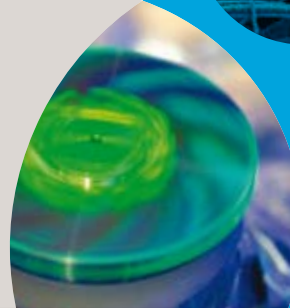
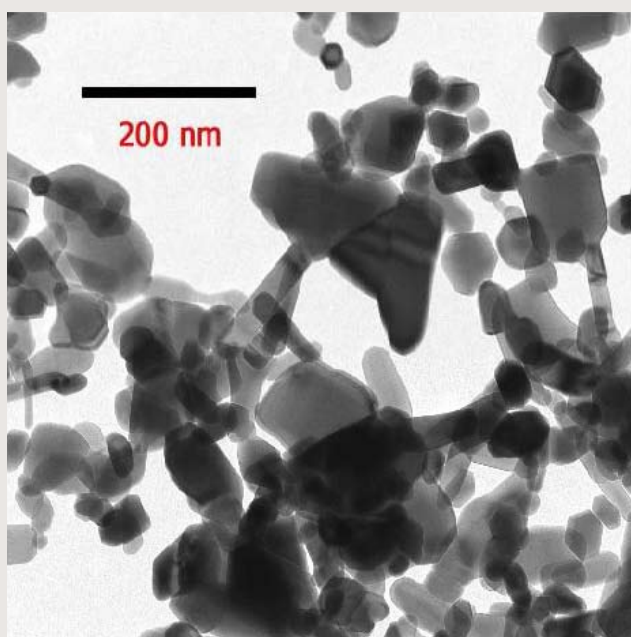


## Guidelines and Protocols for Sampling



18 May 2010

IN SUPPORT OF PROSPECT:

Ecotoxicology Test Protocols for Representative  
Nanomaterials in Support of the OECD Sponsorship Programme

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Cover photo: BASF SE Z-COTE® zinc oxide nano particles (Image courtesy of BASF SE)

## FOREWORD/PREFACE

PROSPECT<sup>\*</sup> is the UK's contribution to the OECD Sponsorship Programme<sup>†</sup> to examine the environmental safety of nanomaterials in accordance with the agreed OECD WPMN 'Guidance Manual for Sponsors of the OECD Sponsorship Programme for the Testing of Manufacture Nanomaterials'.<sup>[1]</sup> It will provide crucial data to the OECD work, by addressing gaps in the current level of knowledge on the physico-chemical and ecotoxicological properties of these materials, followed by fundamental scientific research leading to establishing scientific test methodologies to study those endpoints that may not be assessed through standard tests used for bulk chemicals.

PROSPECT is a public-private-partnership dedicated to supporting the safe and responsible exploitation of nanomaterials, and developing a better understanding of their impact on humans, and the environment. PROSPECT has been created and is lead by the Nanotechnology Industries Association (NIA). More Information on PROSPECT is available at the following websites: PROSPECT: <http://www.nanotechia-prospect.org/home/home> and NIA: <http://www.nanotechia.org>.

This document is to provide guidelines and protocols for the sampling of nanomaterials. It is to be submitted as part of the third deliverable of the PROSPECT Project.

Any mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use.

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<sup>\*</sup> **PROSPECT: Ecotoxicology Test Protocols for Representative Nanomaterials in Support of the OECD Sponsorship Programme'**

<sup>†</sup> OECD's Working Party on Manufactured Nanomaterials (WPMN) launched a Sponsorship Programme in November 2007. The programme involves OECD member countries, as well as some non-member economies and other stakeholders to pool expertise and to fund the safety testing of specific Manufactured Nanomaterials (MNs). In launching this Sponsorship Programme, the WPMN agreed on a priority list of 14 MNs for testing (based on materials which are in, or close to, commerce). They also agreed a list of endpoints for which they should be tested. Much valuable information on the safety of MNs can be derived by testing a representative set for human health and environmental safety.

# 1.0 Introduction

According to ISO/IEC 10725, “sampling” is defined as “a procedure whereby a part of a substance, material or product is taken to provide for testing or calibration a representative sample of the whole. <sup>[2]</sup> Sampling may also be required by the appropriate specification for which the substance, material or product is to be tested.” In other words, the goal of sampling is to select and obtain a test portion of the material in some manner, such that the sub-sample is representative of the larger amount of material.

Sampling is often a major source of error and if a truly representative sample of the batch is not obtained, then the subsequent analysis will give a wrong figure. <sup>[3]</sup> It is the aim therefore to develop correct sampling protocols, considered to be an essential requirement for obtaining valid results within PROSPeCT.

Two types of sampling errors are possible:

a) **Segregation errors**

This occurs when particles are exposed to gravitational, rotational, vibratory or aeration operations (or other types of mechanical motion), usually resulting in fine particles migrating to the bottom and larger particles being concentrated at the top. <sup>[4]</sup> This type of error is thus dependent upon the previous history of the powder and usually occurs with free or easily flowing powders, having a significant range of particle size. This type of error can be minimised by suitable mixing and building up the sample from a large number of increments.

b) **Statistical errors**

This type of error is caused by observing a sample instead of the whole population. Although this type of error cannot be prevented, it is likely that the size of statistical errors can generally be controlled by taking a large enough random sample from the population. <sup>[4]</sup>

In this document, we will review the following information found in the current literature:

- a) various sampling methods currently available (for both powders and those dispersed in liquid media); the pros/cons of each method will be compared.
- b) summary of the “golden rules of sampling”.
- c) protocols recommended for sampling, developed from guidelines found in ISO related documents. [Note: It is important to realise that in the current state of development, the testing/standardisation of measurement methods specific for nanoparticles is very much limited; most of the current literature is geared towards sampling of particulates/ powders in general. Nonetheless, such existing guidelines will provide a good basis to be further developed for the specific nanoparticles under study.

The protocols presented here are designed for laboratories equipped with a limited range of equipment.

## 2.0 Background to Sampling

### 2.1 Sample Reduction for Powders

With reference to powder samples, numerous methods are available for sampling with the main purpose to minimise systematic errors introduced both in collecting the gross sample and in reducing its size to the analytical sample. Common sampling devices used to reduce sample size to a manageable quantity, include <sup>[4,5,6]</sup>:

a) **Scoop sampling**

This technique is the simplest for sample division and involves an operator, using a scoop, to extract laboratory samples from some portion of the bulk sample.

b) **Cone and quartering**

This technique involves placing the sample on a flat surface in the form of a conical heap. The heap is then spread out and fattened into a circular cake, which is then divided into approximately equal quarters. One pair of opposite quarters is removed, combined and formed into a new cone for the process to be repeated (with the other two quarters discarded). The process is repeated as many times as is necessary to obtain a sample of the required size.

c) **Table sampling**

This utilises tilted surface (in which there is a series of holes and splitting prisms) over which a powder sample is allowed to slide. The prism break the stream into fractions and some of the powder will fall through the holes (and then discarded). Ultimately, at the bottom of the plane, a decreased quantity of sample is collected.

d) **Chute riffler**

This utilises chutes i.e. funnelling or channelling device, to divide the powder. Unlike the spinning riffler, the chute riffler has no moving parts.

e) **Spinning riffler** (also called rotary sample divider)

This utilises a series of smaller receivers (or collection tubes), mounted in such a way so as to collect a flowing powder stream over a very short time period. The powder flows from a “hopper” to a “vibratory chute” and then to a “receiver” that holds the containers, which are rotating in a circular motion at a constant speed.

In the work done by Allen and Khan, using sand-sand mixture (in particular a mixture of coarse sand (420 – 500  $\mu\text{m}$  in size) and fine sand (120 – 250  $\mu\text{m}$  in size), with volume fractions of 60% and 40 % respectively ; the coarse and fine binary mixtures were prepared by sieving and blending.), the different sampling methods were evaluated and compared. <sup>[7]</sup> The data presented in Table 1 shows the relative standard deviation (r.s.d.) values obtained via each method; reproducibility of the technique was based on the observed r.s.d. between the different sub-samples generated from the primary sample. In addition, the table shows the corresponding advantages/disadvantages of the various sampling techniques. <sup>[5,7]</sup>

Sampling Device	Advantages	Disadvantages	r.s.d (%)
Scoop Sampler	Reliable for sample that are homogeneous and exhibit poor flow characteristics	Not suitable for heterogeneous sample. All of the bulk material does not go through sampling process. Operator dependent; operator decides where to scoop and what quantity to extract. Sampling more likely to be atypical due to segregation of the material	5.14
Cone and Quartering	Good for powders with poor flow characteristics (and minimal segregation)	Operator dependent; errors can occur due to differences in the manner the heap is formed and sub-divided	6.81
Table Sampler	Ability to separate large quantity of material	It is necessary that the incoming powder to be uniform and consistent. Hence, dependent on the initial feed being uniformly distributed and a complete mixing after each separation, a condition not in general achieved.	2.09
Chute Riffler	Ability to reduce powder samples in half after one pass	The technique is subject to error and operator bias if segregation is allowed to occur in loading the bulk-sampling trough.	1.01
Spinning Riffler	Reliable for free-flowing powder	Inability to do large quantity of powder efficiently.	0.125

Table 1. A Summary: Comparison of Powder Sample Reduction Techniques. <sup>[5,7]</sup>

From Table 1, the collection of methods used in obtaining the sample can be sub-divided into two:

- a) operator dependent methods.
- b) non-operator dependent methods.

In general, the spinning riffler seems to be the most reliable method for sampling as it seems to show little operator bias. <sup>[8]</sup> Although spinning riffles are generally recommended, it is important to understand that this may not be suitable for every nanoparticle material. For example, according to Table 1, this method is only suitable for free-flowing powders. If a spinning riffler is to be used for the PROSPeCT project, then the riffler must be properly validated for all of the PROSPeCT nanoparticles and appropriate guidelines from ISO 14488 should be incorporated into the development of the sampling protocol. <sup>[9]</sup>

Spinning riffles are available in different sizes, in which common commercial systems can provide samples ranging from about 0.5 g to 300 g. <sup>[5]</sup> Spinning riffing is often suitable for the first sample reduction, a step that divides kilograms to hundreds of grams. The next step is to further subdivide in the order of a few grams and this is often referred to as a “laboratory” size sample; again spinning riffing may be suitable for this step. This laboratory

sample size however may not be suitable to be introduced to the instrument and so, the sample size has to be further reduced, and in which milligrams worth of sample is often required. In the last step of sample reduction, the scoop sampling method is considered best, even though this reduction process will almost always will introduce some operator bias, which will be highly dependent on the actual spread in the distribution.

## 2.2 Sample Reduction for Nanoparticle Dispersion/Suspension

According to ISO14488, there are two possible options for sample splitting i.e. either using a pipette or by multiple capillary tubes.<sup>[9]</sup> The latter is achieved by allowing the sample to flow through a set of capillary tubes (capillary tubes typically have a bore size of 0.7 mm) spaced symmetrically in a vessel and is suitable only if there is a requirement to sample multiple samples simultaneously. The advantages/disadvantages of the two types of liquid sampling methods are summarised in Table 2.

Technique	Advantages	Disadvantages
Sample splitting by Pipette	Quick and simple to do Best suited for powders with a maximum particle size of about 20 – 40 µm	Not well suited for powders having a large variation in particle density, shape or size. Method may cause systematically low contents of coarse particles if used for powders containing coarse and/or dense particles.
Sample splitting using Multiple Capillary Tubes	Allows multiple suspension samples to be obtained simultaneously.	Laborious/time consuming method. Method prone to contamination and blocked capillary tubes.

Table 2. Comparison of Suspension Sampling Techniques.<sup>[9]</sup>

From Table 2 it is evident that the pipette method is not only simple but is less prone to contamination and therefore recommended for the PROSPeCT project.

According to ISO14488, prior to liquid (of dispersion) sampling, it is vital that the powders should have been sufficiently mixed with a dispersant to give a homogeneous dispersion.<sup>[9]</sup> It is important for the operator to check the dispersion stability using appropriate characterisation tools, particularly to see evidence of nanoparticle instability (such as aggregation and sedimentation) in the sampling vehicle. In the PROSPeCT project, sample splitting may not always be carried out with a stable dispersion; if this is the case, then this will incur a much higher sampling error.

## 3.0 Protocols Recommended For Sampling

### 3.1 The Golden Rules

Although the final sampling protocols will ultimately vary from one material to the next, it is important to always adopt the “golden rules of sampling” [10,11,12].

- a) Do not overfill sample containers, preferably no more than three-quarters full
- b) Use sufficient amount of material
- c) If using a spinning riffler, validate spinning riffler [†] to show that this method is suitable for the various PROSPeCT nanoparticles
- d) Sample where the material is best mixed, in motion if possible. For powders, don't stop powder flow; the use of specially designed instruments like a rotary/spinning riffler is recommended. If scooping, mix the sample beforehand and take several sub-samples across the bulk.
- e) Use clean instrumentation and containers
- f) Storage. Store in properly sealed containers. Avoid extreme temperatures, pressures and light exposure. If possible, for powders store under inert gas, such as argon or nitrogen.

As the reliability of any measurement will ultimately depend on the degree of representativeness of the test sample, it is important to adopt the golden rules of sampling when ever possible into our protocol.

### 3.2 Powder Sampling Using “Scoop Sampler” [13]

The “scoop sampling” method for powder division should be used when:

- a) the operator has no access to the spinning riffler OR
- b) when the nanoparticle properties is considered as to not being suitable for riffling e.g. exhibit poor flow characteristics OR
- c) when it becomes impractical to use spinning riffler because of insufficient sample material e.g. sub-sampling powders onto carbon pads for SEM (Scanning Electron Microscope) imaging purposes.

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† If a spinning riffler is to be used, ISO 14488 recommends the following steps to be incorporated in the development of sampling protocol:

Step 1. Before purchasing a riffler ensure that the design is such that: a) the hopper results in a constant/non-segregating and even mass flow of the sample material b) no material is to be lost outside the sample holders or remains trapped within the hopper, vibratory feeder, dividing ring or any part of the sampler c) material is not retained between the sample holders.

Step 2. Before using a spinning riffler, ensure that the sampler is clean

Step 3. During sampling, ensure that the material is carefully mixed before being placed in the feed hopper. Actuate the vibratory feeder and leave to operate until the entire sample has been divided.

Step 4. To clean the riffler. The hopper and vibratory feeder are to be cleaned while the riffler is still running, so that no material remains in the hopper or in the vibratory feeder.

Step 5. Possible Validation Method. The sampling method is to be validated for all new materials. The simplest validation to be performed when the riffler splitting technique is used is a mass validation. The following is recommended: (a) first, measure the mass of the gross sample together with the masses of all the increments; repeat this three times b) then, calculate the mean loss of the material. If the mean loss of material is larger than 1 %, then the riffler is deemed to be not working properly and this shall be corrected before further use. If the coefficient of variation in the mass of the increments is larger than 1%, then the riffler is also deemed to be not working properly.

Although the protocol below is written for sampling splitting of a large batch of nanoparticle powder ~250 g into 10 g aliquot, the method can easily be accommodated for sub-sampling to even smaller batches e.g. 10 g aliquots into 500 mg aliquots.

### **Materials**

Homemade “Isolator box” <sup>[§]</sup>, a cheaper alternative to a conventional glove box that can be easily introduced into or removed from a fumecupboard.

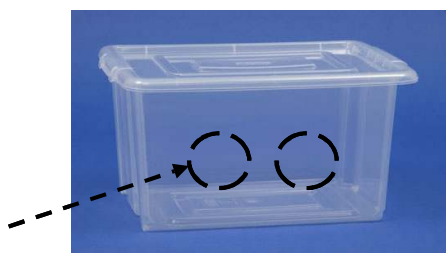
- Bottle of 250 g nanoparticles
- “Spatula spoon” (one end spoon and one end flat) and spatulas of various sizes – all made of stainless steel. These should be cleaned beforehand\*\*
- 25 pre-cleaned glass vials
- Dessicator with silica gel or appropriate desiccant or Vacuum packaging equipment <sup>[††]</sup>

### **Method:**

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#### § **Homemade “isolator box”**

This is a plastic storage box that has “hand holes” (made by cutting appropriate holes on one side of the box – see picture below):



Cut holes to allow  
your hands to get in

This box will prevent powder flying away, as under normal circumstances, the airflow inside a fume cupboard will be turbulent. Hence, this box will prevent loss of powder and contamination of the fumecupboard. It is ideal if the container is made anti-static. If static is problematic then “static bars”, also known as, static eliminators, can be incorporated into the box so as to remove static electricity (ensure that a clean static bar is used for this purpose). The dimension of the box should be large enough to allow sufficient space for the operator to carry out the sampling step with ease; recommended dimension of box LWD of ~ 90 cm x 50 cm x 50 cm.

\*\* Suitable detergent for **cleaning lab wares** can be used for cleaning. Ensure to rinse for a few minutes in running tap water, followed by final rinse with DI water. Tap off excess water, dry using lint free tissue and air-dry at least 1 hour before using.

#### †† **Vacuum packaging equipment**



This is an alternative to storage in a dessicator and highly practical for long-term storage; this method of storage will be suitable **for nanoparticles that are contained in bottles**. This is a convenient method, instead of storing lots of bottles in a dessicator.

Before starting, ensure to conduct full risk assessment i.e. COSHH and RISK assessments, for both normal operating practices as well as for possible spillage/or other accidents. Ensure that the operator has appropriate personal protective equipment to include goggles, anti-static gloves and lab coat. Sub-sampling should be done in the fumehood whenever possible.

- a) Place the following items inside the “isolator box”: clean spoons/spatulas, 25 pre-cleaned glass vials and a bottle of nanoparticles.
- b) Open the large bottle of nanoparticles.
- c) Using a large spoon, stir in the entire mixture several times. While stirring, take a scoop of nanoparticles and transfer to a clean sample glass container. Repeat for all other glass containers.
- d) Repeat step c) until all of the nanoparticles from the bottle has been transferred in roughly equal quantities into the 25 sample glass containers. Note: the size/ shape of the spoon/spatula used at any one time will be dependent on how much material is left inside the bottle of nanoparticles. The operator should use his/her own judgement as to choose the appropriate scooping utensil for such a purpose; the scooping utensil should be big enough to allow sufficient mixing of entire volume but small enough to scoop the sample out of the bottle with ease.
- e) Put the lids tightly on all sample containers.
- f) For long-term storage, air within the container should be replaced with inert gas. One way of achieving this is to put the open containers (that is filled with nanoparticles) inside a glove box and allowing the containers to sufficiently equilibrate with the inert gas (nitrogen or argon) atmosphere. Afterwards, replace the lids and take the sample containers out of the glove box. Vacuum pack individual containers using vacuum packaging equipment. Label each vacuum-packed sample container as appropriate, noting down date, contents, and name of operator. Store the vacuum packed vials inside a cool (~20 °C), dry cupboard; each sample aliquot can be taken out, as required. Once open, the sample vial can be further stored in a desiccator, with silica gel or suitable desiccant.

### 3.3 Sample Splitting by Pippette <sup>[9]</sup>

#### Material

- Nanoparticle dispersion (if possible, ensure that suspension is stable)
- Glass rod (pre-cleaned)
- Glass container (pre-cleaned)
- Glass or disposable plastic pipette

#### Method

During sampling, agitate the suspension (with glass rod, if possible) for a couple of seconds and quickly withdraw aliquots containing the required test portion using a suitable pipette. Use appropriate volume and orifice diameter; it is recommended that the largest particle diameters do not exceed 40% of the pipette diameter. Immediately, transfer the aliquot into a suitable pre-cleaned (glass) container. To avoid contamination, we recommend the use of disposable pipettes.

## References

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