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"VALIDATION AND IMPLEMENTATION OF AN ANALYTICAL METHODOLOGY TO CHARACTERIZE THE FRACTION OF NANO-SIZED PARTICLES IN E171 AND E174"

(Nanofood@, nanoAg@ and EFSA nano projects)

Jan Mast

Service Trace Elements and Nanomaterials

SD Chemical and physical health risks, Sciensano

EFSA Nano Stakeholders Engagement Event, 1 & 2 April 2019, Parma

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OUTLINE

- **Introduction**
- **Analytical methodology**
- **Analysis results of E174**
- **Analysis results of E171**
- **Lessons learnt**

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Characterization of NM in complex matrices in a regulatory context

- Food
- Environment
- Medical devices
- Cosmetics

Characterization of NM in food additives E171, E174 , E175

- Nanofood@
- NanoAg@
- EFSA
- EM unit

Objectives

- Validated methods
- Market study
- Exposure assessment

”EFSA Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain”

“4. Physicochemical characterization of nanomaterial”

- “4.1. Framework for distinguishing nanomaterials and non-nanomaterials”
- “4.2 Pristine material characterization”
- “4.3. Characterization and quantification in matrix” (products)

« Through validated methods, suitable analytical techniques »

- BF-TEM
- SEM-EDX + STEM-EDX
- SP-ICP-MS

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M, Chaudhry Q, Cubadda F, Gott D, Oomen A, Weigel S, Karamitrou M, Schoonjans R and Mortensen A, 2018. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health. EFSA Journal 2018;16(7):5327, 95 pp. https://doi.org/10.2903/j.efsa.2018.5327

Research question: «Materials falls under scope of EFSA guidance (section 1.3)?»

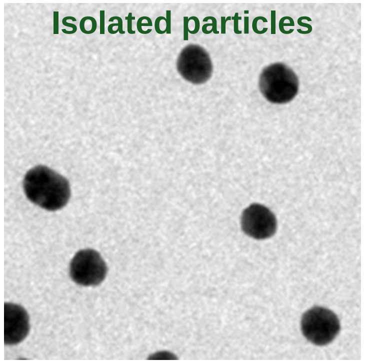
EC recommendation on the definition of a nanomaterial (2011/696/EU):

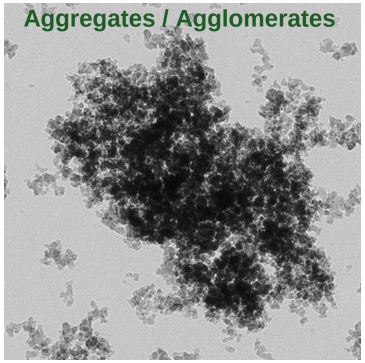
- Natural, incidental, and manufactured
- Refers to constituent particles (primary particles)
- Uses size as the most important defining parameter: "50 % or more of the constituent particles (by number) have one or more external dimensions in the size range 1 – 100 nm."

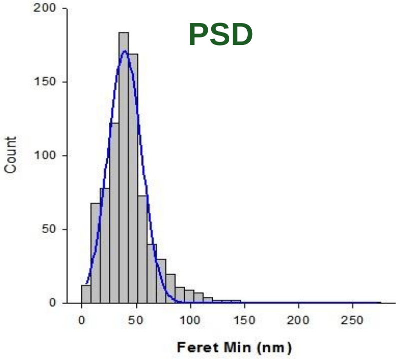
Novel food Regulation (2015/2283):

- Engineered material, intentionally produced to perform a specific function in the food.
- One or more dimensions of the order of 100 nm or less
- It includes also structures, agglomerates, or aggregates, which may have a size above 100 nm but retain properties that are characteristic of the nanoscale.

+ EU Regulation on the Provision of Food Information to Consumers
"all ingredients that are present in food as nanomaterials must be indicated as such on the product label." (refers to Novel food regulation)







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Identification of nano-objects in food

CEN/TC 352: Nanotechnologies — Guidance on detection and identification of nano-objects in complex matrices

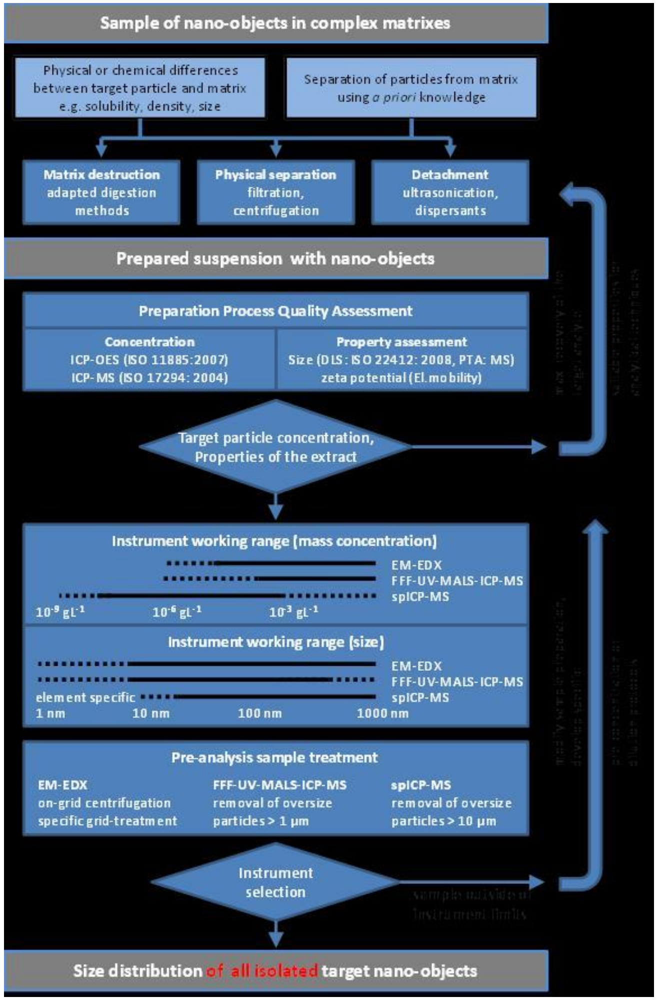
Liquid environmental compartments

- waste water
- consumer products
- Food
- cosmetics

Sample preparation

Size classification +chemical composition

- EM: BF-TEM, SEM-EDX or STEM-EDX
- SP-ICP-MS
- FFF + multiple detectors



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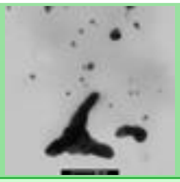
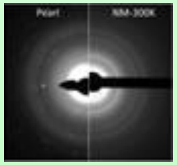
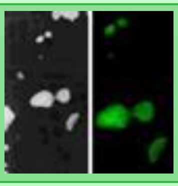
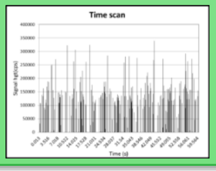
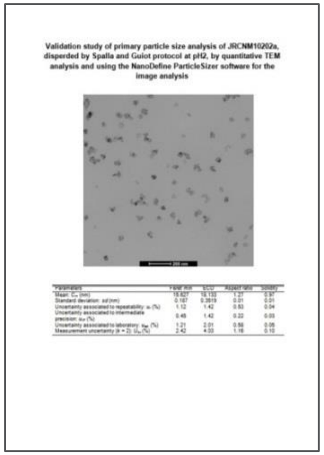
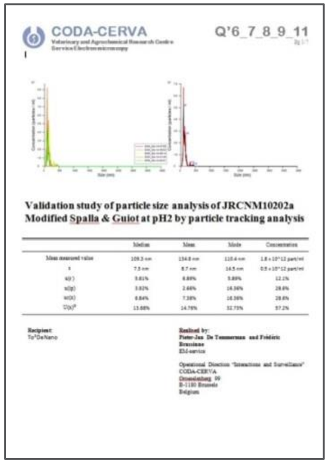
Analytical approach

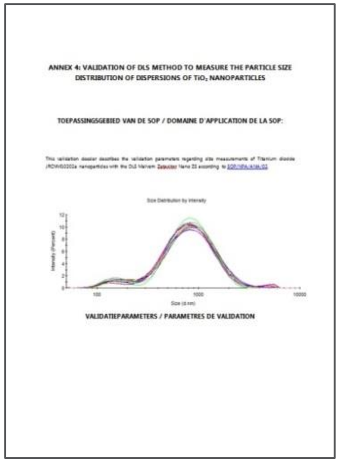
Sub-question	Methodology	Illustration
Particles present?	Descriptive (conventional) EM	
Phase?	Powder electron diffraction	
Chemical composition?	HAADF-STEM-EDX SEM-EDX	
Nano?	Quantitative TEM	
How many?	Total: ICP-MS NP: SP-ICP-MS	 

Quality of the results
Standardised methodology

Validated methodologies







Detailed physicochemical characterization

Technical data sheet of the representative test material JRCNM10202a[1]

Frédéric Brassinne, Pieter-Jan De Temmerman, Jan Mast (CODA-CERVA)

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[2]

Determination methods	TEM[3]
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)[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

Determination method	TEM[7]	
Size measured as	Mean size and standard deviation (Feret Min)	
Conditions[8]	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)[4,5]	46 % (95% CI)[4,5]

Determination method	PTA[9]	
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)[4]	47 % (95% CI)[4]

Determination method	DLS[10]	
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)[4]	45 % (95% CI)[4]

1

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

Frédéric Brassinne, Pieter-Jan De Temmerman, Jan Mast (CODA-CERVA)

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

Determination method	TEM[7]
Size measured as	Mean size and standard deviation (Feret Min)
Conditions[8]	Stable
Mean size	18.1 nm
Standard deviation	2.5 nm
Shape description	Fractal - angular
Measurement uncertainty	28 % (95% CI)[4,5]

Determination method	PTA[9]
Size measured as	Mean hydrodynamic radius and standard deviation
Conditions[8]	Stable
Mean size	Not applicable[10]
Standard deviation	Not applicable
Measurement uncertainty	Not applicable

Determination method	DLS[11]
Size measured as	Mean hydrodynamic radius
Conditions[8]	Stable
Mean size	To be determined nm
Measurement uncertainty	% (95% CI)[4]

Technical data sheet of the representative test material JRCNM10200a[1]

Frédéric Brassinne, Pieter-Jan De Temmerman, Jan Mast (CODA-CERVA)

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[2]

Determination methods	TEM[3]
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)[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

Determination method	TEM[7]	
Size measured as	Mean size and standard deviation (Feret Min)	
Conditions[8]	Stable (pH 7.5)	Meta-stable (pH 2)
Mean size	148 nm	309
Standard deviation	10 nm	64 nm
Shape description	Rounded	Rounded
Measurement uncertainty	24 % (95% CI)[4,5]	61 % (95% CI)[4,5]

Determination method	PTA[9]	
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)[4]	26 % (95% CI)[4]

Determination method	DLS[10]	
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)[4]	93 % (95% CI)[4]

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- Analysis results of E171
- Lessons learnt

Silver (E174) in food

- EC approved food additive (EC 1333/2008)
- Authorized to be used
  - to color the external coating of confectionery
  - for decoration of chocolates
  - in liqueurs
- Applied as a silver-colored powder or as tiny sheets (Directive 95/45/EC)
- Intended and assumed to be present in the bulk form
- EFSA re-evaluation of silver (E 174) as food additive
- Conclusions warrant further characterization of nano-sized fraction using EM

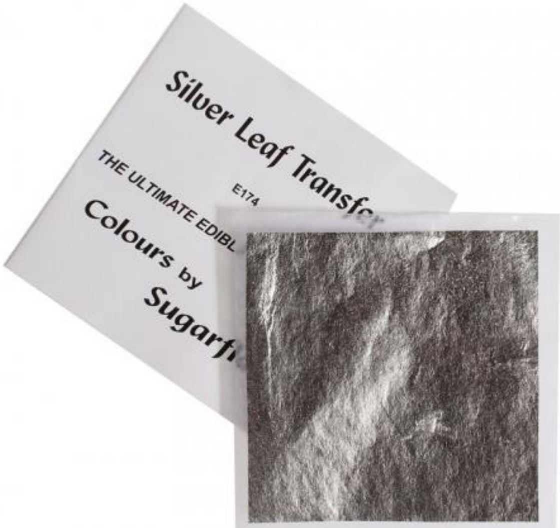
Purchase of E174 materials

Belgian market

- Colloidal reference materials

- Pristine materials purchased from different European websites
  - ➔ Powders, flakes, petals, leaves

- E174 containing food products purchased from several local stores
  - ➔ Sugar beans, candy, cake decoration



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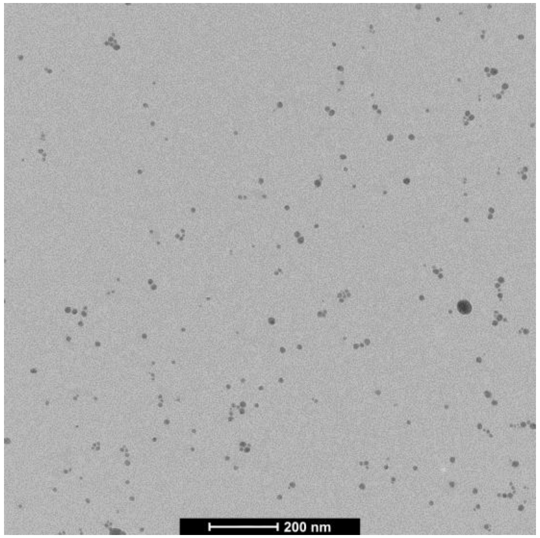
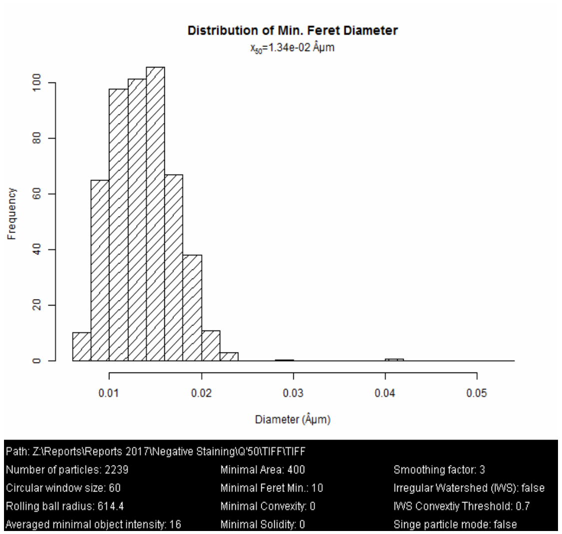
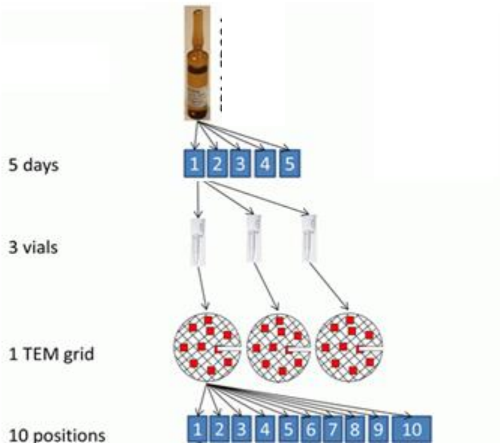
Validation based on reference material: NM-300k







Uncertainty budget of quantitative TEM analysis

- Type A undertainties: top-down estimation of U_r, U_{ip} (5 days, 3 repititions)
- Type B uncertainties: U_{cal}, U_t
- Combined, expanded measurement uncertainty (k = 2): U_{cx} = 8-9%

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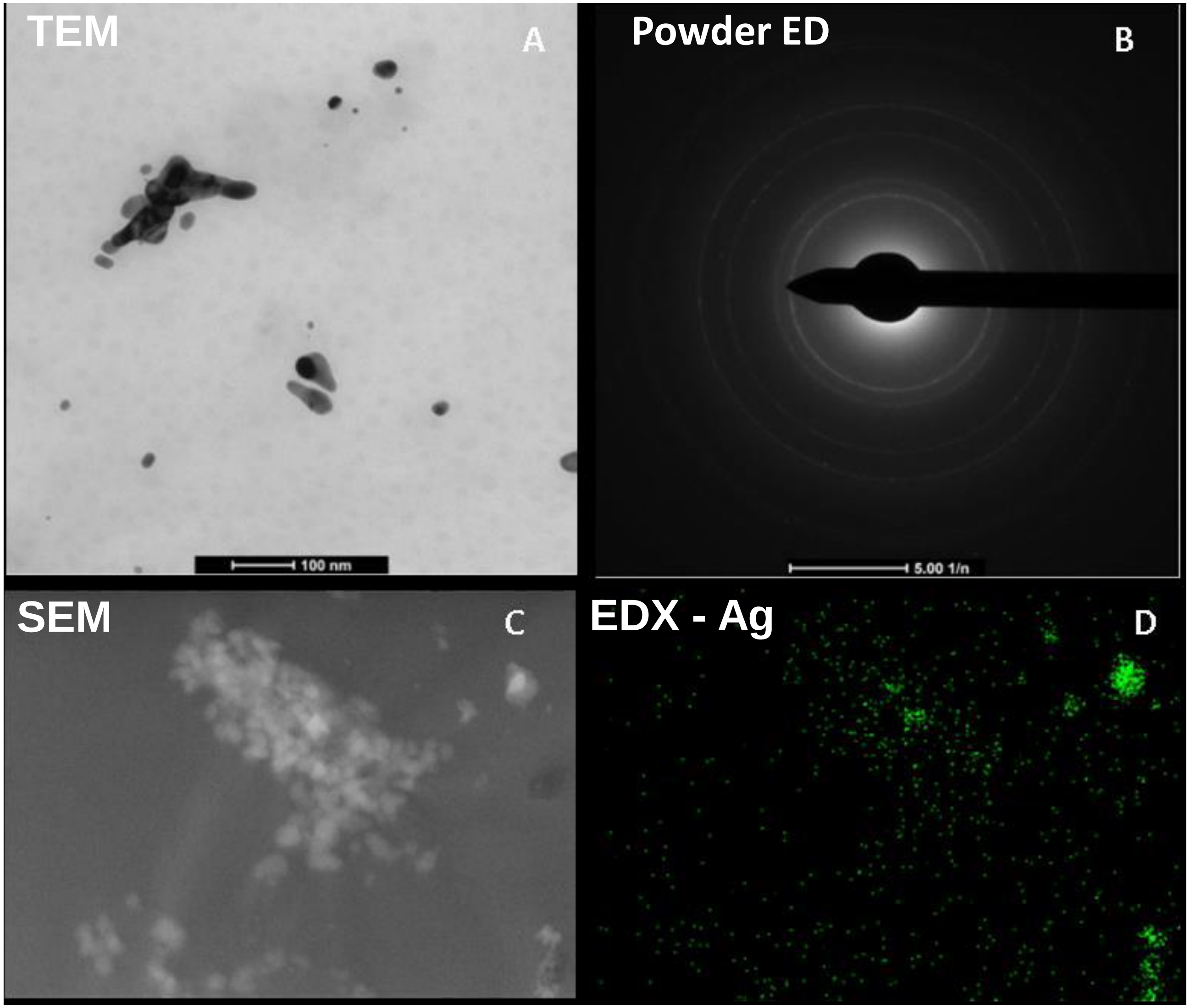
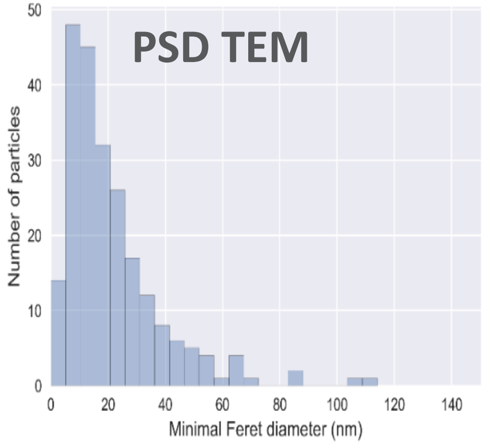
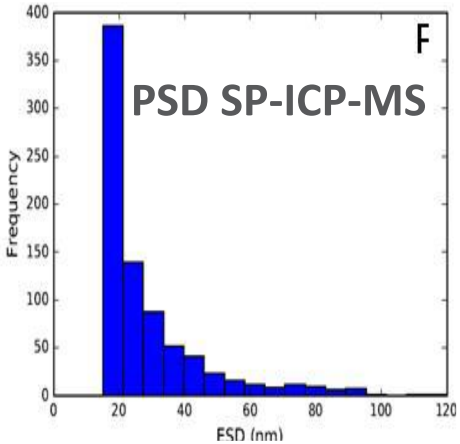
E174 in food products

Mini confetti, silver







E174 food products

Reference	Feret Min (TEM)*		ESD (SP-ICP-MS) *	# NP (<120nm) (/kg)	Mass NP (<120nm) (mg/kg)	Total Ag mass (g/kg)	% Ag NP mass of total Ag mass	#NP/ kg Ag
	Non-sonicated	sonicated	sonicated					
NF@-Mix-Ag-001	19 nm	-	24 nm	$7.1x10^{13}$	21	4.7	0.46	$1,51x10^{16}$
NF@-Mix-Ag-002	17 nm	-	19 nm	$3.5x10^{13}$	6.9	4.7	0.15	$7,45x10^{15}$
NF@-Mix-Ag-003	16 nm	-	22 nm	$4.2x10^{13}$	11	3.4	0.32	$1,24x10^{16}$
NF@-Mix-Ag-004	20 nm	-	22 nm	$4.5x10^{13}$	13	4.1	0.32	$1,10x10^{16}$
NF@-Mix-Ag-005	15 nm	-	23 nm	$7.4x10^{13}$	23	4.7	0.49	$1,57x10^{16}$
NF@-Mix-Ag-006	36 nm	-	16 nm	$1.6x10^{14}$	32	8.7	0.37	$1,84x10^{16}$
NF@-Mix-Ag-007	22 nm	-	22 nm	$3.6x10^{13}$	9.7	4.5	0.22	$8,00x10^{15}$
NF@-Mix-Ag-008	23 nm	-	21 nm	$1.7x10^{13}$	3.4	1.2	0.28	$1,42x10^{16}$
NF@-Mix-Ag-010	12 nm	-	20 nm	$7.8x10^{13}$	22	4.2	0.52	$1,86x10^{16}$
NF@-Mix-Ag-011	10 nm	-	22 nm	$2.0x10^{13}$	6.3	1.9	0.33	$1,05x10^{16}$

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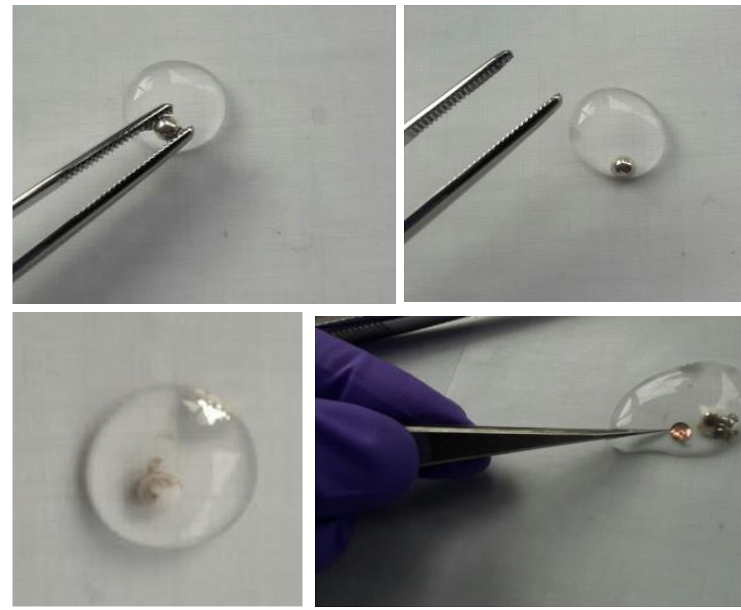
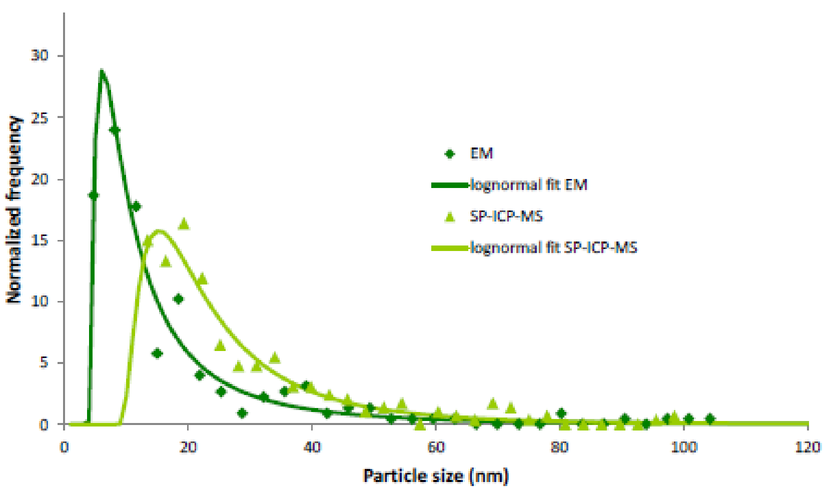
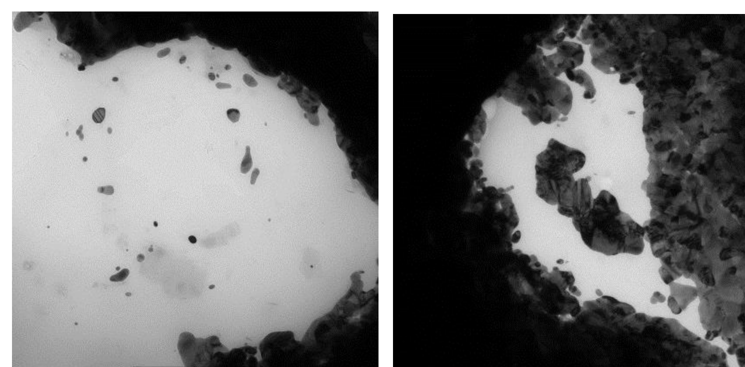
* Median values are reported

Results E174 in food products

- Sample preparation relatively easy
- NPs readily observed by EM
- Flakes with rough, particle-like surface
- Even distribution over EM grid → quantitative TEM
- Representative size distributions, Median values of Feret min values: ± 10-20 nm
- (SP-ICP-MS: 10^{15}-10^{16} NP per kg E174)

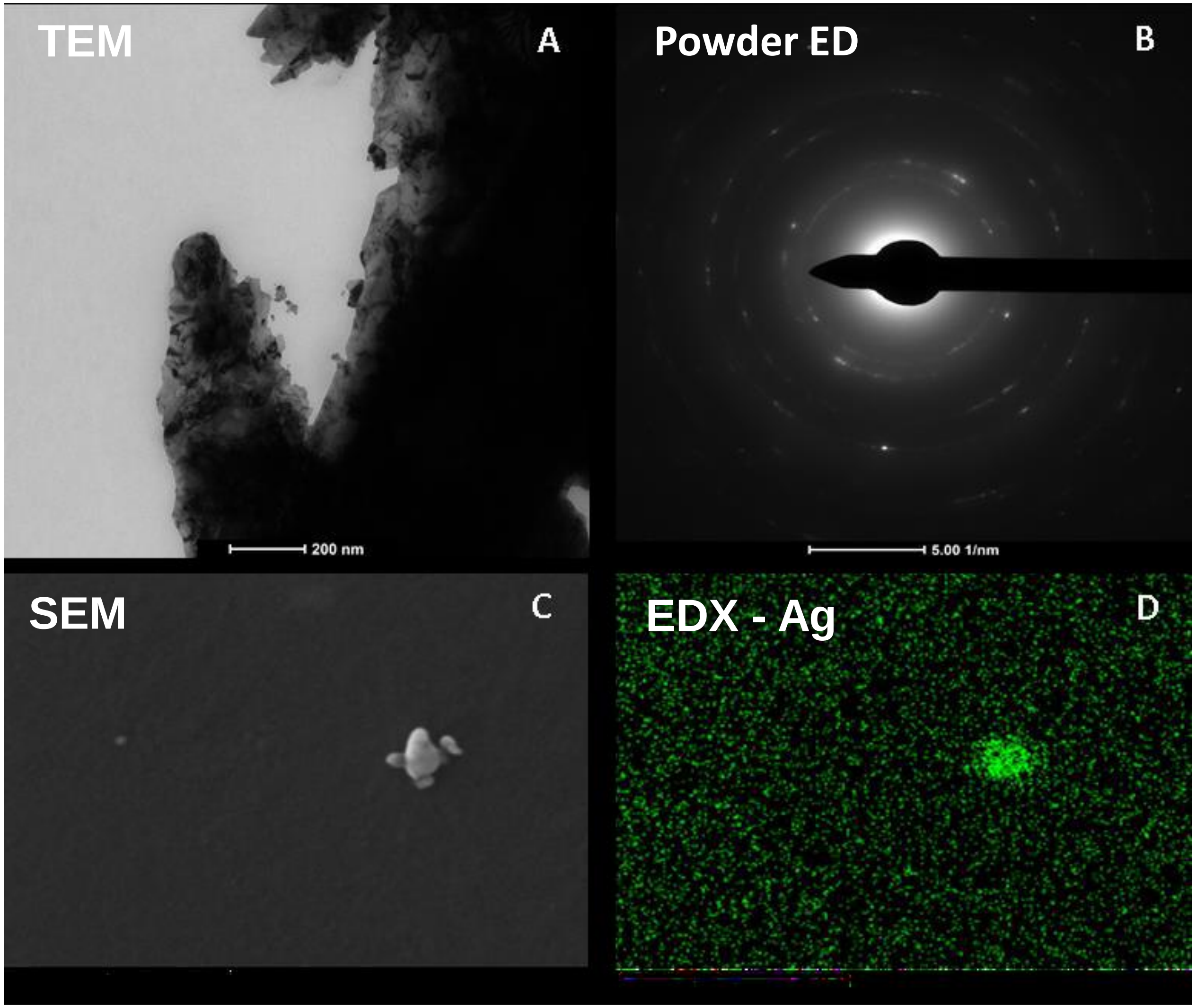


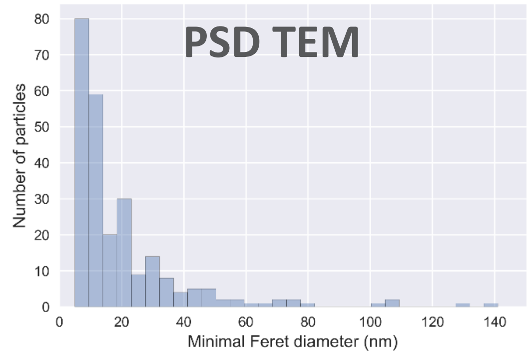


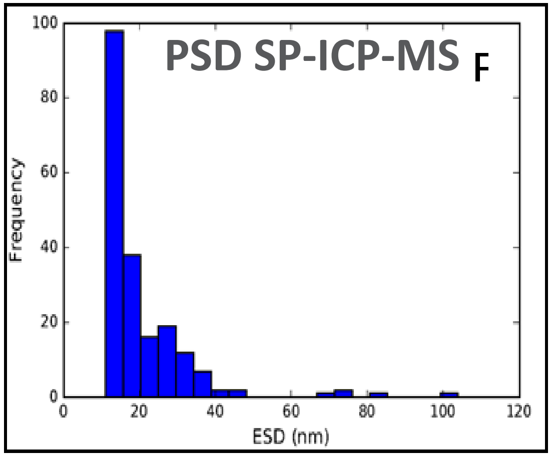


Pristine E174

"Silber-gastro Flocken"







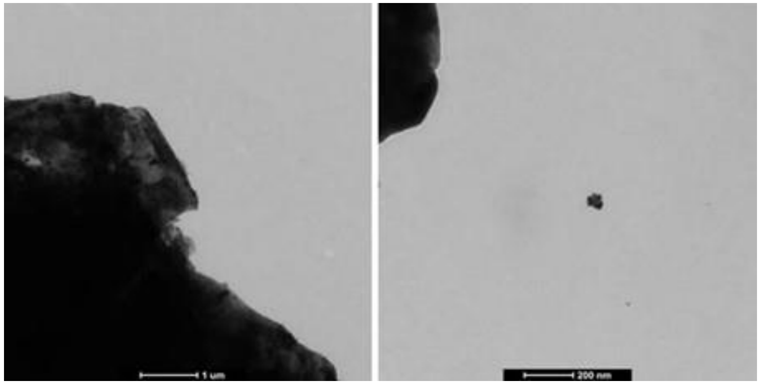
Pristine E174 materials

Reference	Feret Min (TEM)		ESD (SP-ICP-MS)	NP (<120nm) mass (g/kg)	% Ag NP mass of total Ag mass	#NP (<120nm) /kg Ag
	Non-sonicated	**sonicated**	**sonicated**			
NF@-Ag-001	13 nm	13 nm	14 nm	385	38.5	$2.0x10^{19}$
NF@-Ag-002	12 nm	Insufficient number	Insufficient number	Insufficient number	-	$<1.4x10^{15}$
NF@-Ag-003	±20 nm	Insufficient number	16 nm	2.3	0.23	$1.5x10^{16}$
NF@-Ag-004	Insufficient number	10 nm	14 nm	2.5	0.25	$2.8x10^{16}$
NF@-Ag-005	±20 nm	Insufficient number	27 nm	2.7	0.27	$7.1x10^{15}$
NF@-Ag-006	±45 nm	Insufficient number	16 nm	5.0	0.5	$3.8x10^{16}$
NF@-Ag-007	Insufficient number	12 nm	Insufficient number	Insufficient number	-	$<9.5x10^{15}$
NF@-Ag-008	Insufficient number	14 nm	20 nm	2.2	0.22	$1.3x10^{16}$
NF@-Ag-009	7 nm	17 nm	17 nm	2.2	0.22	$1.2x10^{16}$
NF@-Ag-010	14 nm	11 nm	21 nm	4.5	0.45	$2.1x10^{16}$
NF@-Ag-013	5 nm	14 nm	17 nm	6.2	0.62	$3.5x10^{16}$
NF@-Ag-014	12 nm	13 nm	15 nm	3.2	0.32	$2.5x10^{16}$

Results pristine E174

Difficult to obtain representative size distributions

- Few particles
- Non-homogeneous particle distribution
- Flakes are large and smooth
- Some samples contain higher amount of NPs and colloidal fraction



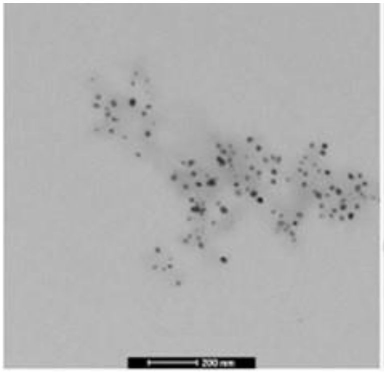
SP-ICP-MS: 10^{15}-10^{16} NP per kg E174

Median values of Feret min values: ±10-20 nm

Reactivity of silver = bottleneck sample preparation

⇒Tailored sample preparation methods for dispersing Ag may lead to artefacts (Demonstrated for pH, PVP, acetone, sonication)

⇒Examined Ag materials react differently



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Conclusions E174

Few nanosized particles in pristine E174

Larger nano-sized fraction in products containing E174

Hypothesis: processing of E174 in products induces formation silver NP

- **More nano-sized particles in the products than in the pristine materials**
- **The surface structure of E174 in products is altered (release associated?)**
- **Mechanisms of formation of NPs :**
  - ***De novo* formation from Ag^{+} by chemical reactions between E174 and reducing agents (PVP, acetone, proteins,…) in the dispersion medium**
  - **Physical treatment, mimicked by (too high levels of) sonication energy,**

For risk analysis:

- **Applied amount of pristine E174**
- **Modification of E174 (particle) properties induced by processing**

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Titanium dioxide (E171) in food

- EC approved food additive (EC 1333/2008)
- Authorized to be used as color in foodstuffs, widely used
- Applications applied on refractive properties (shiny coating, UV protection)
- Several crystal structures, such as anatase and rutile
- Intended and assumed to be present in the bulk form

Purchase of E171 materials

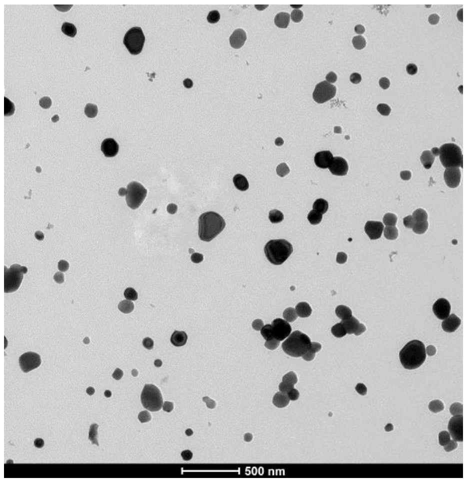
Belgian market

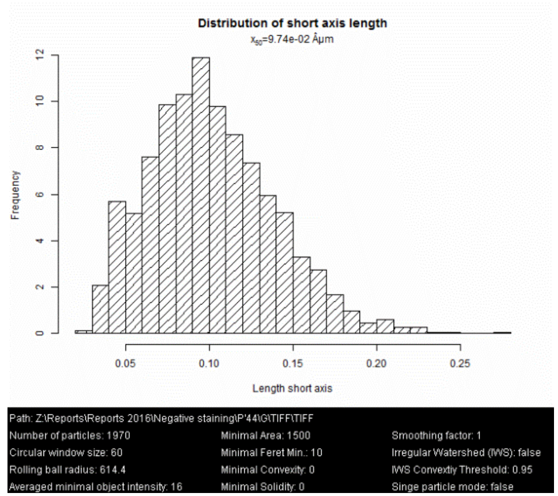
- Reference materials

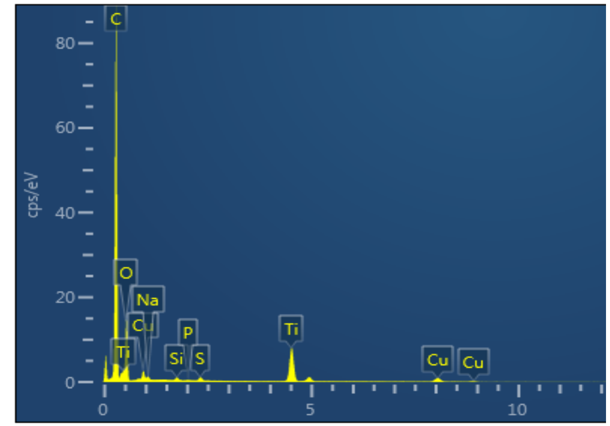
- Pristine materials purchased from different European websites
  - ➔ Powders, flakes

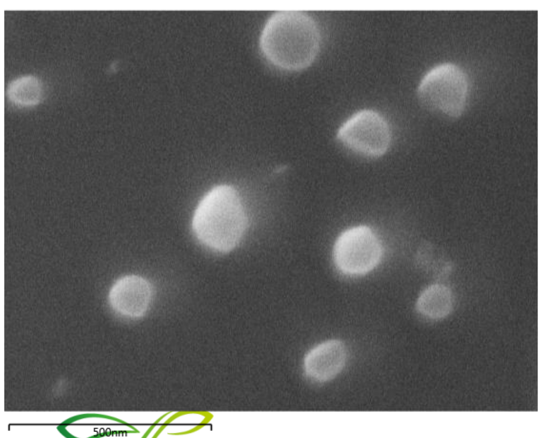
- E171 containing food products purchased from several local stores
  - ➔ sugarbeans, candy, chewing gum, cake decoration, …

Reference materials: NF@-Ti-002, and RTMs NM-100 and NM-103

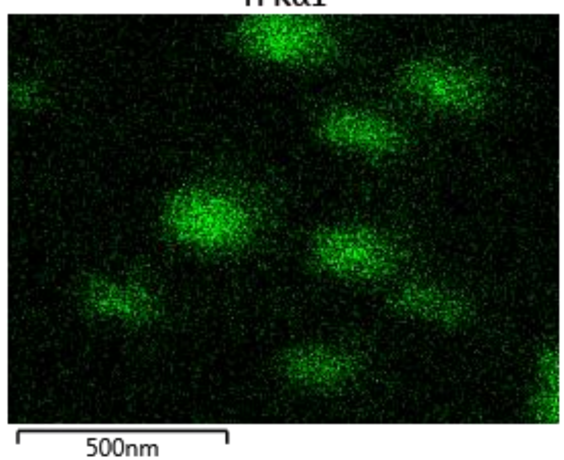




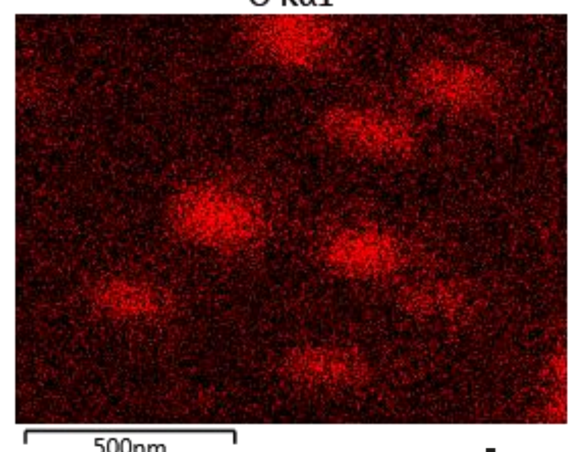




Ti Kα1



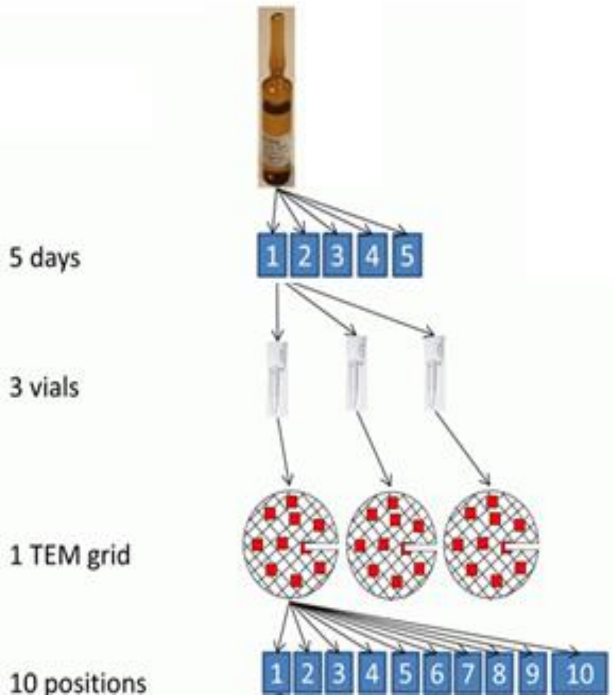
O Kα1

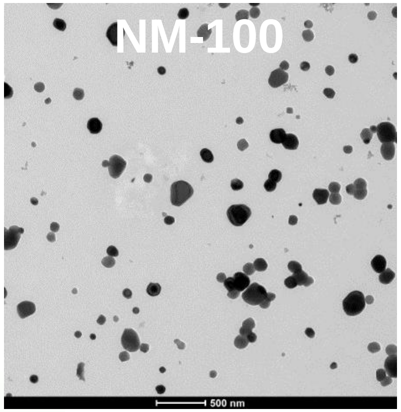


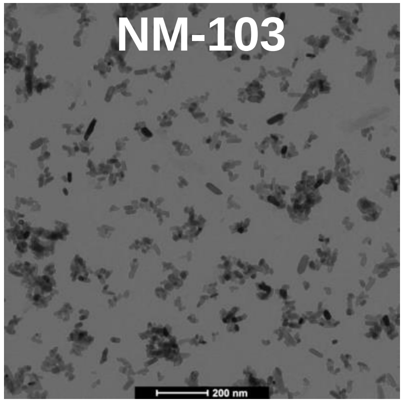
Size: TEM: median Feret min: NF@-Ti-002: 94.8 nm, NM-100: 100.1 nm, NM-103:18.2 nm

SP-ICP-MS: median ESD: NF@-Ti-002: 90 nm, NM-100: 107 nm

Measurement uncertainties based on reference materials





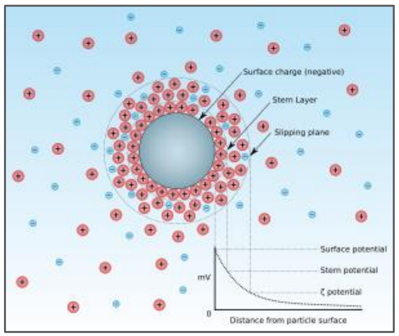


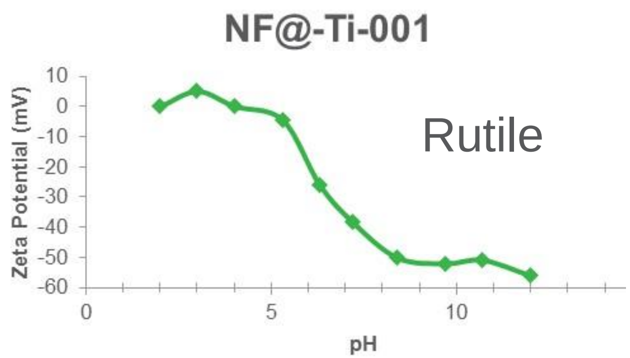
Parameter: median Feret Min	NM-100	NM-103
Mean: C_m (nm)	100.1	18.2
Standard deviation: sd (nm)	2.0	0.4
Uncertainty associated to repeatability: u_r (%)	1.9	2.1
Uncertainty due to day-to-day variation: u_{day} (%)	0.7	1.3
Uncertainty associated to intermediate precision: u_{IP} (%)	2.1	2.5
Uncertainty associated to calibration: u_{cal} (%)	0.8	0.1
Uncertainty associated to trueness: u_{tr} (%)	3.6	3.9
Combined uncertainty: $u_c(x)$ (%)	4.3	4.6
Measurement uncertainty (k = 2): U_{cx} (%)	8.5	9.2

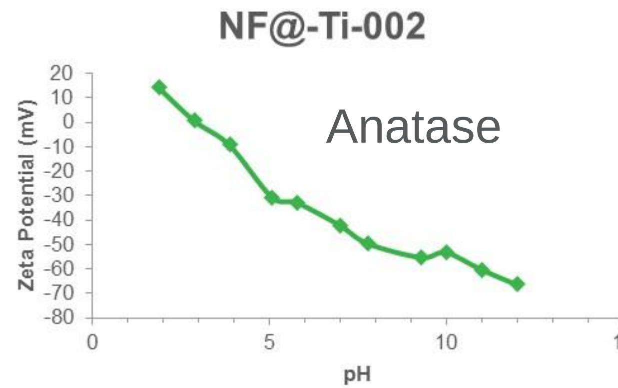
Top-down estimation of the measurement uncertainties:
TEM: measurement uncertainty (k = 2): U_{cx} = 8-10%
SP-ICP-MS: measurement uncertainty (k = 2): U_{cx} = 16%

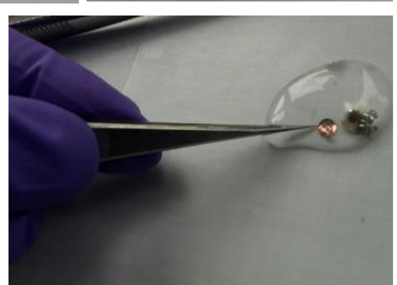
Sample preparation

De-agglomeration by pH adjustment



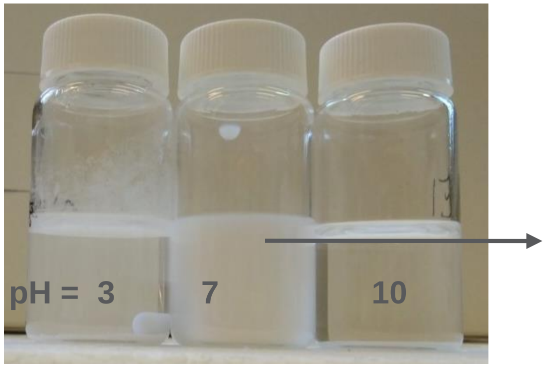




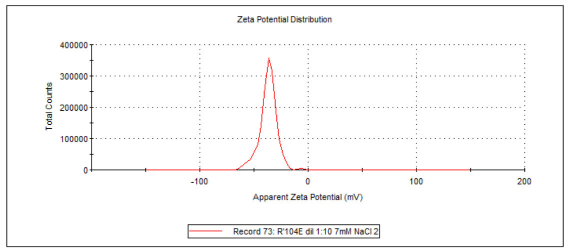


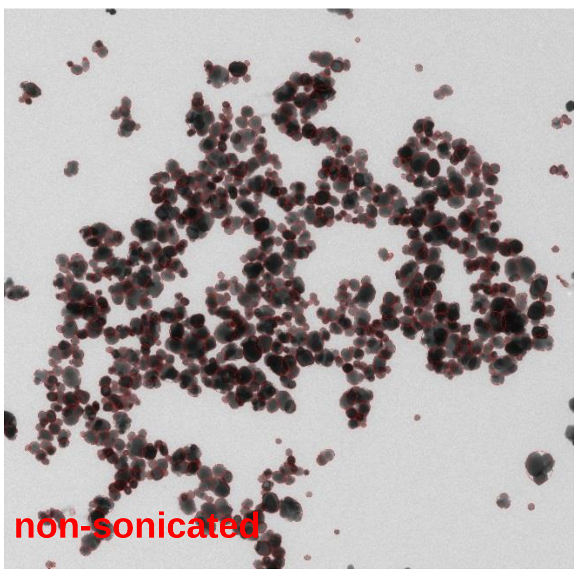
Calibrated probe
-sonication

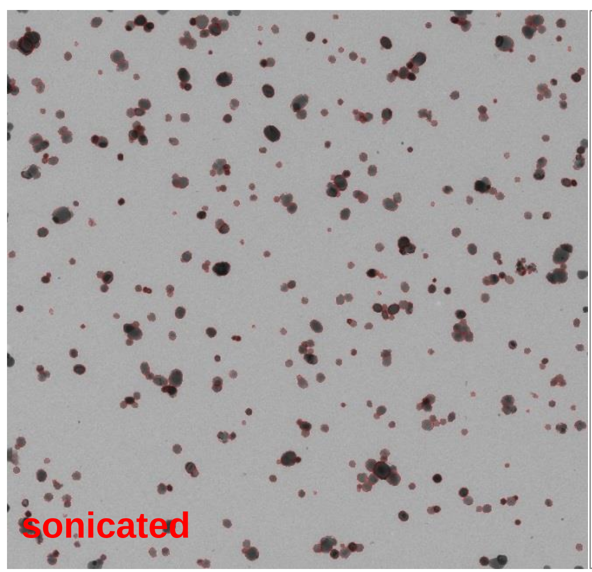
Illustration: pristine TiO2, anatase

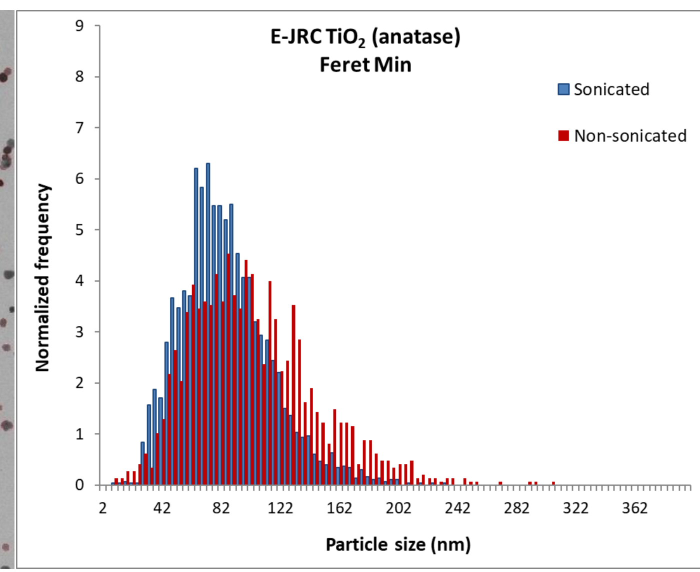


			Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -36.7		Peak 1:	-36.9	99.3	7.66
Zeta Deviation (mV): 8.03		Peak 2:	-7.33	0.7	2.81
Conductivity (mS/cm): 1.06		Peak 3:	0.00	0.0	0.00
Result quality : Good					









COMMISSION REGULATION (EU) No 231/2012

of 9 March 2012

laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council

(Text with EEA relevance)

(OJ L 83, 22.3.2012, p. 1)

E 171 TITANIUM DIOXIDE

Synonyms	CI Pigment White 6
Definition	Titanium dioxide consists essentially of pure anatase and/or rutile titanium dioxide which may be coated with small amounts of alumina and/or silica to improve the technological properties of the product. The anatase grades of pigmentary titanium dioxide can only be made by the sulphate process which creates a large amount of sulphuric acid as a by-product. The rutile grades of titanium dioxide are typically made by the chloride process. Certain rutile grades of titanium dioxide are produced using mica (also known as potassium aluminum silicate) as a template to form the basic platelet structure. The surface of the mica is coated with titanium dioxide using a specialised patented process. Rutile titanium dioxide, platelet form is manufactured by subjecting titanium dioxide (rutile) coated mica nacreous pigment to an extractive dissolution in acid followed by an extractive dissolution in alkali. All of the mica is removed during this process and the resulting product is a platelet form of rutile titanium dioxide.

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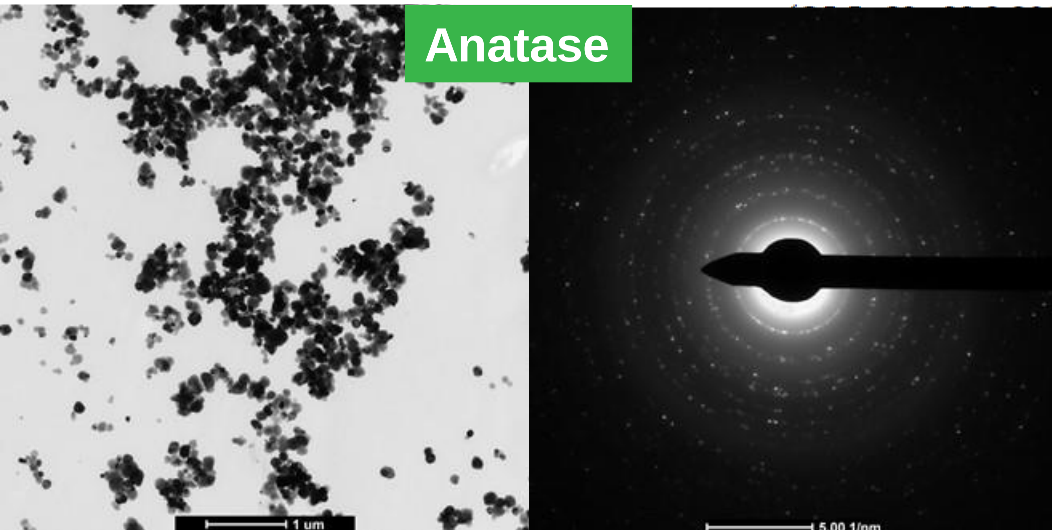
(Text with EEA relevance)

2, p. 1)



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product. The rutile grades of titanium dioxide are
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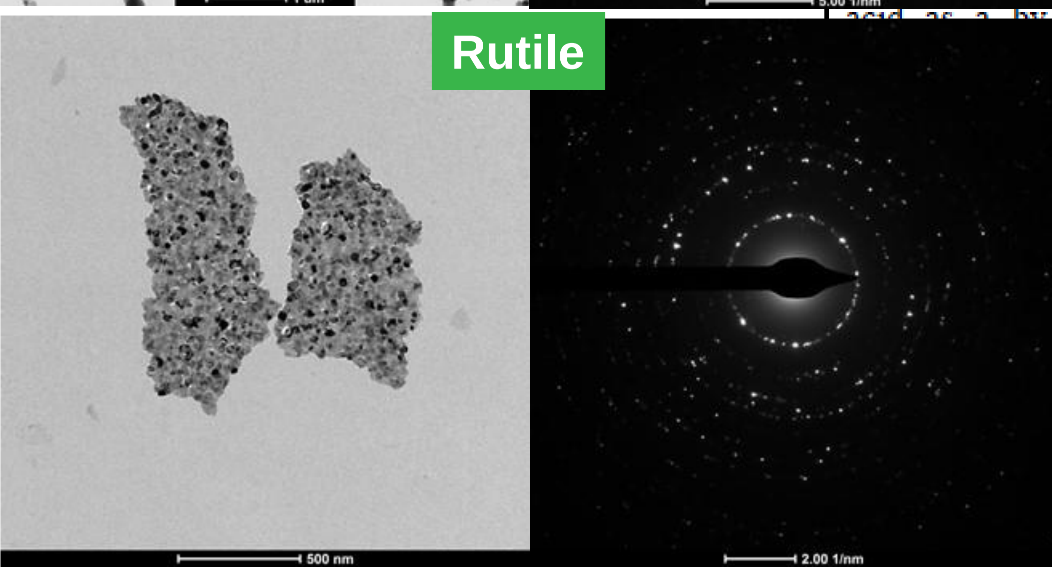

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let structure. The surface of the mica is coated with
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dioxide, platelet form is manufactured by subjecting
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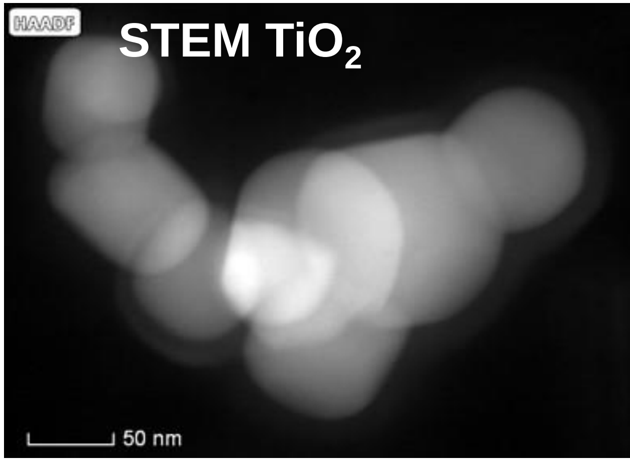
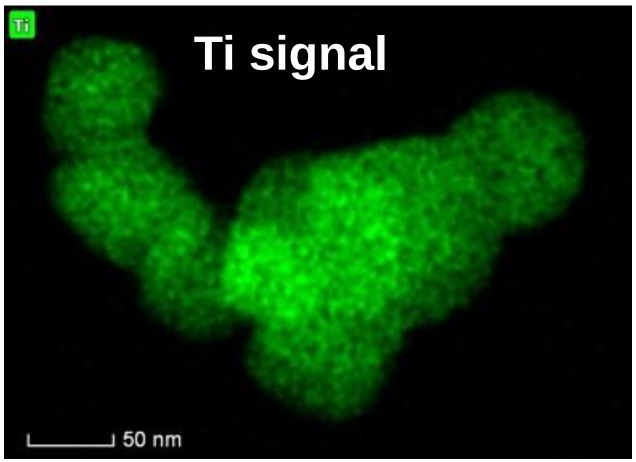
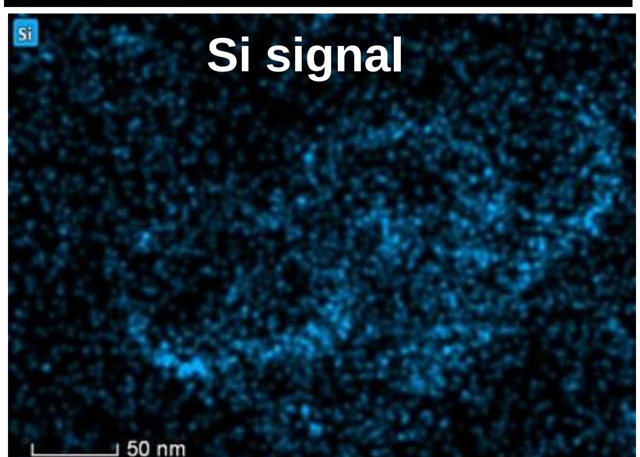






IMISSION REGULATION (EU) No 231/2012

of 9 March 2012

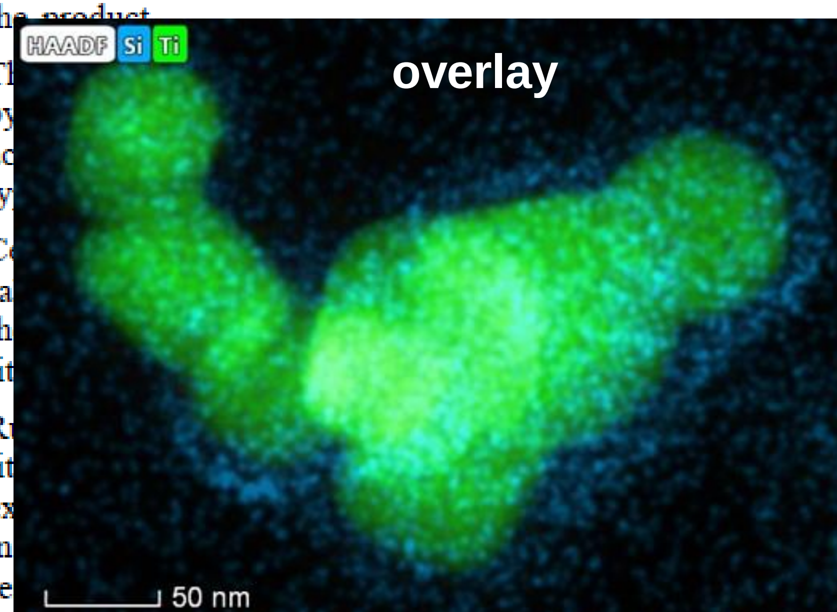
for food additives listed in Annexes II and III to Regulation (EC)
008 of the European Parliament and of the Council

(Text with EEA relevance)

(OJ L 83, 22.3.2012, p. 1)

CI Pigment White 6

Titanium dioxide consists essentially of pure anatase and/or rutile titanium dioxide which may be coated with small amounts of alumina and/or silica to improve the technological properties of the product.



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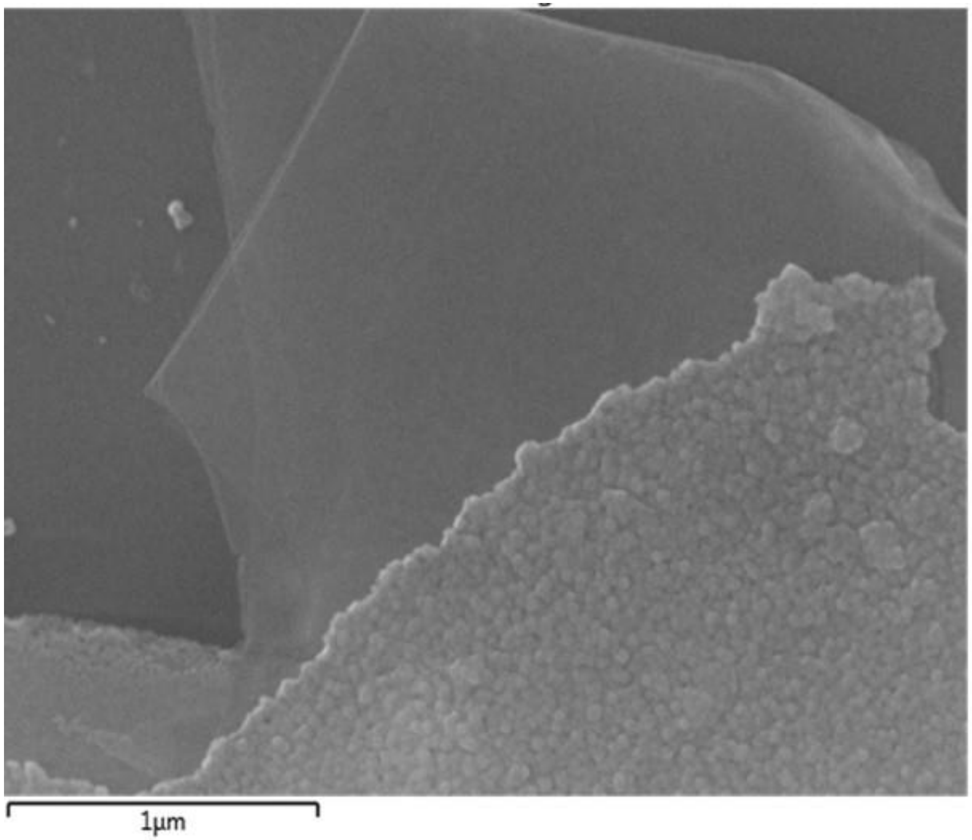

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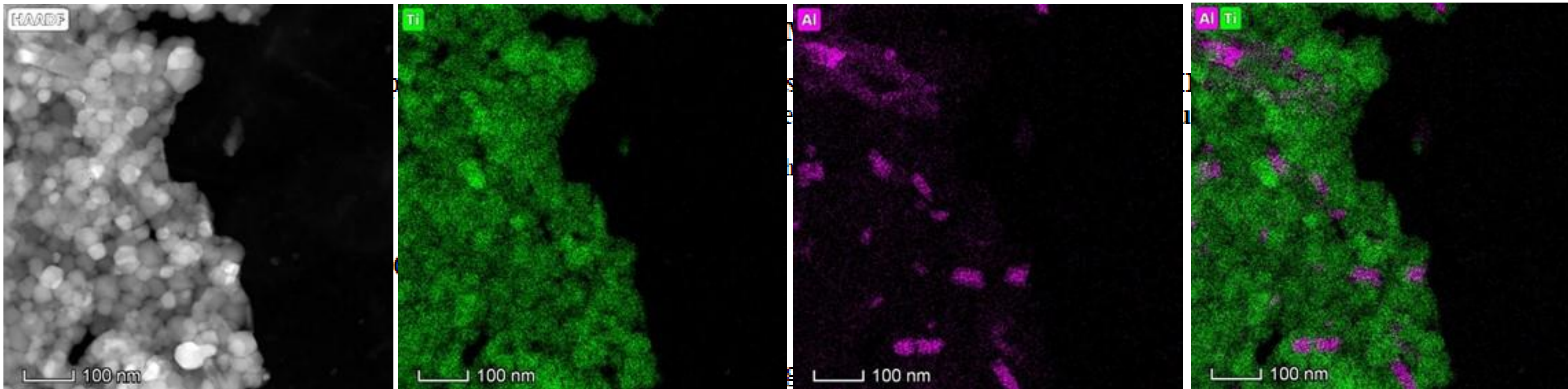
COMMISSION REGULATION (EU) No 231/2012



Definition

Titanium dioxide consists essentially of pure anatase and/or rutile titanium dioxide which may be coated with small amounts of … erties of

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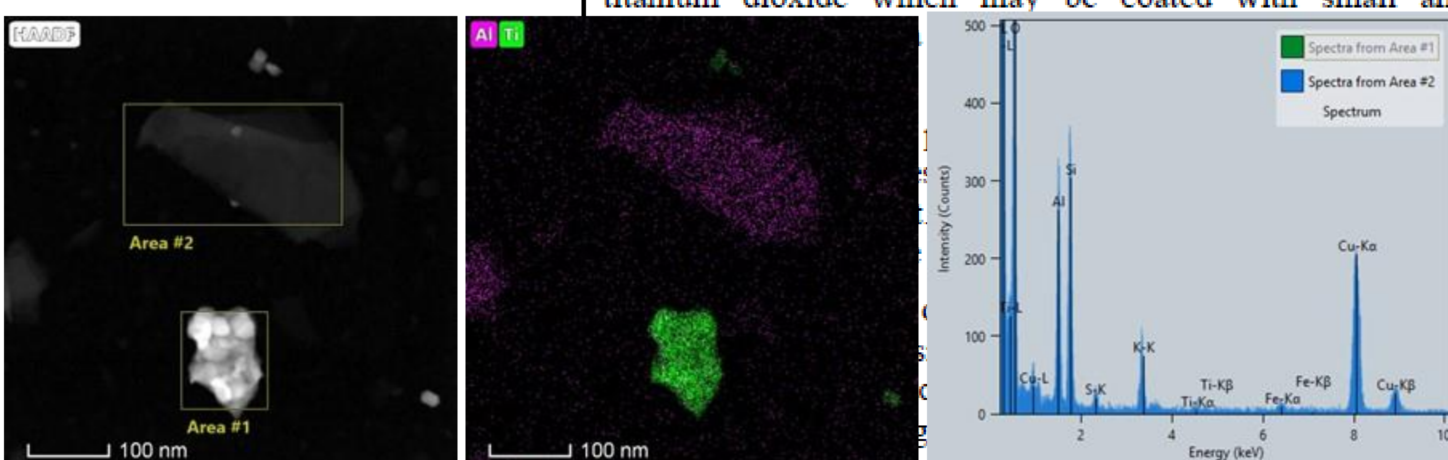

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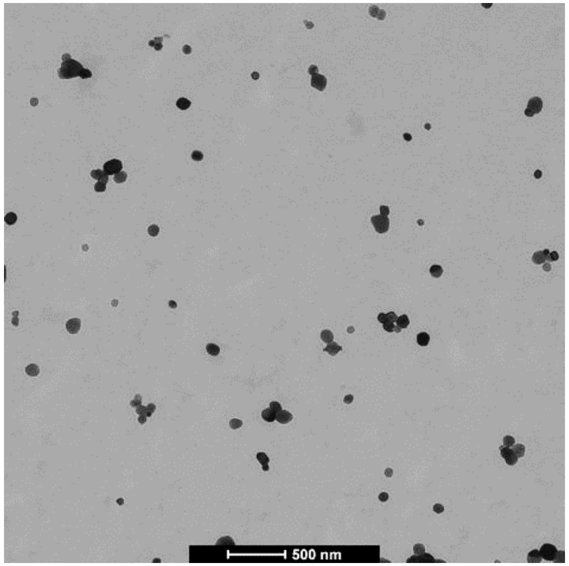
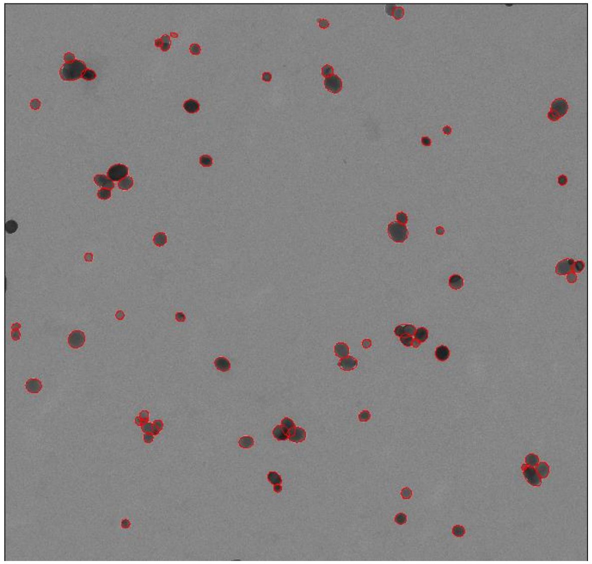
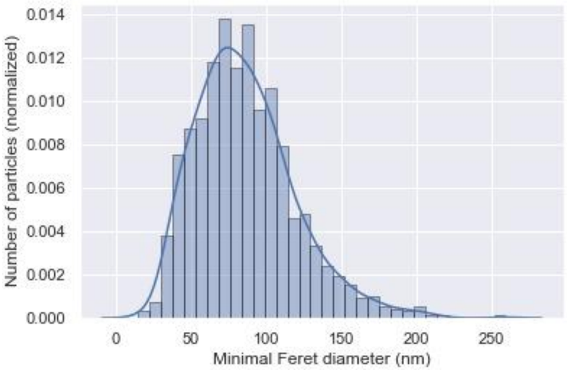
E171 pristine: anatase







E171 Material:	1	2	3	4	5	6
Modal Min. Feret diameter (nm)	83	82	83	82	74	76
Median Min. Feret diameter (nm)	87	88	92	89	83	86

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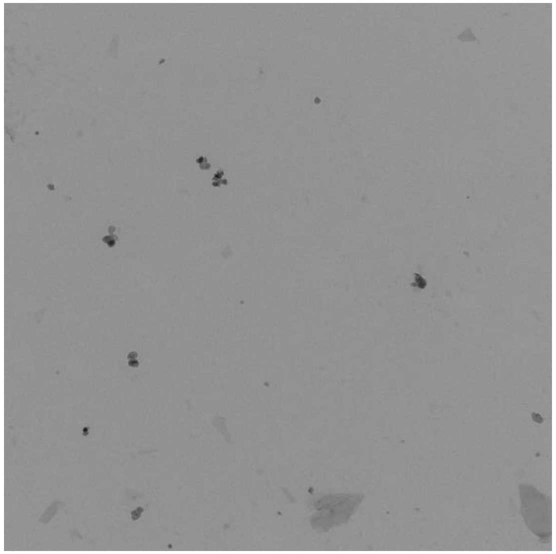
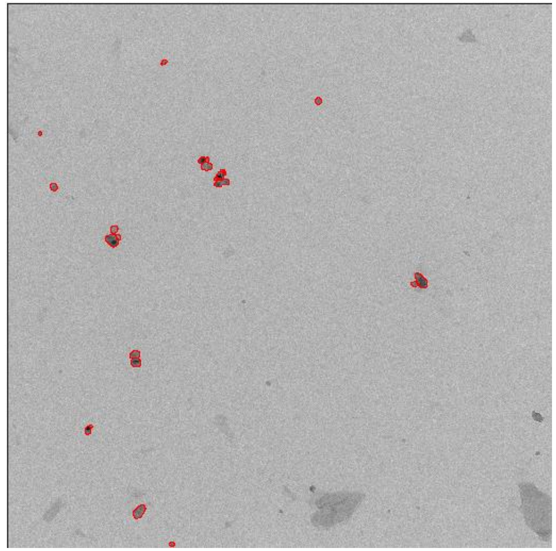
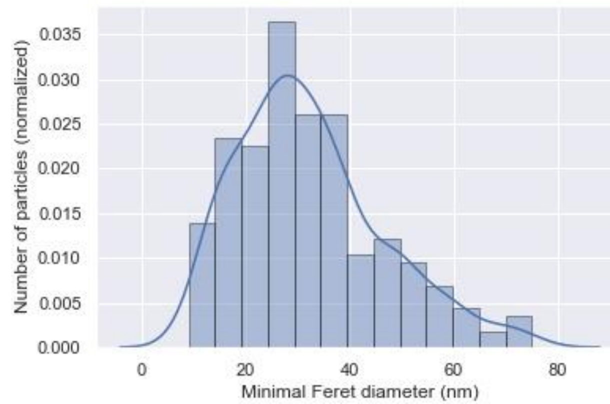
E171 pristine materials: rutile







E171 Material:	7	8	9
Mode Min. Feret diameter (nm)	28	+/-30	+/-30
Median Min. Feret diameter (nm)	30	+/-30	+/-30

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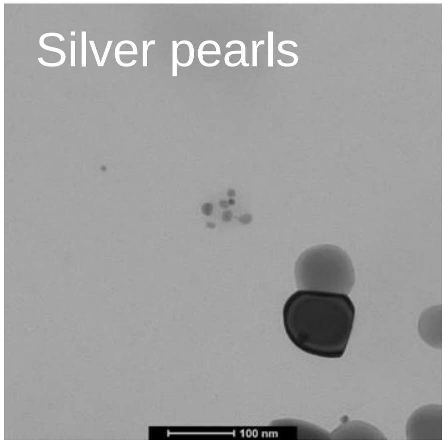
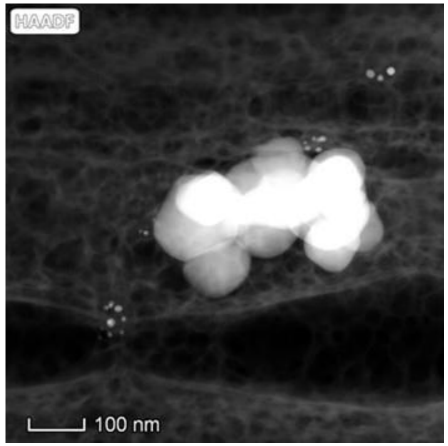
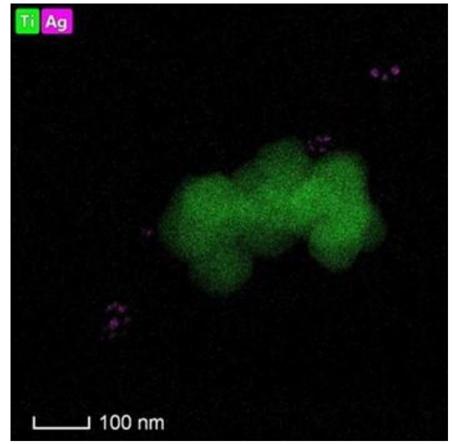
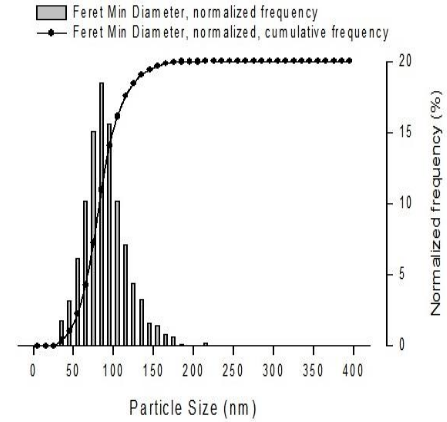
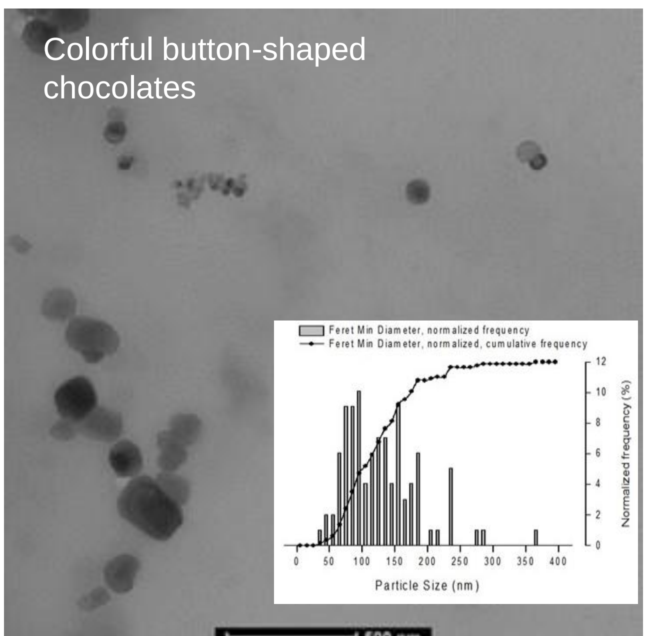
E171 in products: anatase

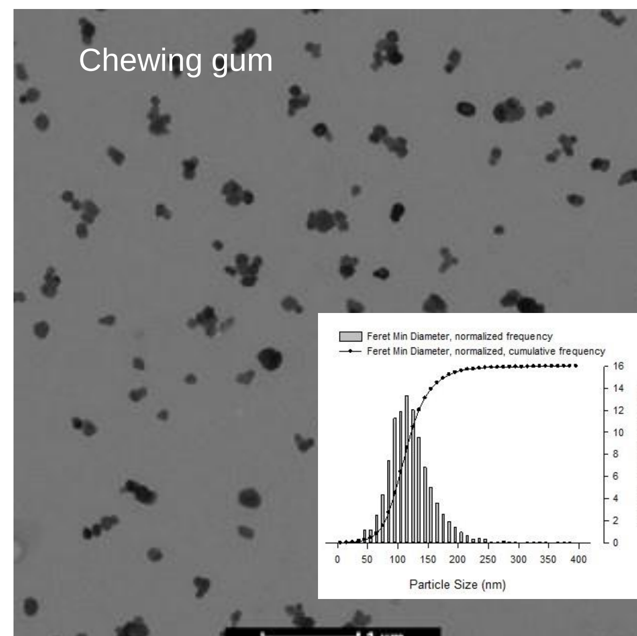






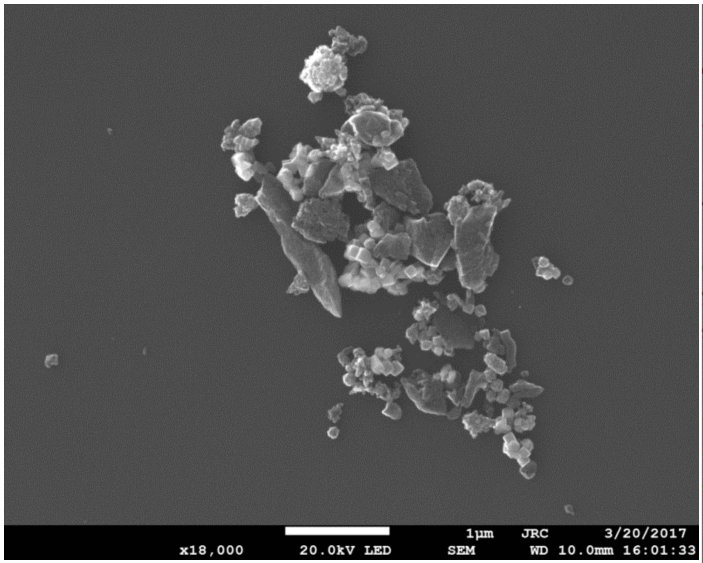


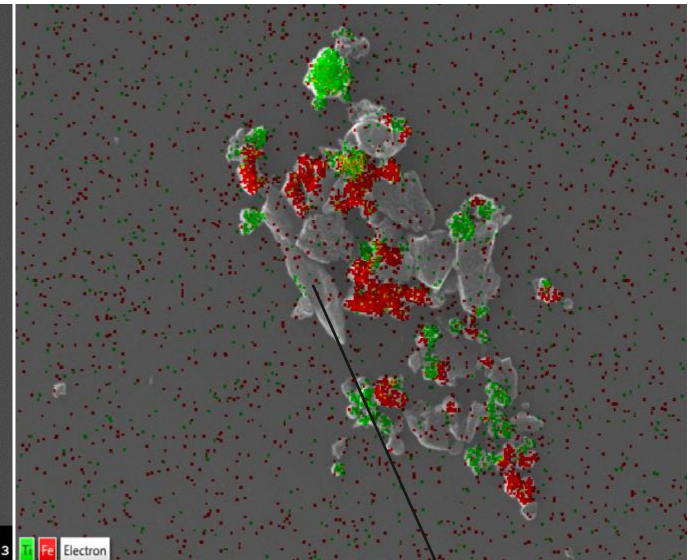




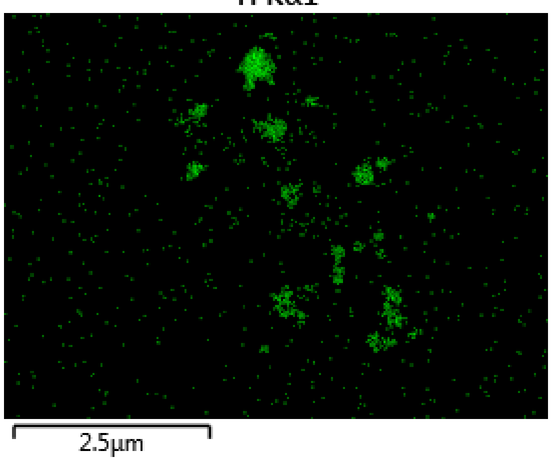
E171 products: rutile

E171 in matrix E172 (iron oxide) and E153 (vegetable carbon)

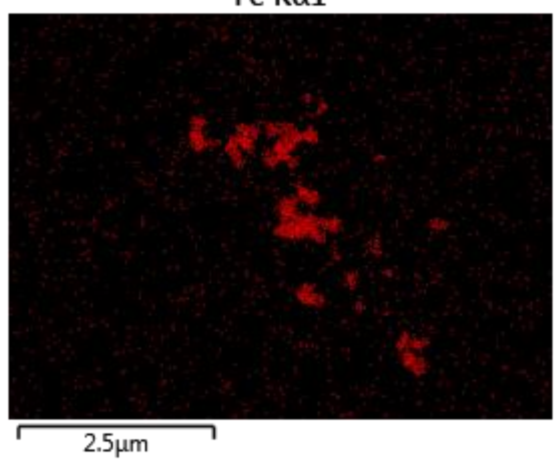


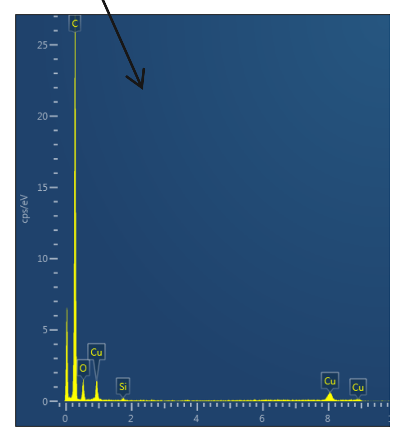


Ti Kα1



Fe Kα1





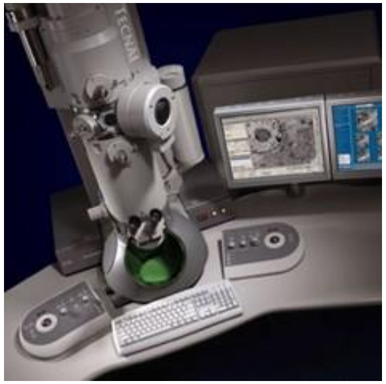
Conclusions E171

- Several types of TiO_2 nano-sized particles are found in pristine E171 and in products containing E171
- E171: Less difficult sample preparation: no transformation between pristine materials and products

OUTLINE

- Introduction
- Analytical methodology
- Analysis results of E174
- Analysis results of E171
- Lessons learnt

Implementation of legislation and guidance



Challenge to build up specialized expertise and analytical capacity

➔ Top-technologies

- Conventional TEM
- Advanced ICP-MS (Sciensano Tervuren)
- Analytical SEM (SEM-EDX, visiting scientist JRC Geel)
- Analytical TEM (Sciensano Ukkel, just installed, 1 mio euro)

➔ Development and introduction of new methodologies (SOPs)

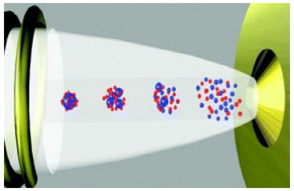
- Sample preparation
- Identification and analysis of individual (primary) particles: size, shape, surface and elemental composition

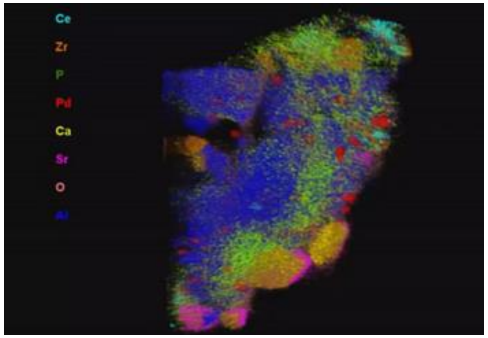
➔ Complex validation approach, with few reference materials

➔ Analytical capacity

- High degree of automation
- Standardized data analysis & reporting of number-based results

➔ Search for collaborations, project opportunities, market





Next steps

Much more analyses…

Further methodology development:

- Automation to increase capacity
  HAADF-STEM-EDX platform: Talos – Velox – Mapps - AVIZO
- Sample preparation

Market study (+ risk analysis)

Compulsary notification to Belgian FAFS

Difficulties applying/interpreting current legislation (definition)

Open communication

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Experience in context of ”EFSA Guidance on risk assessment of the application of nanoscience …

Phys.-chem chapter in EFSA guidance provides general directions.

To be more practical, it could profit from:

- More detailed descriptions of approaches and methods,
- Guidance to «best practices» and later, to standardised methods (CEN)
- Illustrated in examples,
- Aim: to be implemented in MS (control and service) laboratories.

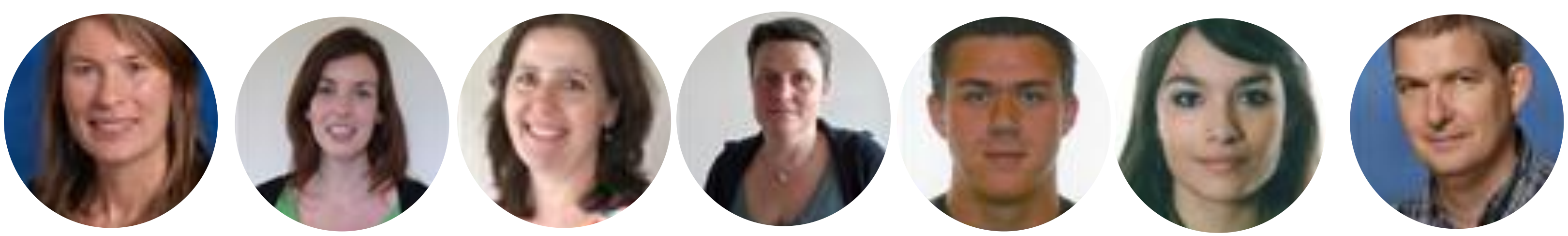
BE proposes WG in context of network for nanotechnologies

(possibly liason with SANTE-JRC initiative « Nano in food: MS laboratories)

? Support of other member states?

Acknowledgements

- FPS Health RCO RF16/6306/nanofood@ 2016-2018: “Implementation and validation of an approach to assess the fraction of engineered nanomaterials in food additives” (nanofood@).
- EFSA 2017-2020: Physico-chemical characterization and exposure analysis of nanomaterials in food additives in the context of risk assessment (EFSAfood@)
- FPS HealthDG4 2016-2018:“Physico-chemical characterization of the fraction of engineered nanomaterials in silver (E174) food additives in the context of risk assessment (nanoAg@)”
- BELSPO 2015-2016: “Method validation of nanomaterial analysis by single particle ICP-MS and DLS” (acronym: Nanoval)
- BELSPO BRAIN.Be (BR/154/A4/To2DeNano) 2016-2018: “Towards a toxicological relevant definition of nanomaterials” (To2DeNano)
- EC Large-scale integrating project FP7 NMP.2012.1.3-3 2013-2017: Regulatory testing of nanomaterials . “A common European approach to the regulatory testing of Nanomaterials” (NANoREG).
- CEN/TC 352: Nanotechnologies — Guidance on detection and identification of nano-objects in complex matrices
- EC Large-scale integrating project FP7 NMP.2013.1.4-3 2013-2017: “Development of methods and standards supporting the implementation of the Commission recommendation for a definition of nanomaterial. (NanoDefine).
- FAFS 2017-2019 NRL Chemistry 1 – parcel 4: Food contract materials – Heavy Metals – Nanoparticles



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Leuvensesteenweg 17, 3080 *Tervuren*