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## Reference Materials: preparation, homogeneity, stability and value assignment

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### Abstract

Reference Materials (RMs) and Certified Reference Materials (CRMs) are widely used in all stages of measurement procedures and in interlaboratory comparisons. CRMs, in particular, play a key role in implementing the concept of metrological traceability of measurement results in chemistry, biology and physics among other sciences dealing with substances and materials and, in this context, laboratories use CRMs as readily accessible measurement standards. In the past decades, an extensive production of RMs and CRMs was carried out. Although some of them are intended for applications in physics, the great majority belongs to the amount of substance related fields. There are various normative references dealing with RMs and CRMs, and broad scientific literature. This work aims at presenting a general overview on the production and characterization of RMs and CRMs, with particular focus on gaseous CRMs. An example of application of gaseous CRMs to support climate change studies is also given.

### 1. Introduction

Reference Materials (RMs) with different characteristics are used in all stages of measurement procedures: the calibration of a measurement system, the value assignment to other materials, the assessment of a measurement procedure, the quality control. They are also used in interlaboratory comparisons either to validate a measurement method or to assess the proficiency of a laboratory.

The demand for new RMs of higher quality is constantly increasing, driven by various issues: there are pressing requirements for more accurate and reliable data, the measuring equipment is continuously improving in precision and a RM can be used only in one stage of the entire measurement process.

Considering the definition of metrological traceability as the *“property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty”* [1], it comes that measurement results need to be traceable to appropriate and stated references to be comparable across borders and over time. In this context, Certified Reference Materials (CRMs) play a key role in implementing the concept of metrological traceability of measurement results in chemistry, biology and physics among other sciences dealing with substances and materials. The property values carried by a CRM can be made traceable to the International System of Units (SI) or other internationally agreed references. Laboratories use CRMs as readily accessible measurement standards to establish traceability of their measurement results to International Standards. CRMs are also used to confirm or establish metrological traceability to conventional scales, such as the octane number, water turbidity and hardness scales.

There are various normative references dealing with RMs and CRMs [2-5], including an International Standard devoted to the general requirements to assess the competence of the reference materials producers (RMPs)

[6]. The production of RMs is a key activity for the improvement and maintenance of a worldwide coherent measurement system. The demonstration of the scientific and technical competence of RMPs is indeed a basic requirement to ensure the quality of RMs. Most CRMs are produced by National Metrology Institutes (NMIs) and Designated Institutes (DIs), but the number of accredited laboratories that produce RMs and CRMs is rapidly increasing.

## **2. Reference Materials and Certified Reference Materials**

A RM is defined as a *“material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process”* [2]. This definition is coherent with the one reported in the International Vocabulary of Metrology (VIM): *“material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties”* [1].

CRMs are a specific type of RMs. A CRM is defined as a *“reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a reference material certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability”* [2]. In the VIM [1], a similar definition is reported; however, attention is paid on the certificate that should be a documentation issued by an authoritative body and to the property value, instead of only to the property of the material itself.

CRMs are measurement standards. According to ref. [1], a measurement standard is defined as the *“realization of the definition of a given quantity, with stated quantity value and associated measurement uncertainty, used as a reference”*. In addition, a *“set of reference solutions of cortisol in human serum having a certified quantity value with measurement uncertainty for each solution”* and a *“reference material providing quantity values with measurement uncertainties for the mass concentration of each of ten different proteins”* are given among the examples of measurement standards [1]. As measurement standards, the key role played by CRMs in implementing the concept of metrological traceability of measurement results is undeniable. Specific procedures must be put in place, as for all laboratory equipment, for handling, transport, storage and planned maintenance, including intermediate checks. In addition, in ref. [7], the use of CRMs provided by a competent producer is explicitly recalled as a means to ensure the metrological traceability of a laboratory measurement result.

On the basis of these considerations, it is straightforward that the principles of classification of measurement standards can be applied to define a hierarchy of RMs. Figure 1 presents a sound hierarchy for CRMs and RMs having property value(s) metrologically traceable to SI, which follows the same principles of a calibration hierarchy [1].

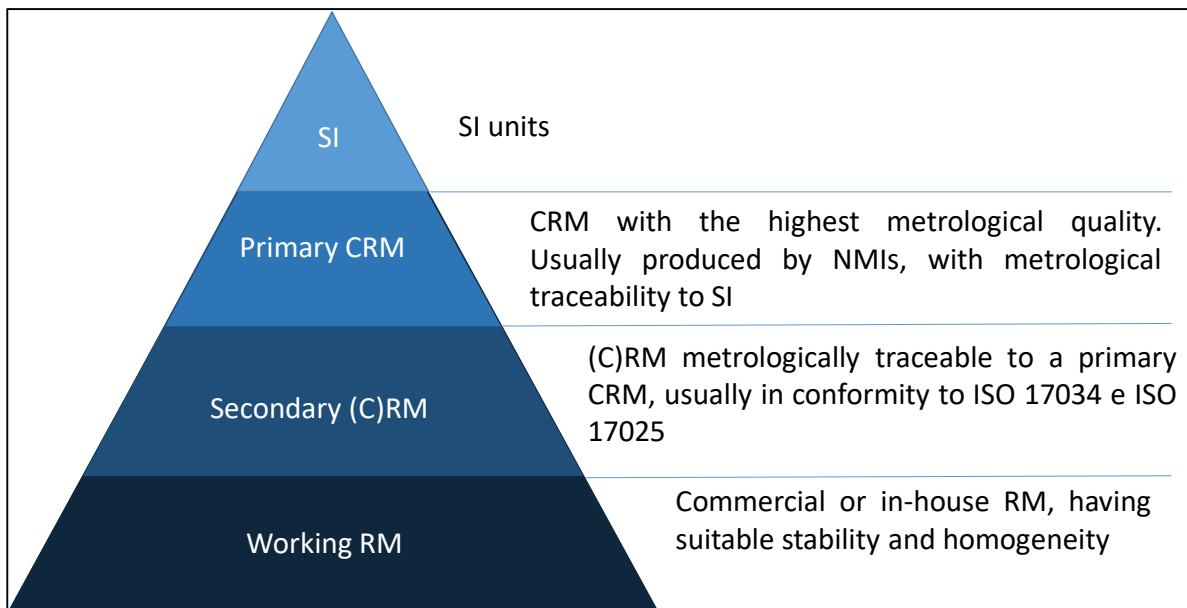


Fig. 1: Hierarchy of reference materials with metrological traceability to SI of property value(s)

The production of RMs and CRMs is a key activity for the improvement and maintenance of a worldwide coherent measurement system. RMs and CRMs are used in various measurements, ranging from chemical composition to biological, clinical, physical, engineering properties, and also in miscellaneous areas such as taste and odour. They may be characterised for one or more specified properties which can be quantitative (*e.g.* amount of specified chemical entity, hardness etc.) or qualitative (*e.g.* identity of substances or species). Different types of RMs are required according to the application. According to their characteristics and uses, the following types of RMs and CRMs can commonly be encountered:

- pure substances characterized for chemical purity and/or trace impurities; they can be used either for calibration or for matrix matching, *i.e.* substances that do not contain the analyte of interest and can be used to prepare blank samples;
- reference mixtures and solutions for calibration, often prepared gravimetrically from pure substances and used for calibration purposes;
- matrix RMs, characterised for the composition of specified major, minor or trace chemical constituents, generally used for procedure steps different from calibration, like method validation, recovery, etc. Such materials may be prepared from matrices containing components of interest at the naturally occurring level or natural materials fortified (spiked) with the components of interest;
- physico-chemical RMs characterized for properties such as melting point, viscosity, optical density;
- reference objects or artefacts characterized for functional properties such as taste, odour, octane number, flash point, hardness.

RMs, particularly matrix RMs and CRMs, play an important role in method development, validation and verification. Their accurate choice and proper use according to the specific application are fundamental, and at least the following aspects should be considered: the measurand (the analyte and its amount fraction), the measurement range, the matrix match and potential interferences, the sample size, the homogeneity and stability of RMs and CRMs, their uncertainty, the procedures used for their characterization and certification. These considerations are particularly relevant in the amount of substance field, where the combinations of analytes, ranges and matrices give an infinite number of measurands and the need of a potentially infinite availability of new RMs and CRMs.

In the last years, an extensive production of RMs and CRMs was carried out. Although some of them are intended for applications in physics [8-12], the vast majority belongs to the amount of substance related fields.

Environmental monitoring is a typical application field of RMs and CRMs, and there is a constant production of new materials suitable, for example, for the determination of contaminants in sediment and soil [13,14] and atmospheric pollutants [15-24].

Due to the importance that RMs and CRMs play in the development of analytical methods in food matrices, many new RMs and CRMs have been developed, ranging from the determination of food constituents and nutrients [25-29] to determination and quantification of contaminants [30,31], drug residues [32] and toxins [33,34] in foodstuff.

Many RMs and CRMs have been developed also in the field of clinical chemistry and bioanalysis [35-48] thus underlining the rising impact of metrological approach to the quality and reliability of measurement results in such areas.

An increasing attention has been paid to the description of the complete RM and CRM development, including homogeneity and stability assessment [13,30,34,35,39,49-52] and characterization and value assignment [10,11,14,27,28,37,39,52-58]. The following sections give some general principles on the various stages of RMs and CRMs production.

### **3. Preparation of RMs**

The preparation of a RM requires a very detailed production planning, which must be carried out before starting any activity related to the production of the RM itself. The planning shall take into account all the relevant steps involved in the production, *i.e.*:

- the definition of the RM (matrix, relevant properties to be characterised, intended use, target uncertainty) and a feasibility study;
- procedures for the sourcing of the material;
- procedures for manufacturing or preparation, including packaging;
- characterisation, homogeneity and stability studies, carried out according to selected and fit for purpose procedures, and combination of the results;
- assessment of commutability, when required;
- preparation of a certificate/report;
- specification of storage and transportation conditions;
- post-production monitoring of the stability.

In the case of CRMs, two additional steps must be added: the establishment of metrological traceability and the measurement uncertainty evaluation of the certified values.

#### **3.1. Homogeneity**

According to the definition [1,2], a RM must be homogeneous with reference to specified properties. Being many RMs prepared as batches of units (bottles, vials, test pieces, etc), homogeneity can refer not only to the variation of a property value within each unit (within-unit homogeneity), but also between separate units of the material (between-unit homogeneity). Such evaluation is necessary to ensure that each RM unit carries the same value for each property. When a RM is not sold as single-use units and subsamples can be taken for measurements, the material within each unit shall be uniform and it is necessary to assess the within-unit variability of the material, or within-unit heterogeneity. As an alternative, instructions for use should be

provided, like remixing of the sample or a minimum sample size in order to have a subsample that is representative for the whole unit.

The assessment of homogeneity might include either the use of prior evidence or an experimental homogeneity study on the candidate RM, or both. In most cases, particularly with materials of natural origin or with complex matrices like foodstuffs, soils, alloys, an experimental study is necessary being such materials typically heterogeneous in composition. Useful approaches and criteria to conduct homogeneity studies are reported in ref. [5].

The homogeneity study results in an uncertainty component that takes into account both between-unit and within-unit homogeneity. Such component has to be considered in the uncertainty budget for the certified value of the relevant property of the RM or CRM. The measurement procedure used for homogeneity assessment should be carefully chosen in order to have good values of repeatability standard deviation and between-run precision; the standard deviation for measurements should be small, ideally less than one third, compared with the expected uncertainty associated with each property value.

### **3.2. Stability**

According to the definition [1,2], a RM should be sufficiently stable for its intended use. This means that the end user can rely on the assigned value at any point within the period of validity of the certificate. In order to fulfil this requirement, the value for each property of interest has to be, at the time of use, consistent with the stated value on the certificate or on other documentation accompanying the material.

The value of each property can change over time for different reasons and causes, to different extents, at different rates. Depending on the nature of the material and on the property, the changes can be extremely diverse, both in terms of form and rate. Hence the stability has to be not only assessed but also monitored in a reliable way. In particular, the stability monitoring is essential for any material intended to remain available for extended periods.

The most important causes of instability can be ascribed to long-term storage conditions at the producer's facilities, transport conditions, storage conditions at the RM user's laboratory and after opening when re-use is permitted.

As for homogeneity, also the stability assessment needs to be carried out either on the basis of prior information on stability under the planned storage and transport conditions or by conducting fit for purpose experimental studies. In the cases of RMs produced in repeated batches, particular care must be put in place to assess the risk of changes in stability from a batch to another. The results of the stability studies have, among their main outcomes, the estimate of a storage lifetime for a RM, also called shelf life, which is the period of time within which the values of the properties of interest are expected to remain acceptable for use.

Stability studies can be classified into various groups:

- according to conditions of measurements;
- according to stability study duration and condition;
- by study objective (transport conditions, long-term stability in storage, etc).

Each kind of study has its own pros and cons, hence the decision on which approach to use has to be carefully evaluated on the basis of the information that the study is supposed to provide.

The classical and isochronous studies belong to the first category. In the classical stability study, the measurements are carried out under intermediate conditions of measurements. Individual samples prepared at the same time, for example coming from the same batch, are measured under the same conditions after

defined time intervals. In the isochronous studies, the measurements are carried out under repeatability conditions. The RM units are exposed to different degradation conditions, like storage and times; then they are measured all together ideally at the same time, practically in a short period of time. Being the analyses carried out under repeatability conditions, the precision associated to the measurements is improved.

The classification of stability studies by duration and conditions leads to real-time or accelerated studies. In a real-time study, the RM is studied under the storage and/or transport conditions that are intended for the RM. In an accelerated stability study, the RM is subjected to more extreme conditions than the intended storage and/or transport conditions, aiming at inducing more rapid degradation with respect to the normal conditions, thus reducing the time required for a real time study. They can be very useful in cases in which an early availability of the RM is required.

Other stability studies can be designed for specific objectives, like expected transport conditions, packaging, lifetime and shelf life of the RM.

Useful information to design stability studies are reported in ref. [5].

All the detectable changes in the property values due to instability must be considered in the claims of the property values themselves and, in case of certified values, the relevant contributions need to be evaluated and included as sources in the uncertainty budget, unless it has been demonstrated that the potential change over the validity period of the certificate is negligible if compared with the certified uncertainty associated with the property value(s).

The stability should be monitored during the entire lifetime of a RM, unless the RM has very short lifetime and early expiry date. An initial monitoring point has to be set, together with appropriate intervals for subsequent monitoring.

### **3.3.Characterization**

The characterization is a fundamental step in the production of a RM, as it leads to the determination of the values of the relevant properties of a RM, as part of the production process. In the case of production in batches, the certified value is the same for the various individual units belonging to the same batch. Instead, if single artefacts are produced, an individual value is assigned to each unit. In the case of CRMs, in addition the associated uncertainty of characterization must be determined and evidence of metrological traceability to a stated reference of the certified values must be provided.

There are several different approaches to carry out the characterization of a RM. Possible examples are [6]:

- a single method, preferably a reference measurement procedure, in a single laboratory: this approach requires the availability of a measurement procedure sufficiently well understood to exclude unknown effects or at least to have systematic errors negligible with respect to the intended use. In order to provide more confidence to the characterization result, a confirmation by an independent measurement procedure should be carried out. This is the generally used approach in the preparation of gaseous CRMs by gravimetry (see section 4).
- two or more methods of demonstrable accuracy in one or more competent laboratories. Such approach is used in the case of non-operationally defined measurands. It requires the availability of different procedures and/or different competent laboratories capable to characterize the material with acceptable accuracy. The property values will be based on the agreement among the results.
- by a network of competent laboratories. In this approach, intended mainly for operationally-defined measurands, the measurand is defined by the procedure itself. A network of competent laboratories that can carry out the measurement by applying the same methods is necessary in order to

randomize possible biases or influence factors thus keeping small enough the uncertainty associated with the property values.

- by value transfer from a reference material (primary CRM) to a closely matched candidate reference material (secondary CRM) using a single measurement procedure performed by one laboratory. This approach requires that the secondary CRM is sufficiently closely matched to the primary to make material-specific bias negligible (*i.e.* same matrix, close quantity level).
- by gravimetric or volumetric preparation data.

### **3.4. Value assignment**

The value assignment is the process of combining the results obtained from the characterization to the ones derived from homogeneity and stability assessments. It allows to determine the assigned values of the specified properties (property values) of a RM or a CRM. These are the values that are issued on certificates or on product information sheets.

For certified values, as the associated uncertainty has to be given in the certificate, the value assignment process comprises also the development of an accurate uncertainty budget and the uncertainty calculation associated with each property value. The following uncertainty sources, at a minimum, need to be taken into account and the relevant contributions evaluated: characterization, between-unit and within-unit inhomogeneity, changes of properties during storage and transport.

### **3.5. Metrological traceability**

Metrological traceability is a property of a measurement result [1]. In the case of a CRM, a statement of metrological traceability to a stated reference of the certified property values has to be provided [1,5,6]. The stated reference can be a definition of a measurement unit through its practical realization, a measurement procedure, a measurement standard. All the parameters that have a significant influence on the certified values or on their uncertainties, need to be metrologically traceable. A specific metrological traceability path must be chosen according to the characterization scheme which has been used. Metrological traceability is a fundamental feature of CRMs as they are primarily used as measurement standards to obtain traceable measurement results. CRMs are of particular relevance in the cases in which traceability to SI units is not technically possible.

## **4. Reference materials and certified reference materials in gas analysis**

Gas analysis is a sector in which the use of CRMs as measurement standards to calibrate instruments and establish metrological traceability is very wide. Gaseous CRMs are currently used in many application fields, like environmental and climate applications [15-24] and energy [59,60].

As for all RMs and CRMs, a detailed production planning is required. An accurate feasibility study has to be carried out taking into account, among other things, safety considerations, possible chemical reactions of the components of the gas mixture, possible reactions of the components with the container materials.

The gravimetric preparation is the most widely applied method to produce gaseous RMs and CRMs in high pressure cylinders [61]. It is a primary direct method that gives direct traceability to mass, providing that the purity of parent gases is assessed and the prepared mixtures are verified against independent reference gas mixtures. It consists of various steps in which pure gases, pure liquids or gravimetrically prepared gas mixtures of known composition are transferred quantitatively into cylinders. The mass of each component is



determined by weighing, before and after each addition, either the supply cylinder or, more commonly, the cylinder in which the gas mixture will be contained. Most weighing procedures require the use of a tare cylinder in order to minimise the correction due to air buoyancy. In this case, it is necessary to select two cylinders made from the same material, having the same internal and external nominal volumes: one will be the target cylinder, *i.e.* the one that will contain the mixture, the other will be used as a tare. In many NMIs, automatic weighing facilities are currently replacing the traditional balances: such systems allow the automatic exchange of the target cylinder and the tare without any intervention of the operator after the weighing process has started, also assuring a better stability of the environmental conditions which are important influence quantities in the mass determination. A detail of the INRIM automatic weighing device hosting the target and tare cylinders is depicted in fig. 2.



Fig. 2: Detail of the INRIM weighing device that allows the automatic exchange of the target cylinder and the tare without any intervention of the operator after the weighing process has started.

At the end of the gravimetric process, the mass fraction of each component is converted into amount fraction by using its molar mass, calculated on the basis of the values of the atomic weights taken from the most recent publication of the IUPAC Commission on Atomic Weights and Isotopic Abundances (CIAAW).

The amount fraction  $y_k$  of the component  $k$  in the final mixture is calculated using the following eq. (1):

$$y_k = \frac{\sum_{j=1}^r \left( \frac{x_{k,j} \times m_j}{\sum_{i=1}^q x_{i,j} \times M_i} \right)}{\sum_{j=1}^r \left( \frac{m_j}{\sum_{i=1}^q x_{i,j} \times M_i} \right)} \quad (1)$$

where:

$M_i$  is the molar mass of component  $i$

$m_j$  is the mass added of parent gas or liquid  $j$

$x_{k,j}$  is the amount of substance fraction of component  $k$  in parent gas or liquid  $j$

$x_{i,j}$  is the amount of substance fraction of component  $i$  in parent gas or liquid  $j$ .

Specific procedures, as a function of the mixture composition, must be in place in order to assure the homogeneity of the mixture before it is analysed or used. In some cases, for example after a long storage period, a re-homogenization might be necessary. Particular care must be paid when dealing with

condensable components, as their condensation may affect not only the preparation but also the homogeneity of the mixture.

After the preparation, each mixture is verified by means of specific analysers, calibrated with independent reference gases that can be either gravimetric or dynamic mixtures obtained by diluting a more concentrated parent mixture with the appropriate matrix gas. An example of a facility to generate dynamic mixtures is presented in fig. 3.



Fig. 3: Dynamic dilution system composed by two Mass Flow Controllers and a mixing chamber.

A direct comparison with an independent gas mixture of similar composition can also serve as verification. Specific criteria must be in place to establish whether the mixture has passed or not the verification. In case of positive verification, the gravimetric value is used as certified property value for the mixture composition.

In order to carry out a stability assessment, a gas mixture has to be analysed immediately after its preparation and then again at regular intervals until either a significant or unacceptable change in composition is observed or an acceptable stability period has been demonstrated. When designing a stability study, knowledge of the composition of the mixture and reactivity of the components must be taken into account. A stability study must be carried out at least on two identical cylinders containing mixtures with the same nominal composition. The greater is the number of cylinders involved in the study, the more confidence can be obtained on the results of the study. The outcomes of such studies can then be used as prior information to assess the stability of similar mixtures.

For certified values, an accurate uncertainty budget must be developed which takes into account the uncertainty sources coming from gravimetry, stability and verification.

The uncertainty component for each amount fraction certified value  $y_k$  of the component  $k$  in the final mixture associated with gravimetry is calculated by applying the Law of Propagation of Uncertainty to eq. (1). According to ref. [61], for each  $y_k$ , the uncertainty contribution associated with the preparation takes into account both the gravimetric process and the stability and is calculated in the following way:

$$u(y_{k,\text{prep}}) = \sqrt{u^2(y_{k,\text{grav}}) + u^2(y_{k,\text{stab}})} \quad (2)$$

The combined standard uncertainty associated with the amount fraction of the component  $k$  in the final mixture is calculated by applying the following eq. (3):

$$u_c(y_k) = \frac{1}{2} \sqrt{u^2(y_{k,\text{prep}}) + u^2(y_{k,\text{ver}}) + (y_{k,\text{prep}} - y_{k,\text{ver}})^2} \quad (3)$$

where  $y_{k,ver}$  is the amount of substance fraction of component  $k$  in the final mixture determined by analytical verification. An expanded uncertainty value is calculated by multiplying the combined standard uncertainty for an appropriate coverage factor and will be reported in the certificate for each certified property value.

#### 4.1. Case study: Gaseous CRMs to support climate change studies

The year 2019 is likely to have been the second warmest in instrumental records, being the global mean temperature of  $1.1 \pm 0.1$  °C above pre-industrial levels. The past five years are the five warmest on record, and the past decade, 2010–2019, is also the warmest on record. Since the 1980s, each successive decade was warmer than any preceding one since 1850 [62].

Global warming is mainly caused by the increase of the concentration of carbon dioxide (CO<sub>2</sub>) and other greenhouse gases (GHGs) in the atmosphere. Atmospheric concentrations of GHGs are at levels that are unprecedented in, at least, 800,000 years. Since the pre-industrial era, anthropogenic GHG emissions have increased, largely driven by economic and population growth and they are among the main causes of GHGs increasing concentration, producing the so-called enhanced greenhouse effect, whose major contributor is CO<sub>2</sub>. Global atmospheric mole fractions of GHGs reached record levels in 2018 with CO<sub>2</sub> at  $407.8 \pm 0.1$   $\mu\text{mol/mol}$ , usually expressed as parts per million (ppm), which constitutes 147% of pre-industrial levels. Early indications show that the rise continued in 2019. [62]. Figure 4 shows the trend of atmospheric CO<sub>2</sub> mole fraction measured in the remote site of Mauna Loa, Hawaii [63].

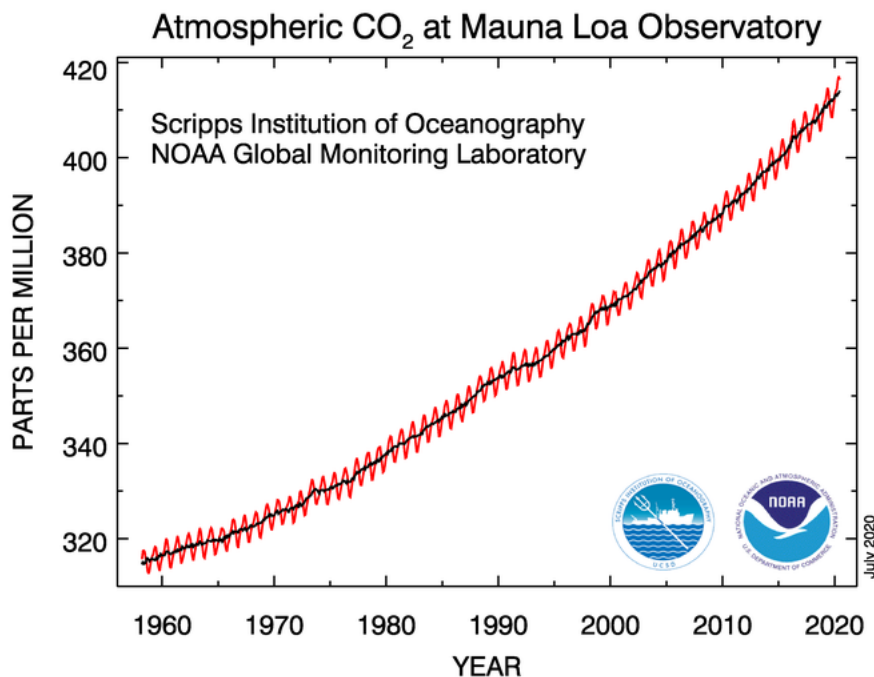


Fig. 4: Atmospheric carbon dioxide concentration measured at Mauna Loa Observatory, Hawaii [63]

The ocean acted as a sink for CO<sub>2</sub>, absorbing over the decade 2009-2018 around 23% of the annual CO<sub>2</sub> emissions and thus reducing the increase in atmospheric concentrations. However, CO<sub>2</sub> absorbed in sea water decreases its pH, thus directly contributing to the process called ocean acidification. Observations from open ocean sources over the last 20 to 30 years show a clear decrease in average pH at a rate of 0.017–0.027 pH units per decade since the late 1980s [62].

The overall risks of future environmental impact and climate change can be reduced by limiting the rate and magnitude of GHG emissions and ocean acidification. In this context, it is necessary to discriminate anthropogenic from natural contributions of CO<sub>2</sub> in the atmosphere, to provide governments and decision makers with reliable emission data. The accurate measurement of the <sup>13</sup>C/<sup>12</sup>C ratio, expressed as δ<sup>13</sup>C according to the convention of reporting stable isotope ratios in terms of a deviation from an international standard (δ-values), is a useful tracer of CO<sub>2</sub> derived from fossil fuel and deforestation sources [64]. The <sup>13</sup>C isotope is stable and heavier than the more common <sup>12</sup>C, which represents the 98.89% of the natural carbon [65]. Plants tend to assimilate selectively the lighter isotopes during the photosynthetic process. For this reason, the admixture in atmosphere of large amount of fossil fuels-derived CO<sub>2</sub>, depleted in <sup>13</sup>C, causes a decrease with time of δ<sup>13</sup>C on a global scale: in the last decades, δ<sup>13</sup>C atmosphere records have shown that the progressive increases of atmospheric CO<sub>2</sub> levels is accompanied by a substantial δ<sup>13</sup>CO<sub>2</sub> decrease, thus confirming the strong correlation between the rising of atmospheric CO<sub>2</sub> level with the anthropogenic changes in the atmospheric carbon reservoir (the so called “<sup>13</sup>C-Suess effect”) [64,66]. The measurement of atmospheric δ<sup>13</sup>C is a means to deepen the knowledge of biosphere-atmosphere interaction, and to adopt appropriate guidelines for climate change management.

The international scale for <sup>13</sup>C/<sup>12</sup>C started as a carbonate laboratory standard for oxygen and carbon isotope ratios. The standard established for <sup>13</sup>C was the Pee Dee Belemnite (PDB) and was based on a Cretaceous marine fossil, *Belemnitella americana*, which originated from the Peedee Formation in South Carolina (US). This material had an anomalously high <sup>13</sup>C/<sup>12</sup>C ratio and was assigned a δ<sup>13</sup>C value of zero. Use of this standard gives most natural material a negative δ<sup>13</sup>C. The original material no longer exists. It was replaced by assigning exact δ-values (both δ<sup>13</sup>C and δ<sup>18</sup>O) to another carbonate (NBS-19) relative to PDB. This new scale is termed VPDB (Vienna Pee Dee Belemnite) in recognition to the role that the International Atomic Energy Agency (IAEA), located in Vienna, played in redefining the PDB scale [67]. At present, NBS-19 is nearly exhausted and IAEA 603 (calcite, CaCO<sub>3</sub>) prepared with the aim to replace it, is certified for the stable isotopic δ<sup>13</sup>C and δ<sup>18</sup>O composition [68].

The measurements of δ<sup>13</sup>C require high precision and accurate analytical setup. It is very difficult to prepare routinely CO<sub>2</sub> from the primary carbonate reference materials across different laboratories to meet the precision of the systems that are currently used. Hence, the atmospheric CO<sub>2</sub> isotope ratio scales are refined scales that use air samples and air in high-pressure cylinders as reference materials. Being the gases volatile, keeping them as reliable references requires a hierarchy of reference gases and a strategy that automatically detects mutual drifts in order to take corrective actions [67].

A lot of research activities are ongoing in order to provide new CRMs of CO<sub>2</sub> having δ<sup>13</sup>C assigned to be linked to VPDB scale [23,24,69] and to set up advanced analytical methods for accurate δ<sup>13</sup>C measurements [70-75]. The European Joint Research Project SIRS – Metrology for Stable Isotope Reference Standards, started in 2017 within the European Metrology Programme for Innovation and Research (EMPIR), operates in this framework. It has, among its objectives, the development of static and dynamic gaseous CRMs for pure CO<sub>2</sub> and for mixtures of CO<sub>2</sub> at 400 μmol/mol in air matrix with uncertainties of 0.1‰ for δ<sup>13</sup>C-CO<sub>2</sub> and of 0.5‰ for δ<sup>18</sup>O-CO<sub>2</sub>. The ultimate goal of this approach is to ensure traceability and consistency with VPDB and work towards meeting the compatibility goals set by the World Meteorological Organization as 0.01‰ for δ<sup>13</sup>C-CO<sub>2</sub> and 0.05‰ for δ<sup>18</sup>O-CO<sub>2</sub> [76].

A follow up of the SIRS project, the European Joint Research Project STELLAR - Stable isotope metrology to enable climate action and regulation” [77] will start in fall 2020. At the end of the STELLAR project in 2023 the improved discrimination between natural and manmade sources of GHGs will support governmental agencies to better attribute emission sources, help demonstrate compliance to national reduction targets and enhance the effectiveness of future abatement strategies.

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