

Chemistry Specifications for Chemistry Services Contractors

National Toxicology Program

Characterization

Final

August 11, 2016

1. *Comprehensive Chemical Analyses (CCA)*

1. General Requirements

1. A characterization plan, including a milestone schedule shall be developed and posted to the NTP IMS for approval by the COR.
 1. The characterization plan shall describe the general analytical approach, and all the steps to be performed for the characterization of the chemical or test article, including, but not limited to the following.
 1. A list of the proposed analytical techniques.
 2. Method parameters, if known, e.g., chromatographic separation mechanism or polarity.
 3. Physical parameters to be determined, e.g., melting point, boiling point, color, optical rotation, density, etc.
 4. Compendial analyses to be performed, e.g., pesticide residues, metals content, nutritional analysis, etc.
 5. Whether Log P, volatiles content, elemental analysis, and/or accelerated stability determinations will be performed.
 6. Literature reference(s) that serve as a starting point for the characterization.
 7. A milestone schedule that includes dates for one or more of the following at the direction of the COR:
 1. Commencement of lab work
 2. Completion of lab work
 3. Completion of Draft Final report
 4. Commencement of QC review
 5. Commencement of QA review
 6. Submission of Draft Final report

2. Analysis Requirements

The contractor shall determine the following for all chemicals and test articles to establish their identity and purity, unless otherwise directed by the COR.

1. *Organics*

1. Determine the unequivocal identity of the chemical or test article using spectrometric techniques (including but not limited to infrared spectrometry (IR), Proton and C-13 nuclear magnetic resonance (NMR) spectrometry, mass spectrometry MS)), and elemental analysis. NMR spectrometry shall include 2D NMR analysis when appropriate.
2. Determine the physical characteristics of the chemical or test article, such as melting point, boiling point, optical rotation, etc.

3. Determine the purity of the chemical or test article using at least two different analytical techniques, for example:
 1. One chromatographic system and differential scanning calorimetry.
 2. Thermogravimetric analysis, elemental analysis, and electron microscopy.
 3. Two chromatographic systems with very different separation mechanisms, e.g., gas chromatography (GC) and thin-layer chromatography (TLC) or high performance liquid chromatography (HPLC), including ultra-HPLC (UPLC).
 4. Two chromatography systems that employ chromatographic techniques with distinctly different polarities, e.g., normal vs. reverse-phase HPLC; polar vs. nonpolar GC, e.g., DB-1 vs. DB-Wax.
 5. Purity analysis methods shall be maximized for separation of all components. Non-selective detectors shall be used (e.g., flame ionization, MS, short wavelength (~200 nm) ultraviolet (UV), charged aerosol, or evaporative light scattering ELS)).
 6. Determine the impurity profile and identify impurities at concentrations $\geq 1.0\%$. The COR may direct that impurities $\geq 1.0\%$ be quantitated against reference standards when they are available. In some instances the COR may require the Contractor to identify impurities at concentrations $< 1.0\%$ (Part 4. Low-Level Impurity Determination (LLID)).
4. Perform compendial analyses when appropriate.
5. When directed by the COR, the Contractor shall determine the log P value of the chemical using a literature method e.g., Gargas et al., 1989.
6. Determine the water content of the chemical using an appropriate analytical method.
7. Using a literature method, determine the volatiles content of all solids.
8. Determine the stability of chemical or test article after storage at temperatures of -20 , 5 , ambient, and $60^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 2 weeks.

2. *Inorganics*

1. Determine the unequivocal identity of the chemical or test article using elemental analysis and spectrometric techniques when appropriate.
2. Determine the physical characteristics of the chemical or test article including but not limited to melting point, particle size, etc., as appropriate.

3. Determine the purity of the chemical or test article using two different analytical techniques (e.g., titration and ion chromatography, or x-ray diffraction).
 4. Determine the impurity profile and identify impurities at concentrations $\geq 1.0\%$. The COR may require identification of impurities at concentrations $< 1.0\%$ (Part 4. LLID).
 5. Perform compendial analyses when appropriate.
 6. Determine the water content of the chemical using an appropriate analytical method.
 7. Using a literature method, determine the volatiles content of all solids.
 8. Determine the stability of bulk chemical after storage at -20 , 5 , ambient, and $60^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 2 weeks.
3. *Complex chemicals, test articles, or chemical mixtures.*
1. Complex chemicals, test articles, or chemical mixtures, e.g., dietary supplements, nanomaterials, asbestos, and organometallics, etc., shall be characterized to the same extent as organics and inorganics (e.g., purity, identity, stability, volatiles, water content, and impurity profile).
 2. Characterization may require the use of one or more purity and identity techniques in addition to the techniques found in Section 1.2.1. *Organics* or 1.2.2. *Inorganics*, above. Examples of requirements for complex test articles are given below:
 1. Herbals and Dietary Supplements
In addition to the requirements given in Sections 1.2.1 and 1.2.2, above, the Contractor shall characterize and/or quantitate dietary supplements for one or more of the following, at the direction of the COR:
 1. Nutritional content, heavy metals, pesticide residues, mycotoxins, and bacterial load at the direction of the COR.
 2. Specific constituents of the dietary supplement, e.g., sennocides in senna.
 3. For dietary supplements that are plant materials, a fingerprint analysis to determine that the test material obtained come from the correct plant species. This analysis may involve , but is not limited to, measurement of chemical marker compounds or polymerase chain reaction (PCR) analysis of DNA.

2. Nanomaterials

In addition to the requirements given in Sections 1.2.1 and 1.2.2, above, the Contractor shall perform one or more of the following analyses of nanomaterials, at the direction of the COR. See the National Cancer Institute, Nanotechnology Characterization Laboratory, Assay Cascade Protocols (http://nci.cancer.gov/working_assay-cascade.asp) for more information.¹

1. Size determination, including diameter and length (for fibers). Size determination may involve, but is not limited to, the use of dynamic light scattering (DLS), atomic force, scanning or transmission electron microscopy (AFM, SEM, or TEM).
2. The Contractor shall determine the chemical composition of nanomaterials, including metal content.
3. The Contractor shall determine surface characteristics, including one or more of the following, at the direction of the COR:
 - Zeta potential
 - Electrolytic conductivity of nanoparticle suspensions
 - pH of nanoparticle suspension

2. Additional Requirements

1. All measured physical parameters, spectra and chromatograms shall be compared to a known standard or standard literature reference value, when available.
2. The contractor shall report the work done in this functional activity following the reporting requirements given in Section 4. Reporting Requirements.

2. *Chemical Identity and Purity Screen (CIPS)*

1. Analysis Requirements

1. At the direction of the COR, the contractor shall determine the identity and estimate the purity of a chemical or test article selected for study. A CIPS may be applied to any test article, including high throughput screen (HTS) chemicals, as a purity and identity check upon receipt, or in lieu of more extensive characterization.

¹ NCI Nanotechnology Characterization Assay

1. Unless directed by the COR, the analysis shall consist of a single analytical system, selected to unambiguously identify the chemical and estimate its purity simultaneously.
2. The system selected for each analysis shall be the simplest and most cost-effective system that can successfully meet the analysis criteria.
3. When a chromatographic method is used, it shall employ temperature or mobile-phase gradients, which achieve satisfactory but not necessarily baseline separation of all chemical components.
4. Typical analytical systems, which may be used for this purpose include the following:
 - NMR (proton and carbon)
 - Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES) or ICP-MS for inorganic compounds
 - HPLC-MS, including UPLC
 - GC-MS

2. Additional Requirements

1. All measured physical parameters, spectra and chromatograms shall be compared to a known standard or standard literature reference value, when available.
2. The contractor shall report the work done in this functional activity following the reporting requirements given in Section 4. Reporting Requirements.

4. *Low-Level Impurity Determination (LLID)*

1. At the direction of the COR, the Contractor shall identify or identify and quantitate impurities in a chemical, test article, or sample present at concentrations $\leq 1.0\%$. Typical analytical systems, which may be used for this purpose include the following:
 - NMR (proton and carbon)
 - Fourier Transform Infrared Spectrophotometry (FTIR)
 - Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES) or ICP-MS for inorganic compounds
 - HPLC-MS, including UPLC
 - GC-MS

2. The contractor shall report the work done in this functional activity following the reporting requirements given in Section 4. Reporting Requirements.

5. *Chemical Reanalysis (CRA)*

1. At the direction of the COR the Contractor shall perform the following analyses on bulk chemicals or test articles.:
 1. The Contractor shall use previously developed methods, as described under Part 6. Protocols Development report (PD) to determine the purity of a chemical or test article relative to a frozen reference standard of the same lot or other reference standard specified by the COR. When the CRA analysis requires the use of a substance-specific SOP, it must be in place prior to its use.
 1. The Contractor shall evaluate the suitability of the analytical system used for the analysis relative to precision, theoretical plates, resolution, and tailing factor.
 2. Analysis will use three replicates; additional replicates may be run at the discretion of the COR.
 1. The contractor shall calculate a relative response factor (RRF) for each replicate analysis of the chemical or test article and the frozen reference sample of the same lot.
 2. A mean and 95% confidence interval (CI) shall be calculated for the frozen reference sample RRF.
 3. The RRF for the chemical or test article is compared to the RRF for the frozen reference.
 4. When the RRF for the chemical or test article, lies outside the 95% CI of the frozen reference standard, the testing lab shall be instructed to contact the study director.
 3. When multiple chemical reanalyses have been performed on the same lot of a chemical, the Contractor shall plot the results, including the acceptability criteria for the analyses as \pm bounds in the graph.
 2. The Contractor shall perform a CRA for chemicals or test articles submitted by NTP testing laboratories within 1 week of receipt. The COR will provide a schedule describing the expected shipment dates for CRA samples from each NTP study for which the Contractor is responsible for CRA analyses.
 3. The Contractor shall perform a CRA of chemicals or test articles prior to shipment of bulk chemicals that have been stored for 6 months or longer since the previous characterization.

2. The Contractor shall report the work done in this Functional Activity following the reporting requirements given in Section 4. Reporting Requirements.

3. *Multiple Chemical Identity and Purity Screen (MIPS)*

1. Analysis Requirements

1. At the direction of the COR, the contractor shall determine the identity and estimate the purity of chemicals or test articles selected for study. A MIPS may be applied to any set of chemicals or test articles, including high throughput screen (HTS) chemicals, as a purity and identity check upon receipt, or in lieu of more extensive characterization.
 1. Unless directed by the COR, the analysis shall consist of a single analytical system selected to unambiguously identify the chemical or test article and estimate its purity simultaneously.
 2. The system selected for each analysis shall be the simplest and most cost-effective system that can successfully meet the analysis criteria.
 3. When a chromatographic method is used, it shall employ a temperature or mobile-phase gradient that achieves satisfactory but not necessarily baseline separation of all chemical components.
 4. When the set of chemicals or test articles is part of a chemical class, the analytical method developed shall be such that it can be applied to all other chemicals or test articles in the class with minimal optimization.
 5. Typical analytical systems, which may be used for this purpose include the following:
 - NMR (proton and carbon)
 - Inductively Coupled Plasma–Atomic Emission Spectrometry (ICP-AES) or ICP-MS for inorganic compounds
 - HPLC-MS, including UPLC
 - GC-MS

2. Additional Requirements

1. All measured physical parameters, spectra and chromatograms shall be compared to a known standard or standard literature reference value, when available.
2. The contractor shall report the work done in this functional activity following the reporting requirements given in Section 4. Reporting Requirements.