

Commercial approaches to the certification of reference materials for environmental analysis

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Introduction

Despite a general acceptance of the role certified reference materials (CRM) play as cornerstones of quality¹ in achieving comparability, facilitating performance-based measurement systems and routine quality control there is a worrying lack of awareness about their availability and proper use.

In most laboratories a mid-1980s revolution in analytical chemistry technology caused a rapid increase in automation and a consequential reduction in the skill base. The result, especially in areas such as food, public health, environmental and clinical analysis, was the development of many standard methods and procedures designed to shore up levels of performance.

The water, wastewater and soil analysis industry is a good example where a heavily regulated infrastructure evolved to control all aspects of the water and waste industries, including the measurement of pollutants in matrices. The inherent variability of the environmental samples to be analysed makes it impossible simply to calibrate the analytical system using traditional pure substance “standards”, so demand grew for both pure substance and matrix RMs and CRMs to cover all needs:

- As tools to help show conformity to the mandated standard procedure or participation in quality system,
- For instrument calibration
- In quality control procedures
- For problem solving
- To demonstrate data quality and conformity to procedures²

To support the standard methods, the National Institute of Standards and Technology (NIST) and the Environmental Protection Agency

Definitions

Reference Material

A material or substance, one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method or for the assigning values to a material.

Certified Reference Material

A Reference Material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realisation of the unit in which the property values are expressed and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

Source: *ISO Guide 30* (1992).²

(EPA) in North America, and the Institute for Reference Materials and Measurements (IRMM) and the Laboratory of the Government Chemist (LGC) in Europe, produced appropriate reference materials (RM) and CRMs for calibration, method validation and the demonstration of traceability. But demand far exceeded traditional resources available to produce RMs and CRMs.

A good example is the unfortunate position of the US EPA in 1991. Traditionally, they supplied both types of CRM. Produced by contractors working for the EPA, the CRMs were paid for by Government. As worldwide demand for EPA Certified Reference Materials grew, the costs incurred by the EPA in maintaining their programme rose to an unsupportable level.

The answer was “de-Federalisation”, that is privatisation, with five contractors (RTC, NSI, Supelco, Ultra and Spex) each taking specific parts of the programme, but with the EPA remaining as the certification body. The privatisation procedure ended up in a welter of litigation and counter litigation, so the EPA walked away in 1993 and the EPA Certified programme was abandoned. However, demand for “EPA Certified Reference Materials” remained. For pure substance CRMs, the various companies began producing many “Certified” reference materials with claims of traceability to NIST, but which were in fact, strictly, neither traceable nor compliant with ISO Guides 30 to 35.³

The production of matrix CRMs is a much more complex process. To meet the need for matrix CRMs user laboratories turned to NIST in the US and BCR and LGC in Europe. Many popular CRMs were quickly sold out. The problems this caused the traditional suppliers was admitted in 1997 by NIST⁴ when the concept of NTRMs or NIST Traceable Reference Materials was first postulated. In the same year, other evidence that supply was not meeting demand appeared in Europe.⁵

Despite these shortages, the economic importance of the availability and proper use of CRMs began to be more widely understood. The huge economic impact of reliable analytical measurements meant that CRMs came to be considered as strategic materials within the EU.⁶

At the same time as the use of CRMs grew, worrying doubts began to develop about the level of understanding of the proper use of CRMs, especially non-use and misinterpretation of data.⁷ A survey, funded by the European Commission in 1993 across

1600 laboratories in 18 European countries, revealed a disturbing picture.⁸ From the responses, it was obvious that many laboratories were unclear about CRM terminology, were unsure about supply and availability, and had unfulfilled needs for CRMs. The main conclusions of the survey were:

- Better education about the proper use of CRMs was needed
- More information about the availability of CRMs was vital
- New methods of production had to be found

At the time, it was believed that the innovative utilisation of existing resources and the creation of associations between commercial, professional and government research organisations would be the answer. The authors of the report failed to take into account the reality that a commercial undertaking must satisfy its customers and make a return on its investments. Institutional producers were offering CRMs at prices that did not reflect the true cost of development and production. This meant that market prices were at levels not generally compatible with normal commercial production. So the challenge was not taken up.

This article looks at two successful commercial approaches. The first is the production and certification of natural matrix CRMs as an integral part of the running of a commercial laboratory proficiency testing programme by a small, highly-specialised company, Resource Technology Corporation (RTC) in the USA. The second is the production of Certified pesticides by a Polish Government agency, in partnership with a private company. The Institute of Industrial Organic Chemistry, Instytut Przemysłu Organicznego (IPO), produces and certifies the materials which are then marketed by Promochem Sp. z o.o.

Candidate CRM selection

Natural matrices

Matrix material CRMs, such as those produced by RTC and agencies including NIST and BCR, must be the same as, or closely mimic, real-world materials. Users need a CRM that is as representative of an actual sample as possible, satisfies the greatest number of end users, meets the needs of regulatory agencies and is applicable to mandated methodologies, including those of the US EPA.

Selection of a candidate batch depends upon the intended analytical

methodology, the analytes of interest and the industrial waste stream that it represents. A candidate material must be homogenisable and stable in the long term. A clear understanding of both the waste stream and the associated industrial processes is also needed. The candidate must be representative of the general type of waste under investigation and, when a specific matrix is under evaluation, it must be representative of the industry. The constituents, the matrix and the physical state of the waste are each evaluated; any candidate material that does not meet all criteria is rejected.

Analyte selection

The choice of analytes is based upon regulatory requirements, the method selected and those typically found in a particular waste stream. This is particularly important in leaching tests, such as the Toxicity Characteristic Leaching Procedure, which measures the long-term natural leaching effects on a contaminated soil. A sample of the candidate material, which must be truly representative of the site, is analysed for the compounds of interest.

Most industrial processes produce a steady and relatively homogeneous waste stream, but for environmental sources, such as municipal incineration, the sample can vary greatly with time, both daily and through the year. A long-term collection scheme may be necessary, with the final candidate material a blend of many sub-samples collected over many months.

The analytical data are evaluated for the number and type of compounds present, their concentrations and the effects of any required stabilisation and sterilisation on the analytes. If it is not possible to find material with the right analyte profile, fortification may be needed. As the worst environmental sites are cleaned up, it is getting more difficult to find representative, naturally-accrued matrices with gross levels of pollution. The careful addition of the analytes concerned to the candidate material to achieve the desired level in the matrix (fortification) may be needed. The number of different analytes and their concentrations in the original waste stream are determined and a mixture is developed, using the analyte or analytes in a chemical form that mimics the actual raw waste that originally caused the pollution.

The goal is to ensure that the fortified material is as representative of naturally accrued material as possible. For example, simply adding a heavy metal in a convenient aqueous salt form or a pesticide in iso-octane is pointless. Spiked analytes will extract from the

matrix in a non-representative way and lead to falsely elevated results.

A bench scale batch is first produced. Selected analytes in the fortification mixture are added to the candidate material over an extended period and at various concentration levels using dry fluidisation and liquid-spray injection to obtain thorough distribution and optimum homogeneity. After addition, the material may be further homogenised before it is analysed to see if the required fortification level has been achieved. This is a somewhat empirical process, especially with organic fortification, because losses during processing are not always consistent and the matrix effect can be significant. Research has shown that analyte recovery may vary after long-term equilibration or ageing of the fortified matrices, so ageing checks are a vital part of the development process. Once a satisfactory procedure has been developed, it is repeated on a 250 to 500 kg batch sample.

All candidate materials, either naturally accrued or fortified and with an acceptable analyte profile, are then submitted for stability testing, with the remainder "aged" for analyte recovery evaluation.

Pesticide reference materials

Calibration CRMs intended for routine use have to be made in large batches and at a commercially realistic cost. To meet these dual objectives and to comply with the various ISO REMCO Guides,³ IPO found it necessary to develop its own, unique procedures.

The main factors for selecting a candidate CRM are demand from the marketplace and availability of candidate materials in sufficient quantity. According to ISO Guide 34 (Annex 1), a pure compound CRM should have a thoroughly established identity and an accurately known purity. Sometimes it is very difficult to achieve both of these requirements when working with large quantities of complex organic molecules such as pesticides. IPO's procedures provide the information needed for selection and certification. Reduced to essentials the procedures require that:

- The identity of the reference material must be confirmed by at least two or more spectral techniques, usually IR, mass and/or NMR spectroscopy.
- The purity of the candidate reference material should be examined by all suitable methods, to determine the levels of as many impurities as possible, so allowing the estimation of purity by difference.



Figure 1. Annular change can heavy duty mixer used for soils and sediments prior to homogenisation.

Sample preparation

Matrix materials

Modification of the matrix material by sieving, grinding or filtration is a major issue in preparing a reference material. Many certification agencies routinely grind candidate materials to a fine, homogeneous powder to improve intra- and inter-bottle homogeneity. However, this does not reflect conditions in the real world, where samples are crushed and sieved to exclude all particles above a certain size. It is well known that the matrix effect can be significant when analysing soils, sediments and other environmental substrates. To have a major physical difference between CRM and sample is inadvisable.

Modification of the matrix can have adverse effects on the material's ability to represent a real-world sample. Extensive testing is needed to ensure that after a material is prepared, it retains important matrix characteristics.

Specialised mixing and ancillary equipment are needed to handle all

types of sample matrices, including mixers for solids, sludge and liquids, as well as sample preparation equipment such as jaw crushers, cone grinders and sieves. Bottle fillers are required to handle liquids, sludges and powders, both flowable and non-flowable. Some examples are shown in Figures 1–3.

The first production stage is particle size reduction, followed by coarse sieving. Sterilisation processes, needed to ensure stability over five years, are carried out, followed by homogenisation. Often it is necessary to evaluate the various processing options to choose the one most appropriate for a particular matrix and the analytes of interest. Factors to consider include:

- Determination of the maximum and minimum particle size for homogenisation
- The ability to obtain a required particle size
- The inherent stability of the candidate material.

Pesticides

The starting point for pesticides CRMs is usually a technical grade commercial pesticide. The exact formulation and detailed analysis of impurities is rarely available, so the active substance has to be purified out using one or more of the techniques in Table 1.

Often it is not possible to obtain suitable commercial material, or the active compound is not robust and breaks down under the conditions outlined above. It is then necessary to synthesise the candidate material directly in accurately controlled conditions, using specially purified substrates. The original process is often found in the literature, so the starting place is at least known, but over the years the process will have been optimised and developed, so the impurity profile of commercial material is very different from the original material. All of this provides some interesting synthetic challenges.

Once pure pesticide material has been produced it must be carefully homogenised, usually by drum mixing in the case of a liquid, or by milling and sieving solids.

Stability

ISO Guide 31 draft, *Contents of Certificates*, contains explicit requirements for an expiry date to be indicated, **in all cases where stability has been demonstrated or is considered possible.**

In pure form, all natural matrices and many modern pesticides can be consid-



Figure 2. Crossfire "V" blender used to homogenise powders. This machine stands 2 m high and 3 m long. It can hold 250 kg of material.

ered potentially unstable, CRMs have to have a stated shelf life. This demand does not pose insurmountable difficulties but adds to cost.

Matrix materials

Microbiological contamination is the main cause of change in analytical values over time. Also, most overseas markets require that all imported soil materials be free from pathogenic bacteria. For these reasons, all samples are checked for sterility and, if needed, sterilised. The sterilisation techniques used are crucial; any procedure must effectively eliminate biological activity without significantly altering the matrix or the analysis of the material.

All finished CRMs released for distribution are subject to a continuous stability-testing programme in which matrices that are considered to be subject to microbial degradation are regularly re-analysed to ensure continued stability.

Pesticides

In their pure form, not all pesticides are inherently stable. During purification and analysis, information about the stability of the molecule is gathered. This is useful in determining the long-

Table 1.

Decolourisation	Using activated charcoal, silica, alumina or Florisil
Crystallisation	With at least two solvents of different polarity
Distillation	Sub-boiling distillation for sensitive molecules, or when impurities have similar distillation characteristics at the normal boiling point
Chromatography	Traditional column or preparative HPLC or together
Extraction	Liquid-liquid, to remove acidic or basic compounds



Figure 3. Variable stroke and bore filler for sludges and liquids (left) and filler for dry flowable and non-flowable materials.

term stability of the certified material and assigning an initial shelf life. All batches of every IPO certified reference materials are monitored on a routine basis to estimate their continuing stability.

Two main procedures for performing these examinations have been established. Once purified, newly developed products are tested using the CIPAC accelerated storage procedure.⁹ The compounds are kept at 54°C for two and four weeks. Quantitative assays are carried out during the test period, and from this data, the shelf life at normal storage conditions can be estimated. This test is widely used in the pesticide industry. Finished and packaged certified reference materials are regularly examined and assayed. From this checking an empirical shelf life is determined, which is amended as further data are accumulated.

Homogeneity

Thorough homogenisation of the candidate CRMs is essential to the production of a quality reference material. Both producers have researched a variety of techniques to homogenise all types of matrices effectively. The process of combining a mixture of materials, such as found in sewage sludge, to produce a homogeneous mixture depends on the characteristics of the materials to be blended, the type of equipment used, blending time and the intensity of mixing, together with a considerable amount of experience. Because most mandated methods specify sample size, variability within units needs to be checked to ensure the recommended sample size will produce valid results.

After mixing, both matrix materials and pesticides material are verified for homogeneity. The material is spatially sampled to obtain a cross-section of the material. This is the best method to

detect inadequate mixing that may produce stratification of the materials and heterogeneous lots. Samples are submitted for single laboratory replicate analysis for selected parameters, based on material composition.

The resultant data are verified for homogeneity by the use of several statistical tests. The tests, which analyse the variances of the analytical data, have been selected as the most valid method to evaluate the homogeneity of bulk lots critically. All candidate CRMs must pass the homogeneity tests prior to packaging; any that fail are either remixed or rejected.

Packaging

Packaging of the homogenised batch into smaller aliquots or sub-samples must be done in a manner that maintains both between- and within-bottle homogeneity.

For matrix samples, specialised filling machines are used to fill the selected containers precisely, which can range from ampoules to five-gallon drums. Selection of the equipment appropriate for a particular filling job is critical to maintain sample homogeneity throughout the filling sequence. Figure 3 shows two types of filling equipment used to fill packs with sludges, liquids and dry material.

The choice of packaging equipment depends on the characteristics of the CRM. For dry substances, the variables include the flowability of the material, its density and/or particle size variations. For liquids and sludges, flow and density characteristics are important factors when selecting both packaging equipment and packaging materials. The type of packaging material also depends on the type of analysis for which the CRM was prepared and the physical characteristics of the material itself. For the determination of organic analytes the CRM must be packaged in glass containers with Teflon lined closures. For inorganic analysis, CRMs may be packaged in polypropylene containers with non-metallic closures.

Pesticides are packed in pre-cleaned brown glass vials under an inert gas atmosphere, for long term stability.

Certification of analytes

Matrix materials

An intensive, single method inter-laboratory round-robin analysis programme certifies analyte values for each CRM. Inter-laboratory testing eliminates the inherent biases of intra-laboratory analysis and provides a broader

statistical base from which to calculate values.

Based on certification requirements a minimum of 20 laboratories are used to establish certification values. Samples are selected at random from the filling sequence to obtain a representative cross-section of the sample lot. The samples are sent to the laboratories for analysis using approved methods. The data are statistically evaluated to ensure normal distribution. Statistical outliers are removed, and calculation of a mean, standard deviation and a 95% confidence window are performed.

Although Referee Laboratories play a role in setting certified values, an intensive single method inter-laboratory round-robin analysis programme is the main source of analyte values used in the certification of a CRM. Experience has shown that using a round-robin analysis programme, with a minimum of 20 laboratories, provides a true picture of laboratory performance, eliminates intra-laboratory bias, the inherent biases of intra-laboratory analysis and provides a broader statistical base from which to calculate values.

Data generated from the round-robin laboratory study are evaluated for distribution and variance as a double check on the homogeneity testing. For certification, data are statistically evaluated to ensure normal distribution. Statistical outliers are removed, and calculation of a mean, standard deviation and a 95% confidence window are performed.

Occasionally data generated by the round-robin analysis are unsatisfactory for certification. When this occurs, a thorough review of all aspects of the batch production, as well as the method, is undertaken to investigate for any possible errors or method bias.

Pesticides

Analysis of candidate pesticides materials poses its own set of problems. Rather than the analysis of a number of analytes within a matrix, the aim is to prove both the identity of the pure substance and the levels of the many trace impurities present. Together this allows an accurate estimation of the purity of the material and proof of identity.

Confirmation of identity

Identification is the most important step of the certification process. The intention is always to use two or more techniques, each based on a different analytical principle, to confirm identity. When the pesticide is a mixture of several isomeric forms or is one of a number of possible isomers, confirmation the structure must be carried out very

carefully and alternative techniques, such as ^1H or ^{13}C NMR, must be used. Even then, there may be some doubt about specificity of the methods used, as it is often necessary to rely on techniques that are based on or related to a similar principle.

Determination of purity

Direct or indirect analytical techniques can be applied for purity determination of organic compounds. Direct techniques include accurate titrimetric methods, but these are rarely applicable to the assay of pesticides. In most cases, the only way to make a purity determination is to separate out and determine the assay of each individual impurity. The purity of the candidate material is then established by difference. The isolation and characterisation of all impurities poses a great challenge. To determine organic impurities, all of the following techniques are needed.

- Gas chromatography with flame ionisation detection, using on-column injection and fused silica “megabore” columns, with a minimum two columns of different polarity
- Gas chromatography-mass spectrometry
- High-performance liquid chromatography in reverse or normal mode, with ultraviolet and/or diode array detection
- Thin layer chromatography as a semi-quantitative method of impurities content estimation
- Differential scanning calorimetry

A thorough knowledge of the advantages and disadvantages of each technique and an understanding how the molecule under investigation will inter-react with each technique is needed to use the various procedures to maximum advantage. For many compounds, only a limited number of analytical methods can be applied, due to the chemical structure or inherent properties of the molecule. In all cases, the nature of the pesticide and the possibilities offered by the various different methods must be carefully considered. As with confirmation of identity, there is often some lingering doubt that enough methods have been used, as the limitations arising from the inherent characteristics of the molecule under investigation often make it necessary to rely on techniques that are based on, or related to, a similar principal, such as HPLC or GC with different columns.

In addition to organic impurities, most pesticide formulations are contaminated with inorganic materials. The most commonly found are sulphated

ash, water content and free acids or bases.

Production of the certificate

The final stage in the production of a CRM is the careful analysis and review of all available data. Although the ISO guides offer assistance, it is very general and there are no systematic rules for the selection of the proper values. Every aspect of the evaluation of the results, especially defining criteria for the exclusion of outliers, requires a complete understanding of the chemistry of the matrix or pesticide and the procedures used for synthesis and purification of the candidate material.

For the matrix materials, reported data from the PT scheme members is used to calculate reference values. The US EPA’s “BIWEIGHT” software, based on Kadafar’s work¹⁰ is used. This is a robust statistical method as recommended in ISO Guide 43.³ The BIWEIGHT program generates a “Student’s T” like statistic for constructing confidence intervals on data sets that may have heavier tails than a classical Gaussian distribution. This is appropriate for symmetric, stretch-tailed curves that are often encountered in the analysis of homogenous samples via inter-laboratory studies. The BIWEIGHT method is also more robust in handling data that results from determinations at, or near, the method detection limit. For data sets that are Gaussian, the BIWEIGHT estimates are comparable to traditional calculation methods. The BIWEIGHT mean, standard deviation and standard deviation of the mean are used to calculate the 95% confidence interval (CI) for the mean and the 95% prediction interval (PI). For normally distributed data, the 95% PI typically represents approximately a 2SD around the mean.

Discussion and conclusions

RTC has certified more than 100 natural matrix reference materials in the last 13 years. Some have been re-issued and are now on their 3rd or 4th lot. Market acceptance of the CRMs has been high. In 20 years, IPO has certified more than 200 pesticides, metabolites and impurities. This represents a significant contribution to the range of CRMs available.

The technique of using data from a laboratory proficiency testing scheme to certify matrix and other materials is

becoming more widely acceptable. In The Netherlands, the Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling, Institute for Inland Water Management and Waste Water Treatment, (RIZA),¹¹ working in an informal cooperative research and development arrangement, produced and certified five matrix CRMs, the first of which is described in the literature.¹² The Institute of Inter Laboratory Studies¹³ has also used data from an existing performance testing schemes to certify six petroleum materials. A Cooperative project between RTC, Promochem GmbH and The Wageningen Evaluating Programs for Analytical Laboratories (WEPAL), at the Wageningen Agricultural University (WAU) in Wageningen, The Netherlands, certified eight soils using data from the WEPAL ILS scheme.¹⁴

Even with all the alternative approaches described, it is not always possible for a NIST or BCR CRM to be included in the analytical procedures employed, usually because there is no suitable CRM available. Therefore the CRMs produced are not always traceable, in the true sense of the word, back to the mole, although they are traceable to a method or a procedure. The manufacturers make great efforts to ensure CRMs are fit for purpose.

When certifying pesticides, there is often a mass of data available, sufficient to prepare a certificate and to offer the material for sale as a certified pesticide reference material. However, the team at IPO is often left with a nagging doubt that they have not yet answered the real questions, which include:

- What does purity really mean in terms of complex organic molecules?
- How should the purity of such molecules be really examined?
- What is the real meaning of traceability to the mole in the commercial analytical world?

These questions raise further questions. Is the concept of traceability to the mole really important in the certification of complex organic molecules? Should we be looking at other ways, for example traceability to the elemental composition, to a method or to a public reference material, spectra or other constant? Elemental analysis might be suitable for a final confirmation of both identity and purity, as discrepancies due to entrapped solvent or other impurities would quickly be revealed. It is worth noting that in other scientific disciplines, such as pharmaceutical analysis, forensic toxicology and clinical chemistry, where the determination of very large mole-

cules is normal practise, certification as defined by ISO is not common and traceability to the mole not a priority.

Certain Institutional, publicly funded producers have expressed doubts about the methods of certification used by RTC, IPO and other commercial producers. The problem seems to be that while they agree that more CRMs are needed, beyond their production capacity, the scientists concerned find it difficult to come to terms with the consequences of commercial pressures. There seems to be a gap between the admission that alternative modes of production are needed and the inevitable compromises this brings.

The adoption of ISO 17025³ as an accreditation standard by the producers of certified reference materials has barely started. It can be argued that the adoption of this standard will bring all producers to the same level, and further harmonise quality of CRMs and improve user confidence.

Another difficulty is that the existing ISO definitions for a CRM and a RM no longer fit the needs of the market. There is clearly a need for a thorough revision of the definitions in the various ISO Guides to reflect commercial reality so that it includes suitable definitions for biological reference materials, pharmaceutical reference substances and reference materials that are fit for purpose, but not produced by conventional routes.

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