



*Subject*  
**Assessment of NMI Report Data Against  
NICNAS Nanomaterial Definition**

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**Title**

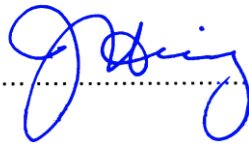
**Assessment of NMI Report Data Against NICNAS Nanomaterial Definition**

**Declaration**

This report/proposal has been prepared in accordance with UniQuest's Quality Management System, which is compliant with AS/NZS ISO 9000:2000.

Signed for and on behalf of UniQuest Pty Limited

**Signed for and on behalf of UniQuest Pty Limited**



**Mr Jim Haig**

**(Manager AMCP)**

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## 1. INTRODUCTION

My opinion has been sought on the assessment of certain particles against the 'working definition of industrial nanomaterial' criteria set by the National Industrial Chemicals Notification and Assessment Scheme – NICNAS. NICNAS published a guidance note on this topic and this has been attached as Appendix A.

Dr Gregory Crocetti of Friends of the Earth Australia provided the following reports:

“DSC measurements and Representative TEM Images of a Sub-set of Sunscreen Samples” RN 123217 issued 26 November 2012 by the Nanometrology Group of the National Measurement Institute.

“DSC measurements and Representative TEM Images of Sunscreen Samples” RN122949 issued 26 November 2012 by the Nanometrology Group of the National Measurement Institute.

“XRD Phase Analysis of TiO<sub>2</sub> Sunscreens” RN122439 issued 23 October 2012 by the Nanometrology Group of the National Measurement Institute.

In addition a series of TEM photomicrographs as high resolution digital tiff images were supplied for each sample – these series having been produced as part of the first two reports above.

In addition to the NICNAS guidance note I, like NICNAS, found the following references helpful in assisting the formation of my opinion:

- ‘Guidance manual for the testing of manufactured nanomaterials: OECD’s sponsorship programme’ ; First Revision, ENV/JM/MONO(2009)20/REV. In: OECD Environment, Health and Safety Publication, Series on the safety of manufactured nanomaterials. OECD Paris, Organisation for Economic Co-operation and Development, No. 25, 92 pp.
- “Requirements on measurements the European Commission definition of the term “nanomaterial” for the implementation of Report EUR 25404 EN” Linsinger T., Roebben G., Gilliland D., Calzolari L., Rossi F., Gibson N., Klein C. European Commission Joint Research Centre, Institute for Reference Materials and Measurements 2012
- ISO-TR\_13014-2012 Nanotechnologies — Guidance on physico-chemical characterization of engineered nanoscale materials for toxicologic assessment

This report seeks to provide an opinion based on clearly defined criteria about certain materials prepared and examined by others. It does not provide further characterisation of the materials and I rely on my experience as an optical and electron microscopist of over 35 years experience.

## 2. NICNAS CRITERIA

The following NICNAS definition served as the criteria against which I assessed the images and data provided to me:

### **NICNAS WORKING DEFINITION OF INDUSTRIAL NANOMATERIAL**

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

[Notes to the working definition:

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

NICNAS goes on to define certain terms:

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

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

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

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

Definitions for some of the terms used in the above NICNAS guide were also found in ISO-TR 13014 :

### **2 Terms and definitions**

For the purposes of this document, the terms and definitions given in ISO/TS 27687, ISO/TS 80004-1, ISO/TS 80004-3, ISO/IEC Guide 99 and the following apply.

## 2.1

### **aggregate**

particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components

NOTE 1 The forces holding an aggregate together are strong forces, for example covalent bonds, or those resulting from sintering or complex physical entanglement.

NOTE 2 Aggregates are also termed “secondary particles” and the original source particles are termed “primary particles” .

[ISO/TS 27687:2008, definition 3.3]

## 2.2

### **agglomerate**

collection of weakly bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components

NOTE 1 The forces holding an agglomerate together are weak forces, for example van der Waals forces, or simple physical entanglement.

NOTE 2 Agglomerates are also termed “secondary particles” and the original source particles are termed “primary particles” .

[ISO/TS 27687:2008, definition 3.2]

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## 2.16

### **nanomaterial**

material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale

NOTE 1 This generic term is inclusive of nano-object and nanostructured material.

NOTE 2 Adapted from ISO/TS 80004-1.

## 2.17

### **nano-object**

material with one, two or three external dimensions in the nanoscale

NOTE Generic term for all discrete nanoscale objects.

[ISO/TS 80004-1:2010, definition 2.5]

## 2.18

### **nanoparticle**

nano-object with all three external dimensions at the nanoscale

NOTE If the lengths of the longest to the shortest axes of the nano-object differ significantly (typically by more than three times), the terms “nanofibre” or “nanoplate” are intended to be used instead of the term “nanoparticle” .

[ISO/TS 27687:2008, definition 4.1]

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## 2.20

### **nanoscale**

size range from approximately 1 nm to 100 nm

NOTE 1 Properties that are not extrapolations from a larger size will typically, but not exclusively, be exhibited in this size range. For such properties the size limits are considered approximate.

NOTE 2 The lower limit in this definition (approximately 1 nm) is introduced to avoid single and small groups of atoms from being designated as nano-objects or elements of nanostructures, which might be implied by the absence of a lower limit.

[ISO/TS 80004-1:2010, definition 2.1]

## 3. **SAMPLES, IMAGES & DATA**

Appendix B presents the images used in the formation of my opinion. The images presented here are meant to be illustrative only; my assessment was made with the original high definition tiffs on-screen with zooming as necessary. A 100 nm circle has been placed upon each image to show the upper limit defined in almost all definitions of nanomaterial. In my assessment I was able to move that circle around the image to make ad hoc decisions on the compliance of any particle or structure with the 100 nm limit.

### **Limitations to Opinion**

The National Measurement Institute reports presented in detail the method of sample pre-treatment used for the samples, both aqueous and non-aqueous (organic). The dispersion, ultrasonication and phase separation procedures are consistent with EU methodologies and will not cause reduction in primary particle size.

The NMI reports do warn, however, about the potential limitations of this pre-treatment:

#### *4. Experimental details*

*To produce sample material suitable for analysis, it was necessary to use a washing procedure to separate the particles from the formulation. In performing the washing procedure, the aggregation/agglomeration state of the particles in the formulation may be changed. Also, particles may be*

*incompletely separated from the formulation, or different particle sub-populations may be present in different liquid phases. i.e., the aqueous or organic phases. Thus the size distributions presented in this report are not necessarily based on the entire particle content of the samples and therefore cannot be used to determine the proportion of particles with sizes below a particular value, such as 100 nm, that may be present in the sunscreen formulation.*

In applying the NICNAS criteria the relative amounts of free primary particles, aggregations and agglomerations is immaterial. In considering these latter forms only the size of the primary particles making them up is considered.

Concern regarding incomplete separation from the formulation or differing populations of particle sizes between separated phases does need consideration. In performing the assessment to NICNAS criteria only 10% of the primary particles need to comply with the size criterion for a finding of nanomaterial. Furthermore, this criterion on quantity is defined by number of particles, not weight or volume. In my opinion the pre-treatment adopted by NMI will not bias the size distribution to any great extent. Should the resulting assessment consider that nanoparticles comprise somewhere between a few percent and say 30% by number, such considerations might warrant further investigation as in my opinion bias might be sufficient, if taken at its worst, to impact upon the final assessment. This further investigation might take the form of varying the procedure, solvents used, temperature, etc and see what effect there has been on the estimate of numbers.

However, if a significant bias were to be present one might expect to see a significant skew in the particle size distribution produced by an appropriate technique. NMI did perform Differential Centrifugal Sedimentation (DCS) on five of the seven samples. While DCS does suffer to some extent from bias caused by aggregation/agglomeration and is therefore not a robust method for absolute sizing, the production of a distribution significantly skewed towards the high end can suggest a problem in the pre-treatment. In this regard I have considered the DCS results and have used them in establishing a level of assurance in my opinion.



### **M122949\_001**

TEM shows ample evidence of agglomeration but primary particles are readily discernible. A small percentage by number, %(n), of large particles were seen. In some cases these larger particles are clearly aggregates formed by twinning of crystals as the adjacent particles have developed out from a crystal face then this has been repeated several times in some instances. However, the agglomerated primary particles show a random orientation indicating they are poorly bonded - in some cases residual vehicle appears to be present. An informal numbers count suggests >>90%(n) of the particles are <100 nm. DCS supports this assessment. It found that >95% of the particles were < 100 nm and there is no evidence of distribution skewing.

### **M122949\_002**

TEM shows weak evidence of agglomeration and primary particles are readily discernible. A trace percentage by number, %(n), of large particles were seen - only 3 or 4 in 10 fields compared to many thousands of small primary particles. Any agglomerated primary particles show a random orientation indicating they are poorly bonded . An informal numbers count suggests >>99%(n) of the particles are <100 nm. DCS supports this assessment. It found that 100% of the particles were < 100 nm and there is no evidence of distribution skewing.

### **M122949\_003**

TEM shows ample evidence of agglomeration and aggregation and primary particles are discernible only occasionally. A significant percentage by number, %(n), of large particles were seen. In some cases these larger particles are clearly aggregates formed by twinning of crystals as the adjacent particles have developed out from a crystal face then this has been repeated several times in some instances. However, agglomerated primary particles show a random orientation indicating they are poorly bonded - in some cases residual vehicle appears to be present. An informal numbers count showed a wide variation in %(n) over the 10 fields supplied as photomicrographs as these notes show:

| Field | Ratio [<100] : [>100] |
|-------|-----------------------|
| 1     | 50:50                 |
| 2     | 70:30                 |
| 3     | 25:75                 |
| 4     | 25:75                 |
| 5     | 90:10                 |
| 6     | 50:50                 |
| 7     | 15:85                 |
| 8     | 60:40                 |
| 9     | 80:20                 |
| 10    | 90:10                 |

My estimate is that there are about 50%(n) sub 100 nm particles. DCS was not performed on this sample.

#### **M122949\_004**

TEM shows evidence of regular but small agglomerates and primary particles are readily discernible. A trace percentage by number, %(n), of large particles were seen - only 2-3 in 10 fields compared to many thousands of small primary particles. Any agglomerated primary particles show a random orientation indicating they are poorly bonded . An informal numbers count suggests >95%(n) of the particles are <100 nm. DCS supports this assessment. It found a similar figure and there is no evidence of distribution skewing.

#### **M122949\_005**

TEM shows ample evidence of agglomeration and aggregation and primary particles are discernible only occasionally. A significant percentage by number, %(n), of large particles were seen. In some cases these larger particles are clearly aggregates formed by twinning of crystals as the adjacent particles have developed out from a crystal face then this has been repeated several times in some instances. However, agglomerated primary particles show a random orientation indicating they are poorly bonded - in some cases residual vehicle appears to be present. An informal numbers count showed a wide variation in %(n) over the 10 fields supplied as photomicrographs as these notes show:

| Field | Ratio [<100] : [>100] |
|-------|-----------------------|
| 1     | 95:5                  |
| 2     | 50:50                 |
| 3     | 80:20                 |
| 4     | 70:30                 |
| 5     | 30:70                 |
| 6     | 50:50                 |
| 7     | 70:30                 |
| 8     | 70:30                 |
| 9     | 50:50                 |
| 10    | 90:10                 |

My estimate is that there are about 70%(n) sub 100 nm particles. DCS was not performed on this sample.

#### **M122949\_006**

TEM shows evidence of regular but small agglomerates and primary particles are readily discernible. A trace percentage by number, %(n), of large particles were seen - only 1-2 in 10 fields compared to many thousands of small primary particles. Any agglomerated primary particles show a random orientation indicating they are poorly bonded . An informal numbers count suggests >>95%(n) of the particles are <100 nm. DCS supports this assessment. It found that 100% of the particles were < 100 nm and there is no evidence of distribution skewing.

#### **M122949\_007**

TEM shows weak evidence of agglomeration and primary particles are readily discernible. A minor percentage by number, %(n), of large particles were seen - only 20-30 in 10 fields compared to many thousands of small primary particles. Any agglomerated primary particles show a random orientation indicating they are poorly bonded . An informal numbers count suggests >>95%(n) of the particles are <100 nm. DCS supports this assessment. It found that 100% of the particles were < 100 nm and there is no evidence of distribution skewing.

#### 4. CONCLUSION

Examination of TEM photomicrographs produced by NMI has been performed and assessment made against the NICNAS working definition for a working material.

Caveats placed by NMI upon their results have been considered and, when placed in perspective against the very large numbers of nanoparticles observed, I consider the caveats to be very conservative. I was able to verify that the techniques and procedures employed and described in their report were appropriate, correctly calibrated and quality assured.

The following samples are unequivocally nanomaterials when assessed against the NICNAS criteria:

M122949\_001  
M122949\_002  
M122949\_004  
M122949\_006  
M122949\_007

These samples have %(n) nanoparticles well in excess of 90% of the particles examined by TEM. This result was supported by NMI's DCS results. Any bias introduced during sample preparation would be insignificant in comparison to the assessments

The following samples are unequivocally nanomaterials when assessed against the NICNAS criteria:

M122949\_003  
M122949\_005

These samples have %(n) nanoparticles in the range 50-65% of the particles examined by TEM. No DCS results were presented in the NMI reports. Any bias introduced during sample preparation would have to be gross to affect the finding. The subjective particle size distribution of the >100 nm particles is relatively tight and does not suggest there is a significant population of large particles sequestered during the preparation. This is a very conservative caveat and I have no evidence at all to suggest that these two samples are not nanomaterials under the NICNAS criteria.

**5. APPENDIX A - EXTRACT FROM NICNAS WORKING DEFINITION USED**

## Guidance for Notifiers Handbook

### REQUIREMENTS FOR NOTIFICATION OF NEW INDUSTRIAL NANOMATERIALS

## **GUIDANCE ON NEW CHEMICAL REQUIREMENTS FOR NOTIFICATION OF INDUSTRIAL NANOMATERIALS**

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

### **NICNAS WORKING DEFINITION<sup>1</sup> OF INDUSTRIAL NANOMATERIAL**

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

[Notes to the working definition:

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

### **EXEMPTION CATEGORIES**

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

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

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

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

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

<sup>1</sup> NICNAS will actively monitor progress of national and international reviews and other scientific developments and regularly re-assess this working definition.

### **PERMIT CATEGORIES**

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

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

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

## CERTIFICATE CATEGORIES

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

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

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

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

## SPECIFIED CONDITIONS FOR REQUESTING ADDITIONAL DATA REQUIREMENTS

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

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

AND

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

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

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

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

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

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

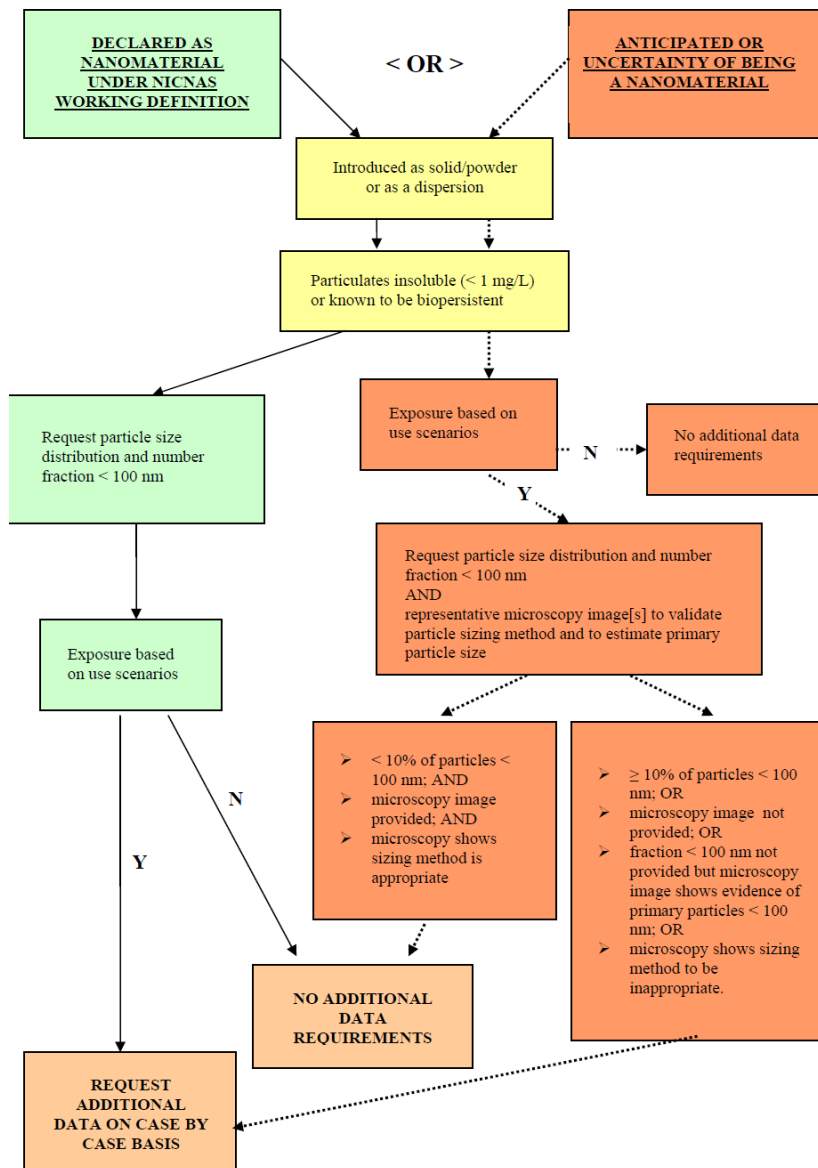
In addition to the particle size information, the following additional data, above that which is normally required for the notification category may also be requested (where applicable) under certain circumstances (see Flow Chart). Specific guidance on physico-chemical characteristics and toxicity testing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

- method of production

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

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

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



**GUIDANCE ON PROVISION OF ADDITIONAL DATA REQUIREMENTS**



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

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

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

In general, all physico-chemical data should specify:

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

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

<sup>2</sup> ISO (2010) Nanotechnologies – Guidance on physico-chemical characterisation for manufactured nano-objects submitted for toxicological testing, ISO/PDTR 13014. The International Organisation for Standardisation,

[http://www.iso.org/iso/iso\\_catalogue/catalogue\\_tc/catalogue\\_tc\\_browse.htm?commid=381983&development=on](http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_tc_browse.htm?commid=381983&development=on), Accessed 17th November 2010.

<sup>3</sup> OECD (2009a) Guidance manual for the testing of manufactured nanomaterials: OECD's sponsorship programme; First Revision, ENV/JM/MONO(2009)20/REV. In: OECD Environment, Health and Safety Publication, Series on the safety of manufactured nanomaterials. OECD Paris, Organisation for Economic Co-operation and Development, No. 25, 92 pp.

<[http://www.oecd.org/document/53/0,3343,en\\_2649\\_37015404\\_37760309\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/53/0,3343,en_2649_37015404_37760309_1_1_1_1,00.html)>, Accessed 17th November 2010.

<sup>4</sup> OECD (2009b) Preliminary review of OECD test guidelines for their applicability to manufactured nanomaterials. In: OECD Environment, Health and Safety Publication, Series on the safety of manufactured nanomaterials, No. 15, ENV/JM/MONO(2009)21. OECD Paris, Organisation for Economic Co-operation and Development, 71 pp.

<[http://www.oecd.org/document/53/0,3343,en\\_2649\\_37015404\\_37760309\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/53/0,3343,en_2649_37015404_37760309_1_1_1_1,00.html)>, Accessed 17<sup>th</sup> November 2010.

*Note: The OECD Working Party on Manufactured Nanomaterials (WPMN) reviewed all 22 OECD test guidelines for physical-chemical properties for their applicability to the testing of nanomaterials<sup>4</sup>. The review concluded that all but two of the current tests may provide information that is applicable to nanomaterials. The two tests not considered to provide useful information are TG 103 Boiling Point and TG 114 Viscosity of Liquids. It was also recognised that some tests would only be applicable to a sub-set of nanomaterials depending on their physical form and chemical composition. For example, it was concluded that the three test guidelines for physical-chemical properties of polymers (OECD TGs 118–120) would only be applicable to polymeric manufactured nanomaterials. The key physical-chemical properties that require characterisation when considering aquatic environmental exposure of chemicals are water solubility, water-soil and water-oil partitioning, hydrolysis and dissociation constants. All of the standard test guidelines for these properties are considered to be potentially applicable to nanomaterials. However, it is noted that the applicability will depend in part on the presence of colloidal dispersions of nanomaterials in water which may complicate both the conduct and/or the interpretation of studies.*

*(i) Particle size and size distribution*

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

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

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

*Fibre-like nanomaterials*

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

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

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

*(ii) Method of production*

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

*(iii) Shape*

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

Recommended test methods: SEM and TEM.

*(iv) Agglomeration/aggregation state*

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

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

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

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

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

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

Recommended test methods: SEM, TEM and DLS.

#### (v) Crystalline phase

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

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

#### (vi) Composition (purity/impurities)

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

Recommended test methods:

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

#### (vii) Surface area

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

Recommended test method: BET gas-absorption method.

#### (viii) Surface charge

Due to their extremely high specific surface area, aqueous dispersions of nanoparticles can easily lose their colloidal stability as a result of changes in the chemistry of the dispersion medium (e.g., ionic strength, pH, level of dissolved organic carbon). Agitation conditions and changes in concentration of the particles can also lead to agglomeration/aggregation. An important predictor of

colloidal stability is the surface charge of particles. The surface charge is usually characterised by measurements of the zeta potential. The measurement of this electrokinetic parameter over a wide range of pH and ionic strengths in water can provide valuable information regarding the tendency of particle size and size distribution to change with time and solution chemistry.

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

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

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

(ix) Surface chemistry (e.g. coating or modification)

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

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

#### GUIDANCE ON TESTING HEALTH EFFECTS OF NANOMATERIALS

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

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

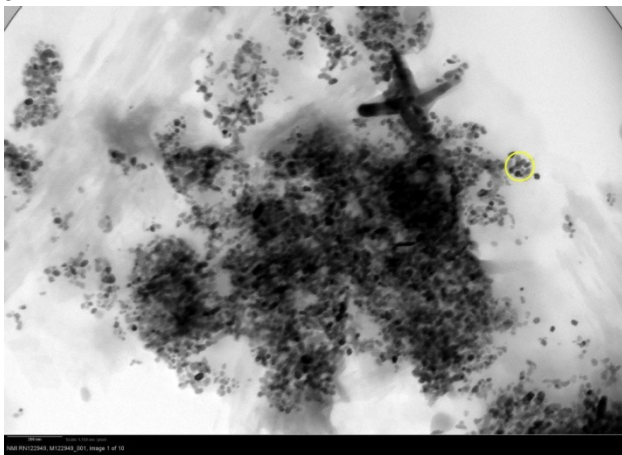
| Summary of preliminary review of OECD test guidelines for their applicability to manufactured nanomaterials OECD Test Guideline | Test  | Comments  |
|---|---|---|
| 417   | Toxicokinetics (Administration-Distribution-Metabolism-Excretion) | <p>Guideline gives only very general guidance. Although this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

## **6. APPENDIX B - IMAGES**

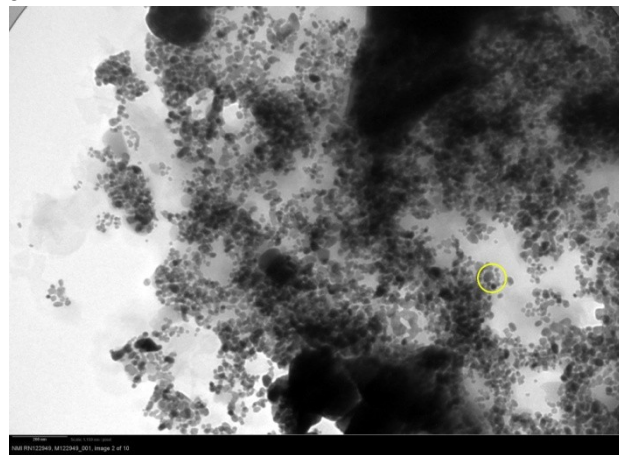


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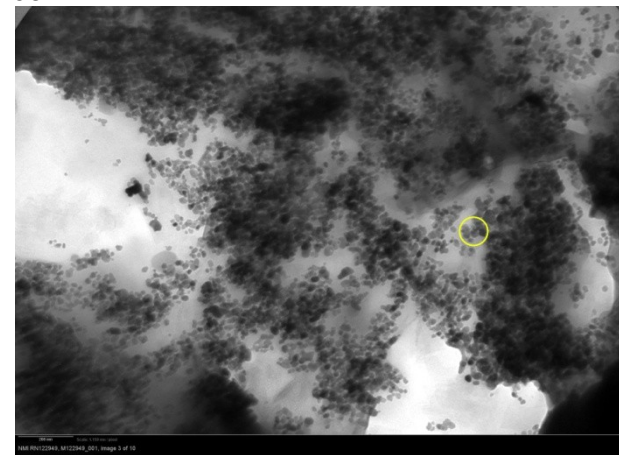
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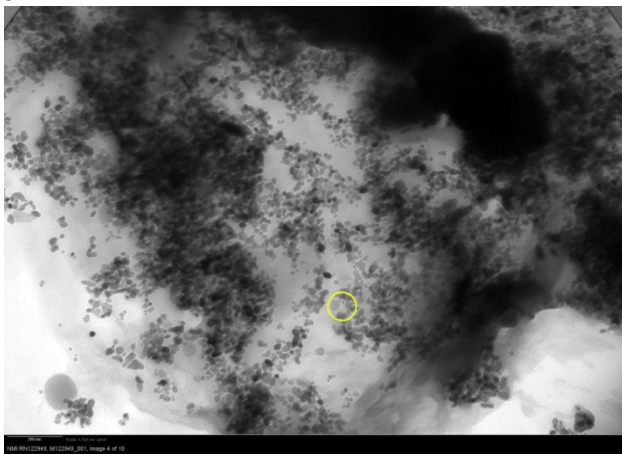
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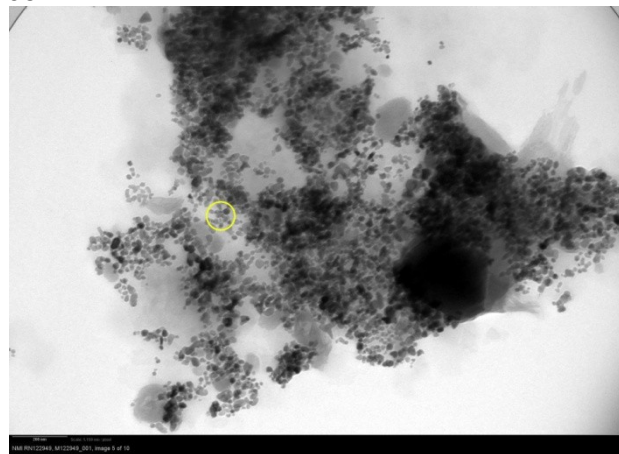
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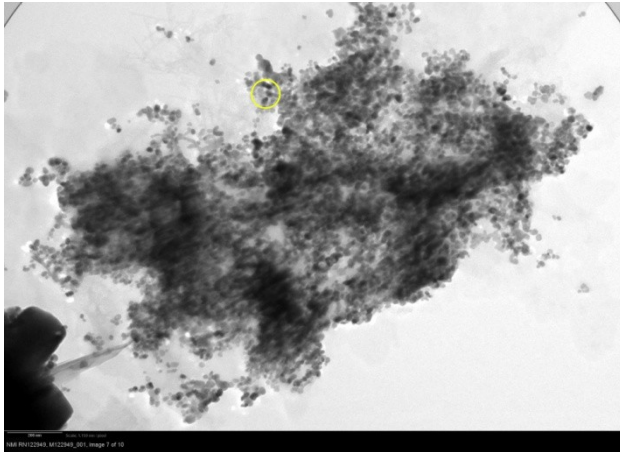


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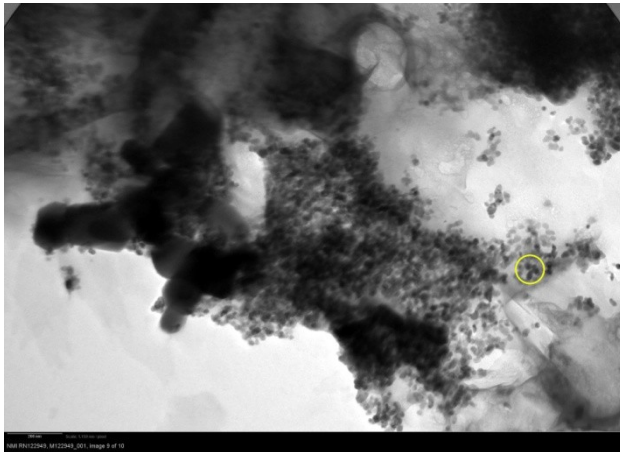
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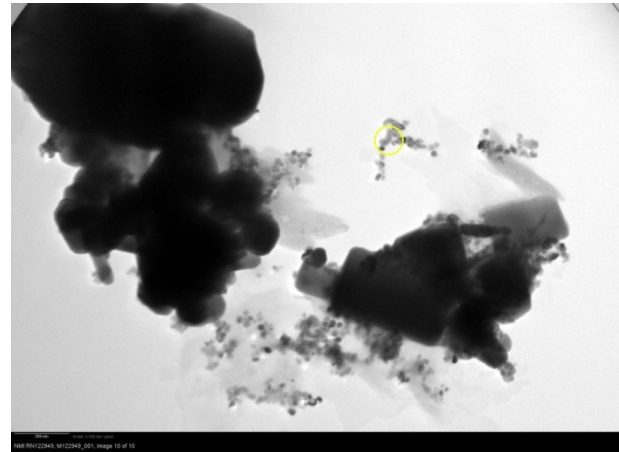
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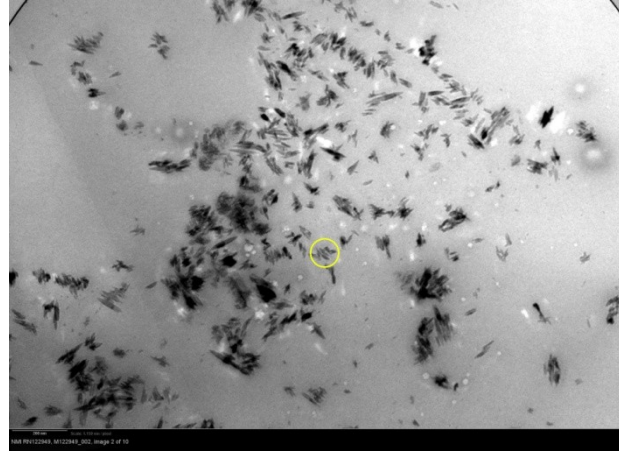


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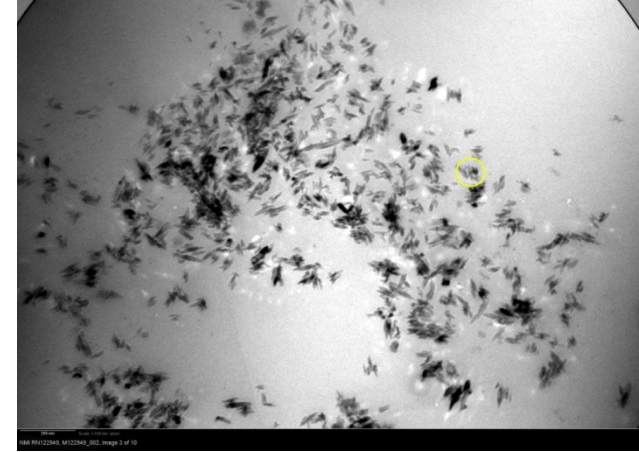
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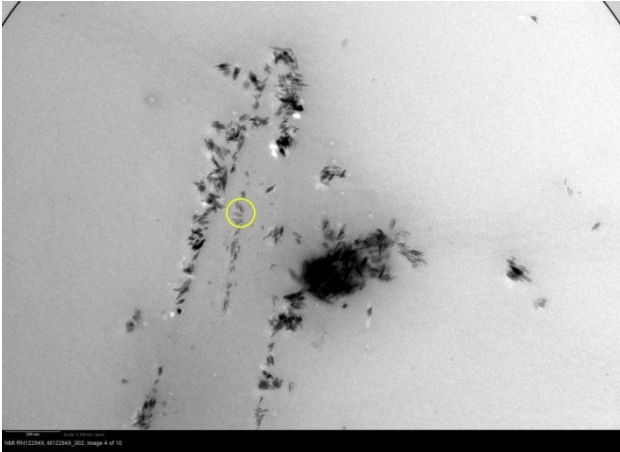
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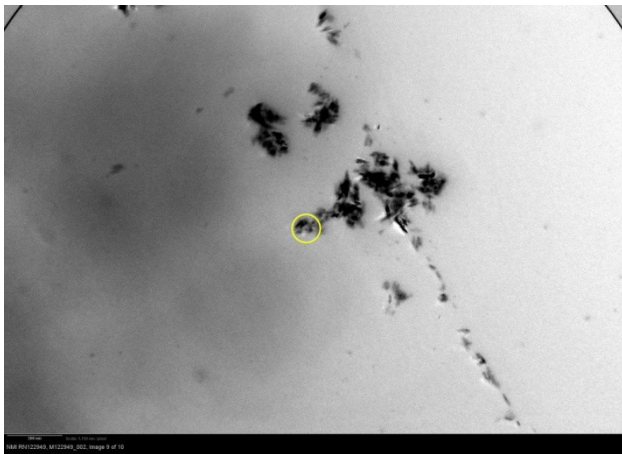
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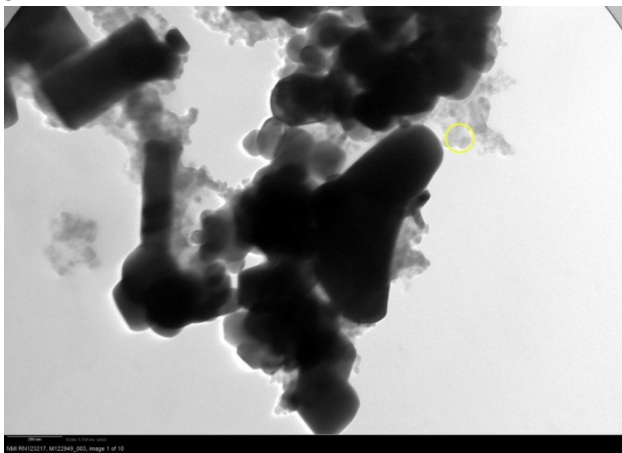
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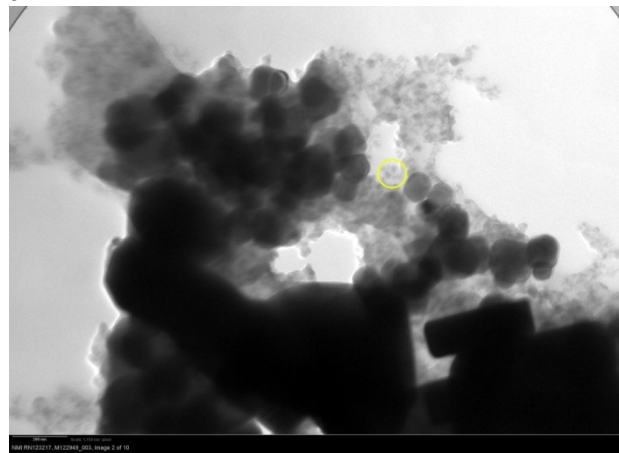


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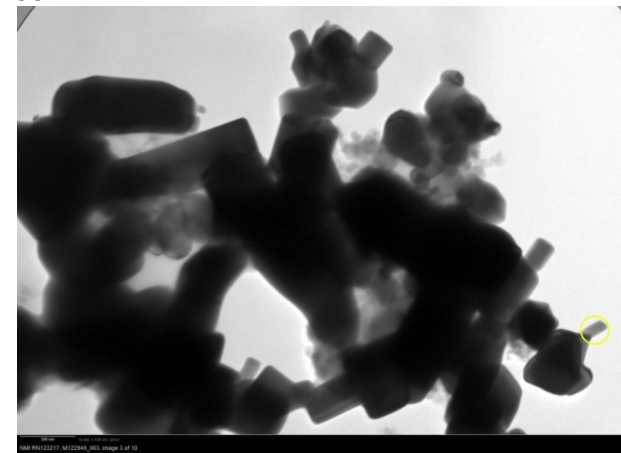
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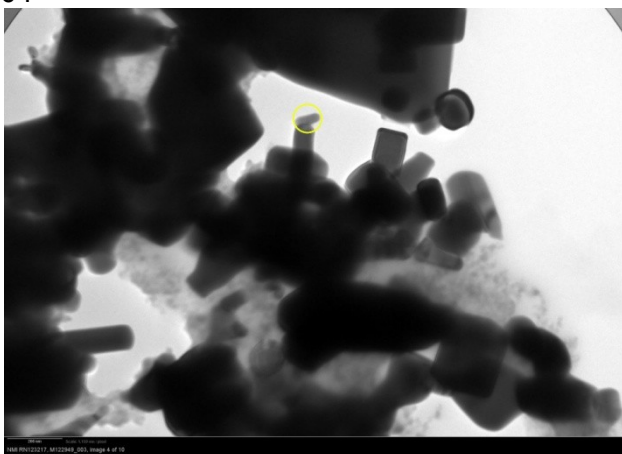
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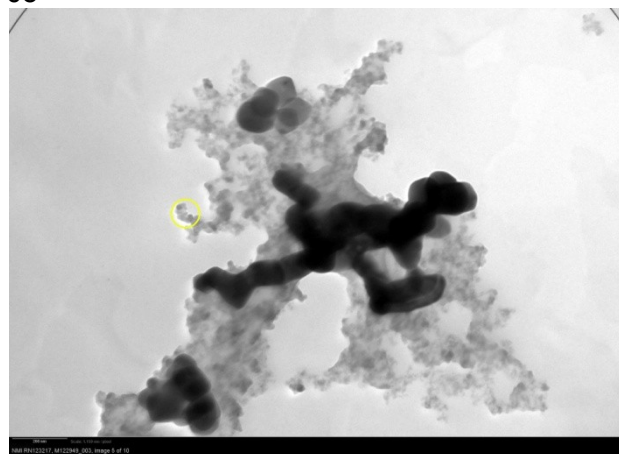
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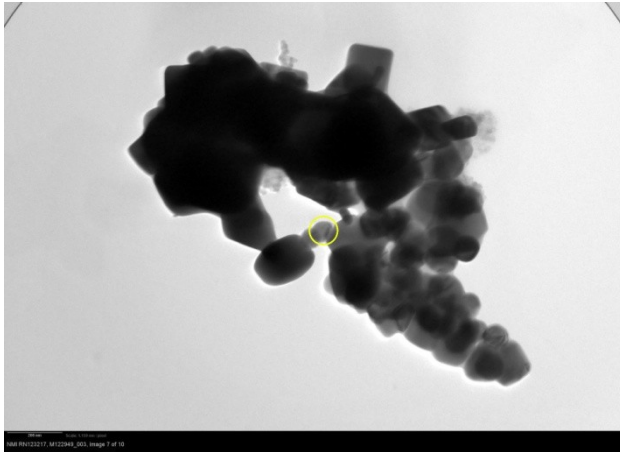


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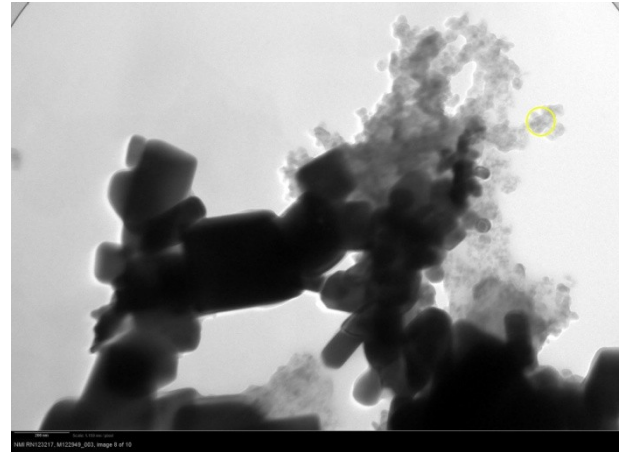


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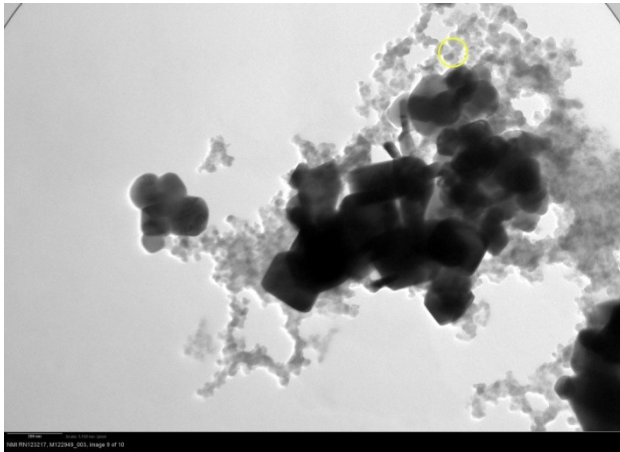
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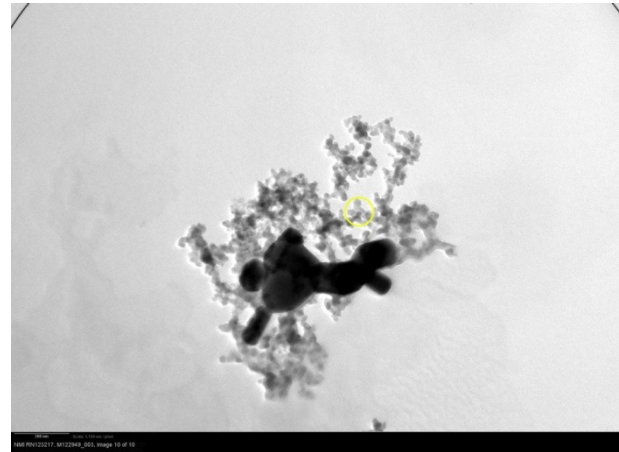
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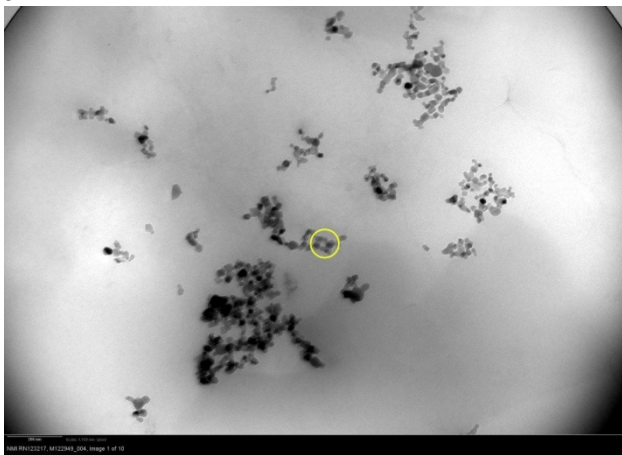


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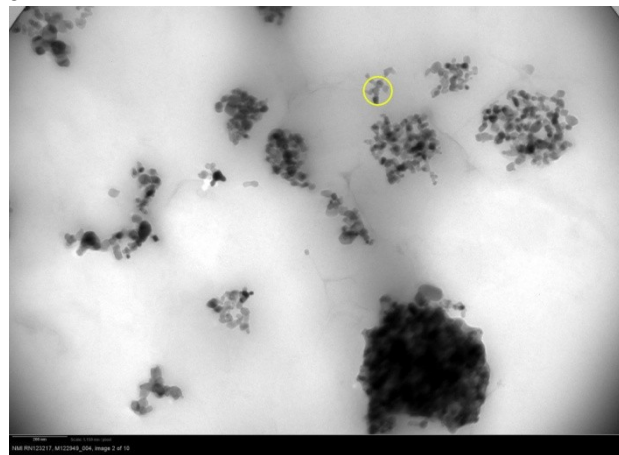


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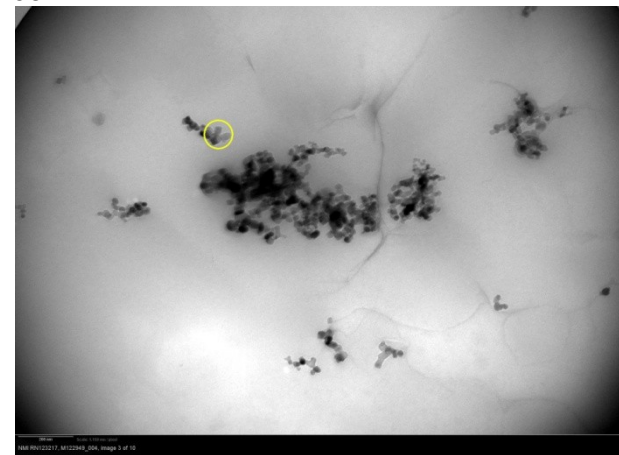
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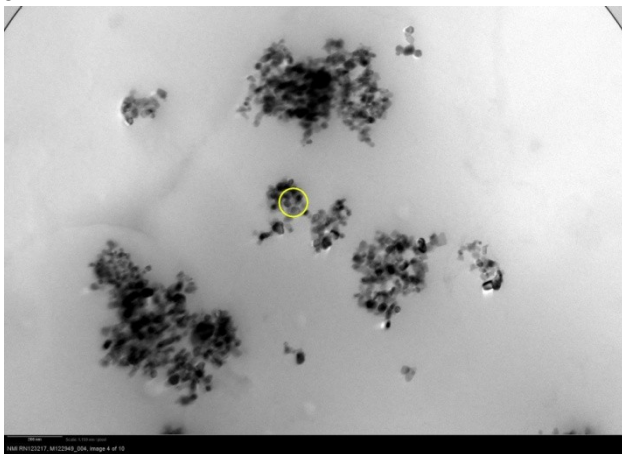
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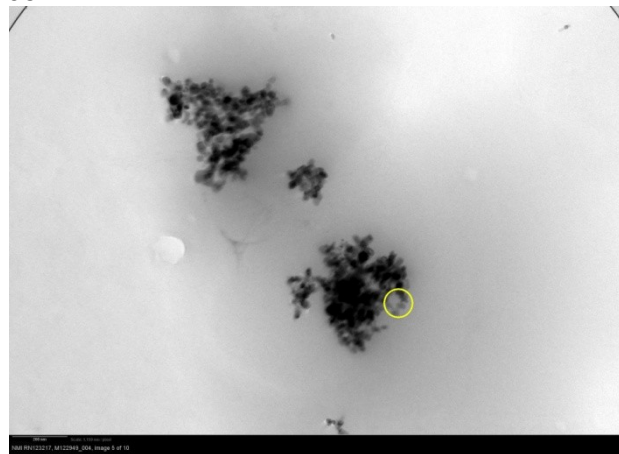
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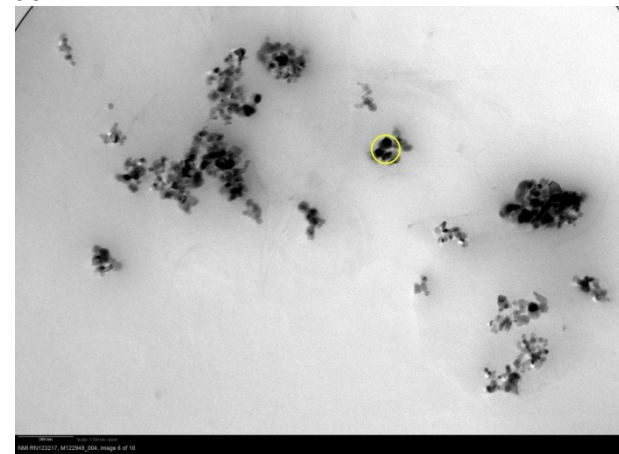
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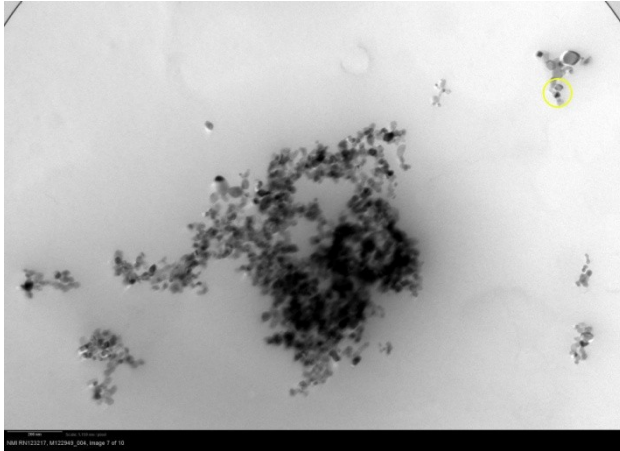
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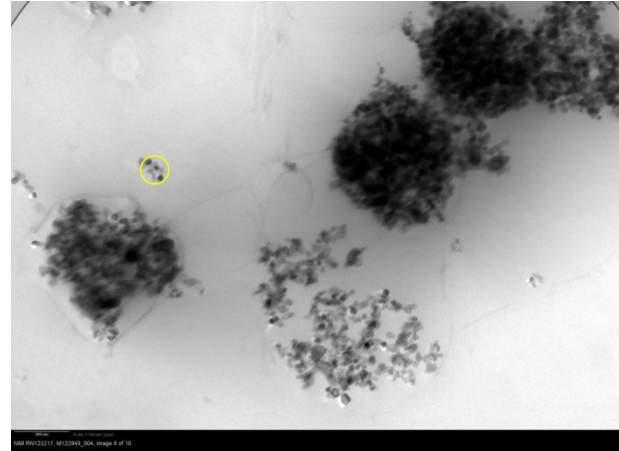


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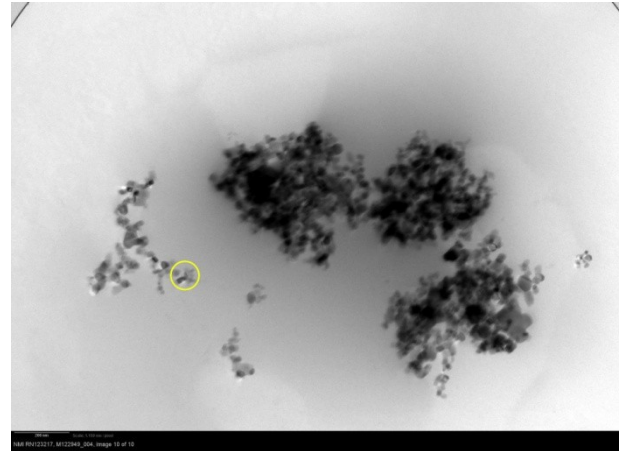
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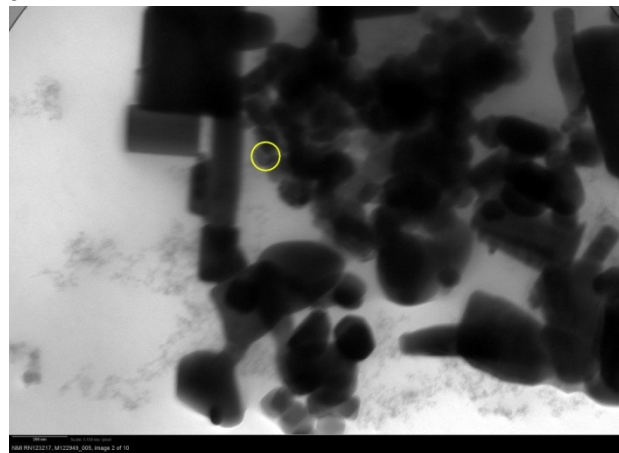


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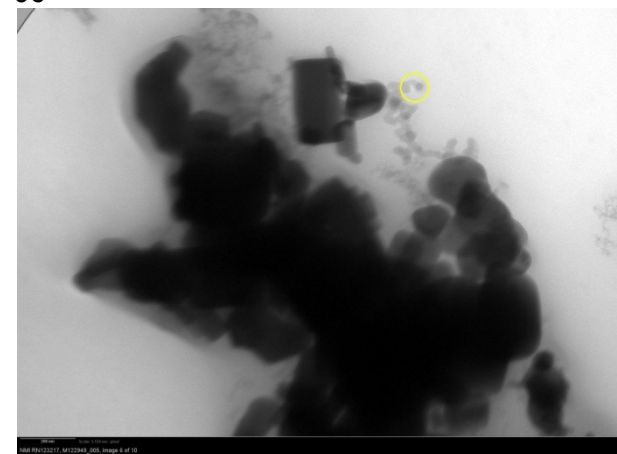
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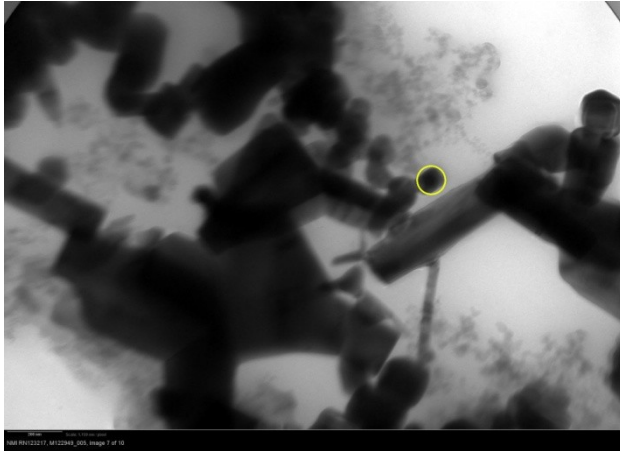


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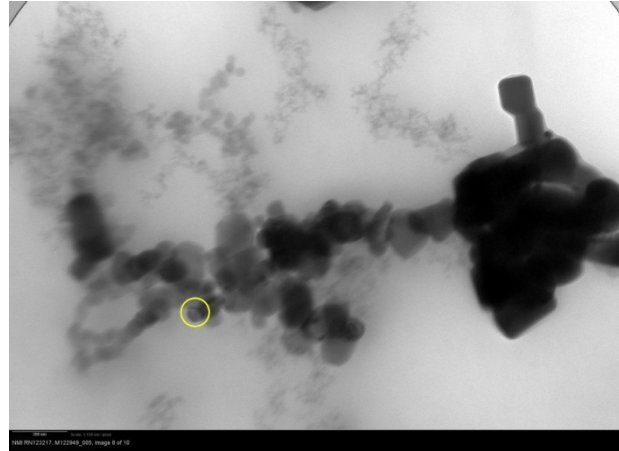


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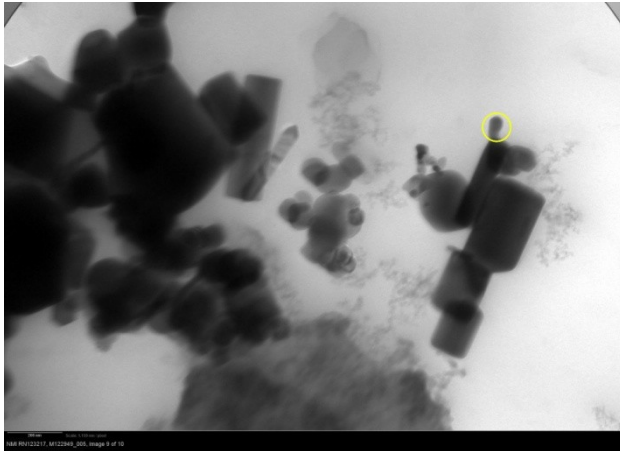
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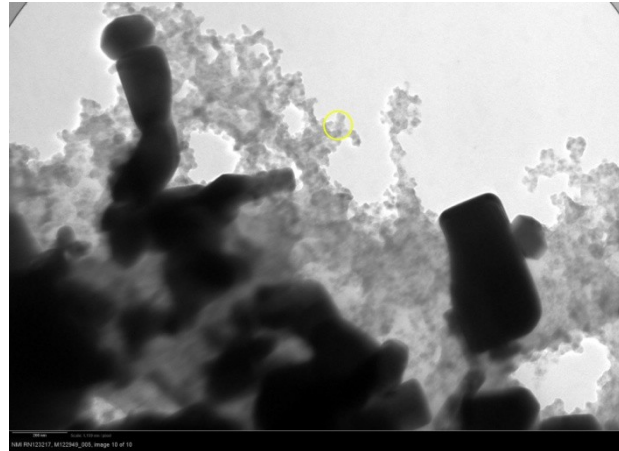
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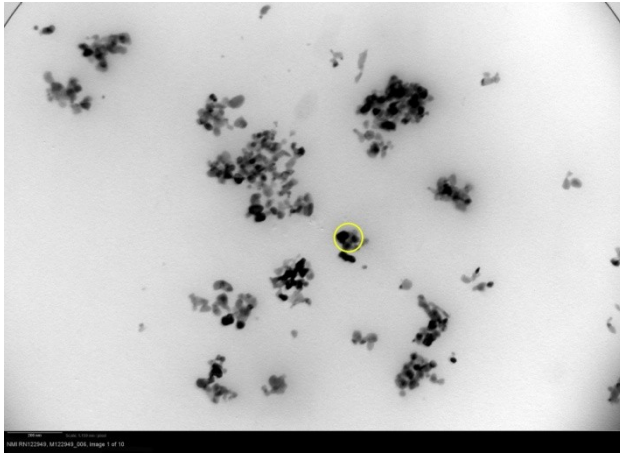
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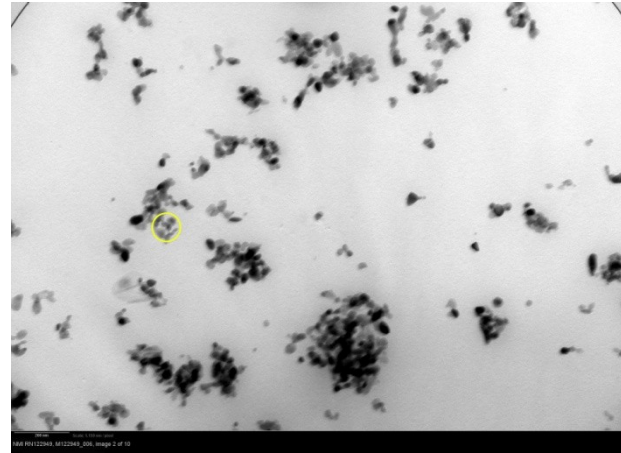


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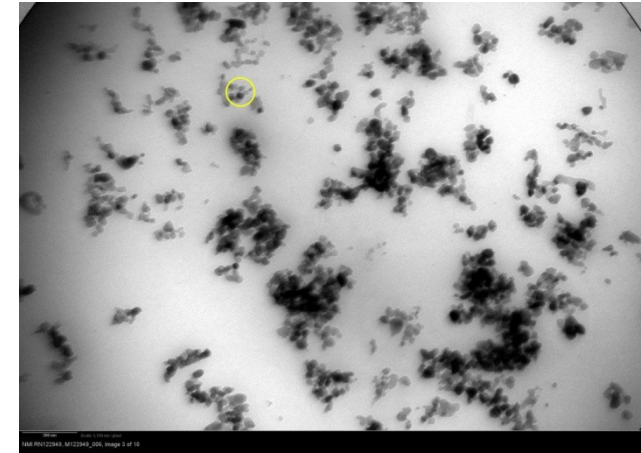
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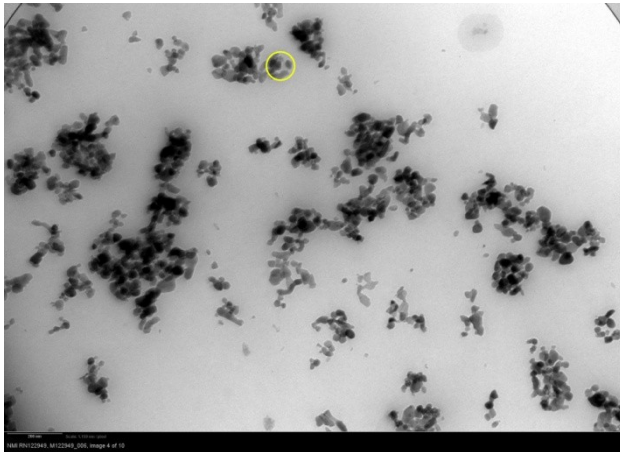
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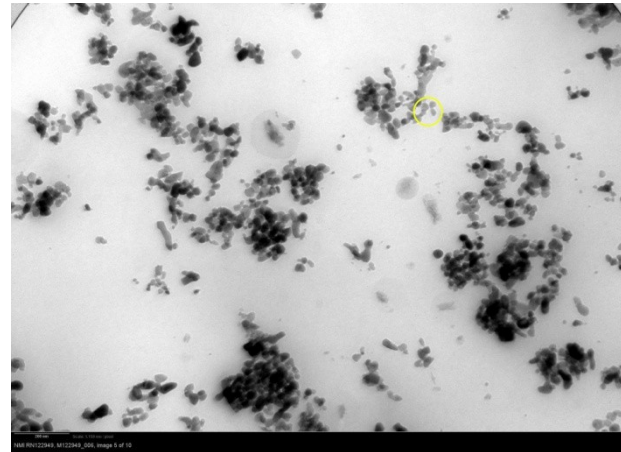
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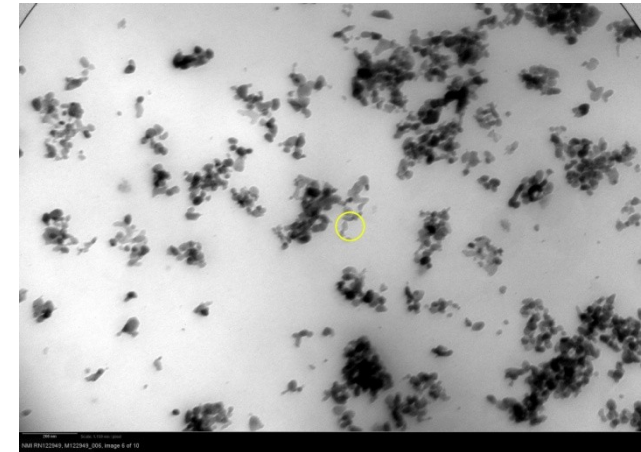
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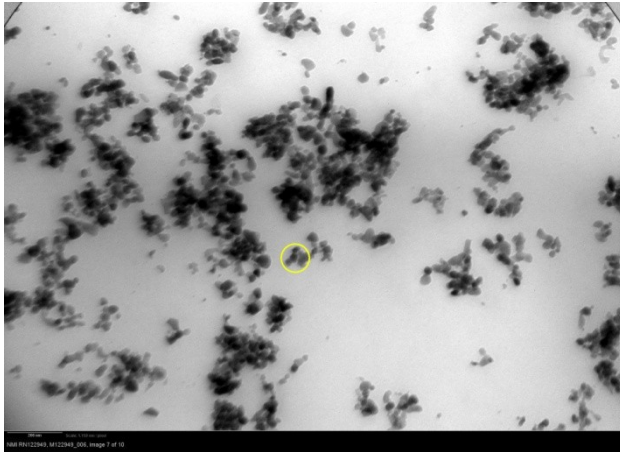


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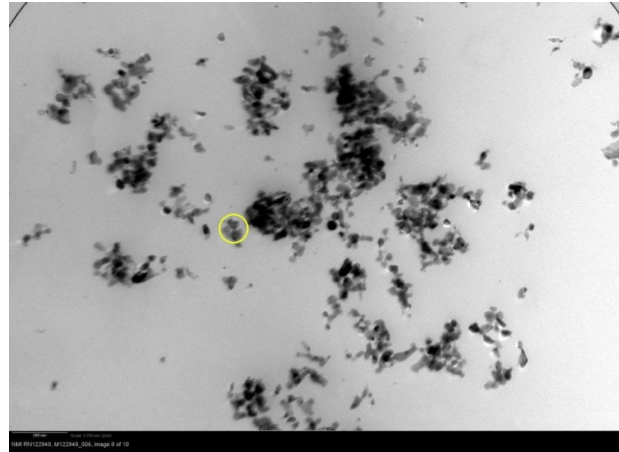


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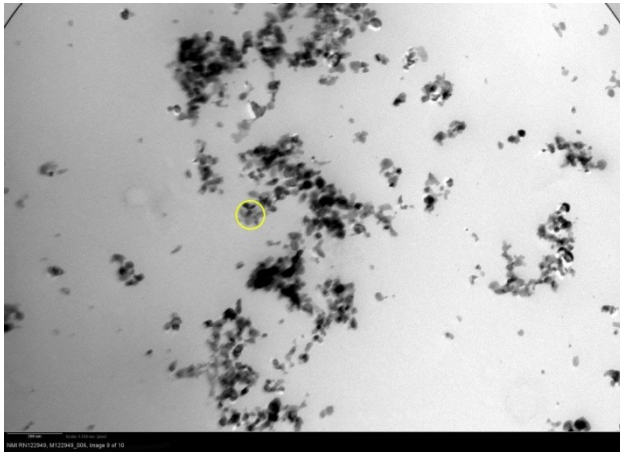
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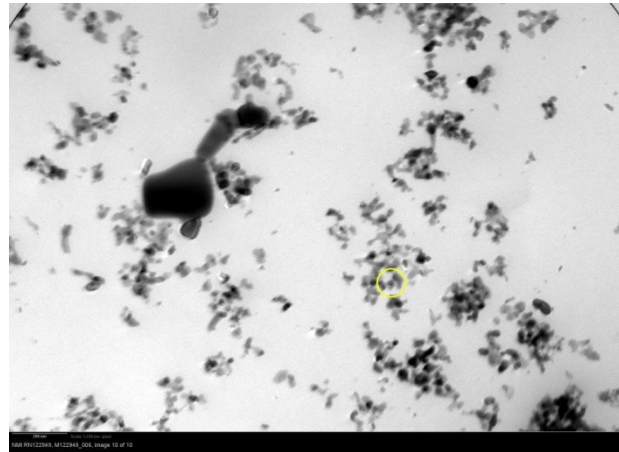
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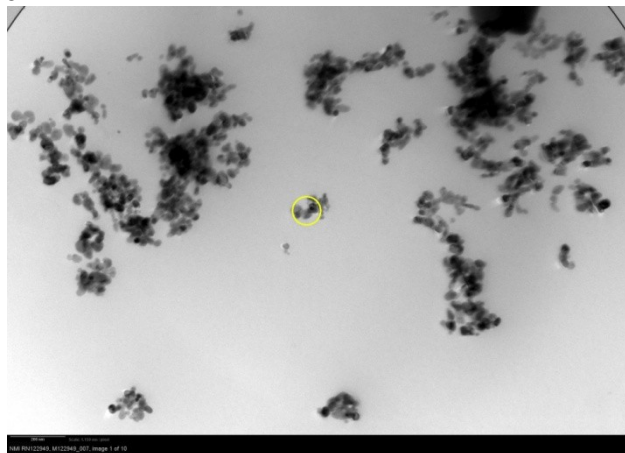


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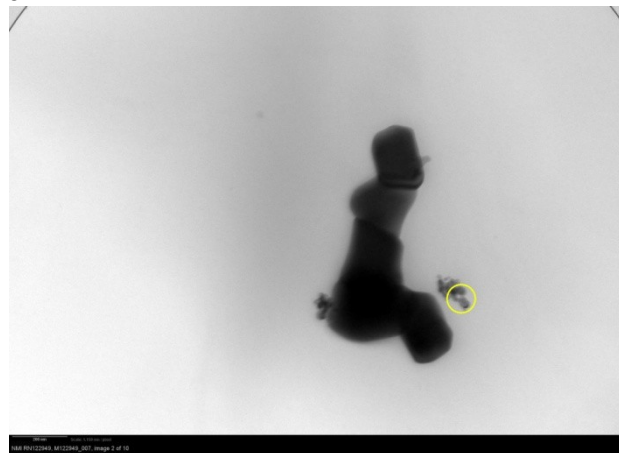


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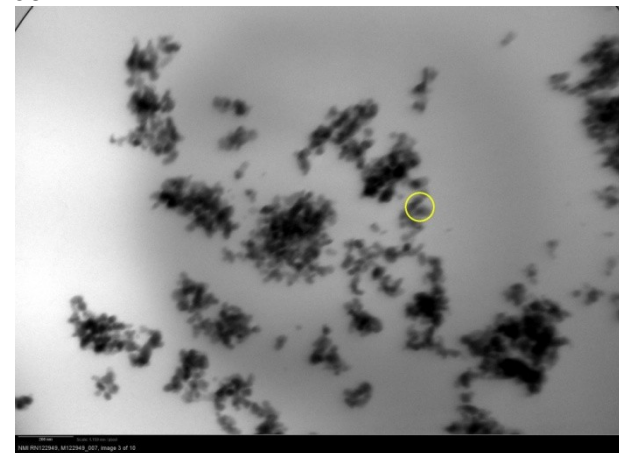
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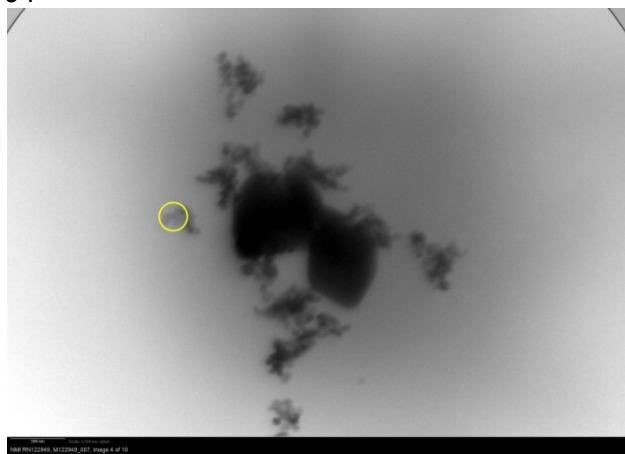
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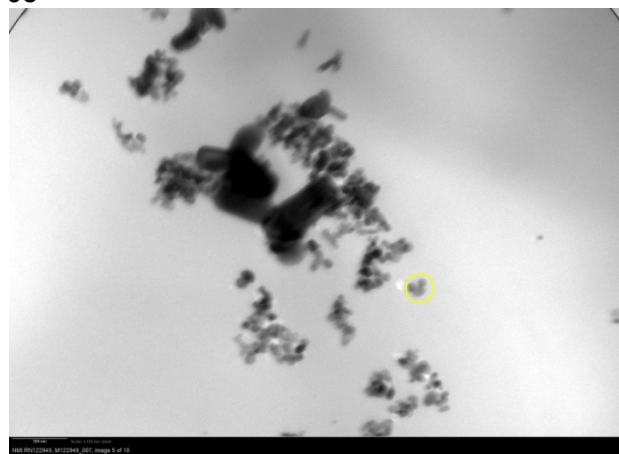
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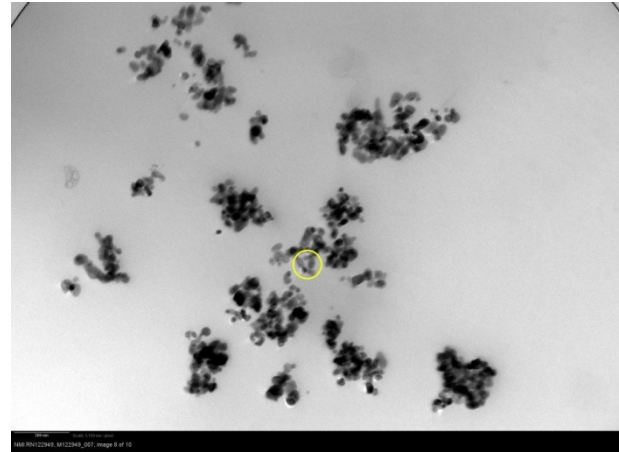


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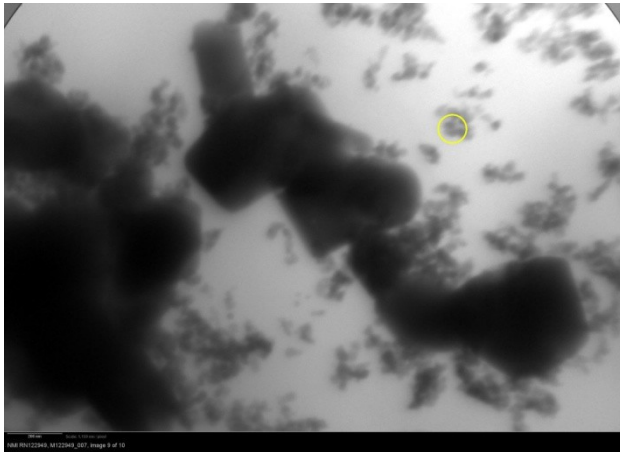
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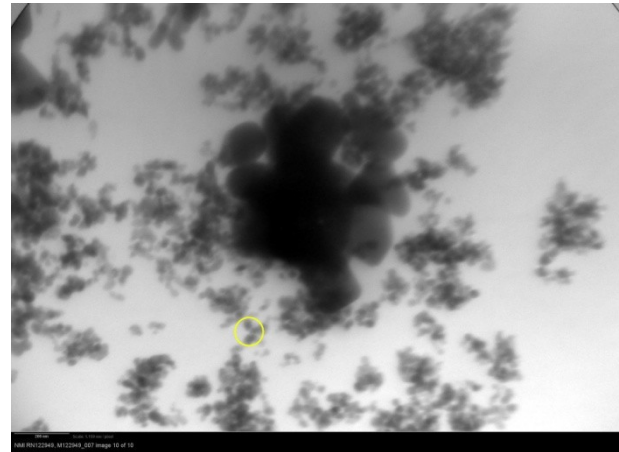
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