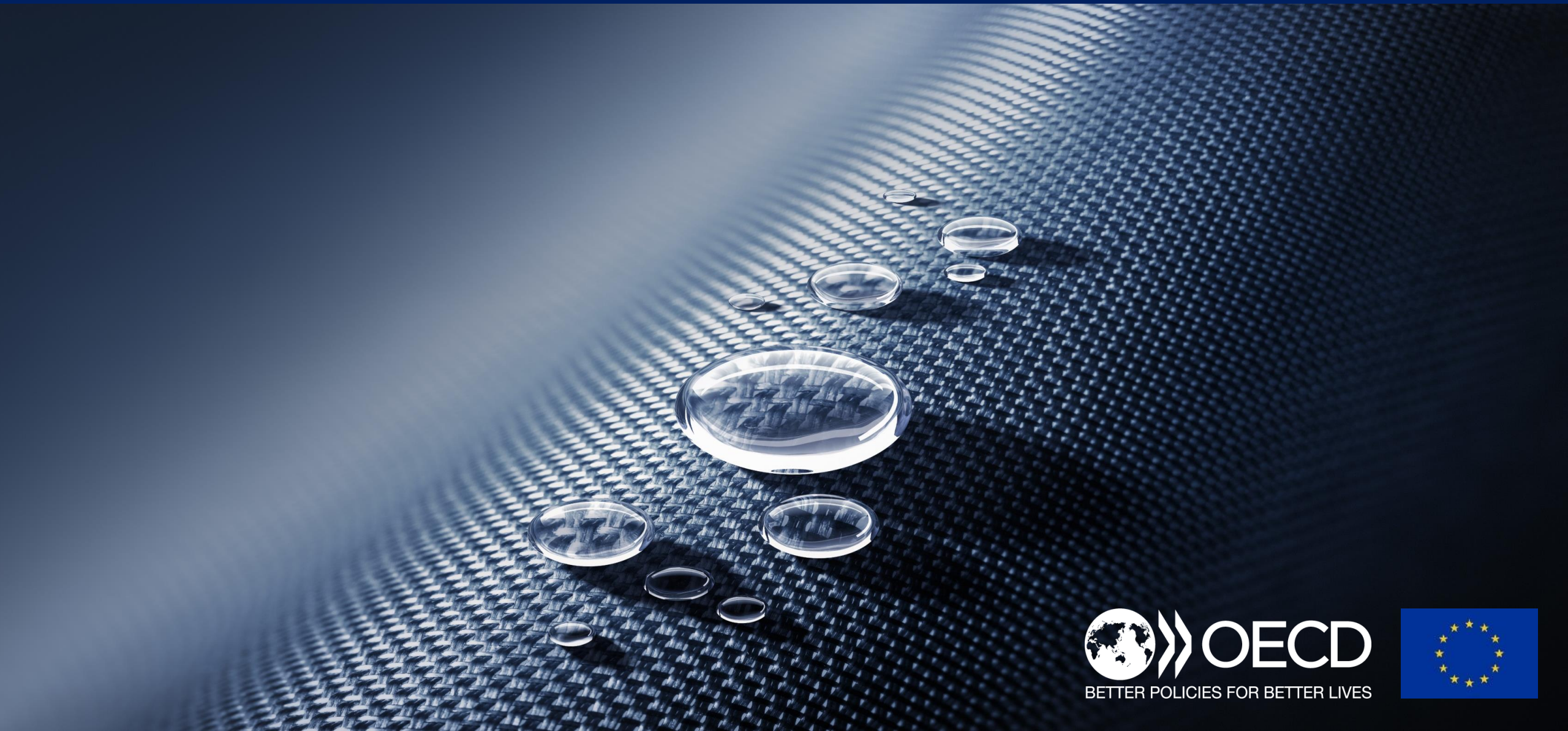
Welcome to the Webinar, we will start in a couple of minutes.

Bienvenidos a este seminario Web de la OECD. Vamos a empezar en unos minutos.



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Interpretation

If you do not see the **“Interpretation” button on the bottom of your screen,** make sure you **download the Zoom app** on your desktop, phone or tablet from the Zoom download center: https://zoom.us/download#client_4meeting or via the direct links available in the chat box.

Interpretación simultánea

Para acceder al botón de **Interpretación**, necesitan tener la aplicación Zoom https://zoom.us/download#client_4meeting , disponible en el **Chat** de este seminario.

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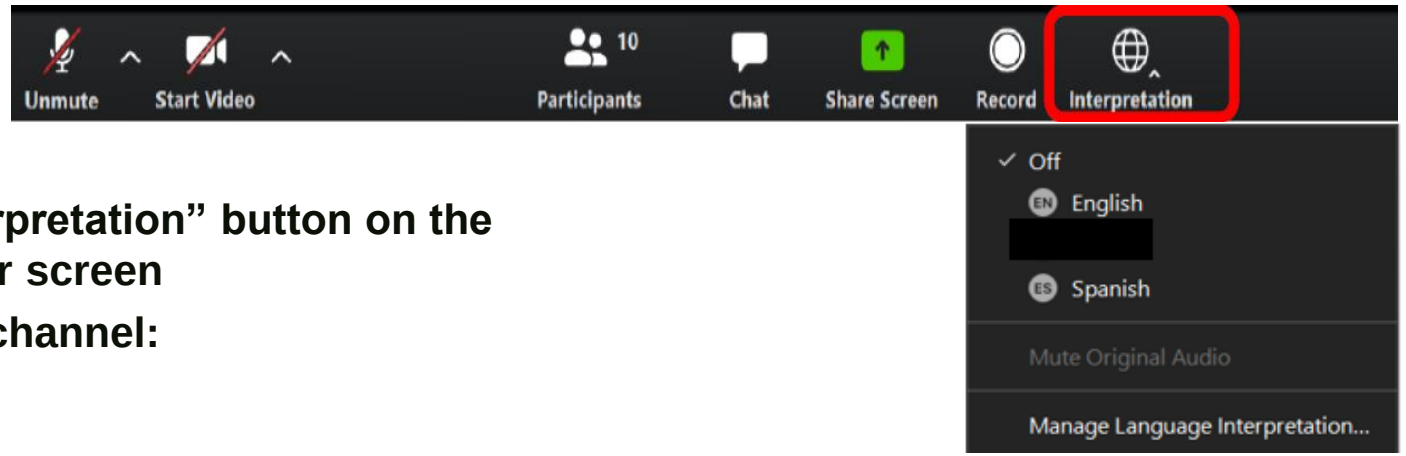

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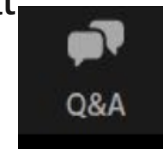
Zoom Instructions

Q&A function:
During the presentation you may have a question that you'd like to be answered.

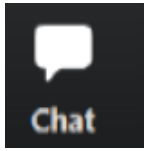


Please send your questions at any time during the talk via the Q&A function in the Zoom control panel so it does not get lost in the Chat.
We will respond to as many questions as possible during the Q&A sessions.

Funcion Q&A:
Preguntas y respuestas: si tiene preguntas durante la presentación, hágalo vía el botón **Q&A**.

Envíe sus preguntas en cualquier momento durante la charla a través de la función Q&A y no en el chat para que los expertos las vean.
Responderemos a tantas preguntas como sea posible durante las sesiones de preguntas y respuestas.

Chat function: During the meeting we will share technical and useful information in the chat box.



Funcion Chat: En el Chat compartiremos información técnica y links útiles para saber mas el tema.

This webinar will be recorded and will be made available in both languages afterwards:
Este seminario web se grabará y estará disponible en ambos idiomas posteriormente:
https://oe.cd/nanomet

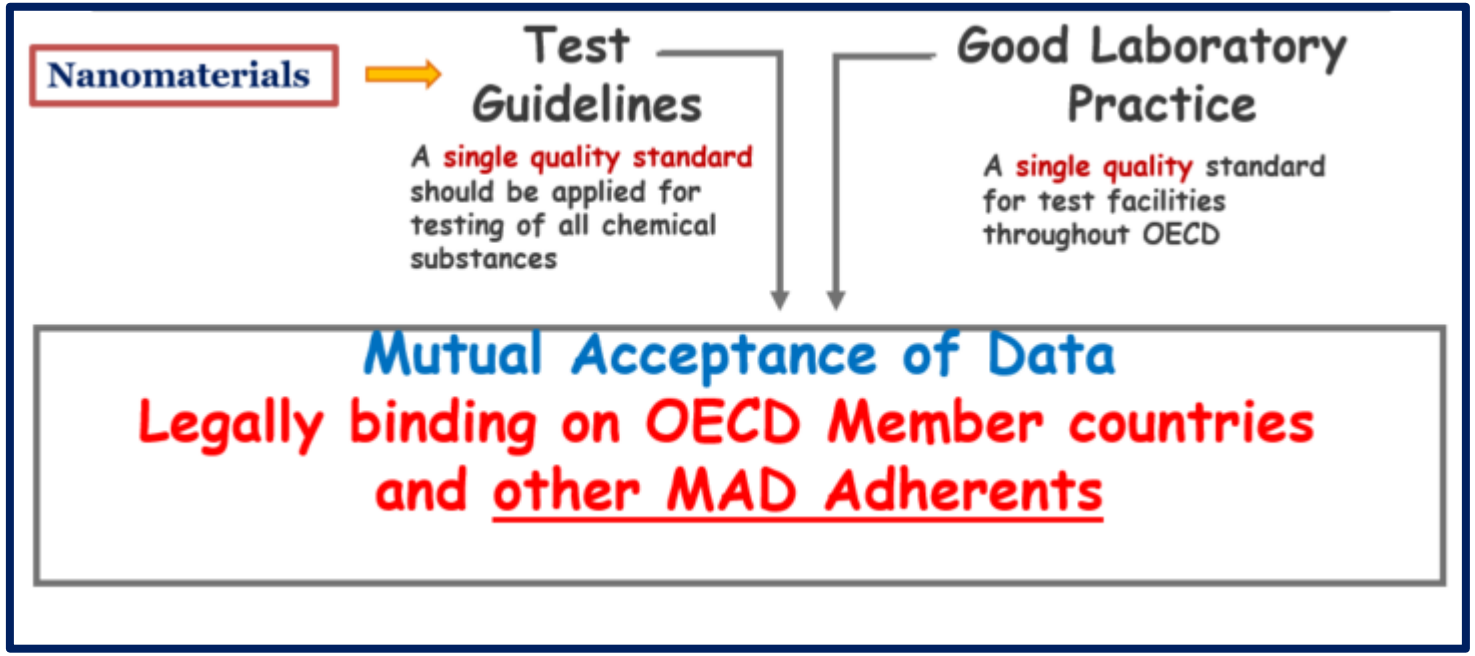
European Commission

THE MUTUAL ACCEPTANCE OF DATA SYSTEM

Data generated using **OECD Test Guidelines (TG)** under **Good Laboratory Practices (GLP)** are accepted across member countries and MAD adhering countries having the same data requirement

"tested once, accepted everywhere."

Saving Costs in Chemicals Management
https://oe.cd/chemicals-costs



HOW ARE TEST GUIDELINES DEVELOPED

National Coordinator(s)

Project proposal submission (SPSF)

Working Party NCs of the TGP (WNT)

Review cycle

Decision

V Project proposal accepted

X Project proposal rejected

(?) Project proposal needs further information

Lead country(ies) + Expert Group (EG)

Evaluate validation status of the method, any additional info/data needed: Transferability and reproducibility?

Supporting documentation

Consolidation of draft TG within EG

Draft Test Guideline

WNT review/comments via written procedure

Submission for approval

Working Party NCs of the TGP (WNT)

TG publication

European Commission

Physical-chemical properties

- Determination of the (Volume) Specific Surface Area of Manufactured Nanomaterials (TG 124)
- Particle Size and Size Distribution of Manufactured Nanomaterials (TG 125)
- Determination of surface hydrophobicity of manufactured nanomaterials (TG 126)
- Determination of solubility and dissolution rate of nanomaterials in water and relevant synthetic biologically media
- Identification and quantification of the surface chemistry and coatings on nano- and microscale materials
- Determination of dustiness of manufactured nanomaterials
- Determination of concentrations of nanomaterials in biological samples for (eco)toxicity studies

Effects on Biotic systems

- **Aquatic and Sediment Toxicological Testing of Nanomaterials**

No. 317 OECD Series on Testing and Assessment
(August 2021, updated March 2022)

Environmental fate and behaviour

- Testing of dissolution and dispersion stability of nanomaterials and use of data for further environmental testing and assessment strategies (TG 318, GD No. 318 OECD Series on Testing and Assessment (August 2021, updated March 2022)
- Leaching in soil columns No. 342 Series on Testing and Assessment, July 2021)
- Removal of nanomaterials in Wastewater Treatment Plants
- Aquatic (Environmental) transformation of nanomaterials *(in draft form in September 2023)*

Human health

- Applicability of TG 487 *in vitro* micronucleus test (genotoxicity) for testing manufactured nanomaterials
- Applicability of TG 442D *in vitro* skin sensitization to manufactured nanomaterials
- (Udpated) TG 412: Subacute Inhalation Toxicity: 28-Day Study
- (updated) TG 413: Subchronic Inhalation Toxicity: 90-day Study

TODAY'S SPEAKERS

Juan Riego Sintes

Cloé Desmet

Francesco Roncari

Andrea Valsesia

European Commission

Joint Research Centre

European Commission
Joint Research Centre
Unit F.2 Technologies for Health

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BETTER POLICIES FOR BETTER LIVES

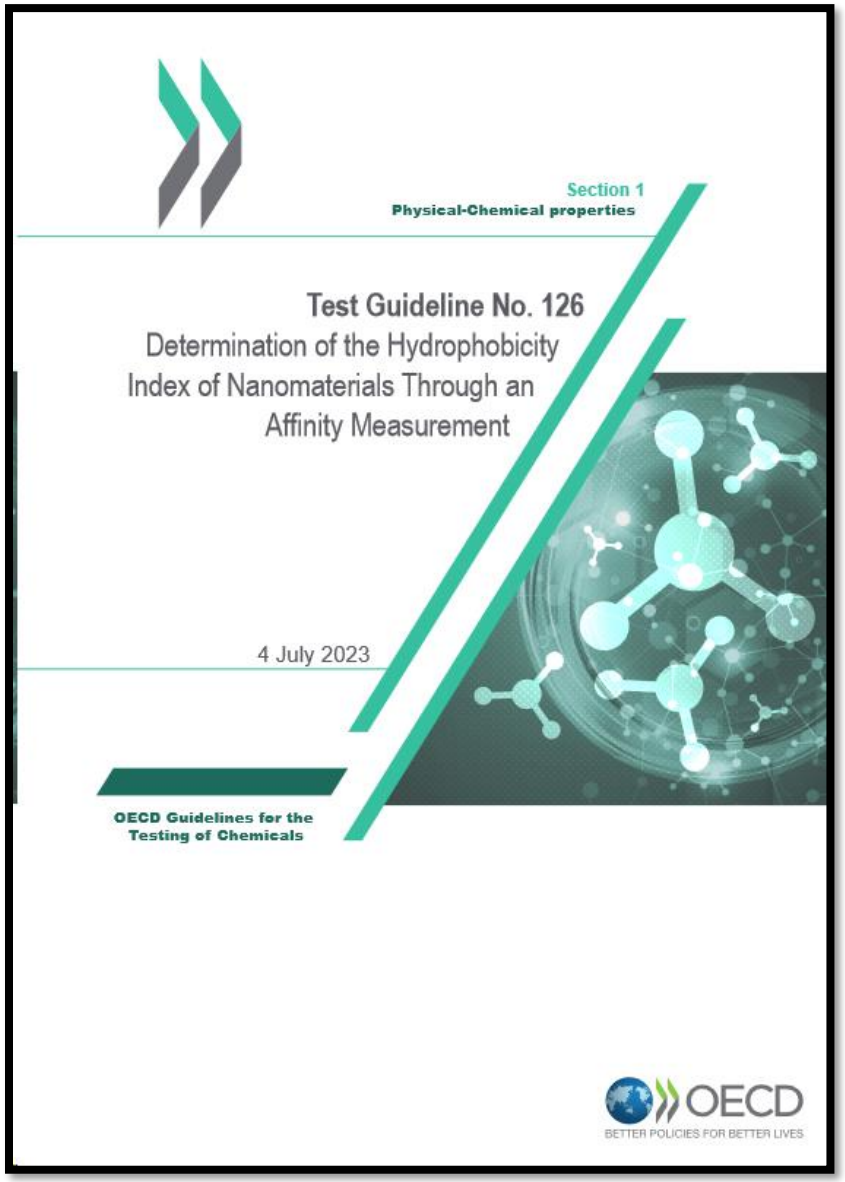
Webinar on test Guideline No 126: Determination of the Hydrophobicity Index of Nanomaterials Through an Affinity Measurement

Cloé Desmet, Francesco Roncari, Pascal Colpo, Juan Riego Sintes, Andrea Valsesia

20 September 2023

Outline

1. Background
2. Method principle
3. Method description
4. The test in practice
5. ILC Validation
6. Method comparison
7. Potential uses
8. Q&A



1. Background (I): **The need**

- **Legislative frameworks** require reporting the octanol/water partitioning coefficient (Kow),
  - indicator of the fate and transport of a chemical in the aquatic compartment
  - key parameter of environmental exposure models
  - human risk assessment

- **OECD & regulatory scientific community**: Kow is not applicable for the assessment of nanomaterials (5,6,7,8)

- **OECD WPMN**: there is a need to develop procedures to acquire information on the behaviour of NMs in fluids (9)

* References 5 to 9 can be found in the introduction of OECD TG 126

1. Background (II): **One Tool**

- **Hydrophobicity** was identified as a relevant parameter in the "*OECD Physical-Chemical Decision Framework for Manufactured Nanomaterials*" (1) and, among other parameters, could provide **information on the interaction of NMs**
  - with aquatic and terrestrial organisms (incl. bioaccumulation and persistence), and
  - with human cell surfaces (incl. transport into the human body through skin and lungs, and consequent accumulation in tissues/organs or clearance from blood circulation) (10)

- **Hydrophobicity** of chemicals influences e.g.:
  - Cellular uptake (2)
  - Toxicity (3)
  - Immune responses (4)/ Immune cells interactions (5)
  - Haemolysis (6)
  - Protein adsorption (7)

(1) https://one.oecd.org/document/env/jm/mono(2019)12/en/pdf. *(2) Zhuet al.Small 8 (2012). (3) Chompoosoret al. Small 6 (2010). (3) Moyanoet al. Journal of American Chemical Society 134 (2012). (4) Shimaet al. Biomaterials 34 (2013). (5) Sahaet al. Materials Horizons 1 (2014). (6) Gessner et al. International Journal of Pharmaceutics 196 (2000). (7)* Shimaet al., Biomaterials 34 (2013).

1. Background (III): **The decision**

- **Hydrophobicity**, size and surface charge are the main parameters influencing **nanoparticle** biocompatibility (1,2,3)

A proposal of the **European Commission JRC to the OECD** Test Guidelines Programme (TGP) to develop a new TG addressing the Hydrophobicity of Nanomaterials was accepted as new item in the TGP workplan in 2019.

The final new **TG 126 Determination of the Hydrophobicity Index of Nanomaterials Through an Affinity Measurement** was adopted in 2023

(1) Shimaet al., Biomaterials 34 (2013). (2) McNeil, NanomedicineNanobiotechnologie1(2009). (3) *Chou et al. Chemical Society Reviews 40 (2011)*

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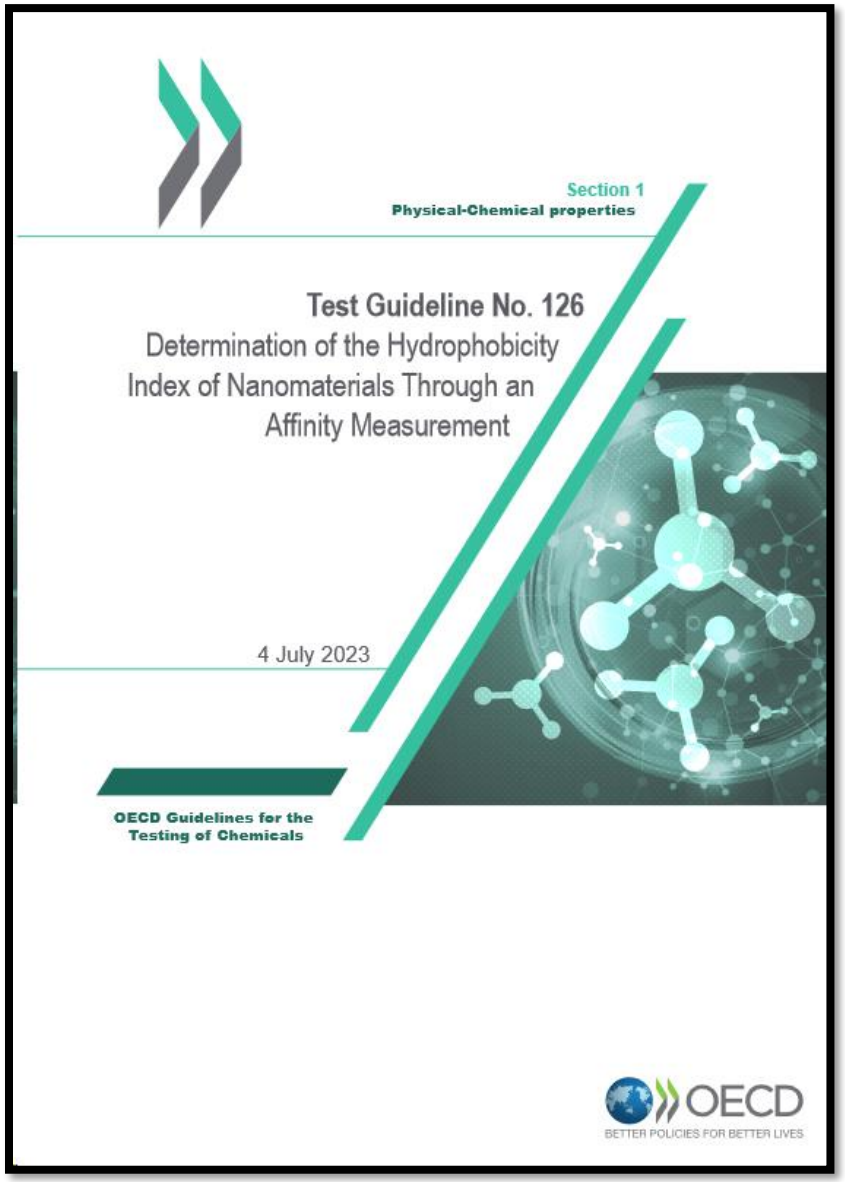
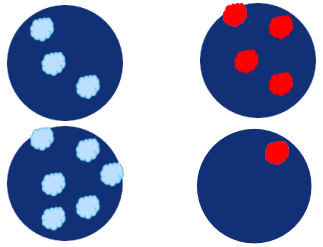


2. Method principle – Existing Methods

Dye Adsorption

Hydrophilic vs hydrophobic dye

Nile Blue Rose Bengal



Hydrophobic

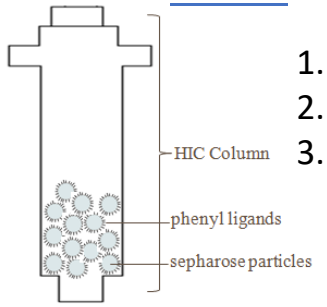
$$\text{Log HR} = \log_{10}\left(\frac{k_{lin,RB}}{k_{lin,NBA}}\right)$$

Hydrophilic

+ Simple
- Not specific

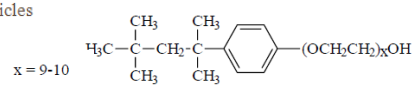
HIC

Column: hydrophobic ligands



1. Butyl FF
2. Phenyl FF
3. Octyl FF

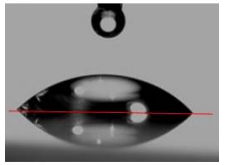
Elution: Triton X-100



+ Accurate
- Expensive, large volume

Contact angle

between liquid & film of NPs

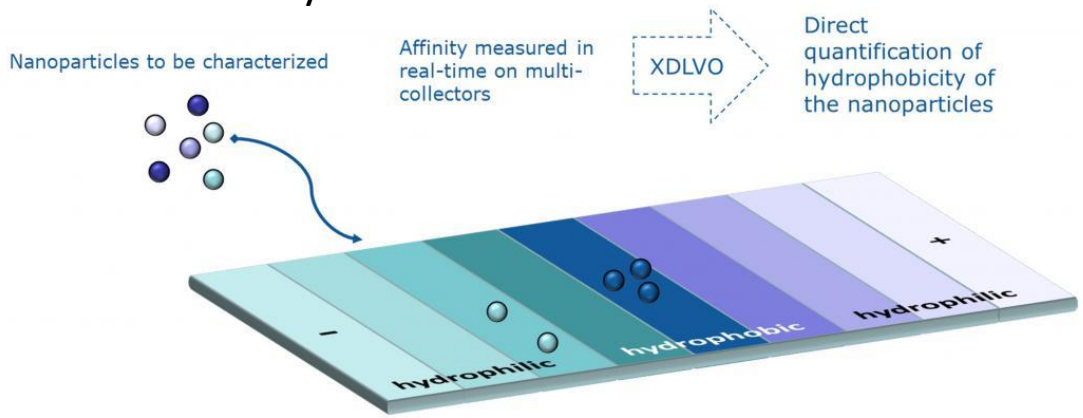


+ Simple
- Large volume, non-accurate

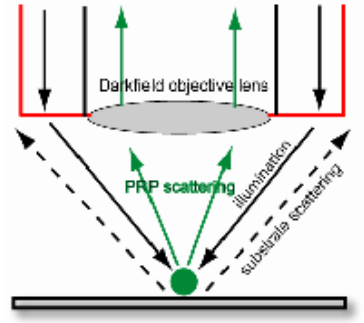
Crandon LE et al (2020) Adaptive methodology to determine hydrophobicity of nanomaterials *in situ*. PLoS ONE 15(6)

2. **Method principle**

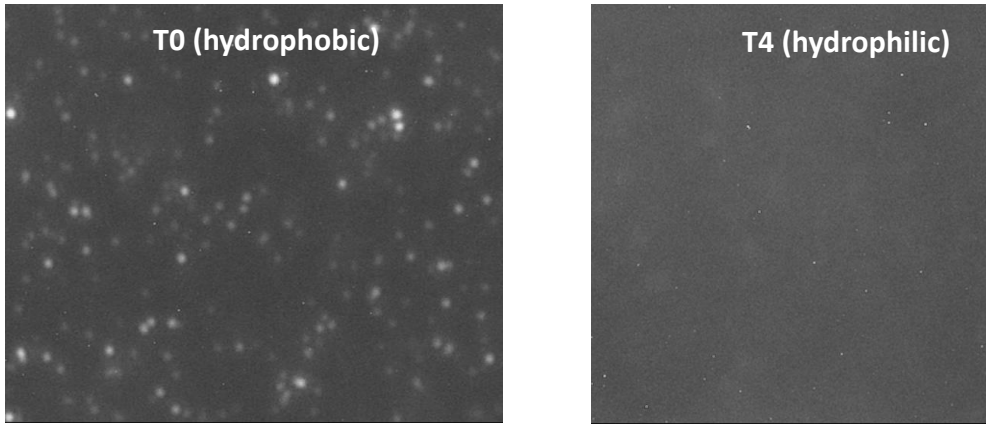
Affinity to collectors

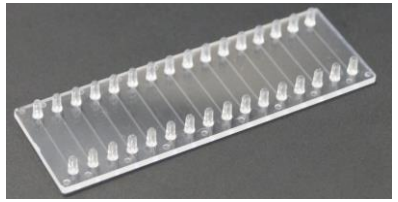


Dark field microscopy



Scattered light:
collected by objective lens
(decays with r^6 → only "big" NP)





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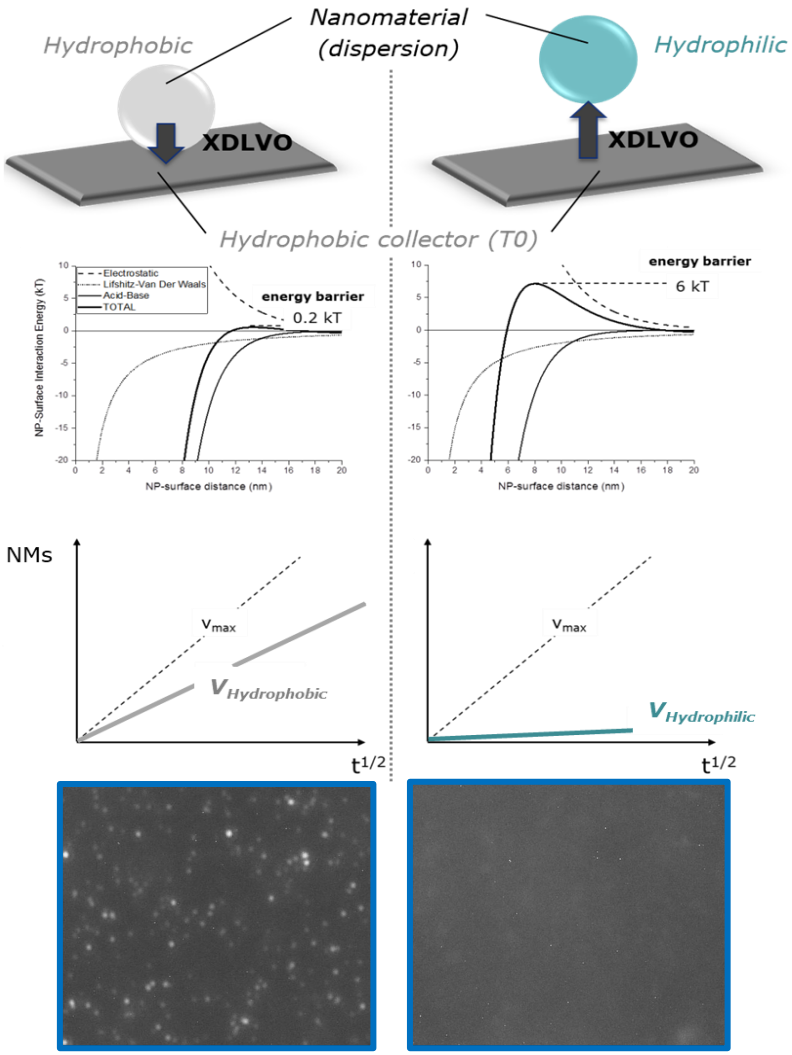
2. **Method principle**

Measurement of the affinity of NMs on engineered surfaces

Determination of the Hydrophobicity Index

➔ **Hydrophobicity characterisation**

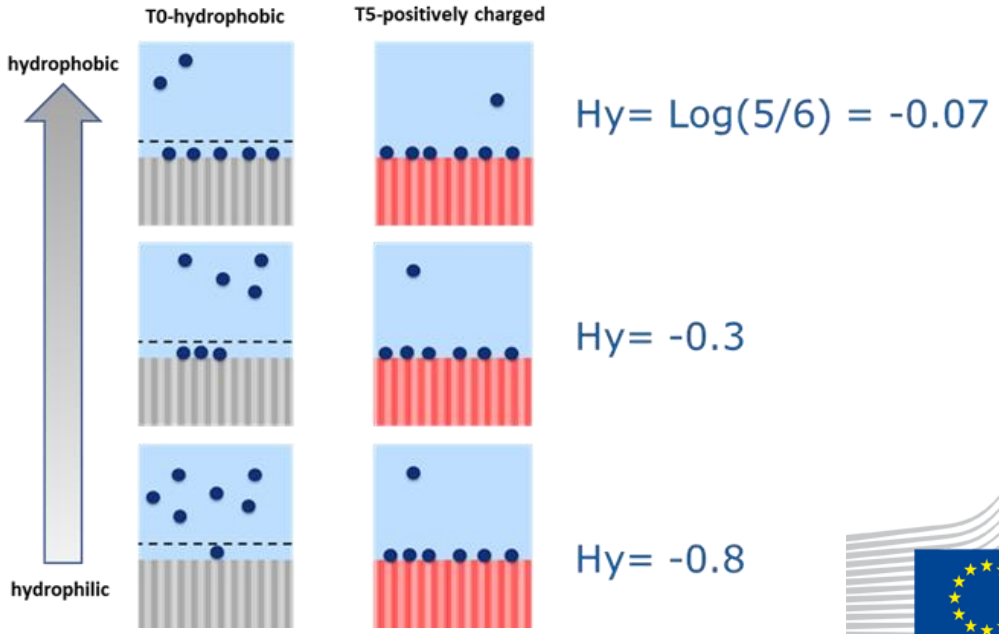
2. **Method principle – The Theory**



v_{max} measured from collector with opposite charge (no barrier, diffusion limited)

v_s measured in the presence of an energy barrier

$$Hy = \log\left(\frac{v_s}{v_{max}}\right)$$



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2. **Method principle – The output**

Hydrophobicity characterisation:

A quantitative answer:

Nanomaterial is

Hydrophobic: Hy close to 0

Hydrophilic: Hy < -1

$$Hy = \log\left(\frac{v_s}{v_{max}}\right)$$

Hy = -1 ➔ 10% of binding on T0 compared to T5

European Commission

2. **Method principle – Hydrophobicity Index**

Hy measured in specific conditions

➔Validity limited to these conditions
➔Depends on: surfactant, pH, salt…
➔Can differ from pristine

Poorly dispersible hydrophobic NMs

➔ Possible use of a surfactant and extrapolation

Broad applicability

Au, Ag, TiO_2, SiO_2, ZnO, GaN, CeO, nanoplastics, liposomes

NM	Hy quantified	Size
Carbon nanotubes	No	/
Cerium oxide	Yes	10-20 nm (primary), ≥100 nm
Dendrimers	No	/
Fullerenes (C_{60})	No	/
Gold Nanoparticles	Yes	≥15 nm
Nanoclays	No	288 nm (primary)
Graphene	No	/
Silicon dioxide	Yes	15-20 nm (primary), ≥50 nm
Silver nanoparticles	Yes	≥20 nm
Titanium dioxide (NM100-NM105)	Yes	21 nm (primary), ≥100 nm
Zinc oxide	Yes	70-90 nm (primary), ≥100 nm

List of representative nanomaterials
OECD overview of the testing programme of manufactured nanomaterials.
The JRC Nanomaterials Repository: Safe handling of nanomaterials in the sub-sampling facility. Cotogno G et al.

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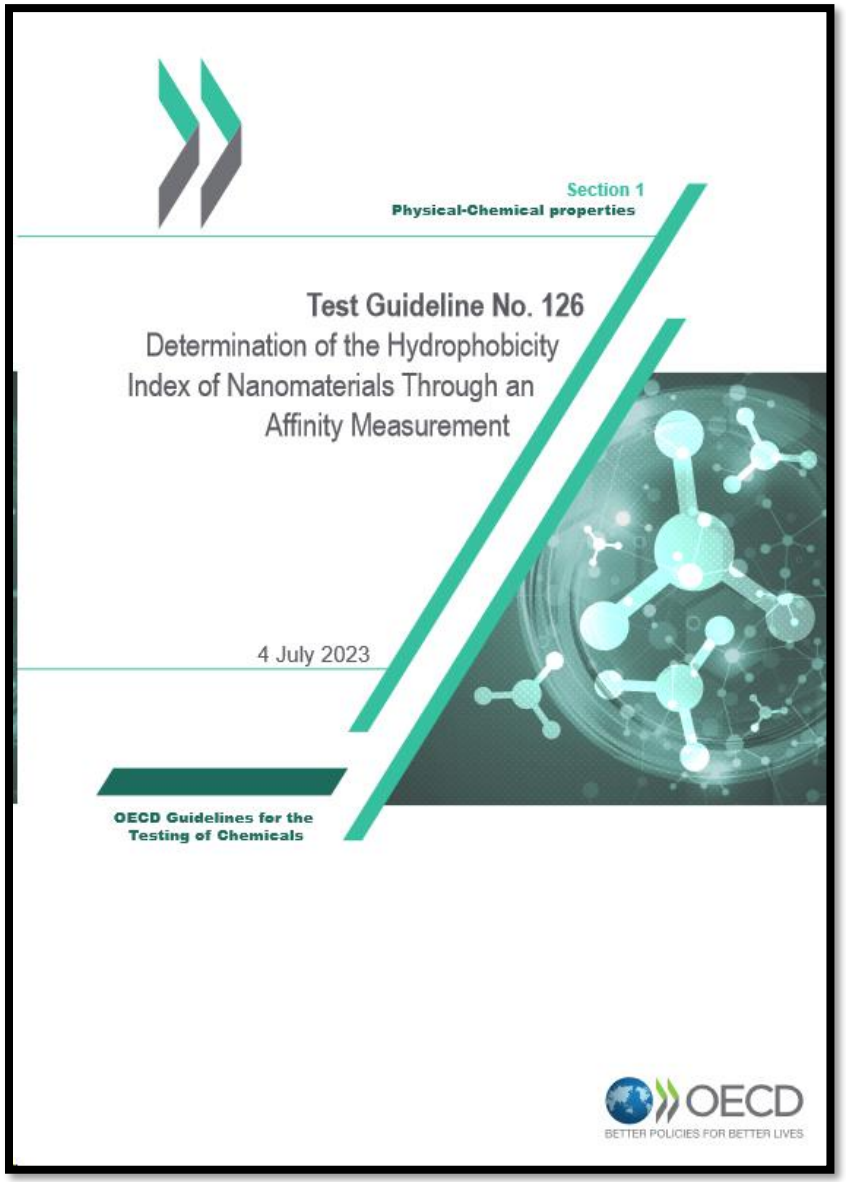
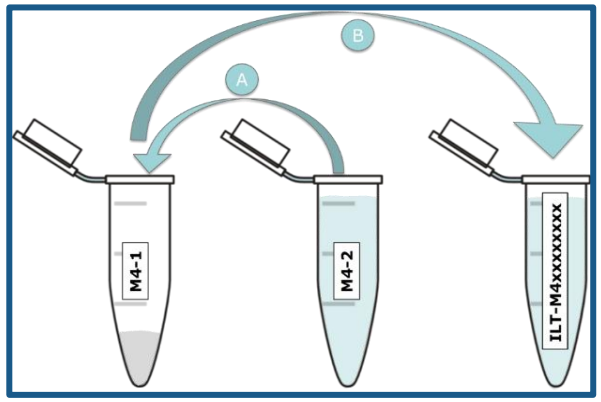


3. Method description

1. Sample preparation

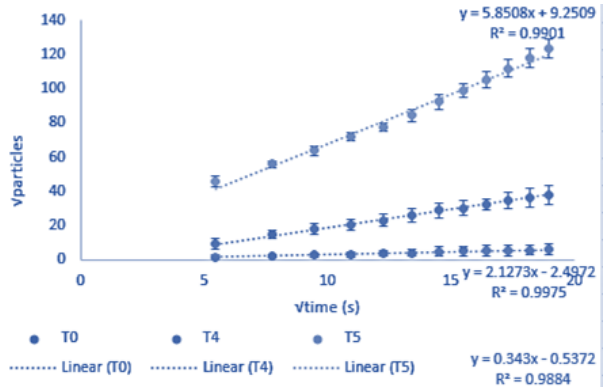


NM sample in stable dispersion

2. Measurement

Counting the particles binding by Dark Field Microscopy

3. Calculation



Affinity on 3 collectors

Hy = …

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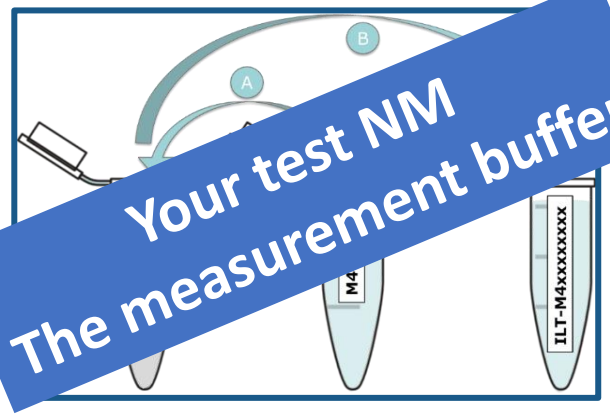
3. Method description

What do I need?

1.



Your test NM
The measurement buffer

2.

The 3 collectors
1 Micropipette
A dark field microscope
A camera + a computer
The TG SOP

3.

ImageJ + TrackMate
(Freely downloadable)
A spreadsheet program
The TG SOP

3. Method description

The samples

Powder

OR

Dispersion

Nanomaterial tested as a dispersion in controlled condition:

Mild dispersion protocol to avoid disruption of potential coating

Buffered condition (known salt concentration and pH)

Optional characterisation:

- Size
- Surface charge (ζ potential)

DLS

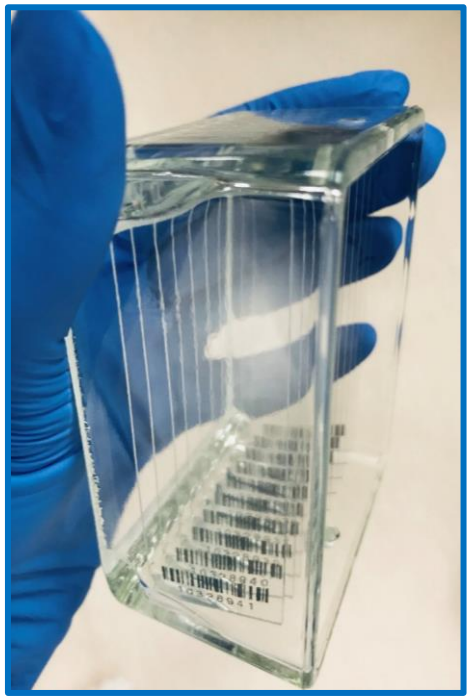
3. Method description

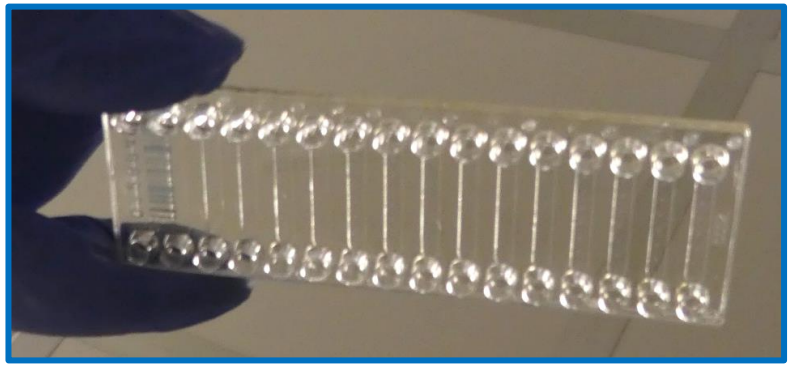
The collectors

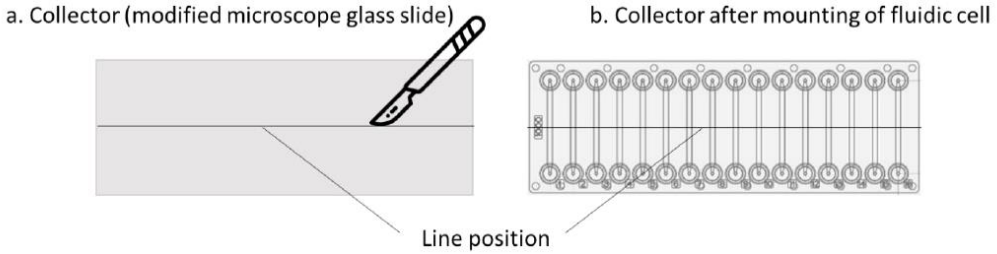
Based on classical glass slide:

- 1 Hydrophobic
- 1 Hydrophilic +
- 1 Hydrophilic -

- 16 channels microfluidic cell

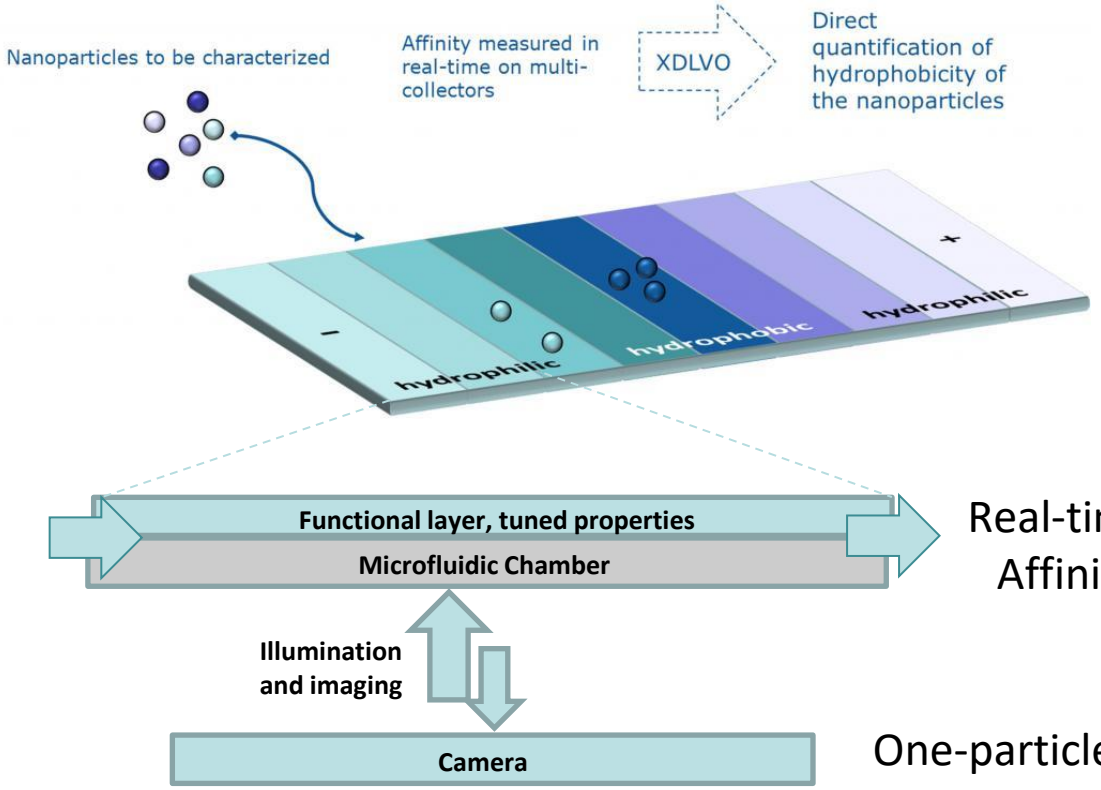






3. Method description

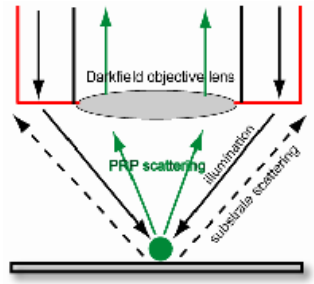
The measurement

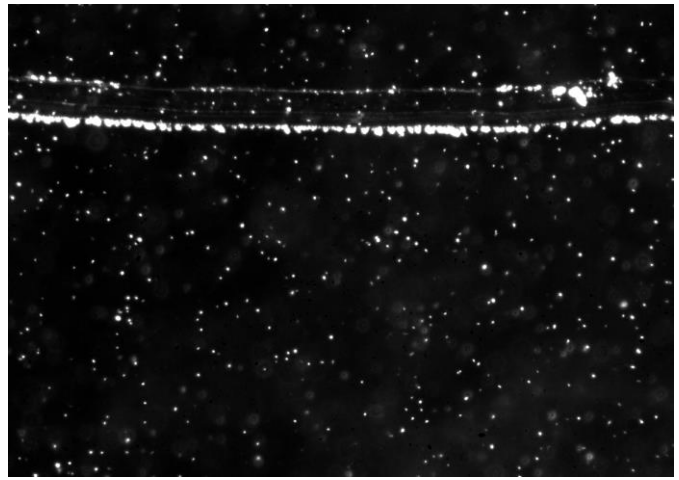


Real-time experiments
Affinity information

One-particle sensitivity

Dark field microscopy





3. Method description

The calculation

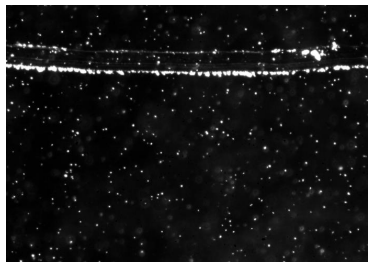
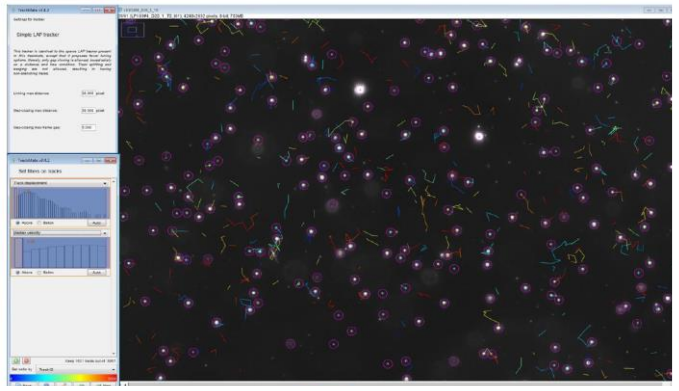
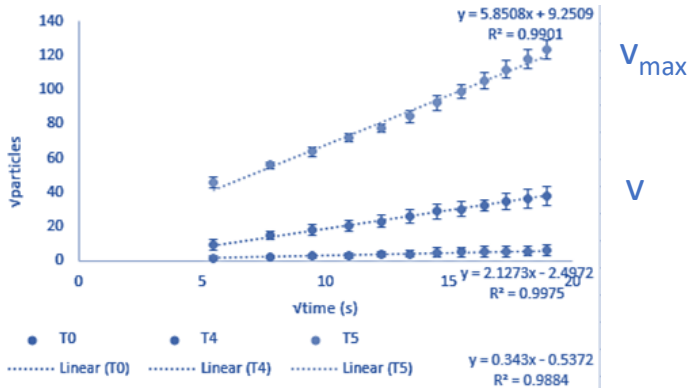


Image Sequence



Automatized particles detection and tracking



V_{max}

V

Binding curves

$Hy = Log (v/vmax)$

Hydrophobicity index determination

Nanomaterial is considered

- **Hydrophobic** **-1 < Hy < 0**
- **Hydrophilic** **Hy < -1**

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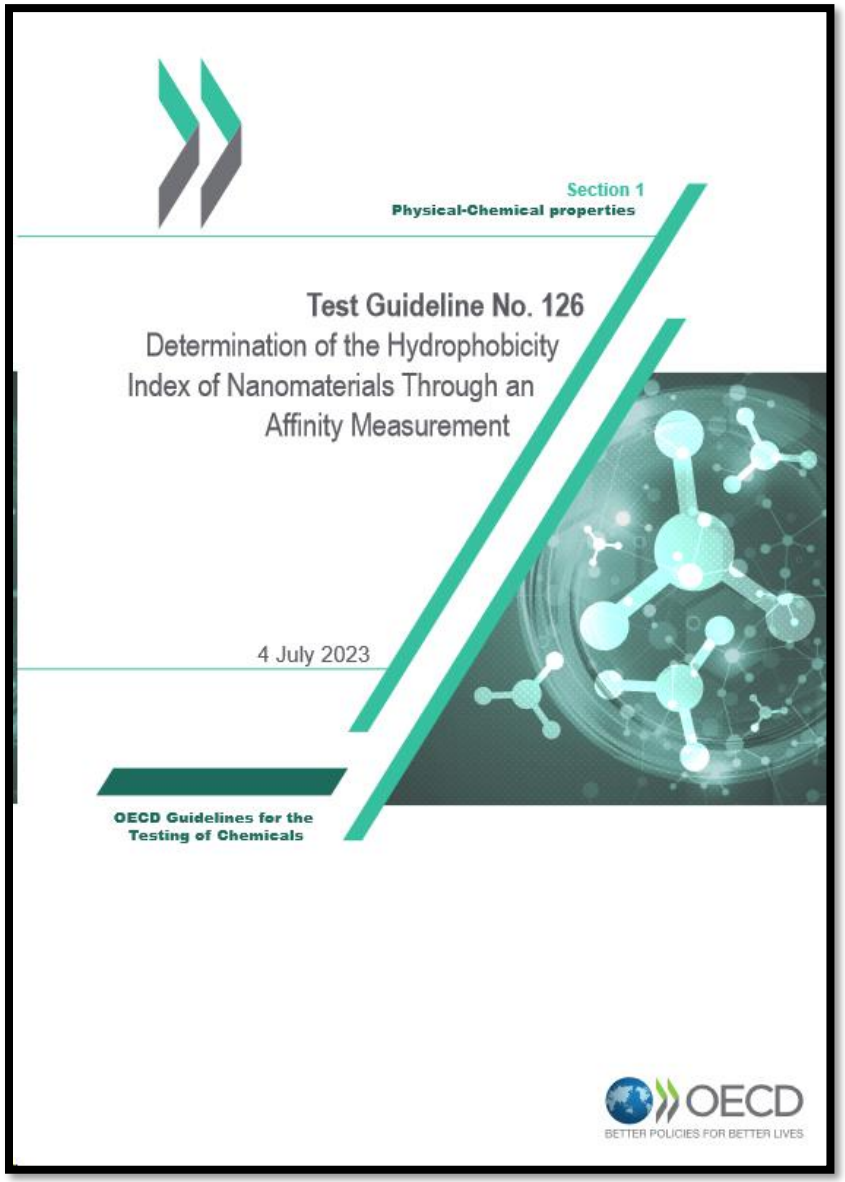
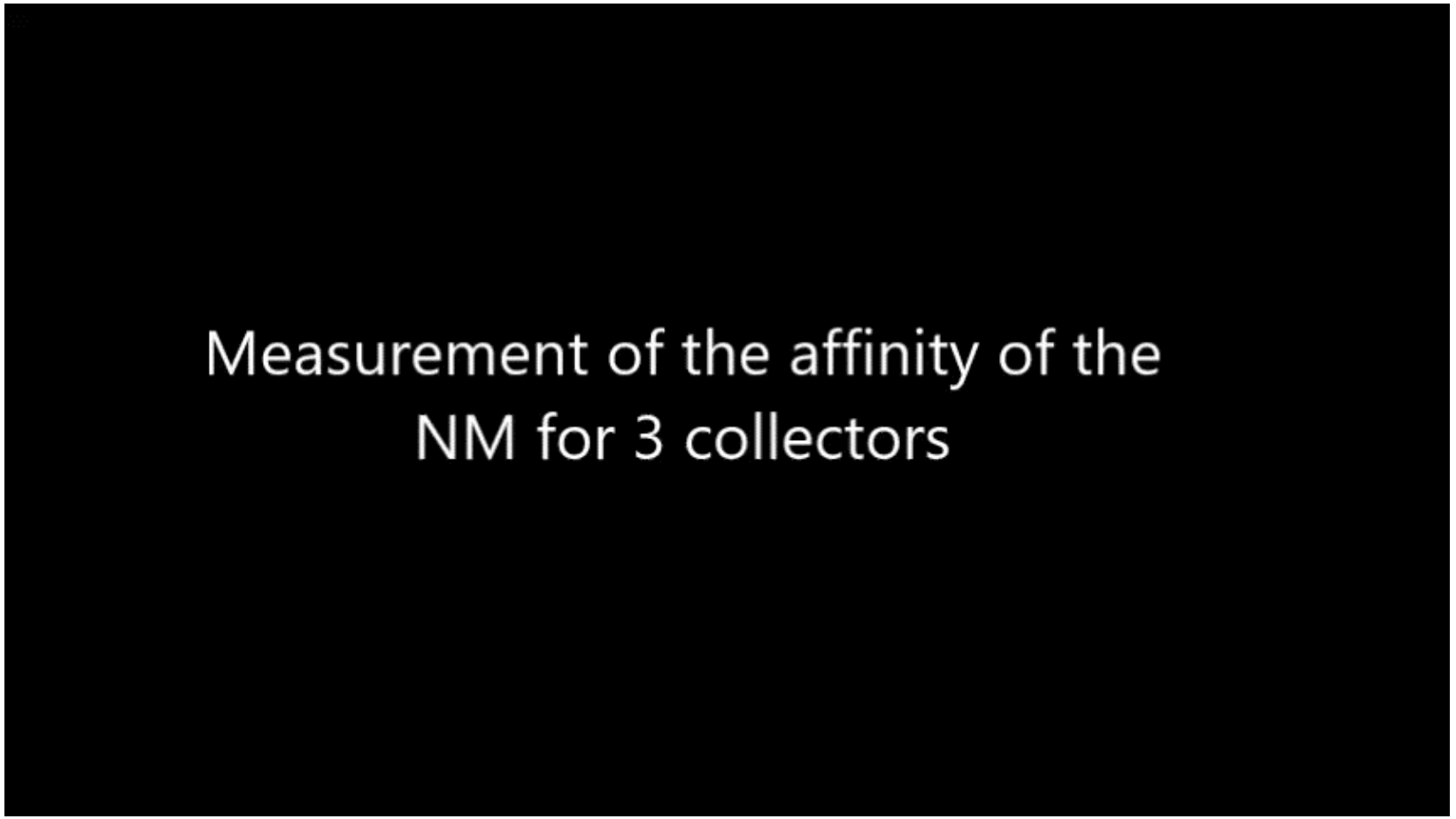


4. The test in practice



4. The test in practice

Dealing with poorly dispersible NM

"Method only applicable to NM in stable dispersion in aqueous medium"

TiO_2
coated with:

Dimethicone
JRCNM62001a

→ Hydrophobic **POORLY DISPERSIBLE**

Size / nm	Zeta Pot. / mV	[Surfactant] / [TiO_2]
148.6 ±6.4	-26.3 ±2.1	0.1
144.0 ±1.3	-26.6 ±1.9	0.5
142.7 ±2.8	-35.1 ±2.3	1
130.9 ± 2.4	-49.2 ±3.5	2

↓ Decreasing hydrophobcity

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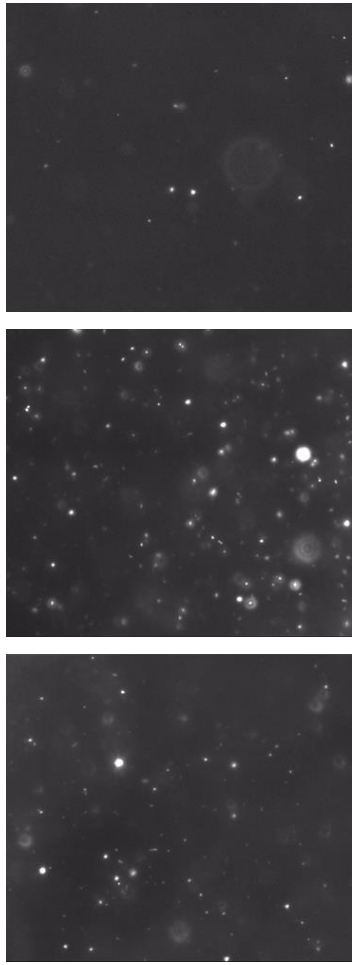
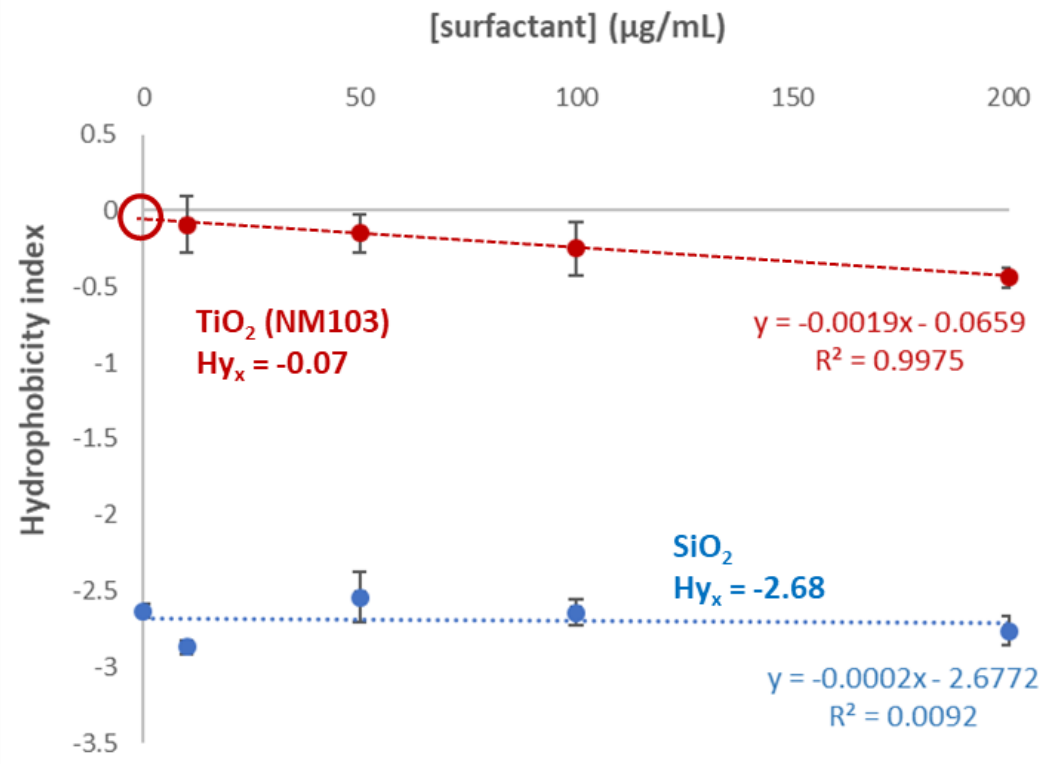
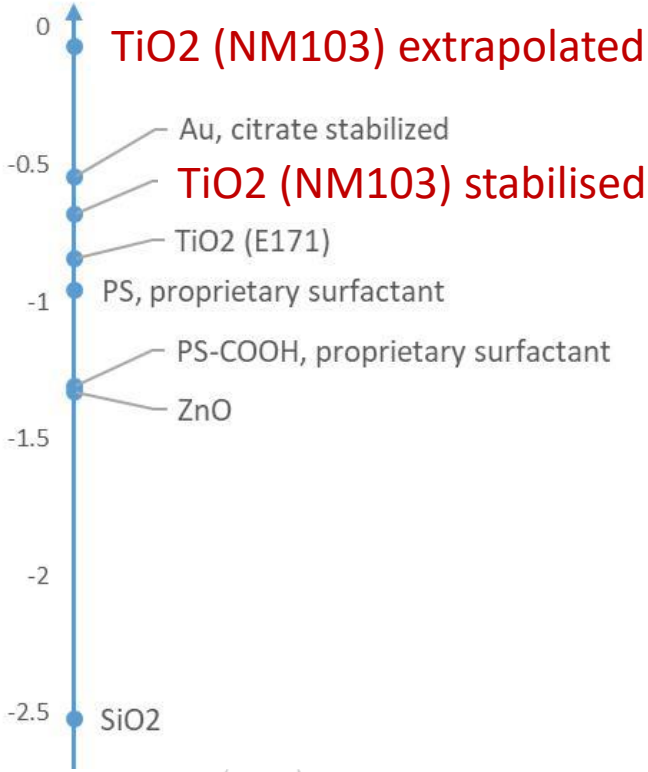
4. The test in practice

Dealing with poorly dispersible NM







➔ **Extrapolation to [surfactant] = 0**

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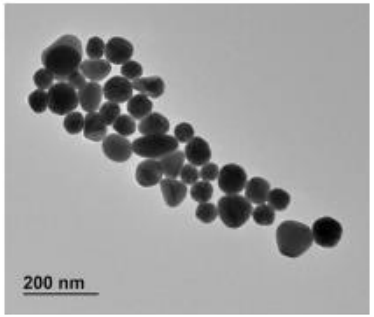
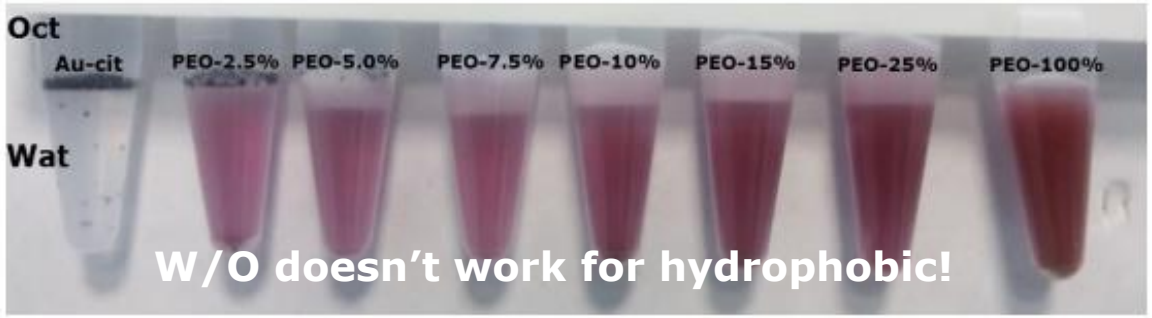
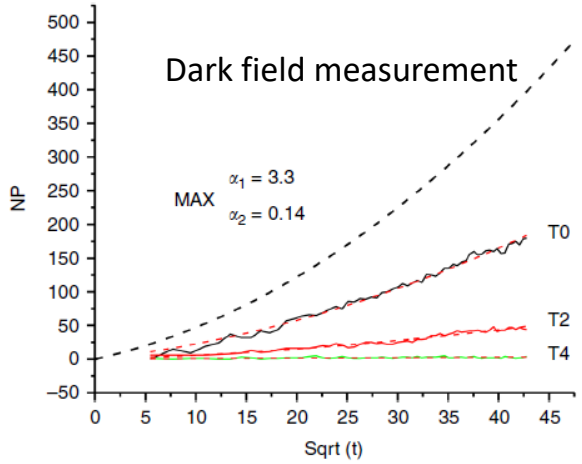
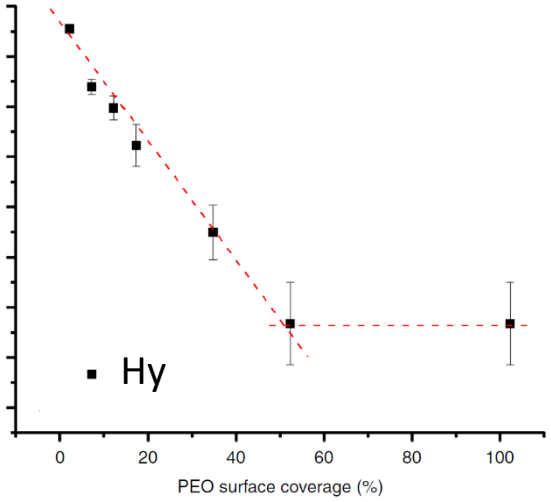
4. The test in practice

An example: PEO coated AuNP









Hy decreases with PEO coating

Valsesia et al. Communication Chemistry, 1, 53 (2018)

Outline

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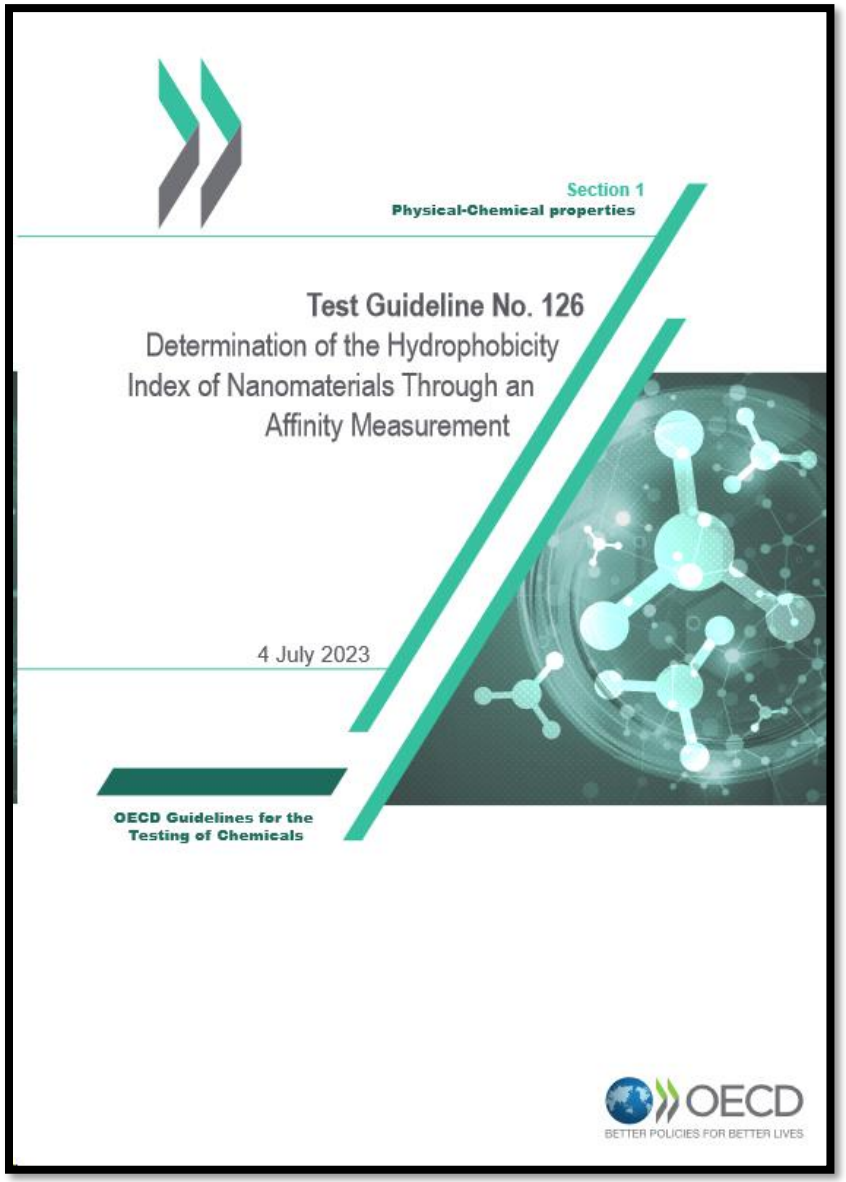
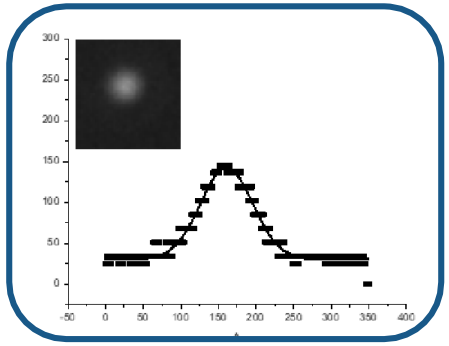
8. Q&A



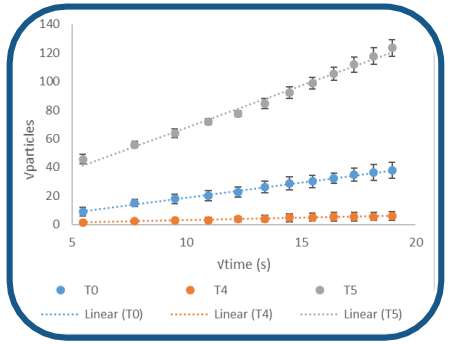
5. Inter-Laboratory Comparison

STEP 1



Detection of 1 control sample

STEP 2



Affinity measurement on 3 collectors

STEP 3

$Hy = Log (v_{T0} / v_{max})$

Increasing hydrophobicity

SiO_2 NM103

Hydrophobicity index determination

ILC: Assessment of
Transferability
Reproducibility
Robustness of the SOP

independently from the DF microscopy setup!

European Commission

5. Inter-Laboratory Comparison

9 Participants

	M1	M2	M3	M4	M5
LAB. 01	x			x	x
LAB. 02	x	x	x		
LAB. 03	x	x	x	x	x
LAB. 04	x			x	x
LAB. 05	x	x		x	
LAB. 06	x		x		x
LAB. 07	x	x	x		
LAB. 08	x			x	x
LAB. 09	x	x	x	x	x

Entities from US, France, Greece, Italy, Germany, Portugal, UK and JRC

3 different materials /laboratory:

M1 (control sample) /all participants

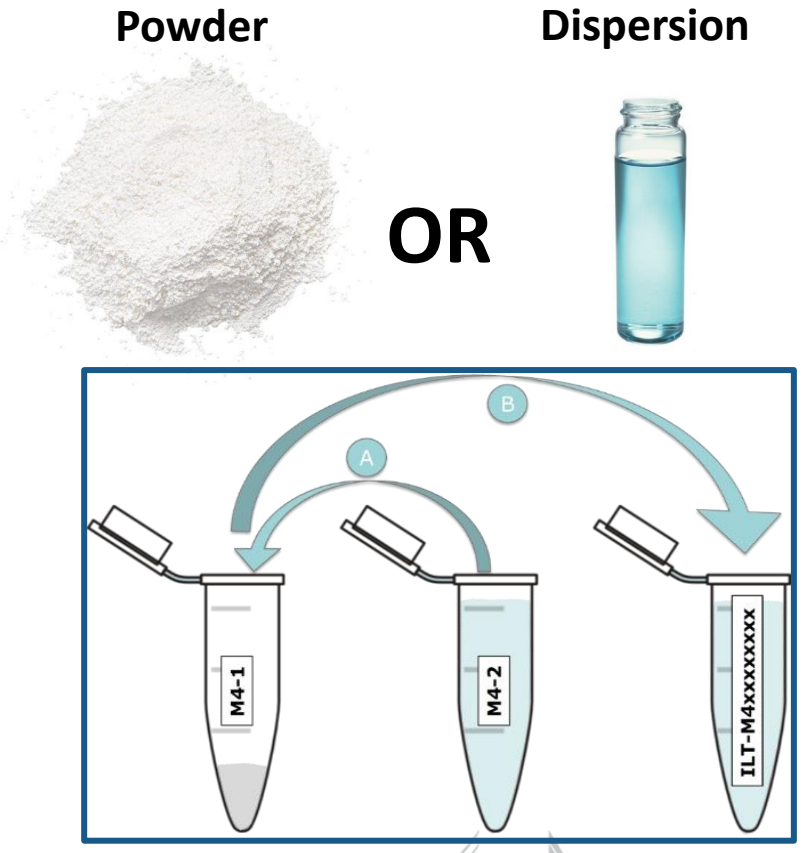
M2-M5 /minimum 5 laboratories

5. Inter-Laboratory Comparison

5 materials tested

SOP provided for dispersion

Sample	Type of material	Size (nm)	Source
M1	Polystyrene (hydrophobic)	500	Polysciences, Inc.
M2	Hydrophobic gold NPs in citrate	70	JRC chemistry lab.
M3	Carboxylate Polystyrene (slightly hydrophilic)	450	Polysciences, Inc.
M4	Hydrophobic TiO_2 Rutile	21 (primary size)	JRCNM62001a
M5	TiO_2 Anatase, E171	20-300	Farmalabor



5. Inter-Laboratory Comparison

TEST KIT containing

- 3 Collectors with 16 fluidic channels ready to use
- 3 material samples to each participant
- Calibration chip
- SOP and tools for data treatment

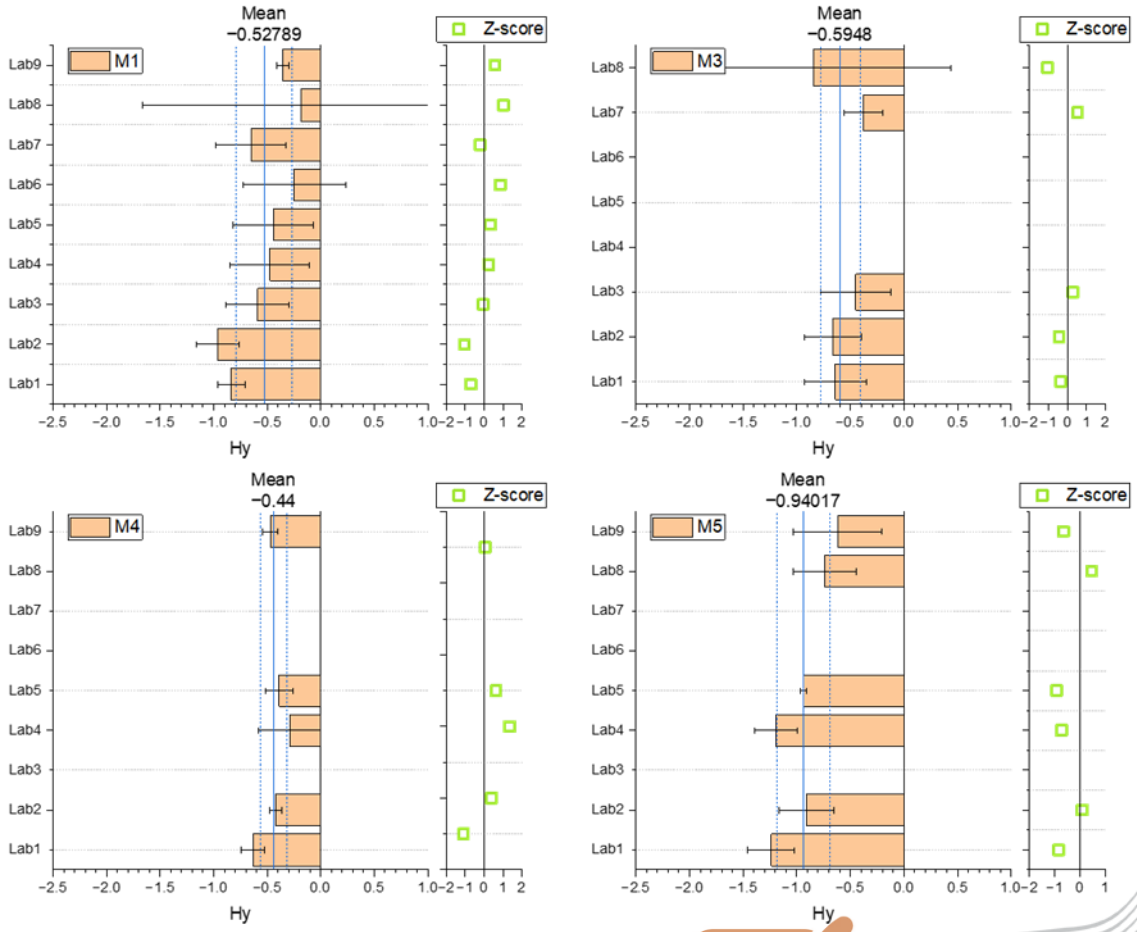
5. Inter-Laboratory Comparison

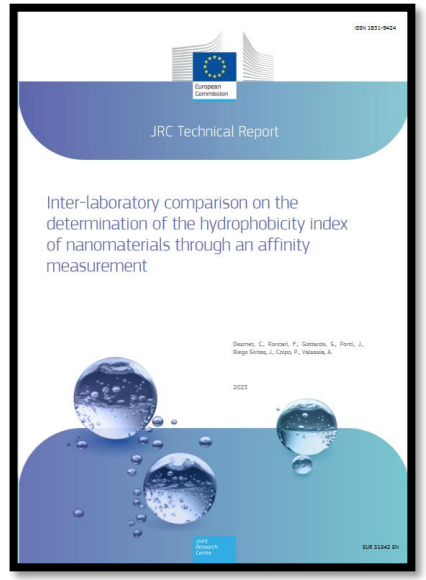
STEP 1

Detection of 1 control sample

STEP 2

Affinity measurement on 3 collectors





Statistical method: ISO 5725-2
Assessment: |Z|<2

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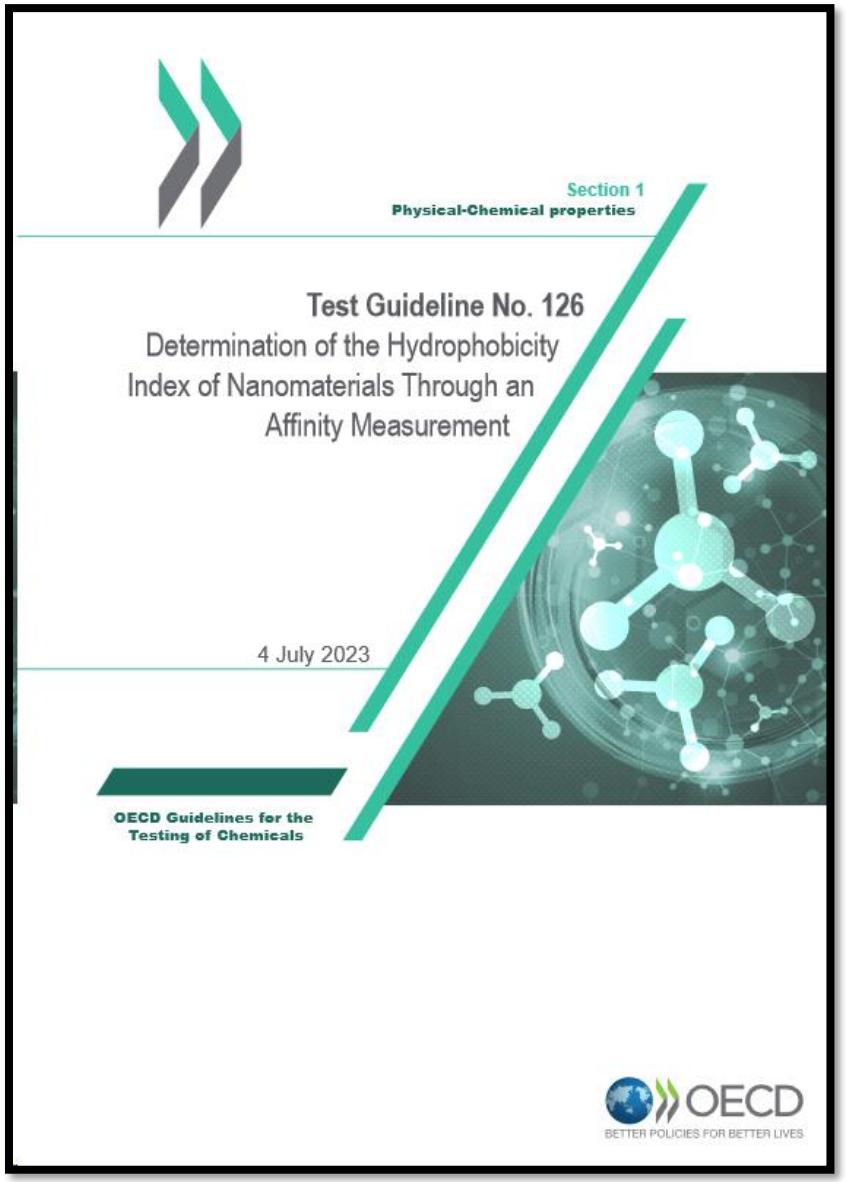


6. Method comparison

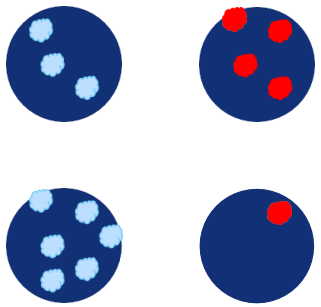
Dye Adsorption

Affinity with chemicals

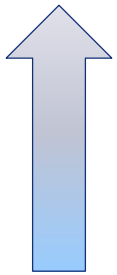
Sensitive to surface topography (roughness, porosity…)

Applicability: Transport of toxicant

Hydrophilic vs hydrophobic dye



Hydrophobic



Hydrophilic

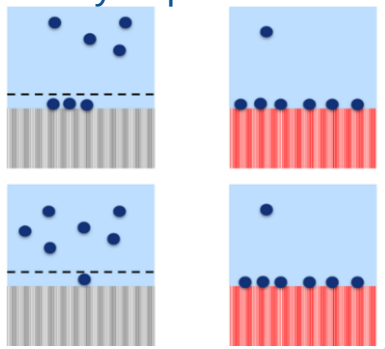
Hydrophobicity Index

Affinity for stationary surface

Less sensitive to surface

Applicability: biodistribution

Water vs hydrophobic surface



Different purposes, both potentially improving environmental fate models

Roncari *et al.*, Front. Nanotechnol 2023

6. Method comparison

Dye Adsorption

AgNPs	Corona	Pristine	Sulfidized
Pristine	No NOM	-0.005 ± 0.003	
Sulfidized	No NOM	0.038 ± 0.012	
	Corona		
Pristine	NOM	0.002 ± 0.12	-0.002 ± 0.007
Sulfidized	NOM	0.09 ± 0.11	0.13 ± 0.04
Pristine	LF-NOM	-0.001 ± 0.2	-0.001 ± 0.005
Sulfidized	LF-NOM	0.05 ± 0.03	0.064 ± 0.002
	Average	0.04 ± 0.08	0.05 ± 0.007

Reference+	
Silica-NH2	1.6
CuO	-1.52
Silica	-1.75

Hydrophobicity Index

		Pristine	Sulfidized
		-1.32 ± 0.07	
		-0.89 ± 0.04	
		Pristine	Sulfidized
Pristine	NOM	-1.17 ± 0.12	-0.32 ± 0.05
Sulfidized	NOM	-0.85 ± 0.11	-0.79 ± 0.06
Pristine	LF-NOM	-1.1 ± 0.2	-0.69 ± 0.11
Sulfidized	LF-NOM	-1.52 ± 0.03	-0.3 ± 0.2
	Average	-1.2 ± 0.2	-0.5 ± 0.2

Reference	
TiO2-h	-0.1
TiO2	-0.7
PS-h	-0.9
PS	-1.4
Silica	-2.6

Different purposes, both potentially improving environmental fate models

Roncari *et al.*, Front. Nanotechnol 2023

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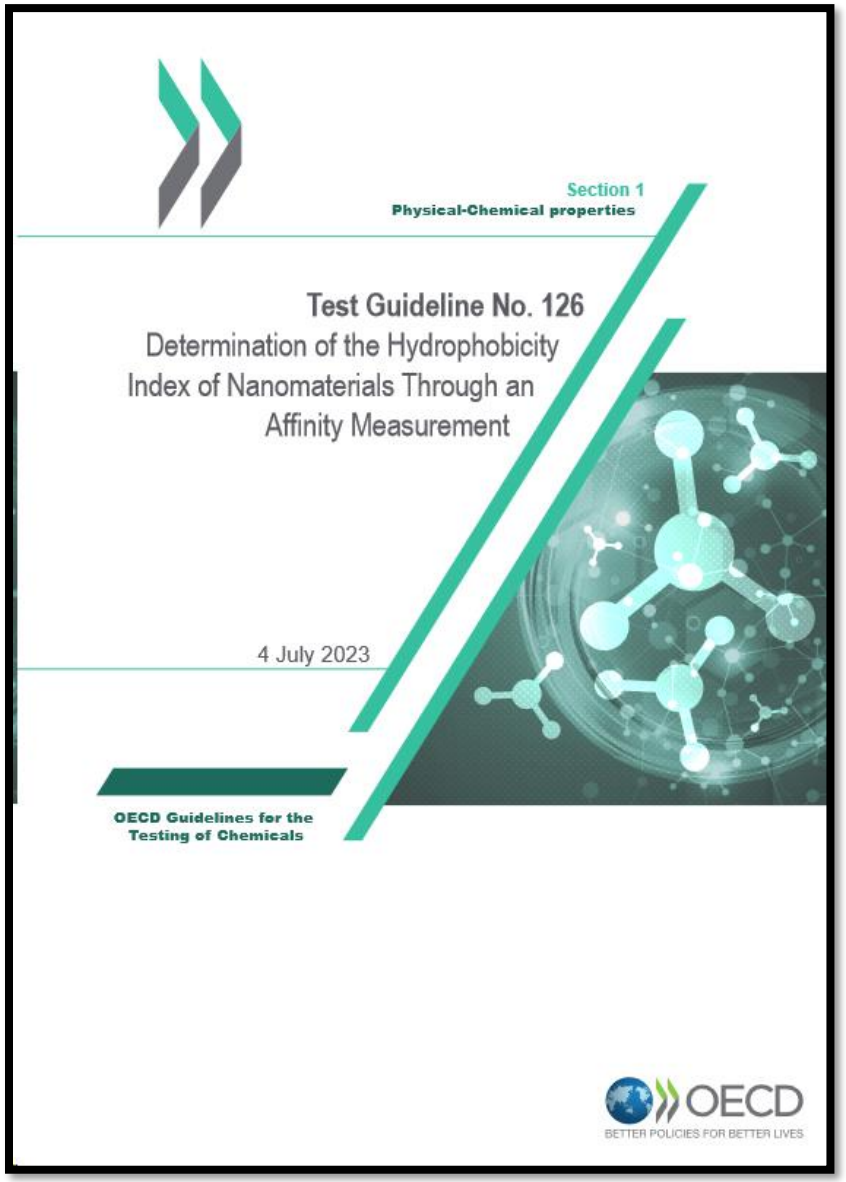
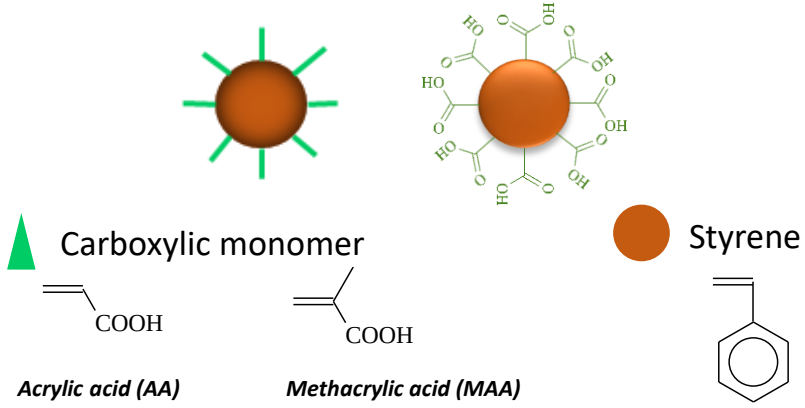


7. Potential uses

I. Fate of nanoplastics

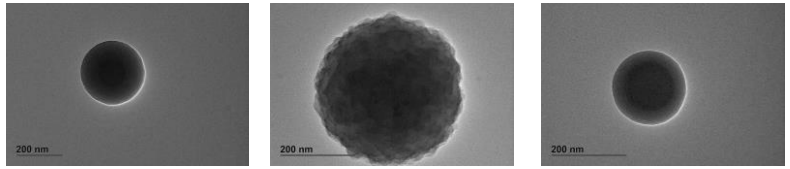
Hy as indicator for environmental fate?

- **Carboxylated polystyrene**



COOH molar content from 9 to 22%

	z-average (nm)	zêta (mV)		
PS9-AA	380	-36	±	5
PS13-AA	350	-26	±	2
PS18-AA	390	-21	±	3
PS22-AA	490	-20	±	3
PS22-MAA	216	-28	±	3
PS9-AA	274	-31	±	2
PS22-AA	333	-20	±	3
Grinded PS	424	-22	±	3

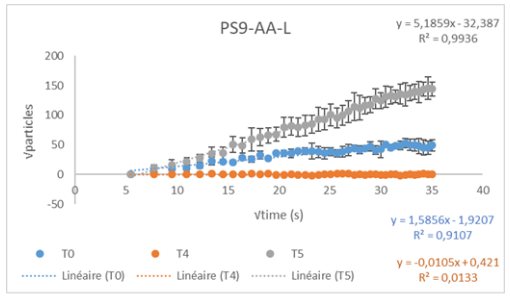


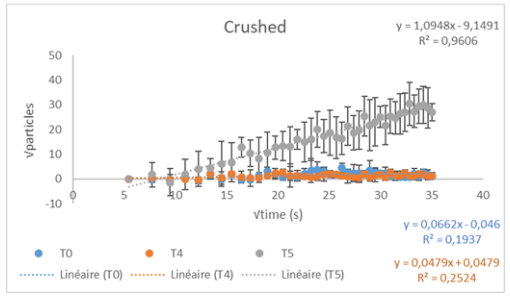
- **Primary plastics from grinded polystyrene**

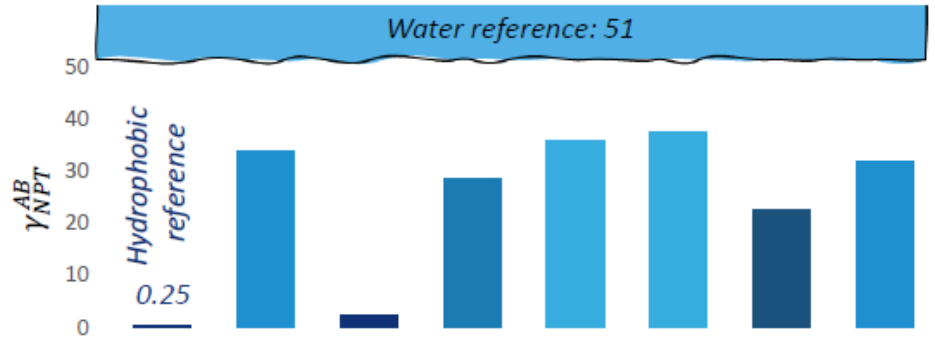
Pessoni L., *et al.* (2019), ES: Nano, 6(7)

7. Potential uses

I. Fate of nanoplastics







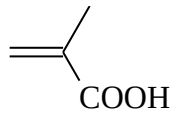
	Std PS	Std PS-COOH	PS9 -AA	PS13 -AA	PS18 -AA	PS22 -AA	PS22 -MAA	PS grinded
COOH (%)	N.A.	N.A.	9	13	18	23	22	N.A.
ζ average (nm)	500	200	274	350	390	333	216	424
ζ potential (mV)	-39 ± 2	-27 ± 2	-31 ± 2	-26 ± 2	-21 ± 3	-20 ± 3	-28 ± 3	-22 ± 3
CA (deg)	98	26	87	36	21	16	46	30

PS500 hydrophobic reference
PSL-COOH hydrophilic reference

Grinded polystyrene is hydrophilic

MAA moiety gives more hydrophobic character than AA

Methacrylic acid (MAA)



Acrylic acid (AA)

Increase in COOH content
➔ Decrease of hydrophobicity of the NM

Pradel et al. Chemosphere 2020, Veclin et al. ACS EST Water 2022

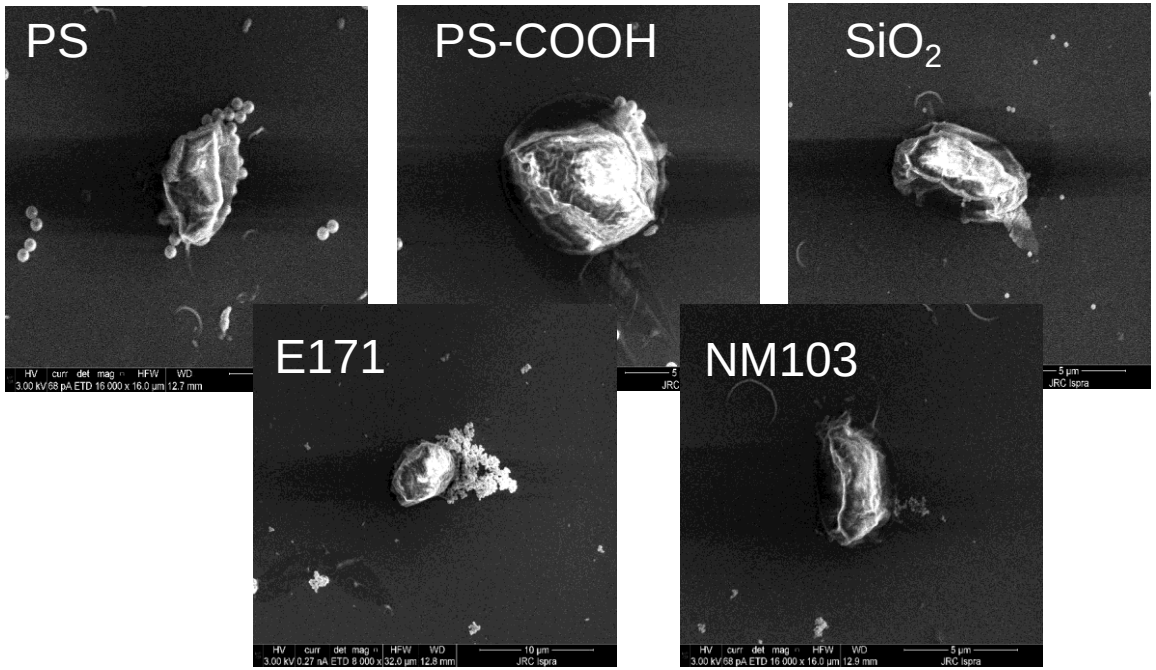
7. Potential uses

II. Bioaccumulation

Hy as indicator for attachment?

2 species of freshwater microalgae (DS and RS)

4 NMs of the ILC + SiO_2 (10^7 part/mL)

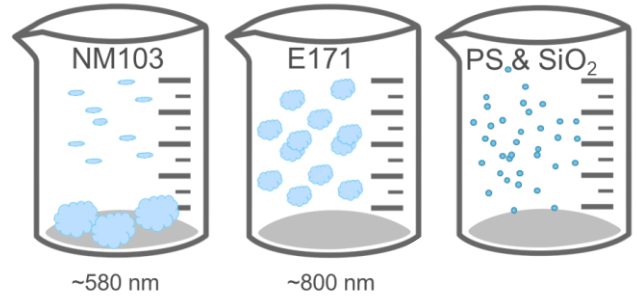


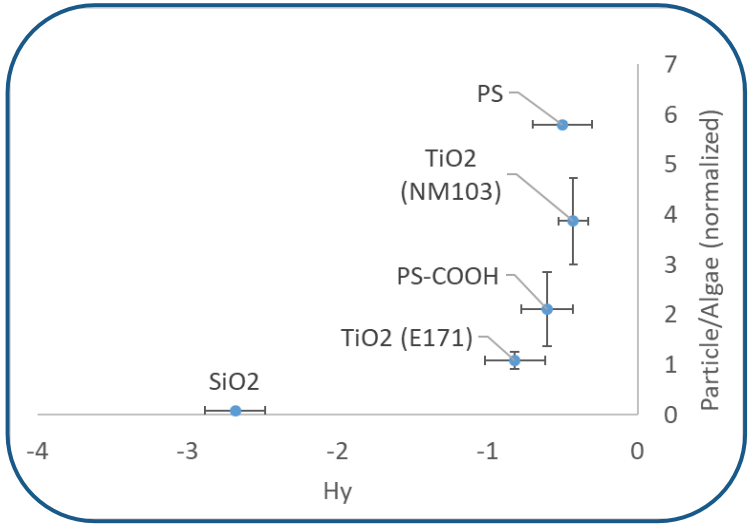
Av. area ctrl algae
Av. area algae + NPs
Av area NPs

➔Number of NPs / algae

+Concentration normalisation

Ongoing work





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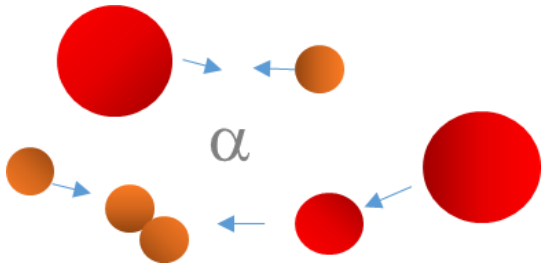
7. Potential uses

III. Heteroaggregation



Hy as a predictor of α?

Aggregation process between particles of different types

e.g. NMs + suspended particulate matter

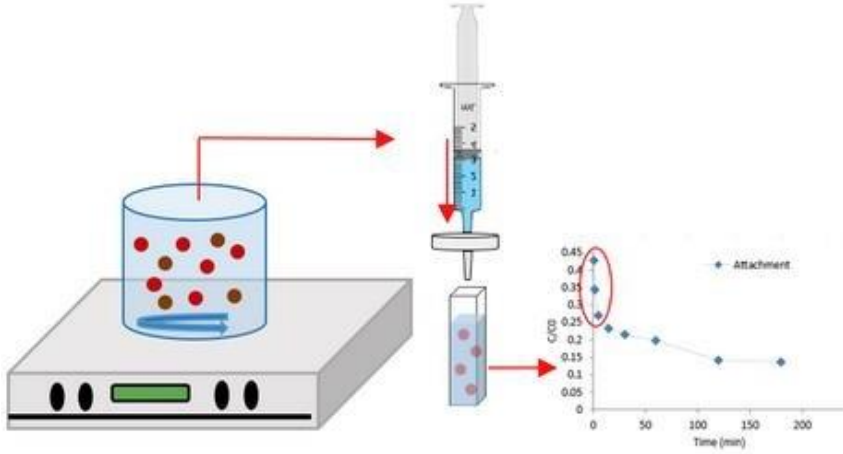
Probability of heteroaggregate formation: attachment efficiency (α)

➔ **Fate descriptor**

BUT no standard, long experimental procedure

Images from Nanofase.eu

Batch test:



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7. Potential uses

III. Heteroaggregation

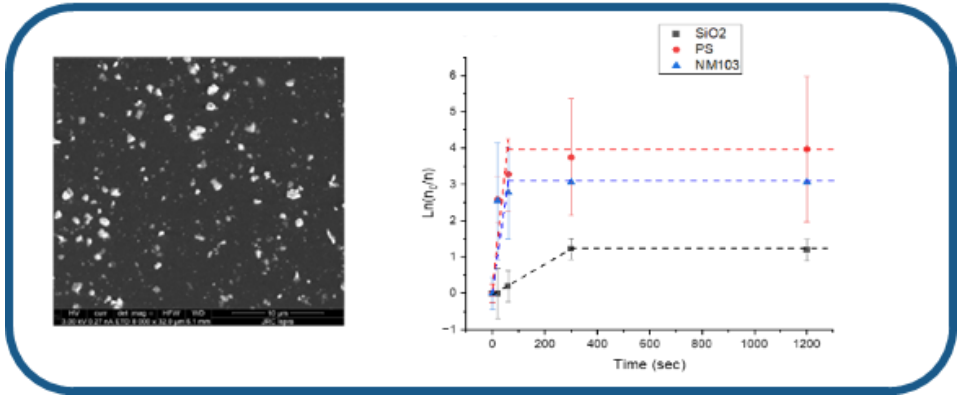
1. EU reference soil in std medium + NM

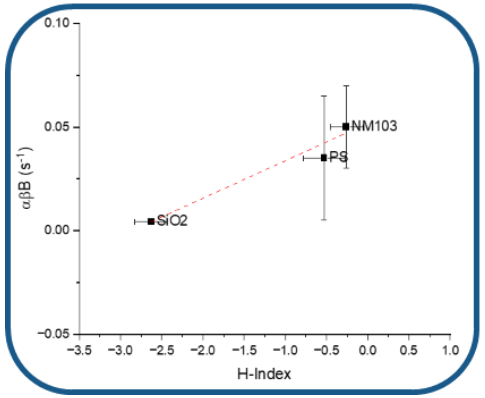
2. Filtration ➔ removal of the aggregates at different time points

(adapted from *Turner et al. Environmental Science: Nano 2020*)

3. Measurement of the concentration of the residual single particles (removal rate)

(Method *Valsesia et al. Microplastics and Nanoplastics 2021)*



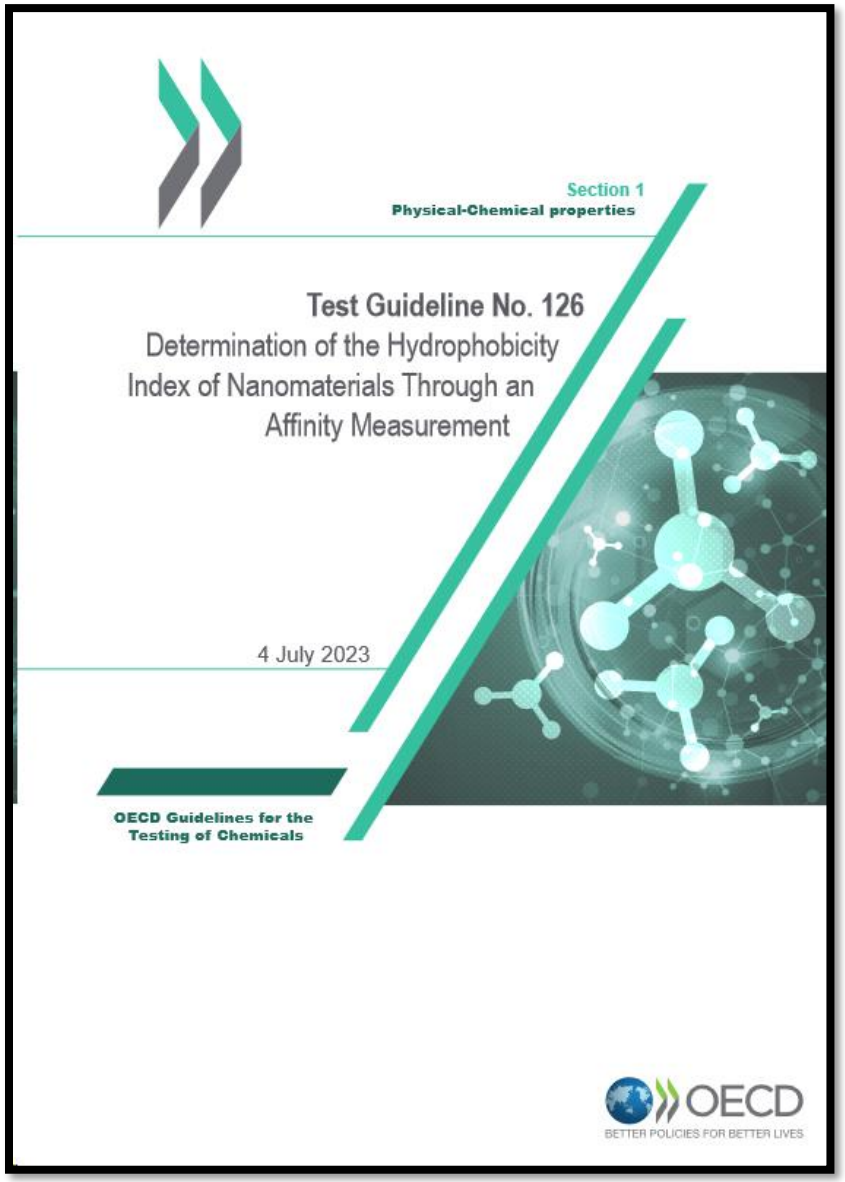


Ongoing work

European Commission

7. Potential uses

Generating data for further assessment



To know more…

Method

Desmet et al. J Nanopart Res. 2017, Valsesia et al. Commun Chem. 2018

JRC technical report JRC133937, **OECD Test Guideline No. 126**

Hydrophobicity of Nanoplastics

Pradel et al. Chemosphere 2020, Veclin et al. ACS EST Water 2022

Hydrophobicity of ZnO and GaN NPs

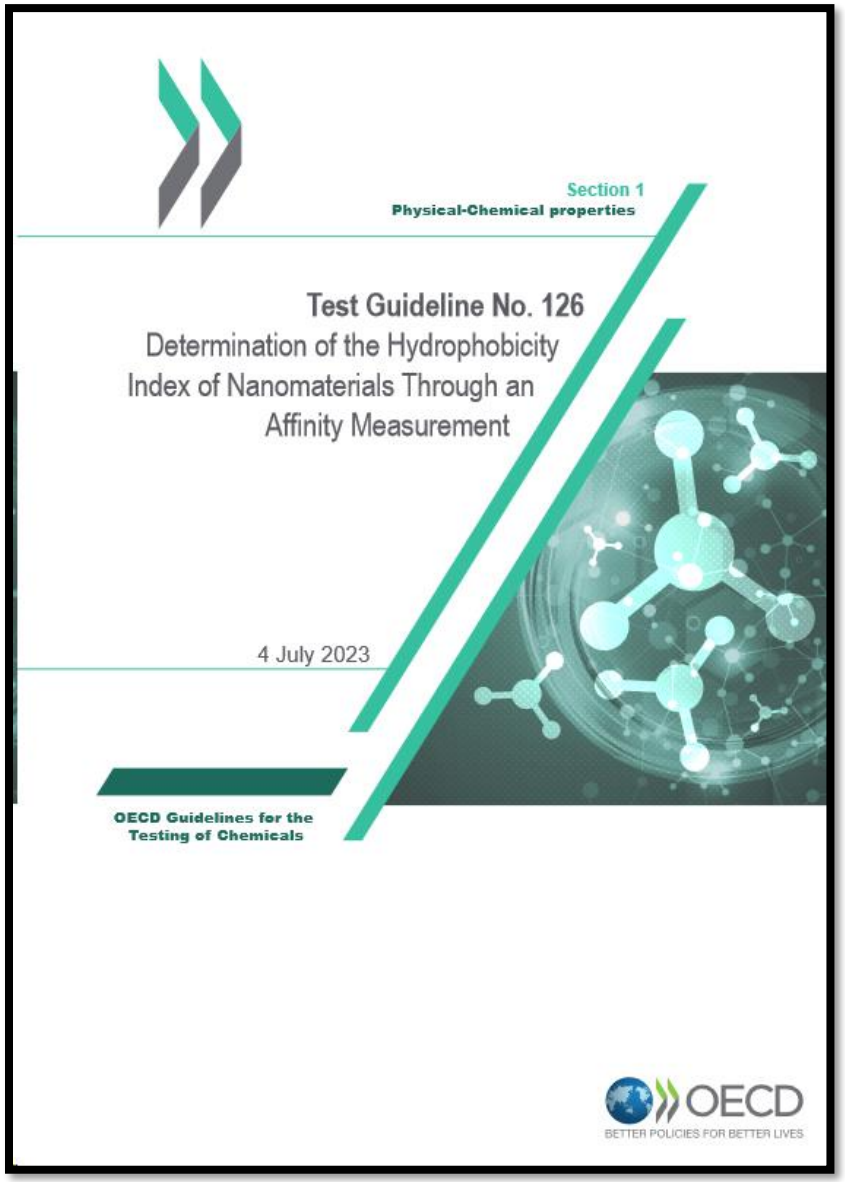
Ciobanu et al. J Appl Biomater Funct Mater. 2022

Comparison with Dye adsorption on PVP-AgNPs

Roncari et al. Front. Nanotechnol. 2023

Correlation with bioaccumulation

Correlation with heteroaggregation ongoing



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- University of Birmingham, UK, Eugenia Valsami-Jones and Nathan Langford
- Foundation for Research and Technology Hellas, Greece, Emmanuel Stratakis and Kostantinos Brintakis

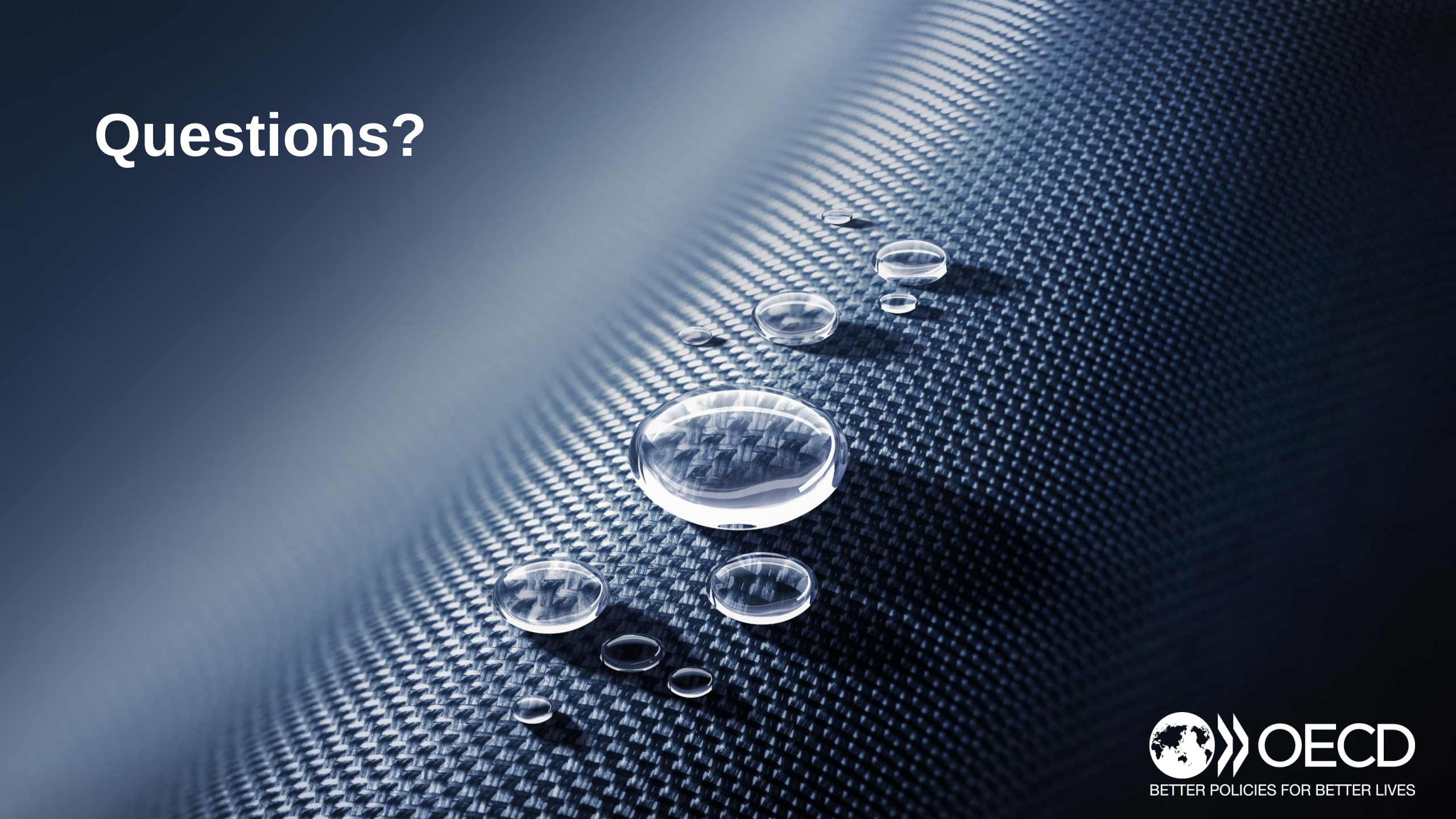
All ILC participants

All collaborations

The OECD National Experts & National Coordinators

OECD Secretariat Mar Gonzalez, Lesley Smith

Questions?



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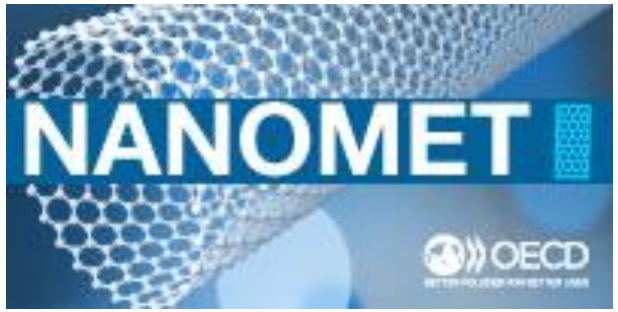

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