slow methods, like the so-called mortar and pestle and sieving, which are laborious and time consuming. To circumvent the logjam, various approaches have been taken to homogenize samples en masse. Due to the availability of different homogenization devices, it is now possible to enhance sample preparation by selecting the appropriate homogenization technique so that this step does not become the bottleneck in chemical analysis.

The classification introduced by Burden[25] is followed here to describe the different homogenization techniques. The main advantages and disadvantages of each technique are indicated in **Table 1**.

1.04.5.1 Manual Homogenization Techniques

1.04.5.1.1 Stirring

A very austere homogenization technique is to transfer the sample to a clean glass or stainless steel bowl and thoroughly homogenize it by stirring with stainless steel spatulas or spoons until textural and color homogeneity are achieved. All gross unrepresentative material (stones, wood chips, plant materials) should be removed. However, this method is far from achieving good homogeneous material, and particle size inhomogeneity may lead to poor reproducibility of replicate analysis.

1.04.5.1.2 Tumbling

The simplest homogenization method is tumbling the sample on a sheet of paper, cloth, or plastic. This method is effective on sample sizes less than 2 kg. However, large differences in size appear, and it is difficult to select a portion of the sample that has total homogeneity. Thus, this procedure is not a good approach and is rarely used in real life.

1.04.5.2 Grinding

Use of mortar and pestle is the best known tool for grinding, but more refined laboratory and automated apparatus systems have emerged. Grinding relies on creating friction by sandwiching the sample between two hard surfaces that slide against each other. Grinding causes tearing and ripping of samples to very fine particles, much like shearing, but differs in that there is direct contact between the sample and the homogenizer. Reduction efficiency depends on the topology of the grinding surfaces. Grinding can be used on wet, dry, and frozen samples. Wet grinding can be used with water, buffer, or solvent, and is generally used for tissues. Dry grinding is popular in the analysis of seeds, plant materials, sediments, and soils. However, depending on the material, friction can occur. Friction generates heat and, therefore, the heat tolerance of the analyte or sample should be taken into consideration when choosing grinding as a homogenization method. To overcome this problem, cryogenic grinding is used for biota samples or tissues, and both the homogenizer and the samples are frozen or deep-frozen with liquid nitrogen. Thus samples become brittle and fracture easily, preserving analytes that are heat labile.

Table 1 Types of homogenizing techniques, and their advantages and limitations

	Strengths	*Limitations*
Grinding		
Mortar and pestle	Use for many different types of materials Easy to use Inexpensive Generates very small particles	Low throughput Generates dust Glass and porcelain can chip High cleaning requirements
Cryogenic grinding with mortar and pestle	Mainly applied to biological samples Generates small particles Inexpensive Available in glass, ceramic, or metal	Small samples can be lost Low sample throughput Pestle and mortar need cleaning and prefreezing between samples
Mixer mills	Useful for hard materials Possibility to cooling with liquid nitrogen Versatile (material, balls and containers) High throughput	Cost
Mortar grinders	Fine ($<$100 μm) comminution Adapted for soft, hard and brittle samples Various materials available Easy to clean and maintain	Cost
Vibratory disk mills	Quick and reproducible grinding of medium-hard, hard, brittle, and fibrous samples Various materials available	Cost Used primarily for minerals and cements
Planetary ball mills	Feed material: soft, hard, brittle, fibrous, dry, wet Grinding to nano range with high efficiency High throughput Several configuration/settings	Cost
CryoGrinder and cryogrinder for small sample grinding	Size reduction, mixing, homogenization, cell disruption Cryogenic, ambient temperature, dry or wet grinding Programmable, high throughput	Cost Sample size must be small ($<$20 ml; $<$100 mg for small sample grinding)
Tissue disruption with glass homogenizers	Inexpensive Easy to use, clean, and decontaminate Generates very fine homogenate	Certain tissues (fibrous and membranous) difficult to disaggregate Low throughput Glass prone to breakage
Shearing		
Waring blender	Easy to use, decontaminate, and sterilize Commercially available Quick procession of large samples Made of stainless steel	Risk of foaming Generates coarse homogenization Not always suitable for efficient extractions
Ultraturrax	Applied for homogenization of soft materials (fruits and vegetables, tissues) Easy to use and maintain Effective	Low sample throughput Limited to small samples
Rotor-stator	Effective at homogenizing a wide array of samples Wide range of volumes Commercially available Generates homogeneous samples	Expensive Shafts are difficult to clean Poor homogenization of fibrous samples Generates heat that denature proteins
Dounce homogenizer	Inexpensive Very effective for mildly lysing cells Easy to use, clean, and decontaminate	Solid tissue not effectively homogenized Prehomogenization of tissues Low sample throughput Fragile and breakable devices
Potter–Elvehjem with PTFE pestle	Inexpensive, except for the motor Easy to use and clean Samples are kept cold during processing Effective for disrupting cells	Not very efficient with solid tissues Poor muscle sample homogenization Multiple steps needed for complete homogenization
French press or French pressure cell	Effective and efficient Very uniform homogenates	Small sample size Low sample throughput Prehomogenization needed Expensive relative to number of samples Clogging
Sealed-system homogenizers	Universal disposable disperser system used for infectious, toxic, or odorous materials Produces size reduction of droplets Safe, no risk of cross-contamination	Small sample volumes ($<$15 ml)

(*Continued*)

Table 1 Types of homogenizing techniques, and their advantages and limitations—cont'd

	Strengths	*Limitations*
Dispersing elements	Used to homogenize tissues No cross-contamination	Ideally, not reusable
Beating		
Bead beating	Used to disrupt cells Simple and effective	Low sample throughput Beads have to be removed and cleaned
Vortex bead beating	Easily available, inexpensive Adapted for all size tubes	Less effective at sample disruption May heat the sample due to friction

Abbreviation: PTFE, polytetrafluoroethylene.

Grinding offers high throughput and versatility, and can be used for a wide array of sample types and sizes. Depending on the used grinding technique and the final size of the material, different types are to be found on the market. The following classification considers the final size of the sample after grinding.

1.04.5.2.1 Coarse Grinding

Jaw breakers. Jaw breakers produce coarse grinding (down to a particle size of 5 mm). In plant homogenization or large tissues, often it is necessary to pregrind the material because the pieces are too large. Jaw breakers are used for materials of medium and extreme hardness and for brittle material or material that is both hard and tough.

Cutting mills. This method of coarse grinding is used for more soft and fibrous and cellulose-containing plant or biological samples.

1.04.5.2.2 Fine Grinding

Mortar and pestle. The classic homogenization with a mortar and a pestle is widely used for small samples in low quantities. The system is very simple. The samples are placed in a mortar, and ground in circular movements and tapping using a pestle. For environmental samples, it is a classic approach to homogenize soils and sediments before sieving biological matter (tissues) or plant materials. Although it has good performance, it requires long grinding times and thorough cleaning between samples, which limits the sample throughput, so alternative tools such as mixer mills are more practical.

Cryogenic grinding with mortar and pestle. Cryogenic grinding with mortar and pestle is a widely used method when samples or analytes are heat labile. The mortar and pestle are precleaned and placed in a Styrofoam tube or cooler chilled with liquid nitrogen. The sample, prefrozen or not, is placed in the mortar and liquid nitrogen is then poured until the set is cooled. Then the sample is ground by slowly pressing the pestle on it with twisting and using circular motion until the sample is shattered into small pieces. Once the grinding is completed, residual sample must be tapped or scraped from the pestle. The sample must then be transferred into a receiving vessel using a prechilled spatula, and if the sample has to be stored frozen, the container or recipient needs to be prechilled.

Mixer mills. Mixer mills are used for size reduction and pulverization of hard, medium-hard, and brittle samples as well as for soft, elastic, or fibrous ones. The sample is placed in the grinding jar of the mill with grinding balls, and is programmed to perform oscillations at a selected frequency and time. The inertia of the grinding balls causes them to impact with high energy on the sample material at the rounded ends of the grinding jar and pulverize it. The grinding jar may be continually cooled with liquid nitrogen from an integrated cooling system before, during, and after the grinding process, to avoid evaporation of volatile components and to evacuate heat produced by the grinding process. It is used for temperature-sensitive materials. Processing for 1–2 min usually homogenizes most samples. To increase sample throughput, several companies, including Retsch, SPEX, and Treomner, introduced a set of vials, a rack of tubes, or even microwell plates locked into a moving platform. These devices, in the simplest form, shake tubes or microwell plates at speeds up to 1600 rpm with one or more grinding balls.

The main areas of application for mixer mills are:

- Agriculture: cereals, grain, oil seeds, soils, straw, tobacco, wood
- Biology: bones, hair, plant and animal tissues, cell disruption, DNA/RNA extraction
- Environmental research: compost, electronic scrap, sewage sludge
- Food: animal feed, cheese, fruit
- Medicine and pharmaceuticals: drugs, tablets
- Mineralogy and metallurgy: alloys, coal, minerals
- Textile and wool, forensic sciences, ceramics and glass, chemicals, plastics

The grinding result is greatly influenced by the grinding tools. The jar and balls should always be made of the same material. This material should be chemically inert toward the reaction mixture. For most applications ceramic materials (e.g., zirconium oxide) are the best choice. They are chemically inert and the material abrasion is comparably low. Some materials possess a high porosity (agate, steel), which could lead to memory effects.

The choice of jar volume, ball charge, and material depends on the type and amount of sample. In order not to falsify the subsequent analytical determination, a neutral material should be selected. In addition, homogenization efficiency, measured as pulverization energy, is determined by the density and weight of the ball material. The higher the ball weight and density, the higher is the pulverization energy. Depending on the material and amount of sample to be ground, the grinding jar capacity, the size of the grinding balls (1–20 mm), the grinding time (1–10 min), and the frequency applied (25–30 Hz) can be optimized. For materials as variable as linings, cardboard, catalysts, hair, wood chips, or silica sand, the final fineness can be of 50–250 μm.

Mortar grinders. Mortar grinders and disk mills are used primarily for fine and very fine comminution of soft, hard, and brittle materials. A final fineness of approximately 100 μm can be achieved with disk mills and <10 μm with mortar grinders. Mortar grinders comminute, mix, and triturate by pressure and friction. The function of the scraper is to feed the material into the area between the mortar and pestle. This forced feed ensures that the whole of the sample is continuously subjected to the grinding and triturating process and is also intensively mixed. The pestle is not located in the center of the mortar but is offset; contact with the rotating mortar and the sample causes it to rotate automatically. The necessary grinding pressure is achieved by the weight of the pestle itself combined with the adjustable spring pressure acting on its axis. Mixer grinders are available in different materials, and are characterized by providing a high degree of homogenization with high reproducibility for dry and wet grinding and high final fineness. They are easy to clean, robust, and of low maintenance.

Vibratory disk mills. Vibratory disk mills are suitable for the extremely rapid and reproducible grinding of medium-hard, hard, brittle, and fibrous materials to analytical fineness. Inside the grinding jar, the grinding tools (usually a puck and a ring) are moved in such a way that the sample is crushed by impact and friction effects. The required reproducible analytical fineness can be achieved after very short grinding times. Grinding sets are typically made from hardened steel, tungsten carbide, agate, zirconium oxide, and steel. Typical materials are cement, cement clinker, ceramics, coal, coke, concrete, corundum, glass, metal oxides, minerals, ores, plant materials, silicates, slag, and soils. These homogenates are primarily used for spectral analysis. X-ray fluorescent analysis requires that the size of the particles to be examined lies within the saturation depth of the X-rays in order to obtain a representative analysis result. Particle size between 50 and 100 μm is usually required. However, to some extent the fineness achieved will depend on the material and how easy it is to comminute since some materials, such as plastic, are very difficult to obtain below 200 μm.

1.04.5.2.3 Extra-Fine Grinding

Planetary ball mills. A planetary ball mill consists of at least one grinding jar, which is arranged eccentrically on a so-called sun wheel. The direction of movement of the sun wheel is opposite to that of the grinding jars according to a fixed ratio. The grinding balls in the grinding jars are subjected to superimposed rotational movements. The jars are moved around their own axis and, in the opposite direction, around the axis of the sun wheel at uniform speed and uniform rotation ratios. The result is that the superimposition of the centrifugal forces changes constantly (Coriolis motion). The grinding balls describe a semicircular movement, separate from the inside wall, and collide with the opposite surface at high impact energy. The difference in speeds produces an interaction between frictional and impact forces, which releases high dynamic energies. The interplay between these forces produces the high and very effective degree of size reduction of the planetary ball mill. Planetary ball mills are smaller than common ball mills, and are mainly used in laboratories for grinding sample material down to very small sizes.

Vibration mill. Twin- and three-tube vibrating mills are driven by an unbalanced drive. The entire filling of the grinding cylinders, which comprises the grinding media and the feed material, constantly receives impulses from the circular vibrations in the body of the mill. The grinding action itself is produced by the rotation of the grinding media in the opposite direction to the driving rotation and by continuous head-on collisions of the grinding media. The residence time of the material contained in the grinding cylinders is determined by the quantity of the flowing material. The residence time can also be influenced by using damming devices. The sample passes through the grinding cylinders in a helical curve and slides down from the inflow to the outflow. The high degree of fineness achieved is the result of this long grinding procedure. Continuous feeding is carried out by vibrating feeders, rotary valves, or conveyor screws. The product is subsequently conveyed either pneumatically or mechanically. They are basically used to homogenize food and feed.

CryoGrinder. As small samples (100 mg or <20 ml) are difficult to recover from a standard mortar and pestle, the CryoGrinder serves as an alternative. The CryoGrinder is a miniature mortar shaped as a small well and a tightly fitting pestle. The CryoGrinder is prechilled, then samples are added to the well and ground by a handheld cordless screwdriver. The homogenization and collection of the sample is highly efficient. In environmental analysis, this system is used when very small samples are available, such as small organisms or organs (brains, hepatopancreas, etc.).

1.04.5.3 Shearing

Shearing homogenization is created by a tangential force being applied to the sample. There are several tools that disrupt by shearing, including blenders, rotor-stators, and some glass homogenizers, such as the Dounce homogenizer, the Potter–Elvehjem homogenizer, and the French press, all named after their inventors.

Waring blender. One of the early innovations applied to sample homogenization was the Waring blender, which made its appearance during early World War II. This simple device was instrumental for protein purification and analyte isolation. Samples are placed in the blender with extraction buffer and then blended. The blades shear and cut tissues, reducing tissues in size significantly. Blenders are used when large quantities of tissue have to be homogenized.

Ultraturrax homogenizer. Ultraturrax is a top-driven food blender used for the production of emulsions and suspensions in continuous operations. During the full continuous process the components to be mixed are fed into the machine at an appropriate rate through various inlet connections. These components are then thoroughly mixed, dispersed, or homogenized within the machine and discharged from the machine through the outlet. Thus, all particles or droplets are treated, producing a narrow particle or droplet size distribution with minimal concentration/quality variations.

Rotor-stator. The rotor-stator, or handheld homogenizer, is one of the most widely used tools for homogenizing plant and animal tissues. Rotor-stators are designed with an outer stainless steel stationary tube (stator) and an inner turning shaft (rotor) connected to a motor. At the bottom of the rotor-stator there are slots on both the tube and shaft. When running at 10 000–30 000 rpm, samples are pressed into the slots and are efficiently sheared, producing a very uniform homogenate in relatively little time. Rotor-stators come in many different widths and bottom slot configurations, and are able to homogenize from 1 to 2000 ml of sample. Like other homogenization techniques, the rotor-stators can generate heat. Thus, some of the most advanced models are equipped with temperature probes that shut down the units if the temperature rise is extreme. They are used for the production of viscous cream and emulsions in the cosmetic (cream, lotion, wax, mascara, gel), pharmaceutical (ointment, dental composite, syrup), food (mayonnaise, dressing, jam, butter, margarine, wasabi), and chemical industries (polyester, synthetic fiber, formulations).

Dounce homogenizer. This homogenizer uses a liquid-based homogenization technique for cell disruption, for small volumes and cultured cells. Cells are lysed by forcing the cell or tissue suspension through a narrow space, thereby shearing the cell membranes. The Dounce homogenizer consists of a round glass pestle that is manually driven into a glass tube. Once the sample is placed in the tube, the pestle is inserted and pressed in an up-and-down motion, which causes the sample to be sheared repeatedly. The shearing force can be controlled by using different pestles with different diameters. The larger the diameter of the pestle, the tighter is the fitting and the greater the shear created, while the opposite is true for the smaller pestle. The Dounce homogenizer is most effective at lysing tissue culture cells and small pieces of tissue in order to generate lysates with intact subcellular particles.

Potter–Elvehjem homogenizer. This device is used to disrupt tissues. Similar to the Dounce homogenizer, a cylindrical glass or hard polymer pestle (generally PTFE) rotates in a close-fitting tube, and a suspension of the tissue particles is subjected to shearing forces as the pestle moves up and down and presses the suspension through the space between the rotating pestle and the tube. It is used for soft tissues such as brain and liver. Both Dounce and Potter–Elvehjem homogenizers can be obtained in a variety of sizes to accommodate a range of volumes.

French press. A French press consists of a piston that is used to apply high pressure to a sample volume of 40–250 ml, forcing it through a tiny hole in the press. Only two passes are required for efficient lysis, due to the high pressures used with this process. The equipment is expensive, but the French press is often the chosen method for breaking bacterial cells mechanically.

Sealed-system homogenizers. This homogenizer is a universal disposable disperser system with hermetically sealable disposable sample tubes used for infectious sample materials, toxic substances, or high-odor substances in human medicine, pathology, veterinary medicine, foodstuffs testing laboratories, toxicology, chemistry, and many other fields. Sealed-system homogenizers are more effective at reducing the size of droplets in a pre-emulsion than they are at creating an emulsion from two separate liquid phases. To resolve this issue, a coarse emulsion is usually produced using a high-shear rotor-stator mixer, which is then fed directly into a high-pressure valve homogenizer. The homogenizer has a piston pump that pulls the coarse emulsion into a chamber on its backstroke and then forces it through a narrow valve at the end of the chamber on its forward stroke. As the coarse emulsion passes through the valve, it undergoes intense disruptive forces that result in the breakdown or size reduction of larger drops into smaller ones. It has the highest level of user safety, with no risk of cross-contamination. It is hygienic and clean, and is suitable for individual use and use in series. In general, it is used for volumes of 2–15 ml.

Dispersing elements. Plastic dispersing elements are ideal for those applications where absolutely no cross-contamination is permitted. The element is disposable and is designed for one-way use. However, it can be reused several times in applications where this is permitted. If deciding to reuse the element, the cleaning instructions have to be followed carefully. It is used basically for homogenizing tissue samples.

1.04.5.4 Beating

Beating is in many ways similar to grinding, but with an impact factor to smash the sample. As before, there are several systems available on the market.

Bead beating. The most widely used method relying on smashing the sample is bead beating, which consists in placing the sample and beads in a tube and rapidly shaking them back and forth. Bead beating has been used for years for the disruption of microorganisms, originally using small glass beads and dental amalgamators (i.e., little shakers that dentists use to mix up the components of metal fillings). Bead beating is simple and effective, although it can process a limited number of samples. Bead beating requires the careful addition and subsequent removal and cleaning of the beads, which can be time consuming and expensive.

Vortex bead beating. Though not their intended use, vortexes are routinely used to disrupt samples. This method relies on the addition of grinding beads to the tube and then repeatedly vortexing the sample. Typically used for the lysis of microorganisms, vortexes have been used to disrupt larger tissues by using large grinding beads (>2 mm) made of zirconium and stainless steel. Homogenizing samples by vortexing generates significant amounts of heat due to the friction created by the grinding balls. Many protocols call for bursts on the vortex interspersed with cooling on ice. Several vortex models are available, which hold multiple microfuge tubes that pulse.

1.04.5.5 Mixing Devices

Homogenization also may be achieved through the use of mechanical mixing devices, including a spiral mixer, a cement mixer, and a twin-shell V-blender.

Spiral and cement mixer. The spiral mixer involves the rotation of the bottled sample in both horizontal and vertical planes. The cement mixer or similar devices involves the rotation of the sample in a chamber with a series of internal baffles that cause the materials to be thoroughly tumbled and mixed. These two methods are useful for samples ranging from 100 g to kilograms.

Twin-shell V-blender. The twin-shell V-blender involves the rotation of two cylinders about a horizontal axis such that the apex describes a circle in the vertical plane. Twin-shell blenders are available commercially in sizes ranging from 4 to 16 quarts internal capacity.

1.04.5.6 Splitting Techniques

Within homogenization the sample is comminuted to small particles, only a part of which will be used for analysis. There are further techniques used to obtain this portion of the sample.

Random subsampling. Once the material is homogenized and sieved to a desired particle size (generally <2 mm), a portion is collected by random sampling for further analysis. This method assumes that the initial material is ground without preference to any given factor, such as color, and that during grinding and sieving the sample becomes sufficiently homogenized.

Spooning. To further eliminate possible heterogeneity within the homogenized sample and to collect the sample size to the desired quantity for a given analysis, subsamples may be obtained by spooning,[26] which involves the random insertion of a spoon or another sampling device into the previously ground and sieved sample. This method is preferably performed rapidly and without extensive visual examination of the sample that could lead to a processor preference in certain cases, e.g., light catching the shiny surfaces of mica flakes leading to preferential inclusion or exclusion of that part of the sample. The spooning type of subsampling markedly reduces the time of sample processing in comparison with multiple and successive splitting operations.

Scooping. Another method used to collect a portion of sample is scooping. A scoop of ground and sieved materials is divided among four containers. The process is repeated continually, changing the filling order of the containers until the sample has been quartered or an appropriate sample size has been obtained.

Cone and quarter technique. Perhaps the best-known sample-splitting method is the classical cone and quarter technique. This technique involves pouring the sample into a cone, flattening the cone, dividing the flattened cone into four equal divisions (quartering), and then removing two opposite quarters. The remaining two quarters are re-plied into a cone and the process is repeated until the desired sample size is obtained. Variations on the process are possible that can enhance the speed of sample size reduction by using just one quarter (chosen at random) to continue the splitting process. Several sources of error for this method have been identified. There is a danger of unequal segregation of heavier materials during the flattening and coning of the sample. Dusting is also a possible source of error during cone formation. Sample loss from the inability to recollect all the material, the ability of the sample to aggregate via static charges, and sample embedment are all further sources of error.

Riffle splitter. The riffle splitter is the most common mechanical method for sample homogenization and/or sample size reduction. The riffle splitter also provides one of the best general methods of sample mixing to obtain bulk sample homogeneity. The riffle splitter is a device having an equal number of narrow sloping chutes, with alternate chutes discharging the sample in opposite directions into two collection bins. Sample homogenization is achieved by repeated pouring of the material through the splitter and combining the halves between passes. The use of the riffle splitter as a subsampling device is done in a similar manner, with the exception that after the sample is passed through the splitter, one collection pan is replaced with a clan pan. The material in the replaced pan, which contains about one-half of the original sample, is then passed through the riffle splitter again, thereby reducing the volume in the clean pan to one-quarter of its original sample volume. This process of sample reduction is repeated until the desired weight or sample size is obtained. The use of riffle splitters and their variations are valuable in splitting samples that range from less than 10 g to several kilograms. The major source of error involved in using a riffle splitter is the loss of fine particle sizes by dusting when processing air-dried or oven-dried samples, which contain fine particle sizes.

Other sample-splitting devices. Several other sample-splitting/homogenizing devices found in the literature include a rotary splitter, a brass disk microsplitter, and a multiple-cone splitter. The rotary splitter involves the pouring of the sample into a feeding hopper located above a rotating disk containing sample bottles or pans. The disk is mechanically rotated and the sample is divided among the collecting bottles or pans. The brass disk microsplitter was designed by Brewer and Barrow[27] for subsampling small particulate samples, which range up to 1 g in the particle size range between 10 and 200 μm. The multiple-cone sample splitter consists of a series of powder funnels and inverted brass cones mounted alternately in a vertical column over a tray containing small sector-shaped pans, shortening the time necessary to reduce samples to grain-counting size by about 75%.

1.04.6 Application to Food Matrices

Meaningful residue data can only be obtained if careful sampling and sampling preparation has been performed. In the case of food samples, the sampling procedure can be responsible for further invalid analytical results. Therefore, the sampling plan should be well studied in relation to the type of contaminant or parameter to be measured and to the matrix to be studied. The sample should be representative of a whole population, and should be accommodated to the level of contaminants that have to be determined.

For such reasons, special care should be taken on sample collection, to avoid nonrepresentative or spoilt samples. The correct procedure is to collect random grab samples of a whole population, mix the grab samples, and reduce the sample size to a final laboratory-size proportion. However, the nature of the matrix will influence the sampling plan. The *Manual of Pesticide Residue Analysis*[28] reviews the sampling procedures and analysis of pesticides in different types of food. It is established that for homogeneous products, 0.5–1 kg is enough for analysis. For products consisting of small units, sampling should be performed depending on the weight of each unit, e.g., 50 units (1 kg) for items less than 25 g, 30 units (1–3 kg) for items of 25–100 g, 15 units (2–5 kg) for items from 100 to 250 g, and at least 10 units for items bigger than 250 g. However, these values are only approximate, because sampling will depend on the sample characteristics.

In whatever case, samples should be transported immediately after collection to the laboratory, under adequate conditions (normally refrigerated) in order to avoid degradation of the pesticides or other contaminants or spoilage of the sample matrix.

Upon receipt, samples should be processed immediately, or otherwise stored at 4 °C or preferably at −20 °C. Before analysis, it is a common practice to check the food sample and notice the appearance, aroma, possible deposits, etc. All the physical characteristics should be described, which will aid in interpretation of the final result. Moreover, at this stage impurities such as stones, insects, or rotten parts should be removed. The sample should by no means be washed before processing. Samples should be weighed afterwards and the number of units should be recorded. In some cases nonedible parts, e.g., stems, peel, leaves, and roots, should be removed and the concentration of residues then established for the weight of the edible portion. In other cases, it is important to analyze the residue levels of contaminants in the outer sample surface. Of course, the proportion of the analyzed sample will depend on the aims of each study.

Freeze-drying is a common method for preparing food samples prior to their homogenization. Freeze-drying destroys cell membranes, and thereafter the contact between the cell tissue and the extraction solvent is increased. On other occasions, food samples might be homogenized as wet weight.

At this stage the gross laboratory sample should be firstly composed in such a way that a representative analytical size sample is obtained from it. This step will depend on the type of sample: whenever possible, material should be wet or dry homogenized, ground, and mixed; products consisting of small units should be quartered and two opposite quarters should be homogenized. For large samples units (cheese, melons, etc.) that cannot be homogenized entirely, aliquots should be gathered and finally homogenized. In any case, from the gross laboratory sample several analytical size samples will be gathered, from which one or two will be analyzed and the rest will be kept as a reserve. These samples should be kept at −20 °C and wrapped in aluminum foil or glassware to prevent further decomposition or contamination.

Once the sample has been homogenized and mixed, the contaminants should be extracted. Food samples are complex to analyze, due to their versatility and differences in the matrix composition (presence of fats, sugars, etc.).

Several techniques are used for homogenizing food samples, depending basically on their hardness and texture. For pulpy foods such as strawberries, tomatoes, peppers, etc., the sample is extracted and homogenization continues in a single step, usually using an ultraturrax.[29,30] Another study uses a similar technique to extract pesticides in lettuce samples.[31] Pressurized solvent extraction followed by gas chromatography coupled with mass spectrometry (GC-MS) was also proposed to determine emerging and priority organic pollutants in leafy vegetables. In this case, 500 g of sample were comminuted with liquid nitrogen and 0.5 g were transferred to a porcelain mortar, then Florisil, sodium sulfate, NaCl, and Hydromatrix were added and the mixture blended to obtain a thoroughly homogeneous mixture.[32]

In the case of hard material such as seeds, nuts, and cereals, the material is ground and processed with the ball mill without solvent. The key factor in dry grinding is that the samples tend to be very hard and as such require a disproportionately large grinding ball. For instance, a grain of rice is only about 20 mg, and easily fits in a well of a deep-well plate, but the 5/32-inch grinding balls used in that format have insufficient mass to crack the rice. To smash the rice requires a 4-ml polycarbonate vial with a 3/8-inch grinding ball.

For meats and fish, the material can be homogenized as dry or wet tissue. Furusawa reports the analysis of sulfamethazine in pork tissue by homogenizing the tissue with an acid solution using an ultrasonic homogenizer, followed by centrifugation and high-performance liquid chromatography (HPLC)–photodiode array detection, without the need to use solvents.[33]

1.04.7 Application to Environmental Matrices

In view of the importance of environmental analytical data, it is essential that the quality of the sampling strategy, sample preparation, and analysis are assured, so that the data generated can be guaranteed. Similar to food analysis, there are unavoidable errors associated with the sampling and sample pretreatment procedures. The concentration of organic contaminants can be falsified if samples are not collected in adequate recipients (e.g., volatile or semivolatile organic compounds), if cleaning is not strictly performed, if storage conditions are not taken into consideration, and if samples are not properly homogenized and split.

For the determination of organic contaminants in environmental matrices, the sampling procedure, sample handling between sampling and analysis (storage conditions, pretreatment, homogenization, subsampling), and analytical methodology and validation should be properly described. In this section, relevant environmental compartments such as water (river, seawater, groundwater, wastewater), sediments, soils, sludge, air, vegetation, and biota are discussed with regard to sample preparation and homogenization. The methods used for several environmental matrices are summarized in **Table 2**.

Table 2 Homogenization techniques used for the analysis of contaminants in different environmental samples, and their extraction and analysis techniques

Matrix	*Compound*	*Homogenization*	*Sample preparation*	*Analysis*	*Reference*
Contaminated marine sediment interstitial water	PCBs	Hand mixing (stainless steel spatula)	Sonication with 50:50 acetone/hexane	GC	Burgess and McKinney, 1997[60]
Sediments	Fenvalerate, cypermethrin, 1,2,4-trichlorobenzene, tributyltin oxide, triphenyltin oxide, and di-*n*-butylphthalate	Freeze-drying and sieving, hand mixing	Not indicated	Not indicated	Clark et al., 1987[46]
Sediments	Toxic compounds	Freeze-drying and sieving with mechanical mixer (jar-rolling apparatus)	Not applied	*Rhepoxynius abronius* bioassay	Ditsworth et al., 1990[45]
River and estuarine sediments	Polybrominated diphenyl ethers	Sieving through 250-, 100-, and 50-μm sieves	Soxhlet extraction in hexane/CH_2Cl_2 (1:1)	GC-NCI-MS	Lacorte et al., 2003[56]
Sediment samples	Organic pollutants	Grinding (mortar & pestle)	Sonication	GC-EI-MS	Lacorte et al., 2006[58]
River sediments	Pesticides	Grinding (mortar & pestle)	Soxhlet, ultrasonic extraction, PLE	GC-EI-MS	Villaverde et al., 2008[57]
Sediments	PAHs, PCBs, organochlorinated pesticide	Mechanical stirrer, wet sieving, and ball mill	Sonication	GC-MS	Opel et al., 2011[55]
Sediments	PAHs, PCBs	Freeze-dried and sieved <63 μm	Soxhlet	GC-MS, GC-ECD	Barra et al., 2004[42]
Soil	PAHs	Air- and freeze-dried/fresh extracted, Retsch planet mill	PLE	GC-EI-MS	Wilcke et al., 2003[59]
Soil	Metals	Mortar & pestle, roller mix, pulvisette	Digestion	Induced coupled plasma-MS	Felt et al., 2008[61]
Beach sand	PAHs	Dry sieving	PLE	GC-EI-MS	Bernabeu et al., 2009[63]
Soil	Trinitrotoluene	In-field homogenization, coning and quartering, mortar & pestle	Salting out and solid-phase extraction	LC-UV	Jenkins et al., 1997[65]
Soil/aquifer	Different solutes and organic carbon	Pulverization in a shatterbox	Wet oxidation	Infrared detection	Ball et al., 1990[66]
Sludge	Alkylphenols	Pulverization and sieving	Ultrasonic liquid solid extraction	GC-EI-MS	Fernández-Sanjuan et al., 2009[71]
Pine needle	PAHs		Soxhlet , ultrasonic extraction, PLE	GC-EI-MS	Ratola et al., 2006[73]
Plant matrices	Trace elements	Drying and homogenization	Not indicated	Not indicated	Markert, 1995[72]
Pork fat tissue, beef muscle, human liver	Trace elements	Brittle fracture technique (ball mill, disk mill)	Not indicated	Neutron capture prompt gamma activation analysis Instrumental neutron activation analysis	Zeisler et al., 1983[77]
Grass shrimps and amphioxus	Different organic chemicals	Hand mixing	Not indicated	Not indicated	Clark et al., 1987[46]
Bovine liver	Trace elements	Brittle fracture technique	Not indicated	Neutron activation analysis	Iyengar and Kasperek, 1977[76]
Fish tissue	Organic compounds	Grinding with a meat grinder	Soxhlet	GC-EI-MS	Araki et al., 2001[78]
Fish tissue	Persistent organic pollutants	Wet blending, freeze-drying, and homogenized with mortar & pestle	PLE	GC-EI-MS	Lacorte et al., 2006[58]
Cell lines and animal tissue	Vesicular fractions	Balch homogenizer	Not applied	Not applied	German et al., 2009[79]
Chicken eggs and tissues	Salinomycin	Vortexing and microwave extraction	Microwave-assisted extraction	LC with postcolumn derivatization and UV	Akhtar, 2005[80]

Abbreviations: ECD, electron capture detection; EI, electron impact; GC, gas chromatography; LC, liquid chromatography; MS, mass spectrometry; NCI, negative chemical ionization; PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls; PLE, pressurized liquid extraction; UV, ultraviolet.

In environmental procedures little attention has been given to sample homogenization procedures, corroborated by the very little information available in articles published in scientific journals, with the exception of the mortar and pestle procedure.

1.04.7.1 Water

Water is theoretically a homogeneous matrix. However, salinity or conductivity differences, organic matter content, humic and fulvic acids, presence of particulate material, temperature, oxygen content, nutrients, chlorophyll, etc., make environmental water highly inhomogeneous. These differences should basically be taken into consideration during sampling and analysis, to obtain a representative sample. The need of water homogenization depends on the type of analysis to be performed and, above all, the type of water to be analyzed (seawater, river, lake, groundwater, wastewater, etc.). Although the methods described above do not apply to homogenized water samples, other procedures are compulsory in preparing the sample before extraction, and indirectly also produce a homogenization of the water. For the determination of organic contaminants, filtration is a widely used procedure to separate the particulate phase and dissolved phase. In general, filters of 0.45 μm are used to filter surface waters while filters of 0.7 μm are used for seawaters. Depending on the organic carbon partition coefficient of a given contaminant, partitioning can occur between the two phases and therefore, both should be taken into consideration in obtaining the total concentration of the contaminant in the sample. On the other hand, wastewaters have large amounts of gross, particulate, and organic matter and thus, the lack of homogeneity between replicates can lead to large differences in the parameter to be measured. Because of the difficulty in filtering the samples, centrifugation is a good alternative to separate the dissolved from the particulate phase. Again, according to the physicochemical properties of the parameter to be measured, the dissolved, the particulate, or both fractions should be considered. Other studies suggest homogenizing well the raw sample before extraction to obtain the total concentration of contaminants in water, i.e., the free and particle-bound contaminants. This is only possible if samples do not clog the extraction system, and is performed in groundwater, rain water, ice, snow, and seawater.

Some analytical methodologies have emerged to determine the concentration of a contaminant using a few (e.g., 1–10 ml) of water. An example is the direct injection of wastewaters (100–500 μl) by liquid chromatography–mass spectrometry. In these cases, the analyst must ensure that the volume analyzed is representative of the water mass to be monitored. In other cases, online solid-phase extraction can reach good sensitivity by extracting only 10 ml of water. Again, sample homogeneity and representativeness have to be ensured.

Overall, bibliographic data refer to sample preparation techniques (water filtration or centrifugation) to improve extraction procedures and analytical performance, but not with the aim of homogenizing the sample. This lack of homogenization may contribute to the large differences in the analytical determination of organic contaminants, as recently stated for the determination of nonylphenol in a round-robin test, which comprised sampling through to final analytical determination.[34]

1.04.7.2 Solid Matrices: Soil, Sediment, Sludge

Grain size can affect the outcome of sediment and soil analysis. For heavy metals and other trace elements, the finest grain-size fractions (<63 μm and <20 μm) accumulate polar analytes due to the ion-exchange properties of clay minerals.[35,36] Nonpolar contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and other organohalogen compounds accumulate in the organic fraction and adsorb onto particles due to their hydrophobicity and molecular mass.[37] However, to date it is not clear what fraction should be analyzed. Several authors have found that PAHs accumulate in the <63 μm fraction but also in coarser grain-size fractions.[38–41] However, the <63 μm is widely used for the analysis of organohalogen compounds in salt-marsh sediments[42] or estuarine sediments.[43]

Sediment/soil homogenization is a common practice in toxicity testing and chemical analysis protocols, with the aim of reducing inter-replicate homogeneity.[44] As a general procedure, freeze-drying and sieving with a mechanical mixer[45] or- more commonly, hand mixing[46–48] are the most used techniques to homogenize soils and sediments. Hand mixing can be performed by blending with a spatula, rolling the sediment on a sheet of plastic or precombusted foil, and tumbling by raising each corner of the sheet in succession, or by coning or caking, and quartering.[49] Regardless of the mixing method (i.e., mechanical or by hand) the efficacy of the method should be demonstrated. In general, homogeneity is achieved when the material becomes uniform in color and texture. However, in some cases this is not enough, and the homogenization method and mixing time needs to be standardized to ensure consistency.[45,50,51] The mixing time required to achieve a homogeneous mixture should be minimized because prolonged mechanical mixing can alter the particle size distribution in a sample. Oxidation of the sediments can also occur with prolonged mixing. Partitioning of the homogenized sediment and soil among the test containers should be done in such a way that it does not affect the material, whether the technique be coning or caking and quartering, or other recommended methods for partitioning the test sediment prior to storage. Sediment material intended for chemical analysis should preferably be kept frozen after homogenization. The recommended storage sample size is 100 g.

In the analysis of sediments and soils, determination of both grain-size distribution and organic matter content is a standard procedure.[35] Grinding of soil samples is a common sample preparation step that is being recommended by the US Geological Survey,[52] the Canadian Society of Soil Sciences,[53] and the US EPA,[54] and refers to grinding alone or when combined with drying and sieving.

Homogenized and sieved samples are generally used to guarantee the quality of the analytical data and to make comparisons among sites. Figure 2 shows the result of sediment and soil sieving through different sizes. Analysis of organic contaminants in

sediments and soils is usually performed at the <63 μm fraction or at the fraction of <120 μm or higher. In a study carried out by Opel et al.,[55] bulk sample aliquots were taken after homogenization of the samples with a mechanical stirrer for mass recovery calculations. Then samples were wet-sieved and separated in 5 grain-size fractions (<20 μm, 20–63 μm, 63–100/125 μm, 100/125–200/250 μm, and 200/225–2000 μm) using stainless steel balls instead of agate balls to reduce sieving time and enhance destruction of agglomerates. The smaller fractions contained the highest total organic content (TOC) and the highest concentration of PAHs, PCBs, and organochlorine contaminants. Also, this fraction produced the best comparable results. Polybrominated diphenyl ethers were analyzed in river and estuarine sediment samples from a catchment site in Portugal[56] and the transport of these contaminants was evaluated. In another study, PAHs and organochlorine pesticides were analyzed in agricultural soils from Spain and Portugal.[57] In these studies, it was found that freeze-dried sediments sieved through 500 μm, 250 μm, and 120 μm produced high homogeneity within each fraction as measured by the TOC, and since TOC was highest in the 120-μm fraction, this fraction was further used to carry out chemical analysis.[58]

Another factor affecting the concentration of contaminants is the pretreatment procedure. Wilcke et al.[59] reported that PAH concentrations in air-dried and freeze-dried soils were lower than in in-field fresh extracted samples, which reduced volatilization losses and contamination risks. However, the same authors indicate that drying, by whatever method, results in standardization of a well-defined water content and facilitates homogenization.

Burgess et al.[60] studied the geochemical effects of sediment homogenization by measuring the concentration and distribution of PCBs in contaminated marine sediment interstitial waters. It was found that homogenization, prior to isolation of interstitial waters, significantly increased the concentration of PCBs in the dissolved and colloidal phases in comparison with undisturbed phase. These authors demonstrate statistical differences in the organic carbon content in interstitial waters in sediments stored for 3 months undisturbed and homogenized, and suggest that the practice of sample homogenization may alter the bioavailability of contaminants.[60]

Felt et al.[61] reported the effect of three grinding procedures on the concentration of metals in three types of soil (mortar and pestle, roller mill, and pulvisette). It was found that there was a slight increase in concentration for several metals in ground soils and that the standard deviation decreased, although roller mill grinding was not effective.

Rasemann et al.[62] performed a soil survey to estimate the environmental risk of contaminated sites caused by hazardous materials – mercury in this case. The study quantified the different sources of variation of the measured mercury content caused by global variations, local heterogeneity, sample preparation, sample pretreatment, and chemical analysis. The study indicates that the type of sample pretreatment influences the analytical results and introduces systematic quantification errors.

To estimate the capacity of beaches for oil burial after the Prestige oil spill, samples from eight beaches were collected using suction cores at low, mid, and high intertidal zones. Once in the laboratory, samples were analyzed for textural and geochemical parameters, and grain-size distribution was measured by dry sieving to have a homogeneous sample, which was thereafter analyzed for PAHs.[63]

Ng et al.[64] applied the homogenization theory to evaluate the diffusion of volatile organic compounds during soil evaporation extraction in the vadose zone composed of aggregated soils.

The presence and control of explosives in soils is a subject of concern and needs careful monitoring of the contaminated site. Jenkins et al.[65] have compared the analytical error and sampling error in discrete and composite samples, and indicate that the standard deviation due to sampling was greater than the error associated with analysis by a factor of 2.6 to 22.8, due to the great spatial heterogeneity. In this study, it is recommended to use composite sampling, in-field homogenization, coning and quartering, and colorimetric analysis to obtain accurate and precise mean concentration estimates of trinitrotoluene (TNT) representative of an area.[65]

The need for sediment and soil homogenization is demonstrated also for sorption experiments. In sorption studies several factors are involved, such as particle density, porosity, pore size and distribution, specific surface area, and carbon content. Ball et al.[66] indicate that mineral characterization of sieved fractions serves as an indispensable complement to the physical characterization of soils, and indicates that pulverization of samples in a shatterbox is useful for homogenizing samples and reducing the variability. This process is used to understand the transport of solutes in aquifer systems. In another study, the sorption of PAHs to natural sediments was studied by isolating organic fractions, including demineralized organic matter, condensed organic matter, and black carbon.[67] The organic carbon normalized distribution coefficient measured from demineralized organic matter was higher than that of the bulk sediment; thus, these fractions have to be taken into account in explaining the partitioning of PAHs.

Studies of contaminant availability are also affected by the sediment/soil fraction. The homogenization of the soil might affect the availability and, therefore, the outcome of a bioassay or chemical analysis might not reflect the field conditions.[68] In this study the uptake kinetics of PAHs by a depletive passive sampler exposed to ground and nonground field-contaminated soil indicate that ground samples reached a steady state much faster than nonground, and thus this affects the exposure of organisms to these soils.[68] Similar findings were found in sediments by Van Hoof et al.,[69] who indicate that sediment homogenization, aging, and contamination history affect the bioaccumulation of PAHs.

Bioremediation is another type of activity where sediment/soil homogenization is needed to obtain highest efficiency. In a study dealing with bioremediation of contaminated soils, the use of slurrying is suggested, since it provides homogenization and uniform nutrient distribution as compared with solid-phase treatment.[70]

On the other hand, sewage sludge accumulates organic contaminants during the water-treatment process and has become an interesting matrix for monitoring organic contaminants. The analysis of contaminants in sludge is intricate because of the complexity of the matrix, as regards composition (high levels of organic matter) and morphology (amorphous and fibrous

material). These large inhomogeneities have to be eliminated to obtain accurate and precise quantification of target compounds. Fernández-Sanjuan et al.[71] freeze-dried and sieved the sludge material sequentially to <63 μm particle size to obtain a homogeneous material that was used to prepare a reference material for alkylphenols. In this study, the effect of the temperature on the preparation of the material was also studied, indicating that when drying at 100 °C, 40 °C, and freeze-drying, the best homogeneity was observed in the latter condition.

1.04.7.3 Vegetation

Vegetation has the capability of trapping contaminants in their waxy, lipid-rich leaves, and in the last years it has been used as a bioindicator of airborne contaminants. Vegetation is characterized by large morphological and physiological heterogeneity, and homogenization procedures should be optimized according to each species. Markert[72] developed a cleaning, drying, and homogenization protocol for the trace element analysis in plant matrices.

Ratola et al.[73,74] demonstrated the usefulness of pine needles as passive samplers of atmospheric contaminants such as PAHs and polybrominated diphenyl ethers (PBDEs). Collection of sample was performed in second-year branches from each tree, and 1–5 g of sample were extracted. Needles were cut in 1-cm portions with precleaned scissors, which turned out to be preferable in comparison with blending because less matrix interference was observed in the GC-MS chromatogram.[73]

1.04.7.4 Biological Tissues

Biological matrices have long been used for cytological, toxicological, and chemical characterization. As early as 1977, large inhomogeneities were observed when sampling a 1-g test portion of a human liver, despite its macroscopic homogeneity.[75] Iyengar et al. introduced a cryogenic homogenization technique for biological sample preparation, where the tissue is ground at near liquid nitrogen temperature in an oscillating ball mill of PTFE.[76] The system was constructed with PTFE material and was recommended as an effective, contamination-free device for size reduction and homogenization of biological tissues. This method was only tested for small amounts of sample (20 g). To hold larger samples (e.g., whole organs), new brittle fracture mills patterned as modifications of Iyengar's device were designed. Ball and disk mills constructed from virgin Teflon permitted to hold sample volumes of 50–1000 g and were used to homogenize pork fat and beef muscle; standard deviations were lower than 5% ($n = 10$), indicating good sample homogeneity.[77]

In other studies directed to analyze organohalogen compounds, fish samples were freeze-dried, homogenized, and kept frozen to avoid degradation or spoilage of the matrix.[58] Recommended storage sample size was of 30–50 g, for future analysis.

Araki et al.[78] reported the homogenization procedure for the analysis of semivolatile organic contaminants in fish. Whole fish, fish fillets, and eggs were homogenized in a commercial meat grinder. Large fish samples were first cut into cubes of 2.5 cm, and the cubes from all the fish comprising the composite were combined and ground. After the first grinding, the ground material was divided into quarters, the opposite quarters mixed together, and the halves then mixed together. The grinding and mixing was repeated until the composite sample appeared to be homogeneous (minimum three times). In another study carried out by Lacorte et al.,[58] whole fish (carp and other freshwater fish) was blended and then the sample was freeze-dried and homogenized, again using a mortar and a pestle, to a fine powder. This material was used to determine persistent organic pollutants on a river basin scale (**Figure 3**).

In other fields, it is necessary to obtain vesicular fractions from cell lines or animal tissue for localization and biochemical analysis. This procedure is time consuming and technically intensive. A study reported by German et al.[79] used a Balch homogenizer and differential centrifugation to obtain two distinct vesicular fractions along with purified nuclear, cytoplasmic, and ghost fractions within a 3-h period without the use of density gradients. It is indicated in this study that excessive homogenization causes the formation of plasma membrane microsomes that contain vesicular fractions and nuclear disruption, altering sedimentation and also contaminating subcellular fractions. However, this technique has not been reported for the analysis of organic contaminants.

Conventional homogenization and vortexing techniques were compared with microwave-assisted extraction for the determination of salinomycin in chicken eggs and tissues.[80] It was found that homogenization requires further cleanup while microwave extraction is a reliable, reproducible, and economical substitute for routinely used homogenization and vortexing.

1.04.7.5 Reference Materials

Nowadays, analytical laboratories must guarantee the accuracy of the results generated using a validated methodology. In addition, many laboratories must be accredited according to UNE-EN ISO/IEC 17025, which is the main standard used by testing and calibration laboratories.[19] Technical requirements of this guide indicate that the laboratory must have quality control procedures to guarantee the validity of the assays and calibration methods. Data generated has to be recorded for traceability and, whenever possible, statistical methods must be used to analyze the results. In these quality control measures, the use of reference materials is compulsory to guarantee the quality of the results of a laboratory.

A reference material is, according to ISO 30, a "material or substance that has one or various properties sufficiently well established for calibrating an instrument, validate an analytical method or assign a value of a material or system."[81] Reference materials are characterized by their high homogeneity and stability, and can be certified (certified reference materials (CRM)) or not (quality control materials (QCM)). CRM are materials that are properly characterized using a validated analytical methodology, and are accompanied by a certificate that provides a value of the property or parameter to be measured, its uncertainty, and its

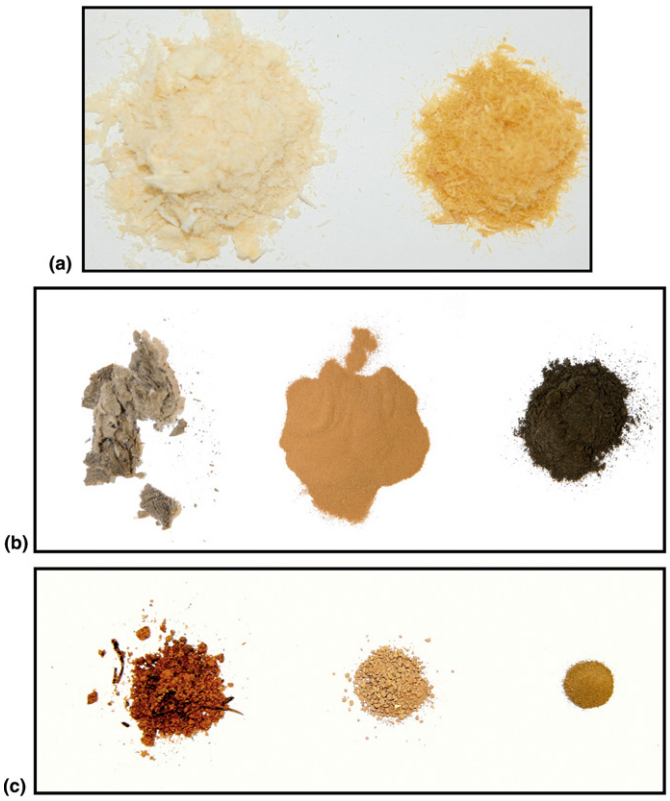

Figure 3 Homogenized materials including: (a) fish (left, freeze-dried and homogenized with a mortar and pestle; right, fish sample reference material); (b) sludge (left, freeze-dried, unhomogenized; middle, sieved through 120 μm; right, grind milled) and (c) soil (left, freeze-dried, unhomogenized; middle, sieved through 2 mm; right, sieved through 120 μm).

traceability. They are used to evaluate the accuracy and traceability of an analytical method, based in the certified values. On the other hand, QCM are homogeneous and stable materials that are used in interlaboratory exercises or to evaluate the repetitiveness and long-term performance of an analytical procedure.[82] In the field of environmental chemistry there are few CRM available, considering the large matrix variability and compounds being routinely measured.[83] This is in part attributable to the difficulty in preparing a certified reference material.

In the preparation of reference materials, it is important to ensure that the content of each bottle in the batch is the same (between-bottle homogeneity) and that the same mean chemical composition exists within the individual bottles (within-bottle homogeneity). At this point, the material can be analyzed for the constituent to be measured. If the standard deviation is within tolerable limits and there are no significant differences between bottles or batches analyzed at different times, the material can be considered homogeneous. Also, the material has to be characterized by different accredited laboratories to assign a reference value. The results provided by all the laboratories have to be statistically equal and the uncertainty value has to be assigned. At this stage, the material can be distributed or sold, and serves to validate a specific procedure or parameter.

Standard Reference Materials (SRM) from the National Institute of Standards and Technology (NIST) aim to monitor contaminants in several environmental matrices, such as fish and marine mammals,[84] air and diesel particulate matter, coal tar, marine and river sediment,[85] mussel tissue, fish oil and tissue,[86] and human serum.[87] These materials are available for the analysis of PAHs, PCBs, chlorinated pesticides, dioxins and furans, organotin compounds, and metals, among others. These SRM can be used for development and validation of analytical methods and as quality control materials for the determination of contaminants. They constitute an appropriate tool to improve quality assurance standards. NIST has also developed a suite of food-matrix SRM characterized for nutrient concentrations. These SRM include infant formula, baby food, diet composites, meat homogenates, oysters, mussels, and fish tissue, chocolate, peanut butter, and spinach.[85]

In NIST SRM, biological materials are generally cryogenically homogenized by milling of large samples (up to 1 kg, previously stored at cryogenic conditions) at liquid nitrogen vapor-phase temperature (−160 °C) in a large-capacity oscillating milling device made of Teflon, leading to a homogeneous powder-like frozen material with a subsampling error <2% if 1-g (wet mass) test portions are used.[88]

On the other hand, bulk site reference materials (BSRM) have also been manufactured from hazardous waste sites. These materials are useful in preparing stabilization/solidification formulations for soils. BSRM are large-volume samples that are representative of the physical and chemical characteristics of a site soil that contains contaminants at reasonably high levels.

A successful BSRM has to be extremely homogeneous and well characterized, meaning that it maintains good fidelity to site matrices and contaminants, and exhibits lowest possible RSD.[89]

Point et al.[88] have developed and applied an ultratrace method for speciation of organotin compounds in cryogenically archived and homogenized biological materials. Cryogenically archived biological materials were maintained in an uninterrupted cryochain from storage conditions through homogenization and bottling. It is indicated that cryogenically processed and stored biological materials are a suitable alternative to conventional freeze-dried materials for organotin speciation, because these conditions are the best for minimizing degradation of thermolabile species and for long-term archival. The proposed method reduces the detection limits and was validated using the European reference material mussel tissue produced by the Institute for Reference Materials and Measurements.[88]

1.04.7.6 Environmental Specimen Bank

Several years ago, several institutions launched the program of Environmental Specimen Banking (ESB), an initiative aimed to collect, preserve, and store relevant environmental samples for scientific studies that might take place at present or any time in the future, with the aim of confirming historical data, investigating a specific parameter, or carrying out temporal trends. Institutions involved in such programs are indicated herein, each having specific objectives and knowledge.

In an early program, the Pilot National ESB program undertook the chemical characterization of a bulk sample, the human liver.[90] Effective homogenization was performed to eliminate sample inhomogeneities that would lead to large uncertainties, which would reduce the usefulness of highly precise and accurate analytical technologies.[90] In this pilot survey, cryogenic homogenization using milling and grinding of frozen tissues (temperature $<140\ ^{\circ}C$) using a PTFE disk was an effective and contamination-free method of particle reduction and homogenization of this tissue.

Modern trace and retrospective analysis of ESBs requires surplus material prepared and characterized as reference materials. Before the sample is analyzed and stored for long periods at cryogenic temperatures, the materials are precrushed, milled, and homogenized. Koglin et al.[91] used a grinding device cooled with liquid nitrogen at $-190\ ^{\circ}C$ to treat deer liver and bream muscles, and the samples were considered homogeneous when at least 90% of the particles were $<200\ \mu m$ (measured with a laser particle sizer).

The NIST Environmental Specimen Banking system consists of two ESBs: the National Biomonitoring Specimen Bank, established in 1979 and the Marine Environmental Specimen Bank, established in 2002. Both facilities were specifically designed to store environmental specimens over 50–100 years to be used for future researchers to establish temporal and spatial trends, verification of past results and changes in the ecosystem structure, health status of marine animals, etc.[92] NIST samples have been prepared in a clean room to minimize inadvertent contamination, and environmental specimens collected in the frame of marine and coastal monitoring programs are cryogenically homogenized using a large-volume vibrating cryomill to generate a fresh, frozen powder material; finally the samples are banked.[77,93]

The ESB of the Federal Republic of Germany has been collecting and preparing specially selected biological reference materials since 1982 to help to control representative ecosystems.[94] The preparation and homogenization of the samples is carried out at cryogenic temperatures, $<150\ ^{\circ}C$, and are characterized for trace elements, bulk elements, PAHs, and chlorinated hydrocarbons. Because of the need and lack of fresh reference materials for organic analysis, the authors indicate that ESB reference materials can be used for such a purpose.

The Groupe Interface Chimie Biologie des Ecosystèms Marins (GICBEM) consists of an evaluation of the ecosystem health status in the Mediterranean Sea based in combining chemical (PAHs, PCBs, metals) and biochemical approaches (mixed function oxygenase parameters) in representative coastal organisms (fish, mollusks, plants) and matrices (water and sediments).[95] Aiming to initiate an environmental banking program including retrospective analysis of both chemical pollutants and biochemical indicators, the study by Garrigues et al. describes the procedures of sample collection and preparation for the short-term storage of samples on board a ship and representative analysis.

1.04.8 Conclusions

In environmental and food analysis, most of the effort is given over to sample preparation and chemical analysis, with the aim of ensuring high-quality data. At present, analytical techniques and methods offer excellent performance in achieving high sensitivity, selectivity, and identification capabilities for a wide range of contaminants. However, analytical chemistry is useless in samples that have not been properly sampled, if they are not representative of an area, or if they have not been perfectly homogenized. As environmental monitoring or food quality control programs are being launched to comply with current legislation and/or to minimize the exposure and effects of contaminants to the population, the need for representative and homogeneous samples increases. As has been exemplified in this chapter, several homogenization techniques are available on the market, each with unique properties and performance. These techniques permit one to comminute and homogenize samples of different texture, volume, hardness, and size to a representative sample. Including sampling and sample homogenization in the whole analytical methodology has imminent advantages, since the original sample inhomogeneity will be transformed into representative, comparable, and reliable data. As most laboratories are implementing validation methods and the use of reference materials to ensure the quality of their data, implementation of quality control procedures in the sampling and homogenization protocols is recommended to ensure the quality of the overall analytical processes.

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See also: Legal and Forensic Sampling; Methodologies for Sample Preservation and Stabilization; Assessing and Controlling Sample Contamination; Equipment for Water Sampling Including Sensors; Passive Sampling of Organic Contaminants in Waters; Seawater Organic Contaminants; Sampling Approaches for Trace Element Determination in Seawater; Sampling of Fish, Benthic Species, and Seabird Eggs in Pollution Assessment; Food Contaminants; Sampling Strategy for Process Control; Sorbent-Phase Sample Preparation in Environmental Analysis; Recent Advances in Sample Preparation for Pesticide Analysis; Sample Preparation of Complex Biological Samples in the Analysis of Trace-Level Contaminants; Sample Preparation for Food Contaminant Analysis; Sample Preparation Techniques for the Determination of Some Food Contaminants (Polycyclic Aromatic Hydrocarbons, Mineral Oils, and Phthalates)

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