DELIVERABLE REPORT

Deliverable:	D2.2 – 1st stage MNM library: externally sourced materials
Work Package:	WP2: MNM selection, acquisition, engineering, characterisation
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Project full title: " Engineered nanomaterial mechanisms of interactions with living systems and the environment: a universal framework for safe nanotechnology"

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Table of contents

Introduction

NanoMILE aims to develop mechanistic models for the interaction of MNMs with biological systems and the environment; in the light of this approach, the selection of the materials for study will originate from a systematic consideration of physico-chemical properties rather than the more commonly adopted approach of simple chemical compositions, industrial scale or perceived commercial relevance. The final selection of MNMs for mechanistic assessment of their interactions with living systems was based on a consideration of a number of factors, including:

1) The hypothesised mechanism of biological activity.
2) The availability of MNMs via one of the NanoMILE sources (project partners or affiliated projects).
3) The feasibility of systematically modifying key physicochemical parameters for the basic material type.
4) The availability of existing toxicological data (avoiding duplication).
5) The identification of gaps in existing materials evaluation.
6) Special needs such as for MNM detection and labelling (stable or fluorescence).

To achieve this, the MNM libraries to be developed in the NanoMILE project have been selected following careful review of other relevant projects (see review of current literature included in Deliverable Report D2.1), commercial availability, quality as well as expertise and capabilities available within the NanoMILE project. The NanoMILE project aims to be comprehensive in its coverage of MNMs, and thus a wide range of MNMs will be selected from the major classes of materials - metals, metal oxides, carbon based structures, functionalised structures and core-shell structures.

The MNM selection within NanoMILE is intended to be representative of each currently hypothesised toxicity mechanism (e.g. oxidative stress, Trojan horse delivery of elevated concentrations of ionic species, inflammatory responses, signalling impacts from adsorbed biomolecules, etc.), whilst also leaving room for discovery of as-yet-unanticipated mechanisms via the systems biology approaches. The ambition of the project is large, and the combination of high throughput (screening) and more detailed (mechanistic) studies allows for a broad coverage of materials, and also intelligent selection of exemplars for more detailed assessment of mechanisms of action.

Milestone 1 (MS1) consisted of a series of iterations with WP3-WP8 leaders, and a teleconference with WP6-WP8 leaders in order to select a list of MNMs to procure, functionalise & synthesize is also summarised in the present Deliverable Report to illustrate the selection criteria that were applied by the various WPs, and how these were integrated as fully as possible into a coherent final set of MNMs classes for investigation within NanoMILE. The “final” selection of MNMs is described and presented as the main outcome for this Deliverable report, and will also form the basis of the Phase 2 (systematically modified particles) and Phase 3 (bespoke particles designed to demonstrate that toxicity can be designed in or out of MNMs) in order to have a coherent body of data.

Clearly given the scale of NanoMILE, practicality and availability had to be taken into consideration during the selection of the Phase 1 particles in order to ensure that the WPs could get started in a

timely manner. However, we also made significant efforts to build on existing knowledge, and align strategically with other projects such as NanoREG (which has a significant focus on nanotubes) and NanoSOLUTIONS, which is our sister project, offering an alternative approach to the same call.

To counter the risk that the consortium end up collecting a lot of small amounts of disjointed data from which it is hard to draw conclusions, there are some materials that will be common to all WPs, and the screening WPs and biointeractions WPs are specifically intended to ensure a detailed match-up of data across all WPs. However, this integration across the whole project will be an ongoing management issue, and will be further reported upon in the phase 2 and phase 3 materials Deliverable reports. A detailed chart indicating which specific NPs are being utilised in which WPs will be maintained over the lifetime of the project, and will be included in Deliverable D2.3 at the end of Year 1 of the project, and in subsequent periodic reporting.

The NanoMILE approach to systematically varied MNMs

A tiered MNM procurement/production strategy is being adopted within NanoMILE, as follows:

- A first group of MNMs has been sourced from variety of external sources (industry, commercial, research repositories, MNM libraries from other EU funded programs/projects as well as national/international standards organizations) and will be rapidly available to the partners for establishment of test protocols and harmonisation of approaches within and across WPs. **This group of MNMs are described in the present deliverable report (D2.2).**
- A second group of MNMs will be produced by appropriate chemical or physical modification of selected members of the first group of MNMs. Key partners in WP2 will be involved in these modifications, including UoB, JRC and PROM. *This 2nd stage MNM library of engineered modification of externally sourced materials will be described in detail in D2.4, due at Month 18.*
- Finally, a third group of specialised and highly tailored materials will be fully synthesised in-house by selected project partners. Here the focus will be on development of nanoparticle libraries and on systematically designing out key properties linked to toxicity (e.g. dissolution or positive surface charge) and assessment of the effect of such changes on other physico-chemical properties and on biological impact. For example, it is conceivable that changing the dissolution potential of an MNM may introduce other toxicity features such as biopersistence, and these aspects will also be assessed, and recommendations regarding optimal design criteria developed, as shown schematically in Figure 1. *This 3rd stage MNM library of in-house synthesized nanomaterials will be described in detail in D2.4, due at Month 18.*

By adopting this three-pronged strategy NanoMILE intends to produce a sufficiently large matrix of MNMs in a relatively short time period so as to be able to benefit adequately from the availability of the high throughput test facilities which are a key aspect of this project. The procurement and production activities will be conducted in parallel.

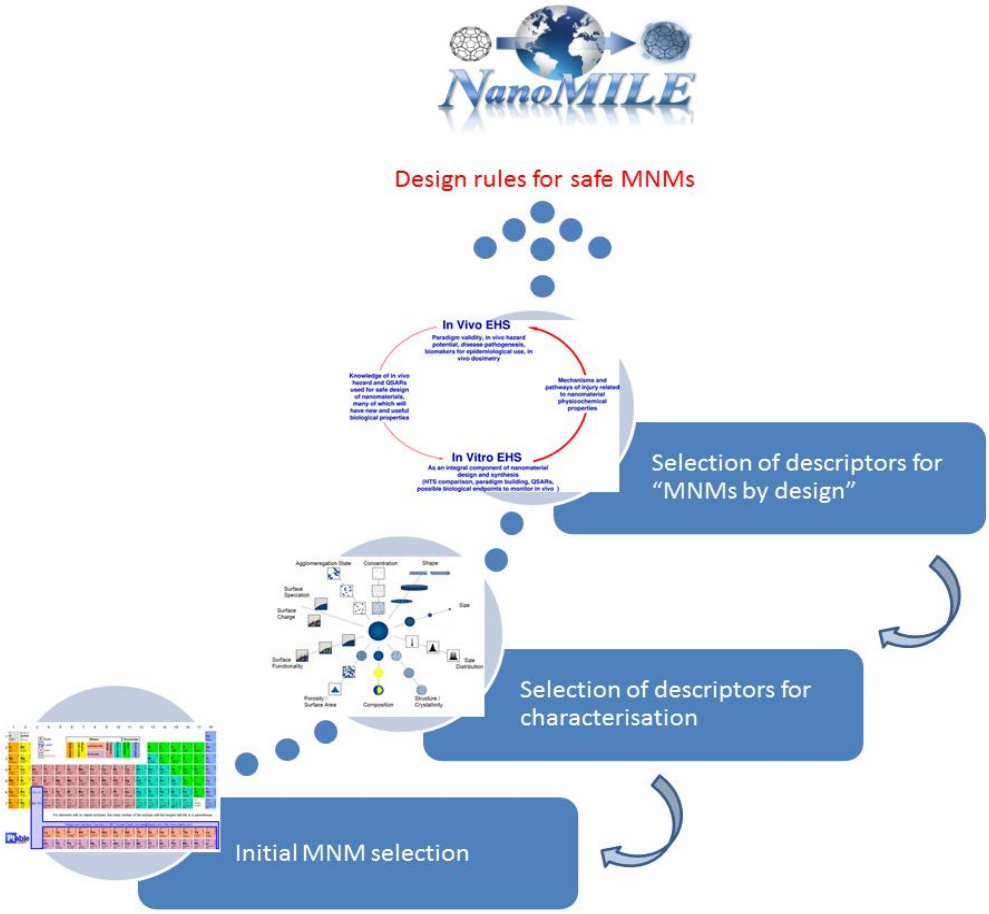

Figure 1: Spiralling-type approach to the NanoMILE MNM classification living document, where later phases are "inspired" by tentative efforts at defining the earlier stages. Note: graphical illustrations for stages 2 and 3 are from Hassellöv & Kaegi, 2009 and Meng et al., 2009, respectively.

Criteria for Selection/Synthesis of MNMs for NanoMILE

Following early work within NanoMILE which will discover systematically the precise mode of action of MNMs properties, key later activities will be carried out towards:

a) practically testing such features by designing them in or out (both at bench and pilot scale);

b) developing models of quantitative structure (property) –activity relationships (QS(P)ARs) enabling predictive work to evolve and feed into risk assessment; and

c) providing an integrated platform for risk assessment.

All materials procured or developed within NanoMILE are being subjected to extensive physicochemical characterization using state-of-the-art methods (imaging, compositional and structural), and following, where possible, established (e.g. QualityNano, NanoValid, NanoReTox) protocols, thus avoiding problems of unreliable cross referencing of experimental results.

Physico-chemical properties that are being characterised and systematically varied

Table 1 below shows the initial list of MNMs chosen for assessment within NanoMILE, and the justification of their selection on the basis of which physico-chemical parameters can be varied systematically in subsequent phases of particle synthesis and re-design (i.e. in the final stage whereby specific factors are *designed in* or *designed out* to validate their role in MNM toxicity as part of the safer by design strategy). An example of the approach is shown schematically in Figure 2.

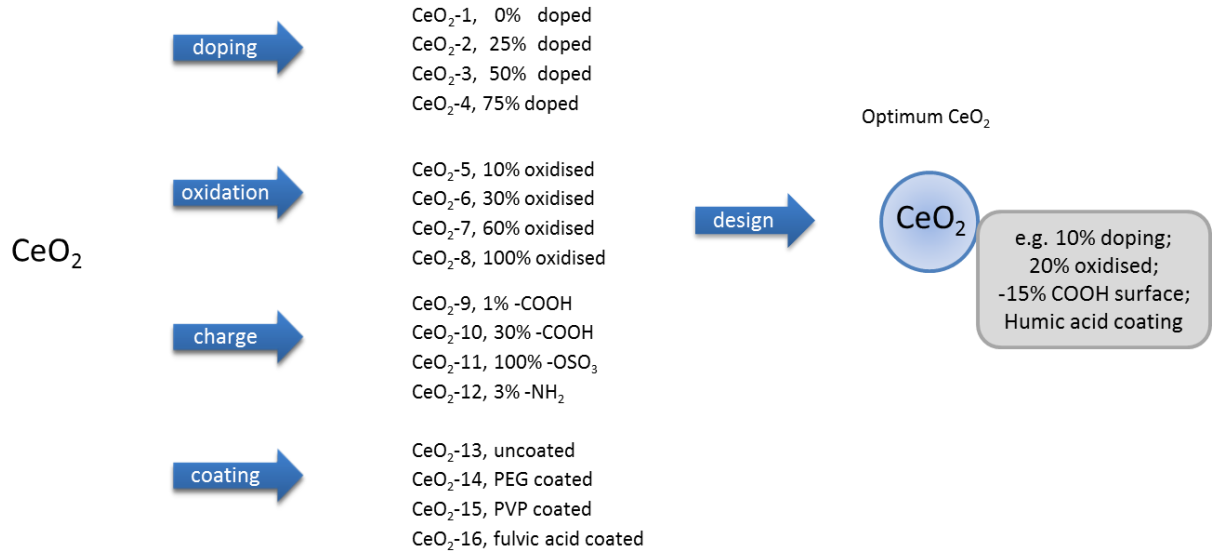

Figure 2: Schematic illustration of the NanoMILE approach to systematically varying physico-chemical parameters (bearing in mind that changing one might impact on others) and utilising this variation and the subsequently generated biological and environmental impact data to determine the "optimal" physico-chemical parameters from a safety perspective that also achieve the functional goal of the material. While this is an ambitious plan, perhaps as a minimum this may allow the NanoMILE consortium to reduce the matrix of options that we proceed to test in WP9 (bespoke safe-by-design MNMs), and thus to home in on the optimised safe MNM), or give input into an appropriate matrix for assessment of principle contributors to toxicity compared to parameters that simply modulate an existing response mechanism.

A key outcome from the NanoMILE project will be an assessment of where / how each of the physico-chemical parameters might impact on the sorts of end-points being assessed in each NanoMILE WP, which will form the basis of design rules for safer-by-design nanomaterials. Even if it is found that in many cases varying only one physico-chemical characteristic is difficult, the emphasis in NanoMile will be placed on obtaining a precise description of the physico-chemical characteristics of NMMs linked directly to a toxicity mechanism, and on untangling the cross-linkage between the different biological tests with respect to the physico-chemical characteristics across a family of MNMs.

Specific requirements for MNMs from the various impact analysis WPs

WP3 – Life cycle evolution of MNMs

The largest limiting factor in terms of particle selection for WP3 is the need to find particles in suitable form to age both in air and water. Furthermore, as aged particles will be used by other WPs, the ageing method needs to be rationalised and standardised, so that, for example, particles are homogeneously aged. A third consideration is relevance, i.e. particles should be akin to what is likely to be ageing in the environment and the ageing process should replicate likely route of environmental ageing.

Particles with a range of different coatings would also be important here, and would allow assessment of the effect of coating on ageing / dissolution.

WP4 – Development of a screening platform for MNMs

This WP will essentially look at all particles, as an initial screening, but requires as much systematic variations as possible, especially in terms of isolating / identifying linked parameters. Initially, WP4 has selected a panel of particles trying to keep size constant and varying material and surface composition.

Some requirements for sufficiently high MNM concentrations to allow dilution into cell culture media and still reach concentrations of 100 µg/mL. This is a challenge for some materials, e.g. silver.

WP4 would also require some fluorescent particles / stable-isotope labelled variants to allow quantification of uptake and comparison with ICP-MS for example.

WP4 needs to ensure using same materials as used in WP5, WP6 and WP7 to allow for comparisons, and also needs at least some MNMs that show a toxicity response.

WP4 will also use a variety of other MNMs but some will be distinct to relevant environmental compartments, considering also the ecology of the organisms as well as the effects of the materials.

WP5 – MNM interactions with biomolecules and environmental factors

Some partners (e.g. LMU) can currently only work with fluorescently-labelled MNMs, although are looking also at labelling serum – however cross-correlation requires both proteins and particles to be labelled. Partners requiring fluorescent labelled particles are initially working on lower priority materials, such as Quantum dots and commercially available silica nanoparticles, while waiting for the phase 2 particles prepared by JRC which involve systematic variation of the charge and coating of silica nanoparticles.

Paramagnetic MNMs are required by NRCWE to facilitate recovery of the MNMs following exposure to whole animals to re-characterise the particles along with their protein coronas.

WP5 also needs information from the ecotoxicology and toxicology WPs on the relevant biological fluids and the relevant timelines for assessing the time-resolved behaviour of the various nanomaterials under the various exposure conditions.

In the final phase of the project it may be clear from the outcomes of the other WPs that additional work may be needed from WP5 to characterise the corona of some specific materials under specific exposure conditions to try to interpret the outcomes and connect them to the corona.

WP6 – MNM bioavailability & biological effects in vitro/in vivo (ecotoxicology)

This WP needs large quantities of particles for *in vivo* work, up to 100g per MNM, and would benefit from testing aged particles.

WP6 will use a series of materials – 2 across all species, and all tests, and linked to WP3 and WP7. Ceria & silver MNMs will be common across all study species.

WP6 will also use a variety of others but some will be distinct to relevant environmental compartment. WP6 is considering the ecology of the organisms as well as the effects of the MNMs.

WP7 - MNM biokinetics and toxicity testing in vitro/in vivo (toxicology)

WP7 has prioritised cerium Oxide (ceria) for a hypothesis-led approach to testing ROS generation. An additional consideration here is the strong need to link to the BASF 2-year study using ceria, and WP7 have thus requested a comparison ceria MNM with a significantly different redox potential, which is currently being produced by PROM.

All studies listed in the DoW for WP7 will utilise at least 3 forms of cerium oxide. Other NMs may be added on basis of the WP4 screening, and material selection will be hypothesis-driven.

WP8 - Systems biology approaches to reveal mechanisms of MNM activity

The main requirement for WP8 is systematic variation of MNMs, comprising of multiple variants around a common core (e.g. 4 core materials with 10 variants of each). It is possible that WP8 will investigate even fewer particles). Another possibility is fewer cores but with more variants of each.

The second important requirement is extensive overlap of MNMs with WPs 6 and 7, and ideally the inclusion of some aged materials from WP3. Good candidate materials for such variations are: 1) Ag NPs (range of sizes, shapes, and surface modifications); 2) ceria NPs with again different oxidation states, doping and surface modifications; and potentially 3) Fe_xO_y NPs with different oxidation states, doping and surface modifications.

Silver will be prioritised. Then ceria, but in this case further conversations are required around what variety of modifications which are available. WP8 also needs to leave some time and funding to respond to the outputs from WP4, so we cannot make the final (4^{th} particle) choice now.

WP9 - data integration / QPARs

While WP9 in largely taken up with the synthesis of the bespoke or designed-to-be-safe MNM variants required to test the specific hypotheses, there are also some specific particle requirements for the QSAR modelling and the data integration. The requirements for data integration and modelling are essential for the experimental design itself, and indeed are feeding into the selection of MNMs for the other WPs and also into the consideration of the sorts of systematic variations that can be designed and assessed as the basis for QSARs. The Month 6 NanoMILE meeting raised some important aspects of the limitations of modelling approaches, as has the final meeting of the ModNanoTox project also coordinated by Prof. Valsami-Jones, where Lazar modelling approaches were demonstrated to be inapplicable for MNMs in an environmental context at present due to significant data gaps that resulted in the training data set being insufficiently offset from the test data set for reliable correlations to be achieved (a public report on the outcomes and challenges is currently being finalised). Some additional discussions within WP9 are required to tease out the detailed particle needs for the QSARs, which will be addressed as a matter of priority over the coming months. Thus, the WP9 particle requirements will be evolved and reported in due course.

First Selection of MNMs for NanoMILE

Table 1: Initial selection of MNMs for assessment in NanoMILE and key descriptors that will be systematically assessed / varied.

Nanomaterial	Justification for selection	Key descriptors	1st group (commercial)	2nd group (modified)	3rd group (bespoke)
CeO_2	Low solubility -> low toxicity Redox variations Isotopic label available Commercial value	Redox state Size Shape Solubility	Sigma JRC repository	Yes (PROM, UoB)	To be decided by Month 12 (MS3)
ZnO	High solubility -> high toxicity Isotopic label available High commercial importance (multiple applications)	Size Shape Dissolution rate / coating	JRC repository	Yes (UoB)	To be decided by Month 12 (MS3)
Ag	Variable solubility -> variable toxicity Isotopic label available High commercial value	Size Shape (including flowers) Dissolution rate / coating Surface defects	Sigma Sciventions Ltd.	Yes (JRC)	To be decided by Month 12 (MS3)
FexOy	Likely low solubility -> low toxicity Multiple structures & Magnetic properties Potential for labelling Medical applications	Crystal structure / phase Magnetic properties Coating Size	Sigma	Yes (PROM, N4I)	To be decided by Month 12 (MS3)
Graphene / other carbon-based MNM	High commercial relevance (e.g. Graphene Flagship) Non-spherical -> potential for alternative mechanisms of action	Aspect ratio Shape / structure C/O ratio / surface groups Surface functionalisation	Thomas Swan	Yes (CEA) (also negotiations with Graphene Flagship partners underway)	To be decided by Month 12 (MS3)
SiO_2	Easily fluorescently labelled	Size	JRC repository	Yes (JRC)	To be decided by

	Multiple synthesis routes Low toxicity generally, though evidence that structural transformations can induce toxicity (e.g. fumed silica)	Porosity	IRMM standards BAM - Federal Institute for Materials Research and Testing		Month 12 (MS3)
TiO_2	Low solubility -> low toxicity Multiple coatings available Different crystal phases Commercial value Photoreactive	Crystal structure / phase Coating (ageing) Size ROS production	JRC repository NIST standards	Yes (PROM)	To be decided by Month 12 (MS3)

Justification for the selection of each MNM class

CeO_2

The inclusion of CeO_2 as one of the chosen classes of materials is based primarily on the redox activity of CeO_2 nanoparticles and the fact that it can cycle between two redox states, Ce^{3+} and Ce^{4+}, which gives it catalytic properties, and suggest a mechanism of activity based on oxidative stress. Indeed literature reports that CeO_2 nanoparticles can act as antioxidant agents or induce oxidative stress, although recent numerous papers have emerged in the literature proposing its use as an anti-oxidative therapy towards a range of inflammatory disorders including diabetes and macular degeneration, as well as its use to sensitise cancer cells to radiation therapy. Doping can be utilised to alter the redox activity of CeO_2 nanoparticles, likely by altering the lattice distortion and/or introduction of defects into the lattice. As part of the NanoREG project, partner BASF are conducting a 2-year inhalation exposure study, and thus aligning the NanoMILE toxicity assays with this has enormous benefits. As a consequence of its use in vehicle catalysts, ceria is an excellent example of a MNM that has a potential for exposure via inhalation, making it especially relevant in this context. Also, the impact of ageing and temperature on the physico-chemical parameters under these conditions (i.e. during combustion) might provide useful insights.

Doping: CeO_2 nanoparticles can be doped with other species, such as zirconium oxide in order to tune the redox activity / ability to cycle between the Ce^{3+} and Ce^{4+} states. In addition, large scale batched (>100g) can be produced by PROM, a project partner, facilitating comparison with the undoped variant, and the BASF study samples (from the JRC repository).

ZnO

The inclusion of ZnO as one of the chosen classes of materials is based on the known toxicity of ZnO nanoparticles which has been shown previously to be linked to its high solubility. A limited amount of work has also shown that doping can control solubility and this, in turn, reduces toxicity, although this paradigm needs to be investigated further. ZnO nanoparticles synthesis is quite complex but a fairly wide range of sizes can be produced and made in order to systematically vary key physicochemical parameters such as charge, and surface chemistry, through doping. ZnO nanoparticles are used in a number of important industrial applications, including production of sunscreens.

Isotope labelling: ZnO nanoparticles can be synthesised to contain a single low abundance non-radiogenic isotope, which is a very effective label for chemical tracing using mass spectrometry based methods (quadrapole or multicollector ICP-MS). Such labelled particles are physically and chemically indistinguishable from unlabelled variants and are particularly suitable for ecotoxicological experiments as they permit the specific detection of zinc from man-made sources in samples which may already contain a high natural background level of zinc.

Ag

The inclusion of Ag as one of the chosen classes of materials is based on the known toxicity of silver particles which results, at least in part, from the release of silver ions by oxidation/dissolution of the metal particles. The rate of release is strongly dependent on the type of silver particle and may be influenced by many factors including size, coating, shape, morphology and dispersant media. Silver nanoparticles can be produced in a controlled manner in a fairly wide range of sizes and shapes and it is possible to systematically vary key physicochemical parameters such as charge, and surface chemistry. The ability to control such key physicochemical properties make it adapted to use in modelling and testing of hypothesis driven studies of the toxicological mechanisms of this highly relevant class of MNM. In terms of industrial consumer products the use of silver nanomaterials in has been in rapid increase in recent years and currently represents on the most widely used type of (intentionally) manufactured nanomaterials.

Chemistry: Silver nanoparticles can be produced with variety of surface modifiers/stabilizer such simple citrate, PEG thiols or polymer coatings such neutral polyvinylpyrrolidone, negatively charged polyacrylic acid or positively charged polyethylenimine coatings, as well as environmentally relevant coatings such as humic or fulvic acids. Such modifications not only influence the release of ionic silver but may be important in determining the interactions between the solid metal particle and their abiotic and biological environments.

Shape/Morphology: Silver nanoparticles can be produced in variety of shapes and morphologies including spheres, triangle wires as well as with highly faceted structures. These variations will clearly change the surface/volume ratio of the particles with being likely to have some influence on ion release while aspect ratio may potentially also affect the uptake behaviour when interacting with living cells.

Fe_xO_y

The inclusion of Fe_xO_y as a priority for assessment reflects a number of considerations, as follows: (1) numerous variants of it can be produced in terms of the oxidation state (ratio of Fe and O), the size and surface coating, and thus more than 40 variants are available for the systems biology approaches; (2) the fact that some of the variants are paramagnetic will allow recovery of the particles from inside cells or living organisms in a non-destructive manner, allowing assessment of the evolution of the biomolecule corona and potentially insights as to the route of uptake and final localisation; and (3) the potential for interference with novel biochemical pathways – iron (Fe) chemistry plays a key role in biology, e.g. iron plays an important role in biology, forming complexes with molecular oxygen in hemoglobin and myoglobin; these two compounds are common oxygen transport proteins in vertebrates. Iron is also the metal used at the active site of many important redox enzymes dealing with cellular respiration and oxidation and reduction in plants and animals. Thus, there is potential for nanoforms of Fe_xO_y to influence some of these biochemical pathways, which might not be observed in standard end-point assays, but where the application of systems biology approaches may shed light on subtle disruptions or amplifications. Additionally, Fe_xO_y nanoparticles are being utilised for applications ranging from environmental remediation (where

they are used as nanosorbents and photocatalysts) and in medicine where they are utilised as contrast agents and for photodynamic therapy.

Magnetite, or Fe_3O_4 is the most commonly studied variant of Fe_xO_y due to its paramagnetic properties which have applications in MRI imaging, and magnetite nanoparticles are commonly referred to as SPIONs (super paramagnetic iron oxide nanoparticles). The primary mechanism of toxicity of SPIONs is thought to be oxidative stress, and several primary sources of oxidative stress in response to SPIONs have been observed, including direct generation of ROS from the surface of the nanoparticles; production of ROS via leaching of iron molecules from the surface degradation of SPIONs by enzymatic degradation; and induction of cell signalling pathways together with their consequence activation of inflammatory cells, which results in the generation of ROS and reactive nitrogen species (e.g., nitric oxide).

Lattice arrangement / crystal face: The existence of multiple variants of Fe_xO_y, varying primarily in the lattice arrangement or crystal face (e.g. hematite (α-Fe_2O_3) and maghemite (γ-Fe_2O_3) or goethite (α-FeOOH), and lepidocrocite (γ-FeOOH)) and/or the Fe to O ratio (e.g. magnetite (Fe_3O_4)) allows systematic investigation of the subtle physico-chemical effects on, for example, ageing and transformation in the environment, or interactions with biomolecules and macromolecules. Here, doping of the MNMs can be used to tune the interactions, and in particular the sorption capacity for other heavy metals, for example.[2]

Graphene / other carbon-based MNM

The inclusion of graphene and other carbon based nanomaterials as a priority for assessment reflects the numerous applications of these materials, as well as their unique structure as two dimensional, extremely thin materials and consequent properties such as having high tensile strength. Additionally, graphene has been selected by the EU as one of its research flagships for the forthcoming 10 years (Graphene Flagship, 2013) and while some safety research is included in the Flagship project NanoMILE offers a unique platform to develop additional mechanistic understanding of the biological and environmental implications of graphene. Surface roughness and edge effects / defects may influence how graphene sheets interact with living species, in addition to known challenges such as inability of macrophages to engulf the material which may lead to persistent inflammatory effects as observed with other high aspect ratio nanomaterials.

Variation of surface chemistry / surface energy: Numerous approaches exist to functionalise the surface of graphene, including use of acetone-water mixtures to tune the surface energy, or the use of pressure to accelerate Diels-Alder reactions to produce precise patterns/arrangements of covalent modifications including biomolecules (e.g. proteins), polymers and others.[3] Many of these reactions simultaneously reduce the graphene, thereby also facilitating tuning of the electronic properties. There are some challenges in terms of surface modification which is usually done in acidic media making it very difficult to maintain the initial length, and indeed dispersion processes in general result in shortening of the initial length. However, as far as possible within current synthetic limitation, systematic sets of materials will be prepared for comparison. Further details of the possible variantion of CNTS will be added to the phase 2 particle list, since no partner has yet requested CNTs or other carbon-based MNMs.

SiO_2

The inclusion of SiO_2 as one of the chosen classes of materials is primarily due to the ease with which key physicochemical parameters (size, charge, and surface chemistry) of the basic material type can be systematically modified. The wide variety of sizes and types of surface chemistry which are obtainable with silica make it particular adapted to use in modelling and testing of hypothesis-driven studies of the toxicological responses of biological systems towards MNM.

Size: By careful control of growth conditions it is possible to produce spherical silica nanoparticles with narrow size distributions and sizes which range from around 15nm to several microns. In the first phase library of nano-materials the particles based on silica will be made as simple size variants selected from within the size range of around 15nm to 100nm.

Chemistry: Starting from as-synthesised (unmodified) SiO_2 it is technically possible to modify the surface chemistry to produce variants of charge, functional groups and variable hydrophobicity/hydrophilicity. In particular, it is possible to modify the charge of the as-synthesised surface of the SiO_2 (Si-OH) by enriching it in amino groups or carboxylic acid groups. Alternatively the hydrophobicity can be increased by addition of hydrophobic aliphatic or aromatic groups. A combination of enhanced hydrophilicity and lower protein absorption can be achieved by addition of polyethylene glycol type groups. The variation of surface chemistry as a systematically varied property will be offered as part of the second and third phase libraries.

Fluorescence: A further important advantage of SiO_2 nanoparticles is that they may be modified with a variety of fluorescent molecules or compounds via external functionalization or internal doping. In the case of internal doping with a fluorescent species, the basic fluorescent silica particle may then be further modified as discussed previously to produce the same range of chemically modified surfaces. These fluorescent particles may then be used to study uptake and localisation in living cells.

The option of fluorescently modified silica will be made available in the first phase library only for silica particles in which size may be varied but surface chemistry will not be modified. In the second and third phase libraries it is foreseen that size controlled fluorescent silica particles with controlled variations in surface chemistry will also be made available.

TiO_2

For use in mechanistic studies this class of materials is highly relevant as it is the prime example of an insoluble nano-material which, on its own, has low toxicity but being photochemically active has the potential to generate highly reactive radical species in biological media. This photochemical activity can be modulated by introducing variations in the doping or crystal structure through modification to the method of synthesis. Alternatively the photochemical behaviour can be altered by covering surface of the particles with inorganic or organic coatings to isolate the surface of the active TiO_2 from the surrounding biological media or living organisms. The inclusion of TiO_2 as a class of material for study in the project is further justified by the wide range of domestic and industrial uses of TiO_2 which, in terms of industrial production scale, make it probably the largest sources of intentional or unintentionally manufactured nanomaterial.

Crystal structure / crystal face: There is conflicting evidence in the literature as to whether crystal phase plays an important role in the toxicity of titania, with some reports suggesting that phase composition of the nanoscale titania plays an important role (e.g. that anatase TiO_2, for example, was 100 times more toxic than an equivalent sample of rutile TiO_2), while other studies have seen no significant difference in toxicity between the two variants and indeed classify both as "nuisance dusts". However, many of these studies are quite old in the sense that the field has matured significantly in terms of approaches and best practice, and indeed re-consideration of the effects of environmental ageing on these particles is needed. Additionally, the application of systems biology approaches may identify subtle differences missed by traditional end-point analyses.

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Appendix 1: Finalised Phase 1 and initial Phase 2 particle list

		Type	MNM	Size	Batch size	Source	Variable Phys-Chem Property	Availability	Phase
	1	CeO2	Micron sized control	< 5 micron (will also ball-mill to make smaller)	1kg powder (will aliquot as required)	Sigma	bulk control	Ordered	Phase 1
			Cerium (IV) Oxide (precipitated, uncoated) NM-211	Primary crystal size 10.3 nm	500 mg (powder - will aliquot before sending)	JRC Repository	Size & Surface area	Immediate	Phase 1
			Cerium (IV) Oxide (precipitated, uncoated) NM-212	Primary crystal size 33 nm	500 mg (powder - will aliquot before sending)	JRC Repository	Size & Surface area	Immediate	Phase 1
			Cerium(IV) oxide (Undoped)	Primary crystal size 20 nm	3.5L of 3.1 wt% dispersion in water (will aliquot)	PROM	Redox (doped versions in preparation)	Immediate	Phase 1
			Ce0.75ZrO.25O2	Primary crystal size 20 nm	Similar dispersion as undoped variant	PROM	redox / variable dopant concentration	Dec. 2013	Phase 2
			Ce0.5Zr0.5O2	Primary crystal size 20 nm	Similar dispersion as undoped variant	PROM	redox / variable dopant concentration	Dec. 2013	Phase 2
			Ce0.25Zr0.75O2	Primary crystal size 20 nm	Similar dispersion as undoped variant	PROM	redox / variable dopant concentration	Dec. 2013	Phase 2
			ZrO2 (Undoped)	Primary crystal size 20 nm	Similar dispersion as undoped variant	PROM	redox / variable dopant concentration	Dec. 2013	Phase 2
			Cerium (IV) oxide	10nm by DLS, 5.5 nm by AFM	0.01 mg/mL dispersion	UoB	Labelled versus unlabelled	Dec. 2013	Phase 2
			Cerium (IV) oxide - stable isotope labelled	10nm by DLS, 5.5 nm by AFM	0.01 mg/mL dispersion	UoB	Labelled for environmentlal tracking	Dec. 2013	Phase 2
			CeO2 - redox ratio 1	~20 nm	0.01 mg/mL dispersion	UoB (research phase)	Redox- range of Ce3+/4+	mid-2014	Phase 2
			CeO2 - redox ratio 2	~20 nm	0.01 mg/mL dispersion	UoB (research phase)	Redox- range of Ce3+/4+	mid-2014	Phase 2
			CeO2 - redox ratio 3	~20 nm	dispersion	UoB (research phase)	Redox- range of Ce3+/4+	mid-2014	Phase 2
	2	Ag	Micron sized control			To be determined	bulk control		Phase 1
			Silver (NM-300K), Solid Contents: 10.16 weight %,	15 nm; D90 <20nm (90% < 20nm)	2000 mg (powder - will aliqot before sending)	JRC Repository	Shape	Immediate	Phase 1
			Ag nanoparticles (spherical, citrate stabilised)	10 nm	0.02 mg/mL (disp. in citrate, 25mL, will alliquot)	Sigma-Aldrich	size	Immediate	Phase 1
			Ag nanoparticles (spherical, citrate stabilised)	20 nm	0.02 mg/mL (disp. in citrate, 25mL, will alliquot)	Sigma-Aldrich	size	Immediate	Phase 1
			Ag nanoparticles (spherical, citrate stabilised)	40 nm	0.02 mg/mL (disp. in citrate, 25mL, will alliquot)	Sigma-Aldrich	size	Immediate	Phase 1
			Ag nanoparticles (spherical, citrate stabilised)	60 nm	0.02 mg/mL (disp. in citrate, 25mL, will alliquot)	Sigma-Aldrich	size	Immediate	Phase 1
			Ag nanoparticles (spherical, citrate stabilised)	100 nm	0.02 mg/mL (disp. in citrate, 25mL, will alliquot)	Sigma-Aldrich	size	Immediate	Phase 1
			Ag-citrate	7nm	0.012 mg/mL (disperson in citrate 0.31 mM)	UoB (in progress)	Size / coating	Nov. 2013	Phase 2
			Ag-citrate	10nm	0.012 mg/mL (disperson in citrate 0.31 mM)	UoB (in progress)	Size / coating	Nov. 2013	Phase 2
			Ag-citrate	20nm	0.012 mg/mL (disperson in citrate 0.31 mM)	UoB (in progress)	Size / coating	Nov. 2013	Phase 2
			Ag-PEG	7nm	0.012 mg/mL (disperson in PEG)	UoB (in progress)	Size / coating	Nov. 2013	Phase 2
			Ag-PEG	10nm	0.012 mg/mL (disperson in PEG)	UoB (in progress)	Size / coating	Nov. 2013	Phase 2
			Ag-PEG	20nm	0.012 mg/mL (disperson in PEG)	UoB (in progress)	Size / coating	Nov. 2013	Phase 2
			Ag Nanoflower (low facet level)	diameter: 130 – 2250 nm; thickness 5 – 50 nm	10mL of 0.1 mM of Ag	Sciventions	degree and depth of faceting (requesred f	Will order based on demand	Phase 2
			Ag Nanoflower (medium facet level)	diameter: 130 – 2250 nm; thickness 5 – 50 nm	10mL of 0.1 mM of Ag	Sciventions	degree and depth of faceting	Will order based on demand	Phase 2
			Ag Nanoflower (high facet level)	diameter: 130 – 2250 nm; thickness 5 – 50 nm	10mL of 0.1 mM of Ag	Sciventions	degree and depth of faceting	Will order based on demand	Phase 2
			Ag nanoclusters with stable sulfide coating (recent literature)	< 5nm	not sure yet	UoB (research stage)	Stability	mid-2014	Phase 2
			Ag Nanoflower (low facet level)	diameter: 130 – 2250 nm; thickness 5 – 50 nm	10mL of 0.1 mM of Ag	UoB (reseach stage)	degree and depth of faceting	mid-2014	Phase 2
			Ag Nanoflower (medium facet level)	diameter: 130 – 2250 nm; thickness 5 – 50 nm	10mL of 0.1 mM of Ag	UoB (reseach stage)	degree and depth of faceting	mid-2014	Phase 2
			Ag Nanoflower (high facet level)	diameter: 130 – 2250 nm; thickness 5 – 50 nm	10mL of 0.1 mM of Ag	UoB (reseach stage)	degree and depth of faceting	mid-2014	Phase 2
			Ag-fulvic acid	7nm	0.012 mg/mL (disperson)	UoB (in progress)	Size / coating	early-2014	Phase 2
			Ag-fulvic acid	10nm	0.012 mg/mL (disperson)	UoB (in progress)	Size / coating	early 2014	Phase 2
			Ag-fulvic acid	20nm	0.012 mg/mL (disperson)	UoB (in progress)	Size / coating	early 2014	Phase 2
			Ag-fulvic acid	20 nm modified Sigma	0.02 mg/mL (dispersion)	UoB (research stage)	Size / coating / stability	early 2014	Phase 2
			Ag-fulvic acid	40 nm modified Sigma	0.02 mg/mL (dispersion)	UoB (research stage)	Size / coating / stability	early 2014	Phase 2
			Ag-fulvic acid	60nm modified Sigma	0.02 mg/mL (dispersion)	UoB (research stage)	Size / coating / stability	early 2014	Phase 2
			Ag-fulvic acid	100nm modified from Sigma	0.02 mg/mL (dispersion)	UoB (research stage)	Size / coating / stability	early 2014	Phase 2
			Ag-humic acid	7nm	0.012 mg/mL (disperson)	UoB (research stage)	Surface chemistry	early 2014	Phase 2
			Ag-humic acid	10nm	0.012 mg/mL (disperson)	UoB (research stage)	Surface chemistry	early 2014	Phase 2
			Ag-humic acid	20nm	0.012 mg/mL (disperson)	UoB (research stage)	Surface chemistry	early 2014	Phase 2
			Ag decahedral particles	50nm	10mL of 0.1 mM of Ag	UoB (research stage)	Size with specific geometry	For consideration	To be confirmed
			Ag decahedral particles	70nm	10mL of 0.1 mM of Ag	UoB (research stage)	Size with specific geometry	For consideration	To be confirmed
			Ag decahedral particles	120nm	10mL of 0.1 mM of Ag	UoB (research stage)	Size with specific geometry	For consideration	To be confirmed
			Ag pentagonal rods	diameter: 45 nm, length: 70 nm	10mL of 0.1 mM of Ag	Commercial (Sciventions)	Rod length with specific geometry	For consideration	To be confirmed
			Ag pentagonal rods	diameter: 45 nm, length: 118 nm	10mL of 0.1 mM of Ag	Commercial (Sciventions)	Rod length with specific geometry	For consideration	To be confirmed
			Ag pentagonal rods	diameter: 45 nm, length: 165 nm	10mL of 0.1 mM of Ag	Commercial (Sciventions)	Rod length with specific geometry	For consideration	To be confirmed
	3a	TiO2	NIST TiO2 (CRM 1898) mixed-phase (anatase and rutile)	~ 70nm primary particle (in water)	15 g (powder - will aliquot)	from NIST	Certified for SSA (55 m(2)/g)	Immediate	Phase 1
			Surface-modified TiO2 - surface 1 (Uncoated)	10 nm primary particles	Aquous dispersion (1.7 wt% particles)	PROM	1st in series of 5 coatings	Immediate	Phase 1
			Surface-modified TiO2 - surface 2 - PVP coated	10 nm primary particles	Aquous dispersion (1.7 wt% particles)	PROM	Surface coating	Immediate	Phase 1
			Surface-modified TiO2 - surface 3 - Pluronic F127 Coated	10 nm primary particles	Aquous dispersion (1.7 wt% particles)	PROM	Surface coating	Immediate	Phase 1
			Surface-modified TiO2 - surface 4 - Dispex AA4040 Coated	10 nm primary particles	Aquous dispersion (1.7 wt% particles)	PROM	Surface coating	Immediate	Phase 1
			Titanium Dioxide (rutile, hydrophobic) NM-103	20 nm (primary crystal size)	2000 mg (powder - will aliquot as required)	JRC Repository	Surface coating	Immediate	Phase 1
			Titanium Dioxide (rutile, hydrophilic) NM-104	20 nm (primary crystal size)	500 mg (powder - will aliquot as required)	JRC Repository	Surface coating	Immediate	Phase 1
			Micron sized control (Titania powder, rutile)	< 5 micron (will also ball-mill to make smaller)	100g powder (will aliquot as required)	Sigma	bulk control	Ordered	Phase 1

Iseult Lynch:
Note these have a high surfactant amount!

Iseult Lynch:
I know this was not 1 of the original 5 NMs selected, but we have it immediately available, so certainly will include in the screening WPs

Iseult Lynch:
Iseult Lynch:
Also not among the final 5 shortlisted, but some WP5 / WP4 partners

	3b	SiO2	Monodispersed SiO2	< 20 nm	100mg/100ml in dispersion	JRC Labs	Size	Immediate	Phase 1
			Monodispersed SiO2	25-30 nm	100mg/100ml in dispersion	JRC Labs	Size	Immediate	Phase 1
			Monodispersed SiO2	50-60 nm	100mg/100ml in dispersion	JRC Labs	Size	Immediate	Phase 1
			Micron sized control (fumed silica)	200-300 nm primary particles, form chains	100g powder (will aliquot as required)	Sigma	bulk control	Ordered	Phase 1
			Monodispersed SiO2-Amine modified	< 20 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -NH2,	Jan. 2014	Phase 2
			Monodispersed SiO2-Amine modified	25-30 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -NH2,	Jan. 2014	Phase 2
			Monodispersed SiO2-Amine modified	50-60 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -NH2,	Jan. 2014	Phase 2
			Monodispersed SiO2 -COOH modified	< 20 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -COOH	Jan. 2014	Phase 2
			Monodispersed SiO2 -COOH modified	25-30 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -COOH	Jan. 2014	Phase 2
			Monodispersed SiO2 -COOH modified	50-60 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -COOH	Jan. 2014	Phase 2
			Monodispersed Fluorescent SiO2 (Ru(bypy)3)	< 20 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size & label	Feb. 2014 if Ru(bypy)3 OK, longer i	Phase 2
			Monodispersed Fluorescent SiO2 (Ru(bypy)3)	25-30 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size & label	Feb. 2014 if Ru(bypy)3 OK, longer i	Phase 2
			Monodispersed Fluorescent SiO2 (Ru(bypy)3)	50-60 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size & label	Feb. 2014 if Ru(bypy)3 OK, longer i	Phase 2
			Monodis. Fluorescent SiO2-Amine modified	< 20 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -NH2	Feb. 2014 if Ru(bypy)3 OK, longer i	Phase 2
			Monodis. Fluorescent SiO2-Amine modified	25-30 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -NH2	Feb. 2014 if Ru(bypy)3 OK, longer i	Phase 2
			Monodis. Fluorescent SiO2-Amine modified	50-60 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -NH2	Feb. 2014 if Ru(bypy)3 OK, longer i	Phase 2
			Monodis. Fluorescent SiO2-COOH modified	< 20 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -COOH	Feb. 2014 if Ru(bypy)3 OK, longer i	Phase 2
			Monodis. Fluorescent SiO2-COOH modified	25-30 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -COOH	Feb. 2014 if Ru(bypy)3 OK, longer i	Phase 2
			Monodisp. Fluorescent SiO2-COOH modified	50-60 nm	Batches 5mg/10ml in dispersion	JRC Labs	Size and Chemistry -COOH	Feb. 2014 if Ru(bypy)3 OK, longer i	Phase 2
	4a	ZnO	Micron sized control - zinc oxide powder	< 5 micron (will also ball-mill to make smaller)	100g powder (will aliquot as required)	Sigma	bulk control	Ordered	Phase 1
			ZnO Uncoated Hydrophilic (NM-110)	150 nm	2000 mg (powder - will aliqot before sending)	JRC Repository	Coated and Uncoated Industrial ZnO	Immediate	Phase 1
			ZnO Coated Hydrophobic (triethoxycaprylyl silane) (NM-111)	140 nm	2000 mg (powder - will aliqot before sending)	JRC Repository	Coated and Uncoated Industrial ZnO	Immediate	Phase 1
			Industrial ZnO - NanoTek from Alfa Aesar	~ 55 nm	<1g	Commercial	Commercial - 40% colloidal dispersion	Immediate	Phase 1
			Industrial ZnO - NanoShield from Alfa Aesar	~20 nm	<1g	Commercial	Commercial- 50% colloidal dispersion	Immediate	Phase 1
			ZnO -Fe Doping 1	50 nm	<1g - dispersions (need to check concentration)	UoB (research phase)	ZnO Solubility 50nm (Fe doping)	mid 2014	Phase 2
			ZnO -Fe Doping 2	50 nm	<1g	UoB (research phase)	ZnO Solubility 50nm (Fe doping)	mid 2014	Phase 2
			ZnO -Fe Doping 3	50 nm	<1g	UoB (research phase)	ZnO Solubility 50nm (Fe doping)	mid 2014	Phase 2
			ZnO -Fe Doping 4	50 nm	<1g	UoB (research phase)	ZnO Solubility 50nm (Fe doping)	mid 2014	Phase 2
			ZnO -Fe Doping 5	50 nm	<1g	UoB (research phase)	ZnO Solubility 50nm (Fe doping)	mid 2014	Phase 2
			Isotope (Zn-67) Labeled ZnO	20-70nm or 100nm	Colloidal or powder route possible	UoB (research phase)	Surface / coating	Dec. 2013	Phase 2
			(67)ZnO Coated	20-70nm or 100nm	Colloidal or powder route possible	UoB (research phase)	Surface / coating	Dec. 2013	Phase 2
	4b	FeOx	Micron sized control						
			Magnetizable NPs Dextran based - Plain	20 nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Plain	50nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Plain	100nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Plain	130nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Plain	250nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Plain	500 nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Amine modified	20nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Amine modified	50nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Amine modified	100nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Amine modified	130nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - Amine modified	250nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - COOH modified	20nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - COOH modified	50nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - COOH modified	100nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - COOH modified	130nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - COOH modified	250nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			Magnetizable NPs Dextran based - COOH modified	500nm	5ml 25mg/ml -dispersion	Commercial (Kisker)	Size & surface charge (& recovery from cel	Will order on request	Phase 1
			FeOx - dextran crosslink 2	20 nm	10g	N4I	Surface modification-Dextran crosslinking	Immediate	Phase 2
			FeOx - dextran crosslink 1	20 nm	10g	N4I	Surface modification-Dextran crosslinking	Immediate	Phase 2
			FeOx - dextran crosslink 3	20 nm	10g	N4I	Surface modification-Dextran crosslinking	Immediate	Phase 2
			FeOx -Dextran Crosslink 4	20 nm	10g	N4I	Surface modification-Dextran crosslinking	Immediate	Phase 2
			Fe2O3 (Hematite)	5nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants: each of 4 redox states, uncoat	Made to order	Phase 2
			Fe2O3 (Hematite)	10nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe2O3 (Hematite)	15nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe2O3 (Hematite)	20nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe2O3 (Hematite)	50nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2

Iseult Lynch:
Labelled with Ruthenium-tris(2,2'-bipyridyl) dichloride Excitation: 452 ± 3 nm with an extinction coefficient of 14,600 M−1cm−1. Emission: 620 nm

JRC can supply interested partners with single size trial samples to see if it works optically for them

Iseult Lynch:
These take 4-6 weeks from ordering. Ready for start Jan if ordered in October

			Fe3O4 (Magnetite)	5nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe3O4 (Magnetite)	10nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe3O4 (Magnetite)	15nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe3O4 (Magnetite)	20nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe3O4 (Magnetite)	50nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)	5nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)	10nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)	15nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)	20nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)	50nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)	5nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)	10nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)	15nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)	20nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)	50nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe2O3 (Hematite)-Dextran	5nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe2O3 (Hematite)-Dextran	10nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe2O3 (Hematite)-Dextran	15nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe2O3 (Hematite)-Dextran	20nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe2O3 (Hematite)-Dextran	50nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe3O4 (Magnetite) -Dextran	5nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe3O4 (Magnetite) -Dextran	10nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe3O4 (Magnetite) -Dextran	15nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe3O4 (Magnetite) -Dextran	20nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			Fe3O4 (Magnetite) -Dextran	50nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)-Dextran	5nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)-Dextran	10nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)-Dextran	15nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)-Dextran	20nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			y-Fe2O3 (Maghemite)-Dextran	50nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)-Dextran	5nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)-Dextran	10nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)-Dextran	15nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)-Dextran	20nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
			FeO(OH)(Goethite)-Dextran	50nm	Batches 10mg (~3 wt% particles in dispersion)	PROM	40 variants (size, coating, redox state)	Made to order	Phase 2
	5	**Carbon based**							
		carbon black	nano-carbon black (Printex90)	14 nm	500g	Degussa?	???	Order as needed	Phase 1
			micro-carbon black (Huber990)	260 nm	500g	Degussa?	???	Order as needed	Phase 1
		CNTs	**Elicarb® SWNT-** low inorganic residue of <5% w/w and typical NTe contents of >90%.	2 nm diameter ~1 micron long	1g	Commercial (Thomas Swa	single or multi-wall	Order as needed	Phase 1
			Elicarb® SWNT (AQ) -low inorganic residue of <5% w/w and typical nanotube contents of >90%.	2 nm diameter, ~ 1 um long	aqueous wet cake - 10g (dispersion in water)	Commercial (Thomas Swa	single or multi-wall	Order as needed	Phase 1
			Elicarb® MWNT: low inorganic residue of <5% w/w and typical nanotube contents of >70%. T	10-12 nm diam., 10s um long	1g	Commercial (Thomas Swa	single or multi-wall	Order as needed	Phase 1
			MWCNT - size as synthesized		1g	CEA	Size(diameter) and chemistry	early 2014	Phase 2
			MWCNT - Size reduced 1		1g	CEA	Size(diameter) and chemistry	early 2014	Phase 2
			MWCNT - Size as synthesised -COOH functionalized		1g	CEA	Size(diameter) and chemistry	early 2014	Phase 2
			MWCNT - Size reduced -COOH functionalized		1g	CEA	Size(diameter) and chemistry	early 2014	Phase 2
			MWCNT - Thermally treated		1g	CEA	Size(diameter) and chemistry	early 2014	Phase 2
			MWCNT Catalyst removed		1g	CEA	Size(diameter) and chemistry	early 2014	Phase 2
			CNT (Radiolabeled)		50mg	CEA	Radiolabeled - ONLY FOR USE BY CEA	early 2014	Phase 2
		Graphene	Graphene Type 1		1g	Graphene Flagship	Surface Functionality	early 2014	Phase 2
			Graphene Type 2		1g	Graphene Flagship	Surface Functionality	early 2014	Phase 2
			Graphene Type 3		1g	Graphene Flagship	Surface Functionality	early 2014	Phase 2
			Graphene Type 4		1g	Graphene Flagship	Surface Functionality	early 2014	Phase 2

Iseult Lynch:
No partner working with Carbon materials until at least 2014. Discuss further at M12 meeting.

Appendix 2: Centralised particles – Summary of particle shipments to UoB for characterisation / distribution

Shipments from JRC repository

JRC record of shipping of MNMs to University of Birmingham

Sample composition	Size	Shape	Form (Select from menu)	Internal code (Batch No.)	Batch size (amount)	No. of batches	Preparation date	Shipping date	Characterisation provided
Titanium, dioxide (hydrophobic, rutile)	20 nm (1 particles)	spherical	powder	NM-103 (vials 1108, 1146, 1256, 1435 and 1521)	5 x 2g	5 sub-samples	unknown	28/08/2013	limited (mean particle size ~ 186 nm); Aluminium oxide coated?
Titanium, dioxide (hydrophillic, rutile)	20 nm (1 particles)	spherical	powder	NM-104 (vials 0560, 0750, 0828,0833 and 0834)	5 x 500 mg	5 sub-samples	unknown	28/08/2013	limited (mean particle size ~ 186 nm); Aluminium oxide coated?
Zinc oxide (uncoated)	42 nm (1 particles)	spherical?	powder	NM-110 (vials 0589, 0676, 0801, 0960 and 2146)	5 x 2g	5 sub-samples	unknown	28/08/2013	mean size 150nm
Zinc oxide (coated with Triethoxycaprylylsilane)	34 nm (1 particles)	flowers?	powder	NM-111 (vials 2689, 2800, 2854, 2939 and 2995)	5 x 2g	5 sub-samples	unknown	28/08/2013	mean size 140nm
Cerium oxide (uncoated, precipitated, cubic)	10 nm (1 particles)	cubic	powder	NM-211 (vials 1967, 2005, 2110, 2163 and 2204)	5 x 500 mg	5 sub-samples	unknown	28/08/2013	slightly hygroscopic
Cerium oxide	10 nm (1 particles)	spherical	powder	NM-212 (vials 3272, 3274, 3339, 3346 and 2420)	5 x 500 mg	5 sub-samples	unknown	28/08/2013	slightly hygroscopic

Shipments from Promethean particles

PROM record of shipping of MNMs to University of Birmingham

Sample composition	Size	Shape	Form (Select from menu)	Internal code (Batch No.)	Batch size (amount)	No. of batches	Preparation date	Shipping date	Characterisation provided
Titanium(IV)dioxide (Uncoated)	10nm (1 particles)	spherical	aqueous suspension	MILE-003	500 mL (1.7 wt %)	1	Aug-13	10/08/2013	200nm by DLS. TEM underway
Titanium(IV)dioxide (PVP coated)	10nm (1 particles)	spherical	aqueous suspension	MILE-002	500 mL (2.0 wt %)	1	Aug-13	10/08/2013	200nm by DLS. TEM underway
Titanium(IV)dioxide (F127 coated)	10nm (1 particles)	spherical	aqueous suspension	MILE-001	500 mL (1.7 wt %)	1	Aug-13	10/08/2013	200nm by DLS. TEM underway
Titanium(IV)dioxide (Dospex AA4040 coated)	10nm (1 particles)	spherical	aqueous suspension	MILE-004	500 mL (2.1 wt %)	1	Aug-13	15/08/2013	200nm by DLS. TEM underway
Cerium(IV)oxide nano	20nm (1 particles)	spherical	aqueous suspension	MILE-005	2.5 L (3.1 wt%)	1 batch (in 3 containers)	Sep-13	14/09/2013	Characterisation underway

Appendix 3: NanoMILE MNM shipping, storage, handling and data reporting guidelines

Draft 1 – Generic form. To be adapted for specific MNMs

Shipping protocol

All shipments of nanomaterials (MNMs) within NanoMILE are logged (what was sent, to whom, and when) and will be reported to the Commission as part of the deliverable reports from WP2.

All shipments will be pre-arranged with the receiving partner to ensure that they are available to receive the samples and store them appropriately, and ready to use them immediately.

Shipments will be by DHL and tracking numbers will be provided to partners, who will then report back to the shipping partner that they were received.

Where deemed appropriate certain temperature sensitive materials such as liquid dispersed silver nanoparticles may be shipped in cool-box containers to reduce the risk of degradation in transit.

Storage protocol

MNMs do not have infinite shelf-life and thus all NanoMILE partners have an obligation to respect the effort involved in synthesising and characterising particles undertaken by the WP2 partners, and make every effort to utilise the MNMs in a timely manner and report back the data generated.

MNMs in powder form are generally more stable than those in dispersion, which can agglomerate, sediment, dissolve (potentially) or become contaminated with bacteria etc. Thus, all NanoMILE particles are shipped as soon as possible after dispersion, and the dispersion date is provided in the shipping record. Optimal storage conditions will be indicated in the shipping protocol, as well as a use-by date indicating when the useable shelf-life of the materials. (These will be updated & refined as the stability and ageing tests progress).

Handling protocol

Each MNM will have a specific set of handling guidelines to be applied to the dispersion prior to making any dilutions or sub-sampling, and prior to measurement, to reverse any settling or agglomeration of particles that may have occurred during shipment / storage. Please apply these as specified.

Should a specific technique recommended not be available in your lab, please report this to the supplier and indicate what you do have available. The supplier will then provide an alternative suggestion, and will undertake limited characterisation (e.g. DLS) using these conditions to allow your results to be comparable to others within the NanoMILE consortium.

WP2 will also undertake characterisation of MNMs in representative media relevant to the project (likely cell culture media; zebra fish embryo medium, and a sea water equivalent). Exact media will be agreed with WP Leaders.

Data reporting protocol

Ensuring accessibility to the data is critical for the success of NanoMILE. We are developing templates for data reporting to ensure maximum utility of the data and to allow all data relating to specific MNMs to be co-localised.

This will likely involve a cloud storage solution (password protected) and living documents where partners can deposit data along with the relevant metadata describing the experimental protocol and conditions (to facilitate cross-comparability of data).

Further information on this will be provided at the M12 meeting in April 2014.

Summary conditions for MNM [*inset details: NanoMILE # and composition*] **handling**

MNM	**Storage conditions**	**Likely stability window**	**Pre-treatment before measuring**
e.g. TiO_2	Room temperature (dark)	6 months	e.g. vortex on maximum for 2 minutes
	Choose an item.	Choose an item.	e.g. treat in a sonic batch at medium power for 5 minutes
	Choose an item.	Choose an item.	
	Choose an item.	Choose an item.	

Appendix 4: NanoMILE partners' available characterisation approaches & responsibilities

The strategy for characterising the materials prior to being supplied to the other work-packages will be based on the two tier system. The first stage analysis will involve a series of basic characterisation techniques which will be applied to all materials. These methods and their availability to the partners are detailed in the Methodologies Table below. In those cases where the required amount of any particular test material is greater than can be produced in single batch it is also noted whether the analysis method need be applied to only to selected representative samples or if analysis of each batch will be necessary. The majority of the characterisation will be undertaken through the laboratories of UoB and JRC while characterisation of CNT materials will be done by the partner CEA who is the producer of this particular class of material, with support from UoB.

Methodologies Table

	Use and Applicability		Availability				
Method	General requirement for single representative sample	Apply method to all synthesized batches of a material type	UoB	JRC	PROM	N4I	CEA
TEM	Obligatory if no SEM	Discretion	Y	Y	N	N	Y
SEM	Obligatory if no TEM	Discretion	Y	Y	N	N	Y
DLS	Obligatory for dispersions	YES	Y	Y	Y	N	Y
NTA	Desirable	Desirable	Y	Y	N	N	N
CLS or Differential Sedimentation Centrifugation	Obligatory for aqueous dispersions	YES	Y	Y	N	N	Y
Zeta-Potential	Obligatory for aqueous dispersions	YES	Y	Y	Y	N	Y
BET	Obligatory if available as solid in sufficient quantity	No	Y	N	N	N	Y
XRD	Obligatory for inorganics	Discretion when relevant	Y	Y	N	N	N

In addition to the above noted analysis measurements there will be additional steps of characterisation methods which will be applied on an "as-required" basis where particular material properties require specific investigation. Examples are the solubility, REDOX potential and

photochemical activity. Detailed protocols for such measurements are under development and will be outlined in future project reports from WP2.