

**ENVIRONMENT DIRECTORATE
CHEMICALS COMMITTEE**

Working Party on Manufactured Nanomaterials

**OECD TEST GUIDELINES AND GUIDANCE DOCUMENTS FOR THEIR APPLICABILITY TO
NANOMATERIALS:
PRELIMINARY STATUS REVIEW**

17th Working Party on Manufactured Nanomaterials

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Test Guidelines are an important component of the system of Mutual Acceptance of Data (MAD), which has legally binding implications for OECD member countries (and those non-members who have adhered to MAD). On a number of occasions, the WPMN has stressed the importance to continue work for ensuring the applicability of Test Guidelines to Manufactured Nanomaterials. In 2009, the WPMN completed a Preliminary Review of OECD Test Guidelines for the Applicability to Manufactured Nanomaterials [[ENV/JM/MONO\(2009\)21](#)]. Since then, a number of efforts, within OECD and other international activities, have contributed to a better understanding on the specific needs, and sometimes on specific amendments, that would be needed for ensuring their applicability.

To date, a number of projects that were initiated within the WPMN have been included in the Programme of Work of the WNT:

- (New) TG on Dispersion Stability of Nanomaterials in Simulated Environmental Media
- (New) TG on Dissolution Rate in Aquatic Environment
- (New) TG for Removal from Wastewater
- (New) GD to Assess the Apparent Accumulation Potential for Nanomaterials
- (New) GD Aquatic (and Sediment) Toxicity Testing
- (New) GD (Decision Tree) on Agglomeration and Dissolution Behaviour in Aquatic Media
- Amendments to Inhalation TGs and GD
 - TG 412 (Subacute Inhalation Toxicity)
 - TG 413 (Subchronic Inhalation Toxicity).
 - TG 403 (Acute Inhalation)
 - TG 436 (Acute Inhalation – Acute Toxic Class)
 - GD 39 (Inhalation Toxicity Testing)
- (New) GD for TG 312 - Leaching in Soil Columns

The current document presents the current status of the TG/ GDs based on the conclusions and recommendations from the OECD expert workshops, as well as from recent publications that evaluated the existing TGs. It is worth noticing that these publications have already identified the amendments that are needed for a number of TGs.

ACTION REQUIRED: ***The WPMN is invited to take note of this document and to agree on the recommendation for the next steps, as presented by SGTA.***

The **number/ letter** mentioned in the table correspond to the following documents:

- A.** Kerstin Hund-Rinke, Anders Baun, Denisa Cupi, Teresa F. Fernandes, Richard Handy, John H. Kinross, José M. Navas, Willie Peijnenburg, Karsten Schlich, Benjamin J. Shaw & Janeck J. Scott-Fordsmand (2016): Regulatory ecotoxicity testing of nanomaterials – proposed modifications of OECD test guidelines based on laboratory experience with silver and titanium dioxide nanoparticles, *Nanotoxicology*, DOI: 10.1080/17435390.2016.1229517
<http://www.tandfonline.com/doi/full/10.1080/17435390.2016.1229517>
- B.** NanoReg Deliverable D 2.9- Revised OECD methods for determination of physicochemical NM properties
- 1.** Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials [ENV/JM/MONO\(2009\)21](#)
 - 2.** Inhalation Toxicity Testing: Expert Meeting on Potential Revisions to OECD Test Guidelines and Guidance Document [ENV/JM/MONO\(2012\)14](#)
 - 3.** Ecotoxicology and Environmental Fate of Manufactured Nanomaterials: Test Guidelines [ENV/JM/MONO\(2014\)1](#)
 - 4.** Report of the OECD expert meeting on the physical chemical properties of manufactured nanomaterials and test guidelines [ENV/JM/MONO\(2014\)15](#)
 - 5.** Genotoxicity of Manufactured Nanomaterials : Report of the OECD expert meeting [ENV/JM/MONO\(2014\)34](#)
 - 6.** Draft report: Evaluation of methods applied in the OECD-WPMN Testing Programme: Methods for Physicochemical Properties Suitability of different methods to characterise nanomaterials, presented at the last SGTA meeting in February 2015
 - 7.** Evaluation of *in vitro* methods for human hazard assessment applied in the Testing Programme [ENV/CHEM/NANO(2016)REV2]

Color code:

	Applicable
	Not Applicable
	Project underway
	Further assessment needed
	Not evaluated
	Unlikely to be use for NM

SESSION 1: PHYSICAL CHEMICAL PROPERTIES		
TG 101 - UV-VIS Absorption Spectra - (Spectrophotometric Method)	might be applicable under some circumstances or to some classes of manufactured nanomaterials further assessment is required (1)	
TG 102 - Melting Point / Melting Range	applicable to manufactured nanomaterials (1)	
TG 103 - Boiling Point	not applicable to manufactured nanomaterials or, though strictly applicable, provide no useful added value (1)	
TG 104 - Vapour Pressure	might be applicable under some circumstances or to some classes of manufactured nanomaterials (1)	
TG 105 - Water Solubility	Alternative approaches exist and can be considered in this revision process. (4) B. Suggestions: <ul style="list-style-type: none"> • Not directly applicable. Requires modifications. <ul style="list-style-type: none"> ○ Kinetic study. ○ Control of variables such as pH or dissolved gases. ○ Protocol for dispersibility determination. • Modifications to solubility protocol will be evaluated and reported in 	

	<p>D2.9. Relevant SOPs for in vitro, in vivo and environmental dissolution testing will also be presented in Task 2.4 (D2.6, D2.7 and D2.8)</p> <ul style="list-style-type: none"> • Dispersibility protocol will be evaluated and proposed as new protocol 	
TG 106 - Adsorption - Desorption Using a Batch Equilibrium Method	Not applicable (3) (B)	
TG 107 - Partition Coefficient (n-octanol/water): Shake Flask Method	<p>B. Suggestions:</p> <ul style="list-style-type: none"> • Not Applicable / From a thermodynamic point of view not possible • It should be replaced by the determination of hydrophilicity/hydrophobicity. • Next needed Evaluation of new methods for testing hydrophilicity/hydrophobicity 	
TG 108 - Complex Formation Ability in Water (Polarographic Method)	<p>B. Suggestions:</p> <ul style="list-style-type: none"> • Not considered adequate for its application to NMs in its current form, but a similar protocol can be useful to determine NM as vector of toxic metals. • Guideline modification for focusing on the adsorption of trace metals on NMs. • Next Protocol evaluation with different metals in several conditions. 	
TG 109 - Density of Liquids and Solids	<p>B. Suggestions:</p> <ul style="list-style-type: none"> • Verification necessary • Protocol evaluation. • Next needed. Evaluation of the measurement of the density of 	

	agglomerates.	
TG 110 - Particle Size Distribution/Fibre Length and Diameter Distributions	B. OECD methods only partially applicable /Completely new protocols (CLS and DLS) are proposed The proposed protocols will be evaluated and reported.	OECD WPMN Project/ Germany
TG 111 - hydrolysis as a function of pH	might be applicable under some circumstances or to some classes of manufactured nanomaterials further assessment is required (1)	
TG 112 - Dissociation Constants in Water	B. Suggestion. <ul style="list-style-type: none"> • Not considered adequate for its application to NMs in its current form • Develop protocols for other more relevant endpoints such as dissociation of water and hydration, surface acidity and isoelectric point. • Development of new protocols, especially for determination of isoelectric point 	
TG 113 - Screening Test for Thermal Stability and Stability in Air	applicable to manufactured nanomaterials (1)	
TG 114 - Viscosity of Liquids	not applicable to manufactured nanomaterials or, though strictly applicable, provide no useful added value (1)	

TG 115 - Surface Tension of Aqueous Solutions	B.Suggestion <ul style="list-style-type: none"> • Modifications necessary. • This protocol is considered relevant only for some NM. • Modifications will be evaluated and the relevance will be analyzed 	
TG 116 - Fat Solubility of Solid and Liquid Substances	applicable to manufactured nanomaterials (1)	
TG 117 - Partition coefficient (n-octal/water) - high performance liquid chromatography method	Not Applicable (B) (4) B. Suggestions: <ul style="list-style-type: none"> • Not Applicable / From a thermodynamic point of view not possible • It should be replaced by the determination of hydrophilicity/hydrophobicity. • Next needed Evaluation of new methods for testing hydrophilicity/hydrophobicity 	
TG 118 - Determination of the Number-Average Molecular Weight and the Molecular Weight Distribution of Polymers using Gel Permeation Chromatography	might be applicable under some circumstances or to some classes of manufactured nanomaterials (1)	
TG 119 - Determination of the Low Molecular Weight Content of a Polymer Using Gel Permeation	might be applicable under some circumstances or to some classes of manufactured nanomaterials (1)	

Chromatography		
TG 120 - Solution/Extraction Behaviour of Polymers in Water	might be applicable under some circumstances or to some classes of manufactured nanomaterials (1)	
TG 121 - Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)	might be applicable under some circumstances or to some classes of manufactured nanomaterials further assessment is required (1)	
TG 123 - Partition Coefficient (1-Octanol/Water): Slow-Stirring Method	Not Applicable (4)(B). B. Suggestions: <ul style="list-style-type: none"> • Not Applicable / From a thermodynamic point of view not possible • It should be replaced by the determination of hydrophilicity/hydrophobicity. • Next needed Evaluation of new methods for testing hydrophilicity/hydrophobicity 	
Dispersion	B. Suggestion: already developed a protocol, next needed is validation	

SECTION 2: EFFECTS ON BIOTIC SYSTEMS		
OECD TG Project Guidance Document on Aquatic (and Sediment) Toxicology Testing of Nanomaterials		WNT Project
TG 201 - Freshwater Alga and Cyanobacteria, Growth Inhibition Test	<p>A. Proposed modifications:</p> <ul style="list-style-type: none"> • The chelating agent EDTA can interfere with metal NMs and a modified version of OECD algal medium (OECD-M) for <i>Raphidocelis subcapitata</i> is proposed. This EDTA-free algal medium should be considered when testing metal nanomaterials. • Measurement of biomass by determination of in-vitro Chlorophyll A (Chla). 	
TG 202 - Daphnia sp. Acute Immobilisation Test	<p>A. Proposed modification:</p> <ul style="list-style-type: none"> • Testing at pH-values where more stable dispersions are obtained and using a very low ionic strength medium with low amounts of divalent cations (e.g. Very Soft EPA medium) under which <i>D. magna</i> can grow and reproduce normally. 	
TG 210 - Fish, Early-life Stage Toxicity Test	<p>A. Proposed modifications:</p> <ul style="list-style-type: none"> • Use of exposure chambers coupled with water changes every 24 hours in order to improve NM dispersion during tests. A prototype exposure chamber is described in Boyle et al. (2015), and our additional modifications include a filter mesh to protect the fish embryos from the risk of mechanical injury and mixing of the water without an air lift. 	

	<ul style="list-style-type: none"> • Conduct tests on hatched larvae only • The TG should include more clearly defined criteria and methodologies for the assessment of sublethal effects. 	
TG 211 - Daphnia magna Reproduction Test	applicable (3)	
TG 216 - Soil Microorganisms: Nitrogen Transformation Test	<p>A. Proposed modifications:</p> <ul style="list-style-type: none"> • For the testing of ion releasing metal NMs, the pH of the soils should be at the lower end of the range accepted according to the test guideline (pH 5.5). • NMs should be added to each replicate, except for low concentrations. • Extension of test duration to 56 days. • Instead of a complex organic nitrogen source such as Lucerne meal, an inorganic nitrogen source (e.g. (NH₄)₂SO₄) is recommended for the testing of nanoparticulate metals or metal oxides. • Multiple short-term determinations of the potential ammonium oxidation activity (ISO Guideline 15685, 2012) during the incubation period instead of the determination of the long-term nitrification process from bound nitrogen to nitrate. 	
TG 217 - Soil Microorganisms: Carbon Transformation Test	<p>A. Proposed modifications:</p> <ul style="list-style-type: none"> • For the testing of ion releasing metal NMs, the pH of the soils should be at the lower end of the range accepted according to the test guideline (pH 5.5). • NMs should be added to each replicate, except for low concentrations. • Extension of test duration to 56 days 	

TG 220 - Enchytraeid Reproduction Test	A. Proposed modifications: <ul style="list-style-type: none"> • For tests in artificial soil, it is recommended to reduce the organic matter content of the standard OECD test soil from 10 to 5 or 2%. • Comparable to soil microflora, the soil pH can be modified where relevant for ion releasing metal NMs. • NMs should be added to each replicate, except for low concentrations. • Extension of test duration to 42 days and measurement of more life stage endpoints, i.e. hatchability, juvenile growth and time to maturity. 	
TG 222 - Earthworm Reproduction Test (<i>Eisenia fetida</i>/<i>Eisenia andrei</i>)		
TG 225 - Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment	A. Proposed modifications: <ul style="list-style-type: none"> • Lower organic matter sediment (2% peat as opposed to 5%). • Applying NMs directly to each sediment replicate. 	

SECTION 3: DEGRADATION AND ACCUMULATION		
<u>Nanoparticle aggregation/ Dispersion stability in water</u>	OECD TG on Dispersion Stability of Nanomaterials in Simulated Environmental Media.	APPROVED BY THE WNT APRIL 2017
Need standard method for measuring particle zeta potential to estimate the degree of particle aggregation and hence the stability of nanomaterial aqueous dispersions. (1)	OECD TG Project New GD (Decision-Tree) on agglomeration and dissolution behaviour of nanomaterials in aquatic media	WNT Project
	OECD TG Project New TG for dissolution rate of nanomaterials in the aquatic environment	WNT Project
	OECD TG Project New TG for Nanomaterial Removal from Wastewater	WNT Project
	OECD TG Project New GD on Assessing the Apparent Accumulation Potential of Nanomaterials	WNT Project
	OECD TG Project New Guidance of OECD312 Soil Column Leaching	WNT Project
	TG 301 - Ready Biodegradability	In principle applicable (1)
TG 301F -Ready Biodegradability	In principle applicable, high conc. (1)	

TG 310 - Ready Biodegradability - CO2 in sealed vessels (Headspace Test)	Applicable, test material need not to be soluble, carriers can be used./ Measures mineralization. (1)	
TG 308 - Aerobic and Anaerobic Transformation in Aquatic Sediment Systems	Applicable, <u>but the bioavailability may limit degradation</u> / Measures mineralization from labeled particles. (1)	
TG 309 - Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test	Applicable / Measures mineralization from labeled particles. (1)	
TG 303A - 303: Simulation Test - Aerobic Sewage Treatment -- A: Activated Sludge Units;	Applicable only with radio-labeled material (1)	
TG 306 - Biodegradability in Seawater	Two methods are presented : a seawater variant of the modified OECD Screening Test (Shake Flask Method) and a seawater variant of the Closed Bottle Test. The latter can be applicable to nanomaterials./ As the closed bottle test is more appropriate an indication of differences in limitations could be useful e.g. in closed bottle method test substance can be less soluble. (1)	
TG 307 - Aerobic and Anaerobic Transformation in Soil	Mineralisation by ¹⁴ CO ₂ measurement possible (1)	

TG 302A -Inherent Biodegradability: Modified SCAS Test	Applicable for mineralisation (1)	
TG 302B - Inherent Biodegradability: Zahn-Wellens/ EVPA Test	Limited use as DOC needed (1)	
TG 302C- Inherent Biodegradability: Modified MITI Test (II)	Not applicable as very high amount of carbon needed. (1)	
TG 304A - Inherent Biodegradability in Soil	Labelled substances needed (1)	
TG 311 - Anaerobic Biodegradability of Organic Compounds in Digested Sludge: by Measurement of Gas Production	Very high amount of test material needed. <u>May not be applicable.</u> (1)	
TG 305 - Bioaccumulation in Fish: Aqueous and Dietary Exposure	applicable (3)	
TG 317 - Bioaccumulation in Terrestrial Oligochaetes	applicable to the testing of ENMs <u>if some additions and changes are added</u> to the TG./ • The application of the ENM must be reported in detail. Wet and dry spiking are feasible./ • A GD that clarifies how the data of the experiments can be interpreted should be developed. (3)	

TG 315 - Bioaccumulation in Sediment-dwelling Benthic Oligochaetes	applicable to the testing of ENM <u>if some additions and changes are added</u> to the TG. /• Wet spiking of the sediment was favoured in the discussion./ • A GD that clarifies how the data of the experiments can be interpreted should be developed (3)	
TG 312 - Leaching in Soil Columns	PROJECT	WNT Project Approved by the WNT April 2017

SECTION 4: HEALTH EFFECTS

TG 401 Acute Oral Toxicity	Not evaluated	
TG 402 Acute Dermal Toxicity	needs to be updated (1)	
TG 403 Acute Inhalation Toxicity	OECD TG Project (inhalation)	WNT Project
TG 404 Acute Dermal Irritation/Corrosion	Applicable (1)	
TG 405 Acute Eye Irritation/Corrosion	Applicable (1)	
TG 406 Skin Sensitisation	Not evaluated	

TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents	needs to be updated to enhance the ability to detect adverse effects (1)	
TG 408 Repeated Dose 90-Day Oral Toxicity Study in Rodents	Not evaluated	
TG 409 Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents	needs to be updated to enhance the ability to detect adverse effects (1)	
TG 410 Repeated Dose Dermal Toxicity: 21/28-day Study	Not evaluated	
TG 411: Subchronic Dermal Toxicity: 90-day Study	Not evaluated	
TG 412 Subacute Inhalation Toxicity: 28-Day Study	OECD TG Project (inhalation)	APPROVED BY THE WNT APRIL 2017
TG 413 Subchronic Inhalation Toxicity: 90-day Study	OECD TG Project (inhalation)	APPROVED BY THE WNT APRIL 2017
TG 414 Prenatal Development Toxicity Study	Applicable (1)	
TG 415 One-Generation Reproduction	Applicable (1)	

Toxicity Study		
TG 416 Two-Generation Reproduction Toxicity	Applicable (1)	
TG 418 Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure	unlikely to be used for nanomaterials (1)	
TG 419 Delayed Neurotoxicity of Organophosphorus Substances: 28-day Repeated Dose Study	unlikely to be used for nanomaterials (1)	
TG 420 Acute Oral Toxicity - Fixed Dose Procedure	Not evaluated	
TG 421 Reproduction/Developmental Toxicity Screening Test 29 July 2016	Applicable (1)	
TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test	Applicable (1)	
TG 423 Acute Oral toxicity - Acute Toxic	Not evaluated	

Class Method		
TG 424 Neurotoxicity Study in Rodents	unlikely to be used for nanomaterials (1)	
TG 426 Developmental Neurotoxicity Study	unlikely to be used for nanomaterials (1)	
TG 427 Skin Absorption: <i>In Vivo</i> Method	Not evaluated	
TG 428 Skin Absorption: <i>In Vitro</i> Method	<p>As deeply discussed in the OECD Expert Meeting on Toxicokinetics of Manufactured Nanomaterials¹⁵, TG 428 should be adopted for testing on MNMs, since there are many critical points in the protocol that may not be adequate for MNMs. In particular, the duration of the observation time, the sampling time, the influence of mechanical process in particles translocation, the MNMs solubility and the compatibility of the receptor fluid with MNMs need to be further explored.</p> <p>some critical factors for the evaluation of skin absorption tests with nanomaterials were not addressed in detail in these studies, and might need to be further explored. These include the optimum exposure duration and time of sampling/evaluation, choice of the exposure concentrations (i.e. stable dispersions without material aggregation should be used), the compatibility of the receptor fluid with MNMs, evaluation of the MNMs cellular uptake, and the influence of mechanical process in particles translocation. Thus, the applicability of TG 428 (Skin absorption: <i>in vitro</i> method) should be further investigated. (7)</p>	

TG 429 Skin Sensitisation	be the most appropriate method for investigating skin sensitization potential of nanomaterial. It has advantages with respect to animal welfare considerations (1)	
TG 430 In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)	Not evaluated	
TG 431 In vitro skin corrosion: reconstructed human epidermis (RHE) test method	needs to be updated (1) as in the case of TG 428, some critical issues related to the protocol are identified (e.g. the lack of the circulation in the subcutis, the relevant length of exposure, surface area of exposure, compatibility of the receptor fluid for MNMs), suggesting that the amendment of this Test Guideline might be needed in view of MNMs testing application. Applicability of TG 431 (In vitro skin corrosion: reconstructed human epidermis (RHE) test method) should be further discussed. (7)	
TG 432 In Vitro 3T3 NRU Phototoxicity Test	Not evaluated	
TG 435 In Vitro Membrane Barrier Test Method for Skin Corrosion	Not evaluated	
TG 436 Acute Inhalation Toxicity – Acute Toxic Class Method	OECD TG Project (inhalation)	

<p>TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants</p>	<p>TG 437 is generally applicable for assessment of eye irritation or serious eye damage potential of MNMs. However, application of TG 437 will require further evaluation of the method and amendment of the protocol to accommodate MNMs related aspects such as through characterization of the MNMs (powders and/or suspensions). As concluded by the SCCS in the Guidance on the Safety Assessment of Nanomaterials in Cosmetics²⁶ “no specific validation has been performed for nanomaterials, although there is no clear scientific basis against the use of the method for nanomaterials. It should, however, be kept in mind that nanomaterials can aggregate/agglomerate in the suspension or can absorb dispersant. These aspects should be verified. Some nanomaterials present in opacity measurements may affect the result, and these should be avoided to allow consistent interpretation of results. Both methods measure the leakage of fluorescein. Possible artifacts due to absorption of the dye to nanomaterials should be verified and eliminated.” Applicability of TG 437 (Bovine Corneal Opacity and Permeability Test Method) should be further discussed. (7)</p>	
<p>TG 440 Uterotrophic Bioassay in Rodents</p>	<p>Not evaluated</p>	
<p>TG 441 Hershberger Bioassay in Rats</p>	<p>Not evaluated</p>	
<p>TG 451 Carcinogenicity Studies</p>	<p>unlikely to be used for nanomaterials (1)</p>	
<p>TG 452 Chronic Toxicity Studies</p>	<p>unlikely to be used for nanomaterials (1)</p>	

TG 453 Combined Chronic Toxicity/Carcinogenicity Studies	unlikely to be used for nanomaterials (1)	
TG 455 The Stably Transfected Human Estrogen Receptor-alpha Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals	Not evaluated	
TG 471 Bacterial Reverse Mutation Test	<p>Applicable (1)</p> <p>The use of the Ames test (TG 471) is not a recommended test method for the investigation of the genotoxicity of nanomaterials. The test guidelines programme should consider modifying the applicability domain within this test guideline accordingly. (5)</p> <p>The bacterial reverse mutation test was used to assess the mutagenic potential of eight types of MNMs. In most cases, the assay was used both with and without metabolic activation (S9 mixture). None of the materials tested showed any mutagenic activity in the bacterial reverse mutation test. However, one study underlined the importance of the lack of uptake of the MNMs into bacteria, and thus the possibility of false negative results. This is in line with the conclusion of the OECD Expert Meeting on Genotoxicity of Manufactured Nanomaterials (ENV/JM/MONO(2014)34) in which one of the conclusion was that the use of Ames test (TG 471) is not a recommended test method for the investigation of the genotoxicity of MNMs²². The TG programme should consider modifying the applicability domain within this guideline accordingly. TG 471</p>	

	(Bacterial reverse mutation test) may be amended with the acknowledgement that it is not applicable for most types of MNMs (no uptake into the bacteria). (7)	
TG 473 In Vitro Mammalian Chromosomal Aberration Test	Applicable (1) The in vitro mammalian chromosomal aberration test was used to assess the mutagenic potential of seven types of MNMs. In most cases the assays were used both with and without metabolic activation (S9 mixture). The majority of NMs were tested in the CHO cell line, but V79 cells, CHL/IU, human lymphocytes, and WI-38 cells were also used. The time of exposure was mostly 4 h (with metabolic activation) and 4 h and 24 h (without metabolic activation), but other times were also used. None of the materials tested showed any mutagenic activity in the in vitro mammalian chromosomal aberration test. No issues regarding potential NMs interferences with the assays were reported. (7)	
TG 474 Mammalian Erythrocyte Micronucleus Test	Applicable (1)	
TG 475 Mammalian Bone Marrow Chromosomal Aberration Test	Applicable (1)	
TG 476 In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes	Applicable (1) In vitro mammalian cell gene mutation tests were used to assess the mutagenic potential of four types of MNMs. In most cases the assays	

	<p>were performed both with and without metabolic activation (S9 mixture). The L5178Y TK +/- cells were used to test all NMs. In addition, CHO cells were used in two studies to test SiO₂ NPs. None of the materials tested showed clearly evident mutagenic activity in the in vitro mammalian cell gene mutation tests. No issues regarding potential NMs interferences with the assays were reported for MWCNTs, SiO₂ NPs, and TiO₂ NPs. For ZnO NPs the increased turbidity was reported at higher concentrations which could potentially influence the conduct of the test.</p> <p>The in vitro mammalian cell gene mutation tests (TG 476) is considered as an alternative for the bacterial reverse mutation test (TG 471), as no report has yet identified specific limitations when testing MNMs with TG 476 (ENV/JM/MONO(2014)34). (7)</p>	
TG 477 Genetic Toxicology: Sex-Linked Recessive Lethal Test in <i>Drosophila melanogaster</i>	Not evaluated	
TG 478 Genetic Toxicology: Rodent Dominant Lethal Test	Not evaluated	
TG 479: Genetic Toxicology: In vitro Sister Chromatid Exchange Assay in Mammalian Cells	Not evaluated	
TG 480 Genetic Toxicology: <i>Saccharomyces</i>	Not evaluated	

cerevisiae, Gene Mutation Assay		
TG 481: Genetic Toxicology: Saacharomyces cerevisiae, Miotic Recombination Assay	Not evaluated	
TG 482 Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells in vitro	Not evaluated	
TG 483 Mammalian Spermatogonial Chromosome Aberration Test	Not evaluated	
TG 484 Genetic Toxicology: Mouse Spot Test	Not evaluated	
TG 485 Genetic toxicology, Mouse Heritable Translocation Assay	Not evaluated	
TG 486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells <i>in vivo</i>	Applicable (1)	
TG 487 <i>In Vitro</i> Mammalian Cell Micronucleus Test	The draft 487 excluded insoluble materials from the applicability domain of the guidelines. The TG has demonstrated utility in testing the genotoxicity of nanomaterials and, in order to prevent any confusion, the relevant sections of the guideline was re-worded carefully so it does not exclude nanomaterials. The approved TG does not contain any nano-specific adaptations. (5)	

	<p>Modification of TG 487 (In vitro micronucleus assay) should be considered to include specific recommendations regarding addition of cytochalasin B when testing MNMs and the verification of intracellular uptake of MNMs. This was also one of the issues discussed in detail at the OECD expert meeting on Genotoxicity of Manufactured Nanomaterials [ENV/JM/MONO(2014)34]22. The JRC is currently leading a project on the development of an OECD Guidance Document that will support implementation of the existing genotoxicity OECD Test Guidelines on in vitro Mammalian Cell Based Genotoxicity when testing manufactured nanomaterials. (7)</p>	
ADME	<p>needs to be considered on a case by case basis (1)</p> <p>The use of the Ames test (TG 471) is not a recommended test method for the investigation of the genotoxicity of nanomaterials. (5)</p>	
Comet assay (single-cell gel electrophoresis)	<p>With the Comet assay, the responses were largely negative or equivocal in the tested cells for most of the investigated MNs. When positive results were obtained, in most cases, dose response relationship could not be established which makes it difficult to conclude on the <i>in vitro</i> genotoxicity of the MNs tested. Therefore, the results from the Comet Assay can give an indication of potential genotoxicity of environmental chemicals. Moreover, the large variability in all the experimental conditions (including differences in cell lines used, concentrations tested, times of exposure, and dispersion protocols) makes the comparability of the results from the various studies practically impossible. (7)</p>	

