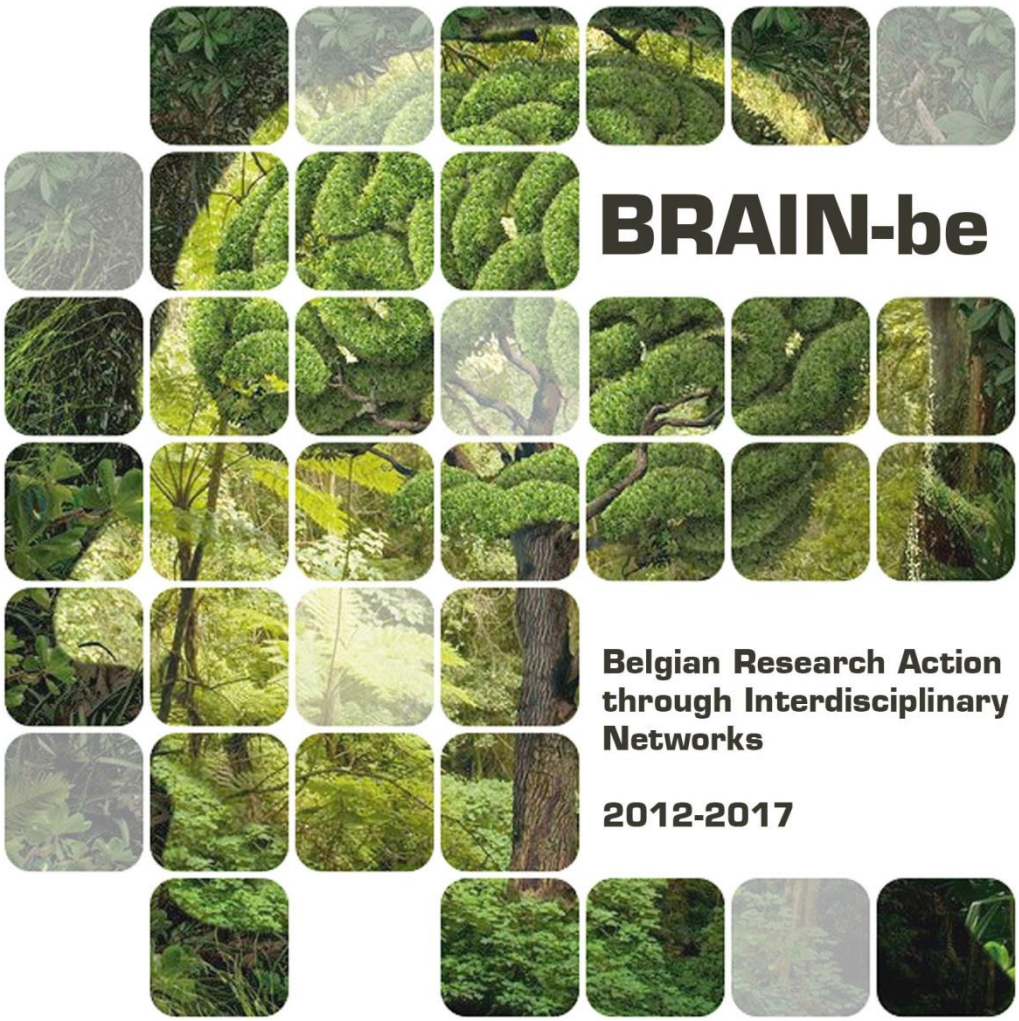

To^{2}DeNano

Towards a toxicologically relevant definition of nanomaterials

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Axis 4: Federal public strategies

ANNEXES

Annex 1: Technical data sheet of the representative test material JRCNM02000a[1]

Frédéric Brassinne, Pieter-Jan De Temmerman, Jan Mast (Sciensano)

Chemical identification of the substance

Chemical name of the substance	Silicium oxide
Chemical Formula	SiO_2
CAS Number	7631-86-9
EC number	231-545-4
REACH registration number	Not available

Particle shape and size[2]

Used determination methods	TEM[3]
Shape	Sphere
Number of dimensions	3
Characteristic dimension	Diameter (Feret Min)
Mean particle size	18 nm
Median particle size	15.9 nm
Standard deviation	0.6 nm
Measurement uncertainty	8 % (95% CI)[4,5]

Agglomerates and Aggregates[6]

Is the substance available in agglomerated form	yes
Determination of the agglomerate size	Together with the aggregated particles
Is the substance available in aggregated form	Yes

[1] In the context of the BELSPO BRAIN-be project "Towards a toxicologically defined definition of a nanomaterial" (To2DeNano), we are characterizing a silicium oxide representative test materials JRCNM02000a in detail using a combination of newly developed methods that were validated in parallel on the same samples. To illustrate that the obtained results are very meaningful in a regulatory context, we introduce these results in the template that is used for the physico-chemical characterization of nanomaterials in the Belgian registry for nanomaterials.It is not our intention to register these products formally, so introduction of the information as a regular application is not aimed for.

[2] Results are described in detail in "TEM Primary Particles validation report JRCNM02000a. In addition to the Feret Min, these reports also describe the ECD and the MICD.

[3] TEM is one of the few methods that allows determining the distributions of characteristics size and shape properties of primary particles present as single particles and in aggregates and agglomerates. TEM does not allow measuring the particles itself but only their projection. Based on experimental data (DLS validation reports and PTA validation reports seen in the annexes of the "Agglomerates and Aggregates description", it is shown that the DLS ensemble method and PTA are not able to characterize the primary particles.

[4] Results are from a top down validation study (see annex) with triplicate measurements on five days within one week.

[5] Traceability is based on analysis of certified reference materials (ERM-FD100, ERM-FD102, ERM-FD304) and calibrated using the cross-grating method based on a 2160 lines/mm optical diffraction-cross grating

[6] Results are described in detail in "Validatiedossier_JRC02000_Aggregates_Agglomerates". In addition to the Feret Min, the TEM based reports also describe the ECD, and the MICD.

Determination method	TEM[7]
Size measured as	Mean size and standard deviation (Feret Min)
Conditions[8]	Stable
Mean size	42 nm
Standard deviation	7 nm
Shape description	Fractal - angular
Measurement uncertainty	32 % (95% CI)[4, 5]

Determination method	PTA[9]
Size measured as	Mean hydrodynamic radius and standard deviation
Conditions[8]	Stable
Mean size	176 nm
Standard deviation	10 nm
Measurement uncertainty	13 % (95% CI)[4]

Determination method	DLS[10]
Size measured as	Mean hydrodynamic radius
Conditions[8]	Stable
Mean size	269 nm
Measurement uncertainty	32 % (95% CI)[4]

Coating

Do the particles have a coating	No

Impurities[11]

Are there any impurities	Yes, indicated below according decreasing concentrations
Used determination method	ICP-OES
#1	Mass concentration (%): 0.1 Impurity: Na (sodium), S (sulfur) Description: higher than 0.1% of mass concentration
#2	Mass concentration (%):0.01 Impurity: Ca (calcium), Al (aluminum) Description: higher than 0.01% of mass concentration
#3	Mass concentration (%): 0.005 Impurity: Fe (iron), K (potassium) Description: between 0.005-0.01% of mass concentration
#4	Mass concentration (%):0.001 Impurity: Mg (magnesium), Zr (Zirconium) Description: between 0.001-0.005% of mass concentration

[7] TEM is one of the few number based methods that allows determining the distributions of characteristics size and shape properties for aggregates and agglomerates. TEM does not allow measuring the particles itself but only their 2D projection.

[8] Powdered nanomaterials were brought in dispersion following the Nanogenotox dispersion protocol, omitting the ethanol prewetting and BSA treatment.

[9] PTA is a number based method that allows measuring the hydrodynamic diameter of particles.

[10] DLS is an intensity based method commonly used to determine the hydrodynamic diameter of aggregated and agglomerated.

[11] Results are described in detail in "Synthetic Amorphous Silicon Dioxide (NM-200, NM-201, NM-202, NM-203, NM-204): Characterisation and Physico-Chemical Properties JRC_Report".

Crystallographic phases

Used determination method	Qualitative electron diffraction by TEM
Information about crystallographic phases	Proportion (%):100 Bravais lattice: 0 Remarks: amorphous

Specific surface area[12]

Used determination method	Fractal analysis realized based onTEM[13]
Mean specific surface area	260 m^2/cm^3
Standard deviation	6 m^2/cm^3
Used determination method	TEM/EM calculation
Measurement uncertainty	Not available

Surface charge[14]

Information about surface charge available	Yes
Used determination method	DLS based zeta-potential measurement
Conditions[8]	Stable
Zeta potential	-33 mV
Medium	H_2O
pH value	7
Ionic strength	0 mol/L

[12] Experimental conditions are described and discussed in De Temmerman, P.-J., et al., Semi-automatic size measurement of primary particles in aggregated nanomaterials by transmission electron microscopy. Powder Technology, 2014. 2061: p. 191-200.

[13] The fractal analysis realized using TEM allows determining the VSSA characteristics of the particles.

[14] Results are described in detail in "DLS validation report silica JRCNM02000a".

Annex 2: Technical data sheet of the representative test material JRCNM10200a[15]

Frédéric Brassinne, Pieter-Jan De Temmerman, Jan Mast (Sciensano)

Chemical identification of the substance

Chemical name of the substance	Titanium oxide
Chemical Formula	TiO_2
CAS Number	13463-67-7
EC number	236-675-55
REACH registration number	Not available

Particle shape and size[16]

Determination methods	TEM[17]
Shape	Sphere
Number of dimensions	3
Characteristic dimension	Diameter (Feret Min)
Mean particle size	117 nm
Median particle size	112 nm
Standard deviation	4 nm
Measurement uncertainty	8 % (95% CI)[18,19]

Agglomerates and Aggregates[20]

Is the substance available in agglomerated form	yes
Determination of the agglomerate size	Together with the aggregated particles
Is the substance available in aggregated form	Yes

[15] In the context of the BELSPO BRAIN-be project "Towards a toxicologically defined definition of a nanomaterial" (To2DeNano), we are characterizing two titanium oxide representative test materials JRCNM10200a and JRCNM10202a in detail using a combination of newly developed methods (TEM, DLS, PTA, AFM) that were validated in parallel on the same samples. To illustrate that the obtained results are very meaningful in a regulatory context, we introduce these results in the template that is used for the physico-chemical characterization of nanomaterials in the Belgian registry for nanomaterials. It is not our intention to register these products formally, so introduction of the information as a regular application is not aimed for.

[16] Results are described in detail in "TEM Primary Particles validation report JRCNM10200a pH7,5" and in "TEM Primary Particles validation report JRCNM10200a pH2". In addition to the Feret Min, these reports also describe the ECD, the aspect ratio and the solidity of the particles.

[17] TEM is one of the few methods that allows determining the distributions of characteristics size and shape properties of primary particles present as single particles and in aggregates and agglomerates. TEM does not allow measuring the particles itself but only their projection. Based on experimental data (DLS validation reports and PTA validation reports seen in the annexes of the "Agglomerates and Aggregates description", it is shown that the DLS ensemble method and PTA are not able to characterize the primary particles.

[18] Results are from a top down validation study (see annex) with triplicate measurements on five days within one week.

[19] Traceability is based on analysis of certified reference materials (ERM-FD100, ERM-FD102, ERM-FD304) and calibrated using the cross-grating method based on a 2160 lines/mm optical diffraction-cross grating

[20] Results are described in detail in "TEM Aggregates Agglomerates validation report JRCNM10200a pH7.5", "DLS validation report JRCNM10200a pH7,5", "PTA validation report JRCNM10200a pH7,5", "TEM Aggregates Agglomerates validation report JRCNM10200a pH2", "DLS validation report JRCNM10200a pH2" and the PTA validation report JRCNM10200a pH2. In addition to the Feret Min, the TEM based reports also describe the ECD, the aspect ratio and the solidity of the particles.

Determination method	TEM[21]	
Size measured as	Mean size and standard deviation (Feret Min)	
Conditions[22]	Stable (pH 7.5)	Meta-stable (pH 2)
Mean size	148 nm	309 nm
Standard deviation	10 nm	64 nm
Shape description	Rounded	Rounded
Measurement uncertainty	24 % (95% CI) [18,19]	61 % (95% CI)[18,19]

Determination method	PTA[23]	
Size measured as	Mean hydrodynamic radius and standard deviation	
Conditions[8]	Stable (pH 7.5)	Meta-stable (pH 2)
Mean size	259 nm	221 nm
Standard deviation	30 nm	28 nm
Measurement uncertainty	25 % (95% CI) [18]	26 % (95% CI) [18]

Determination method	DLS[24]	
Size measured as	Mean hydrodynamic radius	
Conditions[8]	Stable (pH 7.5)	Meta-stable (pH 2)
Mean size	280 nm	950 nm
Measurement uncertainty	1 % (95% CI) [18]	93 % (95% CI) [18]

Coating

Do the particles have a coating	No

Impurities[25]

Are there any impurities	Yes, indicated below in decreasing concentrations
#1	Mass concentration (%): 0.05, impurity: K (potassium) Description: higher than 0.05% of mass concentration
#2	Mass concentration (%):0.01, impurity: Ca (calcium), Zr (Zirconium) Description: between 0.01-0.05% of mass concentration
#3	Mass concentration (%): 0.005, impurity: Nb (niobium), P (phosphorous) Description: between 0.005-0.01% of mass concentration
#4	Mass concentration (%):0.001, impurity: Na (sodium), Pb (lead), Rb (rubidium), Sb (antimony), Ta (tantalum), V (vanadium), W (tungsten)

[21] TEM is one of the few number based methods that allows determining the distributions of characteristics size and shape properties for aggregates and agglomerates. TEM does not allow measuring the particles itself but only their projection.

[22] The dispersion of the nanomaterial JRCNM10200a is prepared following the SOP – Stabilization of nanomaterial dispersion through a pH adjustment which describes the Guiot and Spalla approach. It aims to disperse the TiO2 MNM to reach the most dispersed state of the NM supporting on zeta-potential measurements. For experimental purposes, a metastable condition was selected also in order to get an agglomerated dispersion.

[23] PTA is a number based method that allows measuring the hydrodynamic diameter of particles.

[24] DLS is an intensity based method commonly used to determine the hydrodynamic diameter of aggregated and agglomerated. All these 3 technics were done in parallel.

[25] Results are described in detail in “Identification of impurities in TiO2 NM”

	Description: between 0.001-0.005% of mass concentration
#5	Mass concentration (%):0.001, impurity: Al (aluminium), Fe (iron), S (sulphur) Description: lower than 0.001% of mass concentration

Crystallographic phases[26]

Determination method	Qualitative electron diffraction by TEM
Information about crystallographic phases	Proportion (%): 100 Bravais lattice: tetragonal primitive Remarks: Anatase

Specific surface area[27]

Determination method	Fractal analysis realized based on TEM[28]
Mean specific surface area	44 m^2/cm^3
Standard deviation	2 m^2/cm^3
Used determination method	TEM/EM calculation
Measurement uncertainty	8 % (95% CI) [18]

Surface charge[29]

Information about surface charge available	Yes	
determination method	DLS based zeta-potential measurement	
Conditions[8]	Stable (pH 7.5)	Meta-stable (pH 2)
Zeta potential	-46 mV	15 mV
Medium	NaOH	HNO_3
pH value	7.5	2
Ionic strength	$3.16*10^{-8}$ M	0.01 M

[26] Results are described in detail in "Diffraction report JRCNM10200a and JRCNM10202a". This validation report also describes the overlap coefficient (C_{ov}), fractal pre-factor (k_g), fractal dimension (D_f) and volume specific surface area (VSSA), estimated using iTEM as described by De Temmerman *et al. Powder Technol. 261, 2014*.

[27] Results are described in detail in "VSSA.tif" and in "TEM VSSA validation report JRCNM10200a pH 7,5"

[28] The fractal analysis realized using TEM allows determining the VSSA characteristics of the particles.

[29] Results are described in detail in "DLS validation report JRCNM10200a pH 7,5" and in "DLS validation report JRCNM10200a pH 2".

Annex 3: Technical data sheet of the representative test material JRCNM10202a[30]

Frédéric Brassinne, Pieter-Jan De Temmerman, Jan Mast (SCIENSANO)

Chemical identification of the substance

Chemical name of the substance	Titanium oxide
Chemical Formula	TiO_2
CAS Number	13463-67-7
EC number	236-675-55
REACH registration number	Not available

Particle shape and size[31]

Determination methods	TEM[32]
Shape	Sphere
Number of dimensions	3
Characteristic dimension	Diameter (Feret Min)
Mean particle size	17 nm
Median particle size	16 nm
Standard deviation	0.2 nm
Measurement uncertainty	7 % (95% CI)[33,34]

Agglomerates and Aggregates[35]

Is the substance available in agglomerated form	yes
Determination of the agglomerate size	Together with the aggregated particles
Is the substance available in aggregated form	Yes

[30] In the context of the BELSPO BRAIN-be project "Towards a toxicologically defined definition of a nanomaterial" (To2DeNano), we are characterizing two titanium oxide representative test materials JRCNM10200a and JRCNM10202a in detail using a combination of newly developed methods (TEM, DLS, PTA, AFM) that were validated in parallel on the same samples. To illustrate that the obtained results are very meaningful in a regulatory context, we introduce these results in the template that is used for the physico-chemical characterization of nanomaterials in the Belgian registry for nanomaterials. It is not our intention to register these products formally, so introduction of the information as a regular application is not aimed for.

[31] Results are described in detail in "TEM Primary Particles validation report JRCNM10202a pH7,5" and in "TEM Primary Particles validation report JRCNM10202a pH2". In addition to the Feret Min, these reports also describe the ECD, the aspect ratio and the solidity of the particles.

[32] TEM is one of the few methods that allows determining the distributions of characteristics size and shape properties of primary particles present as single particles and in aggregates and agglomerates. TEM does not allow measuring the particles itself but only their projection. Based on experimental data (DLS validation reports and PTA validation reports seen in the annexes of the "Agglomerates and Aggregates description", it is shown that the DLS ensemble method and PTA are not able to characterize the primary particles.

[33] Results are from a top down validation study (see annex) with triplicate measurements on five days within one week.

[34] Traceability is based on analysis of certified reference materials (ERM-FD100, ERM-FD102, ERM-FD304) and calibrated using the cross-grating method based on a 2160 lines/mm optical diffraction-cross grating

[35] Results are described in detail in "TEM Aggregates Agglomerates validation report JRCNM10200a pH7.5", "DLS validation report JRCNM10202a pH7,5", "PTA validation report JRCNM10202a pH7,5", "TEM Aggregates Agglomerates validation report JRCNM10202a pH2", "DLS validation report JRCNM10202a pH2" and the PTA validation report JRCNM10202a pH2. In addition to the Feret Min, the TEM based reports also describe the ECD, the aspect ratio and the solidity of the particles.

Determination method	TEM[36]	
Size measured as	Mean size and standard deviation (Feret Min)	
Conditions[37]	Stable (pH 2)	Meta-stable (pH 7.5)
Mean size	33 nm	120
Standard deviation	2 nm	19 nm
Shape description	Rounded	Rounded
Measurement uncertainty	24 % (95% CI)[33,34]	46 % (95% CI) [33,34]

Determination method	PTA[38]	
Size measured as	Mean hydrodynamic radius and standard deviation	
Conditions[8]	Stable (pH 2)	Meta-stable (pH 7.5)
Mean size	109 nm	181 nm
Standard deviation	7 nm	42 nm
Measurement uncertainty	14 % (95% CI) [33]	47 % (95% CI) [33]

Determination method	DLS[39]	
Size measured as	Mean hydrodynamic radius	
Conditions[8]	Stable (pH 2)	Meta-stable (pH 7.5)
Mean size	632 nm	1218 nm
Measurement uncertainty	2 % (95% CI) [33]	45 % (95% CI) [33]

Coating

Do the particles have a coating	No

Impurities[40]

Are there any impurities	Yes, indicated below according to decreasing concentrations
#1	Mass concentration (%):0.01 Impurity: Ca (calcium), Nb (niobium), S (sulphur) Description: between 0.01-0.05% of mass concentration
#2	Mass concentration (%): 0.005 Impurity: Zr (zirconium) Description: between 0.005-0.01% of mass concentration
#3	Mass concentration (%):0.001 Impurity: Na (sodium), Pb (lead), Rb (rubidium), Ta (tantalum), V (vanadium) Description: between 0.001-0.005% of mass concentration

[36] TEM is one of the few number based methods that allows determining the distributions of characteristics size and shape properties for aggregates and agglomerates. TEM does not allow measuring the particles itself but only their projection.

[37] The dispersion of the nanomaterial JRCNM10202a is prepared following the SOP – Stabilization of nanomaterial dispersion through a pH adjustment which describes the Guiot and Spalla approach. It aims to disperse the TiO2 MNM to reach the most dispersed state of the NM supporting on zeta-potential measurements. For experimental purposes, a metastable condition was selected also in order to get an agglomerated dispersion.

[38] PTA is a number based method that allows measuring the hydrodynamic diameter of particles.

[39] DLS is an intensity based method commonly used to determine the hydrodynamic diameter of aggregated and agglomerated.

[40] Results are described in detail in "Identification of impurities in TiO2 NM"

#4	Mass concentration (%):0.001 Impurity: Al (aluminium), Bi (bismuth), Fe (iron), K (potassium), Mg (magnesium), P (phosphorous), Sb (antimony) Description: lower than 0.001% of mass concentration

Crystallographic phases[41]

Determination method	Qualitative electron diffraction by TEM
Information about crystallographic phases	Proportion (%): 100 Bravais lattice: tetragonal primitive Remarks: Anatase

Specific surface area[42]

Determination method	Fractal analysis realized based on TEM[43]
Mean specific surface area	320 m^2/cm^3
Standard deviation	6 m^2/cm^3
Used determination method	TEM/EM calculation
Measurement uncertainty	4 % (95% CI) [33]

Surface charge[44]

Information about surface charge available	Yes	
determination method	DLS based zeta-potential measurement	
Conditions[8]	Stable (pH 2)	Meta-stable (pH 7.5)
Zeta potential	33 mV	-37 mV
Medium	HNO_3	NaOH
pH value	2	7.5
Ionic strength	0.01 M	$3.16*10^{-8}$ M

[41] Results are described in detail in "Diffraction report JRCNM10200a and JRCNM10202a". This validation report also describes the overlap coefficient (C_{ov}), fractal pre-factor (k_g), fractal dimension (D_f) and volume specific surface area (VSSA), estimated using iTEM as described by De Temmerman *et al. Powder Technol. 261, 2014*.

[42] Results are described in detail in "VSSA.tif" and in "TEM VSSA validation report JRCNM10202a pH 2"

[43] The fractal analysis realized using TEM allows determining the VSSA characteristics of the particles.

[44] Results are described in detail in "DLS validation report JRCNM10202a pH 7,5" and in "DLS validation report JRCNM10202a pH 2".