GUIDANCE ON NEW CHEMICAL REQUIREMENTS FOR NOTIFICATION OF INDUSTRIAL NANOMATERIALS

A new industrial chemical that falls under the working definition of an 'industrial nanomaterial' will not be permitted to be introduced under some exemption and self-assessment categories. These processes apply to any *new chemical* that meets the following working definition of 'industrial nanomaterial':

NICNAS WORKING DEFINITION[1] OF INDUSTRIAL NANOMATERIAL

> ***… industrial materials intentionally produced, manufactured or engineered to have unique properties or specific composition at the nanoscale, that is a size range typically between 1 nm and 100 nm, and is either a nano-object (i.e. that is confined in one, two, or three dimensions at the nanoscale) or is nanostructured (i.e. having an internal or surface structure at the nanoscale)"***

[Notes to the working definition:

- intentionally produced, manufactured or engineered materials are distinct from accidentally produced materials
- 'unique properties' refers to chemical and/or physical properties that are different because of its nanoscale features as compared to the same material without nanoscale features, and result in unique phenomena (e.g. increased strength, chemical reactivity or conductivity) that enable novel applications.
- aggregates and agglomerates are considered to be nanostructured substances
- where a material includes 10% or more number of particles that meet the above definition (size, unique properties, intentionally produced) NICNAS will consider this to be a nanomaterial.]

EXEMPTION CATEGORIES

New chemical exemptions are underpinned by S 21 (4) and (6) of the Act. S 21AA imposes annual reporting obligations on persons introducing chemicals under S 21 (4) and (6) of the Act.

From 01 January 2011, nano-forms of new chemicals will not be permitted to be introduced under exemption categories where human and/or environmental exposure can reasonably be anticipated, these being:

- Low volume cosmetic and non-cosmetic exemptions (S21(4))
- Low concentration (<1%) non hazardous cosmetic exemption (S21(6c)).

Introducers who advise NICNAS of introductions under these exemption categories will be required to declare on their Annual reporting form, that their chemicals are <u>not</u> nanomaterials, according to the NICNAS working definition above.

The following exemption categories will remain available for nanoforms of new chemicals:

[1] NICNAS will actively monitor progress of national and international reviews and other scientific developments and regularly re-assess this working definition.

- Trans-shipment exemptions – current conditions of introduction remain unchanged (S21(6b))
- R&D exemptions S21(6a)) – with some amendments to the annual reporting requirements. All nanomaterials introduced in volumes over 100g/year will be identified as nanomaterials and their full chemical name provided.

Any substances that meet the working definition of 'industrial nanomaterial' (above) currently introduced under exemption categories will require a NICNAS permit or certificate if introduction is to continue after 01 January 2011. Introducers should contact NICNAS prior to this date to determine the most appropriate notification category for their nanomaterial(s).

PERMIT CATEGORIES

All permit categories under Part 3 of the Act will remain available for use by introducers of nano-forms of new chemicals. Some changes to notification forms and information requirements may apply as follows:

- Addition of a declaration by the notifier on the permit application forms stating that the chemical is a nanomaterial or not.
- More specific information (such as particle size, shape and other specific information on properties) may be required under specified conditions (see "Specified conditions for requesting additional data requirements").

To complement these changes NICNAS may stipulate permit conditions for conventional chemicals where it can be reasonably assumed that a nano-form may be introduced in the future.

CERTIFICATE CATEGORIES

Of currently available certificate categories, all <u>except</u> self-assessment categories will be available for use by introducers of nano-forms of new chemicals. Introducers who annually report introductions under self –assessed certificate categories will be required to declare that their chemicals are <u>not</u> nanomaterials, according to the NICNAS working definition above.

Some changes to notification forms and information requirements may apply as follows:

- Addition of a declaration by the notifier on the certificate application forms stating that the chemical is a nanomaterial or not.
- More specific information (such as particle size, shape and other specific information on properties) may be required under specified conditions (see "Specified conditions for requesting additional data requirements").

Complementing these changes, NICNAS may stipulate specific secondary notification conditions to the assessment of conventional chemicals where a nano-form may be introduced in the future.

SPECIFIED CONDITIONS FOR REQUESTING ADDITIONAL DATA REQUIREMENTS

As a minimum requirement particle size information (**primary particle size and number-weighted size distribution**) will be required in the following cases:

- where the chemical is an industrial nanomaterial
- where it can be anticipated or there is uncertainty that the chemical could be a nanomaterial and exposure to human health or the environment is expected based on use scenarios

AND

- the chemical is introduced as a solid/powder or as a dispersion and is insoluble (e.g. water insolubility < 1 mg/L); and/or known to be biopersistent*.

Note 1: If particle size information cannot be supplied for a chemical which meets certain conditions outlined above (other than where it has been declared as a nanomaterial), the chemical will be assumed to be an industrial nanomaterial for risk assessment and recommendations.

Note 2: The following chemicals that meet the circumstances outlined above may not be subject to the additional data requirements.

- *compounds that dissociate in water to form ions*
- *colloidal polymers*
- *micelles*
- *biological materials*

Please contact NICNAS for advice on notification requirements for these chemicals.

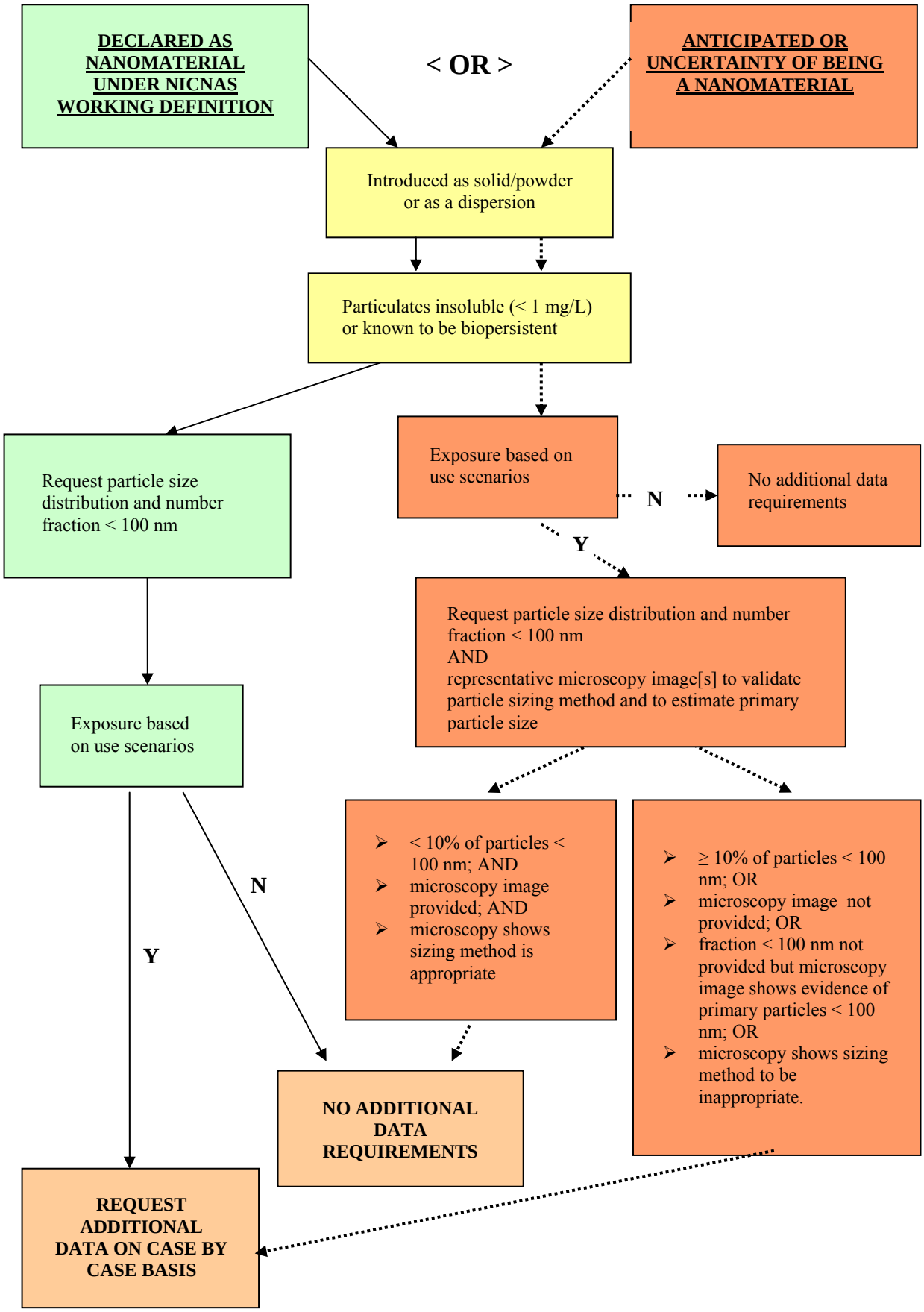
In addition to the particle size information, the following additional data, above that which is normally required for the notification category may also be requested (where applicable) under certain circumstances (see Flow Chart). Specific guidance on physico-chemical characteristics and toxicity testing are provided below:

- method of production
- medium identity
- medium conditions (identity and concentration of stabilizers, ionic strength and ionic composition)
- shape
- crystalline phase
- agglomeration/aggregation state
- composition (purity/impurities)
- surface area
- surface charge
- surface chemistry (such as coatings and modifications)
- toxicity data will be requested on a case-by-case basis

Note 3: These additional data requirements will be determined on a case by case basis and are subject to variation as new knowledge regarding toxicity of nanomaterials is developed.

* "biopersistent" is defined as the ability of a substance to remain in the body in spite of physiological clearance mechanisms

FLOW CHART: Conditions for provision of particle size information and additional data requirements for permit and certificate categories



GUIDANCE ON PROVISION OF ADDITIONAL DATA REQUIREMENTS

The following provides guidance on the physico-chemical characterisation and reporting requirements for the additional data requirements (i.e. above that which is normally required for the notification category). Recommended test methods are identified for the physico-chemical data informed by ISO's Technical Report ISO/PDTR 13014 on Nanotechnologies – *Guidance on physico-chemical characterisation for manufactured nano-objects submitted for toxicological testing*[2] and the OECD Sponsorship programme *Guidance manual for the testing of manufactured nanomaterials*[3]. Please refer to these documents for further details and alternative methods.

Where specific data are requested by NICNAS and it is not feasible or not considered to be applicable to provide the additional physico-chemical data, a scientific rationale for not providing these test results must be provided.

The physico-chemical data should be supplied for the nanomaterial as manufactured (i.e. at the point on completion of manufacture or as the sample is removed from the manufacturer's container) and, where data available, in the end-use product formulation.

In general, all physico-chemical data should specify:

- the grade of the nanomaterial tested, including its purity
- the testing authority or organisation
- the method of preparing the test sample
- the physical conditions used for all test data, for example, agitation method (dispersing aids), pH, ionic strength, ionic composition, temperature or pressure.

The standard of testing to obtain data should be performed in compliance with GLP standards. Notifiers may refer to the OECD Principles of Good Laboratory Practice for information on this matter.

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[2] ISO (2010) Nanotechnologies – Guidance on physico-chemical characterisation for manufactured nano-objects submitted for toxicological testing, ISO/PDTR 13014. The International Organisation for Standardisation, http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=381983&development=on, Accessed 17th November 2010.

[3] OECD (2009a) Guidance manual for the testing of manufactured nanomaterials: OECD's sponsorship programme; First Revision, ENV/JM/MONO(2009)20/REV. In: OECD Environment, Health and Safety Publication, Series on the safety of manufactured nanomaterials. OECD Paris, Organisation for Economic Co-operation and Development, No. 25, 92 pp. <http://www.oecd.org/document/53/0,3343,en_2649_37015404_37760309_1_1_1_1,00.html>, Accessed 17th November 2010.

Note: The OECD Working Party on Manufactured Nanomaterials (WPMN) reviewed all 22 OECD test guidelines for physical-chemical properties for their applicability to the testing of nanomaterials[4]. The review concluded that all but two of the current tests may provide information that is applicable to nanomaterials. The two tests not considered to provide useful information are TG 103 Boiling Point and TG 114 Viscosity of Liquids.

It was also recognised that some tests would only be applicable to a sub-set of nanomaterials depending on their physical form and chemical composition. For example, it was concluded that the three test guidelines for physical-chemical properties of polymers (OECD TGs 118–120) would only be applicable to polymeric manufactured nanomaterials.

The key physical-chemical properties that require characterisation when considering aquatic environmental exposure of chemicals are water solubility, water-soil and water-oil partitioning, hydrolysis and dissociation constants. All of the standard test guidelines for these properties are considered to be potentially applicable to nanomaterials. However, it is noted that the applicability will depend in part on the presence of colloidal dispersions of nanomaterials in water which may complicate both the conduct and/or the interpretation of studies.

(i) Particle size and size distribution

The mean primary particle size and number weighted primary particle size distribution with number fraction < 100 nm should be provided. In addition, a representative microscopy image at a magnification capable of resolving features < 100 nm should be provided to validate the particle sizing method.

When measuring the particle size distribution an effort should be made, for example, through sonication or the use of dispersing aids to fully disperse the nanomaterial, to break down any loose agglomerates including those of fibres. The method of dispersion and sample preparation should be reported.

Where the size distribution and the number weighted percentage of particles < 100 nm have not been provided, the chemical will be assumed to be a nanomaterial under the NICNAS definition if there is evidence of primary particles of < 100 nm in the representative microscopy image.

Fibre-like nanomaterials

For nanomaterials that are fibre-like such as carbon nanotubes, the aspect ratio (fibre length range and diameter range) is required. For guidance on measurement please refer to the OECD technical guidance document No. 10 Particle Size Distribution/Fibre Length and Diameter Distributions.

[4] OECD (2009b) Preliminary review of OECD test guidelines for their applicability to manufactured nanomaterials. In: OECD Environment, Health and Safety Publication, Series on the safety of manufactured nanomaterials, No. 15, ENV/JM/MONO(2009)21. OECD Paris, Organisation for Economic Co-operation and Development, 71 pp. <http://www.oecd.org/document/53/0,3343,en_2649_37015404_37760309_1_1_1_1,00.html>, Accessed 17th November 2010.

Recommended test methods: Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Atomic force microscopy (AFM), Dynamic Light Scattering (DLS)*, Laser Diffraction, Disk centrifugation, Scanning Mobility Particle Sizer (SMPS)

*DLS, although suitable for monodisperse materials, should not be solely relied upon for measuring the primary particle size distribution of nanomaterials with broad size distributions as this method is strongly biased towards larger particles or aggregates which may obscure the presence of nanoparticles.

(ii) Method of production
The method of production must be described including the methods used for purification as these may affect key properties of the nanomaterial including the type and level of impurities and surface chemistry.

(iii) Shape
A detailed description of the physical shape of the nanomaterial should be provided using terms such as spheres, fibres, tubes or plates.

Recommended test methods: SEM and TEM.

(iv) Agglomeration/aggregation state
The agglomeration/aggregation state of a dispersion of the nanomaterial in an aqueous medium should be provided. It is recommended that this data requirement be determined by two different techniques. This would typically include results from a direct observational technique such as transmission electron microscopy (TEM) or scanning electron microscopy (SEM), as well as dynamic light scattering (DLS). The electron microscopic techniques provide information on the structure and size of primary nanoparticles whereas light scattering provides information on the average hydrodynamic radius of agglomerates/aggregates of nanoparticles dispersed in the water phase. The information derived from both techniques is complementary and important to fully characterise the state of nanomaterial aggregation in aqueous media used for environmental fate and effects testing.

In addition, a qualitative assessment of the degree of aggregation/agglomeration in the end-user or finished product should be provided. Where feasible, a representative microscopy image should also be provided.

Agglomerate (definition from ISO TS27687 2008): collection of loosely bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components

- Note 1. The forces holding an agglomerate together are weak forces, for example van der Waals forces, as well as simple physical entanglement.
- Note 2. Agglomerates are also termed secondary particles.

Aggregate (definition from ISO TS28687 2008): particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components.

- Note 1. The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement.
- Note 2. Aggregates are also termed secondary particles and the original source particles are termed primary particles.

Recommended test methods: SEM, TEM and DLS.

(v) Crystalline phase

Crystalline phase refers to the specific space group for a given crystal structure. In certain cases, it is possible to have multiple crystalline phases, such as with silica (i.e. amorphous and different crystalline forms) and titanium dioxide (i.e. rutile phase and anatase phase). A description of the average crystalline phase should be reported.

Recommended test methods: X-ray diffraction, electron diffraction, TEM

(vi) Composition (purity/impurities)

The percentage purity of the nanomaterial together with the identity and percentage of all impurities should be provided. Impurities may arise from incomplete reactions, from reagents used for production (e.g. catalysts) or from post-production handling (such as absorption of endotoxins).

Recommended test methods:

- For metallic impurities: Atomic absorption spectroscopy (AAS), Inductively coupled plasma mass spectroscopy (ICP-MS) and Inductively coupled plasma atomic emission spectroscopy (ICP-AES).
- For organic impurities: UV/VIS, GC-MS or LC-MS.

(vii) Surface area

The exposed surface area per unit mass of the nanomaterial presented as m^2/g should be provided.

Recommended test method: BET gas-absorption method.

(viii) Surface charge

Due to their extremely high specific surface area, aqueous dispersions of nanoparticles can easily lose their colloidal stability as a result of changes in the chemistry of the dispersion medium (e.g., ionic strength, pH, level of dissolved organic carbon). Agitation conditions and changes in concentration of the particles can also lead to agglomeration/aggregation. An important predictor of colloidal stability is the surface charge of particles. The surface charge is usually characterised by measurements of the zeta potential. The measurement of this electrokinetic parameter over a wide range of pH and ionic strengths in water can provide valuable information regarding the tendency of particle size and size distribution to change with time and solution chemistry.

The zeta potential of the nanomaterial in aqueous dispersion should be measured over as wide a pH range as practicable, but any measurements must span the environmentally relevant pH range of 4-9. The test methodology including details of the dispersion medium (such as ionic strength and identity and concentration of any added electrolytes or stabilisers) should be fully described. A full plot of the measured

zeta potential versus pH profile of the nanomaterial should be submitted. The pH for the point of zero charge (PZC) of the nanomaterial should be estimated if there is no net charge on the particles in the measured pH range.

Recommended test method: Measure electrophoretic mobility and calculate zeta potential.

(ix) Surface chemistry (e.g. coating or modification)
The chemical nature of the outermost layers of the nanomaterial, if different to the rest of the material should be provided. This includes the identity of any coatings or stabilisers/surfactants and intentional functionalisation. If the nanomaterial has a functionalised surface, the treating agent must be identified. Unintended functional groups on the surface such as those induced by purification processes may also be identified if feasible.

Surface chemistry will play a key role in determining fate in natural aqueous systems, colloidal stability and exposure. For a given functionalisation or coating it will affect other physico-chemical properties such as agglomeration, surface charge, surface area and water solubility.

GUIDANCE ON TESTING HEALTH EFFECTS OF NANOMATERIALS

The applicability of the OECD Test Guidelines for testing manufactured nanomaterials has been reviewed by the OECD Working Party on Manufactured Nanomaterials[3]. This review found that in general the OECD Test Guidelines are applicable for investigating the health effects of nanomaterials, although it was noted that in some cases there will be a need for a further modification to the OECD guideline. This particularly applies to studies using the inhalation route and to toxicokinetic studies. The following table summarises the key points from this review.

For each test, an adequate characterization of the nanomaterial tested 'out-of-the-bottle' should be reported together with a description of the sample preparation. Where feasible, characterization of the nanomaterial in the dosing medium (i.e. particle size distribution, agglomeration/aggregation state) should also be provided.

Summary of preliminary review of OECD test guidelines for their applicability to manufactured nanomaterials

OECD Test Guideline	Test	Comments
417	Toxicokinetics (Administration-Distribution-Metabolism-Excretion)	Guideline gives only very general guidance. Although this is currently being updated it is questionable whether modifications would be sufficient for investigating nanomaterials. It is likely that specific studies on the absorption and distribution of nanomaterials will need to be designed on a case-by-case basis. In particular, due to the likely property of nanoparticles to translocate whatever the exposure conditions, studies tracking the distribution of labelled nanomaterials in-

OECD Test Guideline	Test	Comments
		vivo at realistic exposure scenarios will be necessary. The main issues associated with ADME studies with nanomaterials are: (1) Ensuring that the label remains with the nanoparticles following route of entry into the body; and (2) Ensuring that the label does not alter the biological activity of the nanoparticle particularly since the changes in surface chemistry of the nanoparticle can significantly influence the physicochemical properties of the nanoparticle and, as a consequence, the toxicity of the nanoparticle. Therefore preliminary studies should be undertaken to certify that the above 2 criteria are met before undertaking a toxicokinetic study.
427	Skin absorption in vivo	No comments.
428	Skin absorption in vitro	The use has been questioned for nanomaterials since it has been claimed that mechanical aspects such as flexing may be important and some further development of this assay may be needed for nanomaterials.
420, 423 or 425	Acute oral	Would be appropriate for initial investigation. It should be recognised that the extent of pathology at autopsy is limited.
403	Acute inhalation	Includes only very limited histological examination at autopsy. Detailed examination of the respiratory tract would be appropriate with consideration of the addition of BAL (broncho-alveolar lavage) and possibly pulmonary cell proliferation endpoints. *The methodology for this test should not be confused with intratracheal instillation, that is commonly used to assess the pulmonary toxicity of nanomaterials. Intratracheal instillation in rats can cause misleading artefactual effects associated with doses that overload respiratory clearance mechanisms.* The OECD guideline is currently being updated, but this does not take into account nanomaterial assessment. Further revision should be planned after adoption or a specific guideline should be developed.
402	Acute dermal	Only requires minimal pathology; it would be desirable to have enhanced pathology when investigating nanomaterials.

OECD Test Guideline	Test	Comments
430, 431, 435	*in-vitro* methods for investigating skin corrosion	May be used but noting that measurement of cell viability using MTT (or other metabolically converted vital dye) may not be appropriate due to marker inactivation.
404, 405	Skin and eye irritation	Appropriate for investigating the irritancy of nanomaterials.
406	Skin sensitization – Guinea pig models	Should not be considered for nanomaterials. TG 429 is the preferred method.
429	Skin sensitization - Local lymph node assay (LLNA)	Appears to be the most appropriate method for investigating skin sensitisation potential of nanomaterials. The test permits an estimation of the potency of the sensitisation reaction.
432	Phototoxicity – in vitro assay	Mainly used for cosmetics – UV filters in sunscreens for phototoxicity.
407 & 409	28 day and 90 day repeat dose oral studies	Appropriate for investigating the repeated dose toxicity of nanomaterials by the oral route. Consideration needs to be given to enhancing the ability of these methods to detect adverse effects that are a particular concern with some nanoparticles (eg: cardiovascular effects with nanoparticles). Recently been updated to enhance their ability to detect neurotoxic and immunotoxic effects and also effects on the reproductive system. TG407 is currently being updated to give enhanced ability to detect effects on the endocrine system.
412 & 413	14-28 day and 90 day repeat dose inhalation	Both guidelines need to be enhanced with respect to neurotoxicity and immunotoxicity when investigating nanomaterials. The TG412 has very limited pathology. Detailed histological examination of the entire respiratory tract would be expected when investigating the effect of nanomaterials following repeated exposure by inhalation. Consideration needs to be given to enhancing the ability of these methods to detect adverse effects that are a particular concern with some nanoparticles.

OECD Test Guideline	Test	Comments
410, 411	21/28 or 90 day repeat dose dermal studies	Little use for chemicals in general. It is likely that any testing of nanomaterials by the dermal route would be limited to acute toxicity and investigation of the extent of absorption through skin.
   471 **473** **476**	In vitro genotoxicity tests Bacterial reverse mutation assay; In-vitro mammalian cell gene mutation test *In-vitro* mammalian cell gene mutation assay, with the mouse lymphoma assay being the preferred assay.	Appropriate for an initial investigation of the mutagenic potential of nanomaterials. However it has been recognised that treatment of mammalian cells in vitro with insoluble particles may lead to misleading results.
474, 475 or 486	In vivo genotoxicity tests	Positive results in vitro would need to be followed up in vivo if the bone marrow or liver were appropriate target organs, and this would be dependent on systemic availability.
477, 478, 479, 480, 481, 482, 483, 484, 485	Genotoxicity tests	Are unlikely to be used when investigating the mutagenicity of nano materials.
451, 452, 453	Chronic toxicity and carcinogenicity	Unlikely to be used for nanomaterials except in very exceptional circumstances.
421, 422, 415, 416	Reproductive toxicity	Appropriate for investigating the reproductive toxicity of nanomaterials via the oral route. Need to be modified if exposure was by inhalation and need careful consideration.
414	Developmental toxicity	

GUIDANCE ON TESTING THE ENVIRONMENTAL FATE AND EFFECTS OF NANOMATERIALS

The assessment of the environmental risks of nanomaterials in Australia will be conducted using the conventional risk assessment paradigm currently applied to all chemical substances, including industrial chemicals. This risk assessment framework involves parallel evaluations of the environmental fate and effects of chemical

substances according to harmonised international test guidelines, followed by a risk characterisation step[5] (EPHC, 2009).

The unique properties of nanomaterials may present some new challenges including technical issues to do with the applicability of harmonised test guidelines for chemicals[6] (Batley and McLaughlin, 2010). However, coordinated global activities by the OECD Working Party on Manufactured Nanomaterials have now identified critical strengths and weaknesses in the current test guidelines as they may apply to testing of the environmental fate and effects of nanomaterials[3]. The results of this review and supporting scientific data for the behaviour of nanomaterials in aquatic systems provides a basis for general guidance on appropriate approaches to characterising the environmental fate and effects of industrial nanomaterials.

Environmental Fate

The OECD test guidelines for environmental fate endpoints have each been reviewed by the WPMN for their applicability to the testing of nanomaterials[3]. According to this preliminary review, several existing test guidelines are applicable for testing the environmental fate of nanomaterials. However, the applicability of individual test methods is dependent on the behaviour of the nanomaterials in the environment, which in turn depends on the physical and chemical properties of nanomaterials in environmental media.

Based on the detailed evaluation carried out by the WPMN on test guidelines related to abiotic and biotic degradation, the tests seem to be applicable to the same extent for nanomaterials as for the comparable bulk materials. However, fully inorganic nanomaterials will not require testing in any of the biotic degradation tests. The conclusions of the WPMN on the current OECD test guidelines for biodegradability to nanomaterials is summarised in **Table 1**.

The potential for bioaccumulation of nanomaterials in aquatic organisms to be assessed using *OECD TG 305 Bioconcentration: Flow-through Fish test* may have some critical limitations in sole testing of bioaccumulation of nanoparticles. For example, it is likely that in most cases the size of nanoparticulate materials (one critical dimension in the range 1–100 nm) limits the uptake of these particles through membranes in fish compared to standard molecular chemical substances. Nevertheless, this test provides a valuable starting point for assessing bioaccumulation potential in aquatic organisms.

[5] EPHC (2009) Environmental risk assessment guidance manual for industrial chemicals. Environment Protection and Heritage Council, Australia, 109 pp, <http://www.ephc.gov.au/taxonomy/term/75>, Accessed 2010 Oct 28.

[6] Batley GE and McLaughlin MJ (2010) Fate of manufactured nanomaterials in the Australian environment. Bangor, Centre for Environmental Contaminants Research, CSIRO Land and Water, 76 pp (Technical report prepared for the Department of the Environment, Water, Heritage and the Arts). <http://www.environment.gov.au/settlements/biotechnology/publications/manufactured-nanomaterials.html>, Accessed 2010 Nov 11.

Table 1. International guidelines (OECD) for assessing biodegradability - applicability for nanomaterials

Test Guideline	Limitations	Applicability for nanomaterials
OECD 301A DOC die way	Test substance has to be soluble, non-volatile, not sorbed to vessel or sludge and non-toxic at test conc.	In principle not applicable as the nanomaterial has to be soluble.
OECD 301B CO_2 evolution test	Test substance must be non-volatile and non-toxic at test concentration.	Applicable, but higher test material concentration needed e.g. compared to OECD 310 (2–40 mg C/L). Measures mineralization.
OECD 301C Modified MITI Test	Test substance has to be non-toxic at test concentration, subject to interference from nitrification.	In principle applicable, but high conc. needed
OECD 301D Closed bottle test	Test substance has to be non-toxic at test concentration, subject to interference from nitrification.	In principle applicable.
OECD 301E Modified OECD screening test	Test substance has to be soluble, non-volatile, not sorbed to vessel or sludge and non-toxic at test conc.	In principle not applicable as the nanomaterial has to be soluble.
OECD 301F Manometric respirometry test	Test substance has to be non-toxic at test concentration, subject to interference from nitrification.	In principle applicable, high conc.
OECD 310 (Headspace test)	Test substance must be non-toxic at test concentration (pH 2 for analysis of CO_2)	Applicable, test material need not to be soluble, carriers can be used. Measures mineralization.
Simulation Tests for Freshwater (Marine) and Sediment Systems		
OECD 308 Aerobic and anaerobic transformation in aquatic sediment systems	Simulates suspended sediment only. Test substance has to be non-toxic, non-volatile and soluble. Site specific with respect to sediment. Sorption to sediment may be misleading if ^{14}C not used.	Applicable, but the bioavailability may limit degradation. Measures mineralization from labelled particles.
OECD 309 Aerobic mineralisation in surface water	No comment provided	Applicable. Measures mineralization from labelled particles.

Environmental effects

There are currently 13 OECD guidelines (**Table 2**) for testing substances for adverse effects on a variety of aquatic life, and these include tests for both acute and chronic effects. These guidelines have each been reviewed by the WPMN to evaluate their applicability to the testing of nanomaterials[3]. In summary, it is likely that the ecotoxicity endpoints described in the current test guidelines are applicable to the testing of nanomaterials. These endpoints generally involve whole-organism responses that integrate many possible modes of toxicity and are thus also likely to be indicators of potential adverse effects of nanomaterials.

However, the review also highlighted a common challenge associated with the application of these test guidelines to nanomaterials: that guidance on preparation, delivery, measurement, and metrology in all of the test guidelines is currently insufficient for testing of nanomaterials. There are coordinated efforts by the OECD to refine and adapt this aspect of the test guidelines for the particular requirements of testing the environmental effects of nanomaterials. In the interim, it is recommended that the design and conduct of aquatic effects tests of nanomaterials should be closely integrated with measurements of the physical/chemical properties of these materials. The particularly relevant properties that should be characterised relate to the colloidal stability and solubility of the nanomaterials under typical aquatic exposure conditions.

Table 2. List of OECD guideline tests for aquatic ecotoxicity reviewed for applications to nanomaterials

Test guideline	Description of test
201	Alga, Growth Inhibition Test
202	*Daphnia sp*. Acute Immobilisation Test
203	Fish, Acute Toxicity Test
204	Fish, Prolonged Toxicity Test
209	Activated Sludge, Respiration Inhibition Test
210	Fish, Early-Life Stage Toxicity Test
211	*Daphnia magna* Reproduction Test
212	Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages
215	Fish, Juvenile Growth Test
218	Sediment-Water Chironomid Toxicity Using Spiked Sediment
219	Sediment-Water Chironomid Toxicity Using Spiked Water
221	*Lemna* sp. Growth Inhibition Test
224	Determination of the Inhibition of the Activity of Anaerobic Bacteria Reduction of Gas Production from Anaerobically Digesting (sewage) Sludge