ANSES Opinion
Request No. 2024-AUTO-0038

The Director General

Maisons-Alfort, 23rd June 2025

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the "analysis of the results of an exploratory study to measure the presence of nanoparticles in plant protection products and biocidal products and to propose, where appropriate, management measures to protect potentially exposed populations and the environment"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health, the evaluation of the nutritional characteristics of food and the protection of the environment by assessing the impact of regulated products.
It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are published on its website. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 23rd June 2025 shall prevail.

On 9 April 2024, ANSES issued an internal request to analyse the results of an exploratory study that sought to measure the presence of nanoparticles in plant protection products and biocidal products and to propose, where appropriate, management measures to protect potentially exposed populations and the environment. Proposals for regulatory changes were also called for, with the aim of improving methodologies enabling a better characterisation of the nanoparticles that may be found in these products.

1. BACKGROUND AND PURPOSE OF THE REQUEST

According to the European Commission's Recommendation of 10 June 2022[1], "Nanomaterial" means a natural, incidental or manufactured material consisting of solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50% or more of these particles in the number-based size distribution fulfil at least one of the following conditions:

a) one or more external dimensions of the particle are in the size range 1 nm to 100 nm;
b) the particle has an elongated shape, such as a rod, fibre or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm;
c) the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm.

In the determination of the particle number-based size distribution, particles with at least two orthogonal external dimensions larger than 100 µm need not be considered. However, a material with a specific surface area by volume of < 6 m^2/cm^3 shall not be considered a nanomaterial.

It should be noted that in ANSES's April 2023 opinion on "Definition of nanomaterials: analysis, challenges and controversies – ANSES opinion, Collective expert appraisal report"[2], ANSES found that this new recommendation was more restrictive and less flexible than the previous one, thus paving the way for a potential regression in the protection of public health and the prevention of health and environmental risks associated with nanomaterials. It recommended henceforth considering a broader definition of nanomaterials, one that was more inclusive than the current European recommendation, so that nanospecific hazard characterisations would concern as many nanomaterials as possible.

An analysis of the data collected as part of ANSES's management of annual declarations of nanoparticle substances[3] showed that since the scheme came into force in 2013, numerous intended uses in "Plant protection products" have been declared (declarations for which the descriptor PC 27 was entered).

In order to analyse these points in detail, additional investigations were carried out by ANSES:

- a detailed analysis of declarations mentioning an intended use in plant protection products,
- letters sent to the main companies marketing these products to check for the presence of nanomaterials in their products.

This found that the reasons given for these declarations in R-Nano concerned almost exclusively the presence of co-formulants that may be in nanoparticle form.

[1] Commission Recommendation of 10 June 2022 on the definition of nanomaterial (2022/C 229/01)
[2] https://www.anses.fr/en/system/files/AP2018SA0168RaEN.pdf
[3] Nanoparticle substances declaration scheme – https://www.r-nano.fr/

Following this observation, ANSES commissioned an exploratory study to analyse plant protection products and biocides and check for the potential presence of nanoparticles.

The expert appraisal sought to carry out the following tasks in order to respond to the questions raised:

1) Analyse the results of the exploratory study carried out on plant protection products and biocides.
2) Based on the results of the product analyses, indicate whether the results obtained on products as sold (undiluted form) can be extrapolated to products as used (generally in diluted form) or to their residues after application.
3) Depending on the results of the analysis conducted for the first two points above, specify whether management measures should be taken for potentially exposed populations and the environment, in order to reduce exposure to nanoparticles from the use and application of plant protection products and biocides.
4) In order to better characterise the distribution of the particles found in the products, indicate the types of additional tests that could be added to the regulatory requirements. The formulation types should be taken into account.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (January 2024)".

The expert appraisal falls within the sphere of competence of the Expert Committees (CESs) on "Plant protection substances and products, biocontrol" (lead CES responsible for validating the work), "Biocidal substances and products" and "Physical agents and new technologies" (associated CESs for information and comments).

It was examined within the Regulated Products Assessment Department (DEPR) by an in-house project team made up of staff from the Unit for Physico-Chemical Assessment and Analytical Methods for Regulated Products (UPCMA), the Unit for the Assessment of the Toxicology of Plant Inputs (UETIV), the Unit for the Assessment of the Toxicology of Biocides (UETB), the Unit for the Assessment, Ecotoxicology and Environment of Plant Inputs (U3EIV), the Unit for the Assessment, Ecotoxicology and Environment of REACH Biocides (U3EBR), the Residues and Food Safety Unit (URSA) and the Scientific Watch and Development Team (CVDS).

The methodological and scientific aspects of the work were presented to the CESs between June 2024 and January 2025. It was adopted by the CES on "Plant protection substances and products, biocontrol" at its meeting on 14 January 2025.

ANSES analyses interests declared by experts before they are appointed and throughout their work, in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public via the website: https://dpi.sante.gouv.fr/.

3. ANALYSIS AND CONCLUSIONS OF THE CESS

3.1. Analysis of the exploratory study

ANSES issued a public call for tenders with a view to the "Analysis, quantification and characterisation of the size and number of particles in plant protection products and biocides". Following this consultation, the offer submitted by France's national metrology laboratory (LNE) was selected. The LNE has over 15 years of experience in the measurement and characterisation of nanomaterials and a multitude of complementary state-of-the-art equipment and platforms for studying their properties.

3.1.1. Methodology

The aim of this exploratory study was to determine the potential presence of nanoscale particles[4] in plant protection products and biocidal products. Quantifying the **primary particles**[5] and their chemical nature in the tested products was outside the scope of the study, because most of these products had highly complex compositions comprising several co-formulants[6] (2 to 17 per product), which may themselves be made up of several constituents. This complexity in the formulations makes it more difficult to analyse the primary particles in the products and would have required a more in-depth analysis. Furthermore, the techniques used to determine particle distributions are unable to measure the primary particles and could lead to a change in the physical state of the particles during sample preparation and analysis. Lastly, the analyses carried out on solid products targeted particle sizes between 20 nm and 570 nm and from 1 to 20 µm, which do not encompass all the particles found in the products. Therefore, the

[4] Particle with one dimension smaller than 100 nm.
[5] Primary particles are the initial (single) forms from which a material is assembled. They may differ from the constituent particles, which are morphologically identifiable within a material as aggregates or agglomerates.
[6] Co-formulants are defined in the document "Definitions and functions of co-formulants in biocidal products", CG 45, February 2021, as any non-active substance or mixture that is intentionally added to a biocidal product.
Co-formulants are described in Article 2(3)(c) of Regulation (EC) No 1107/2009 as being substances or preparations which are used or intended to be used in a plant protection product or adjuvant, but are neither active substances nor safeners or synergists.

proportions given in the LNE report annexed to this opinion are indicative but cannot be taken into account in a quantitative analysis of the results.

The plant protection products selected for this study were mainly products containing co-formulants consisting of clay or silica, the majority of which were found in the R-Nano declarations. These products were also selected because their active substances represent those with among the highest tonnages sold in France. The detailed compositions of the tested plant protection products were provided in the marketing authorisation application dossiers for these products.

The study also included biocidal products that may contain co-formulants or active substances in nanoparticle form. These biocidal products have not yet been assessed and can still be marketed under the "transitional regime" provided for in Regulation (EU) No 528/2012. Indeed, certain biocidal products contain active substances that are currently being assessed at European level. Because these products do not yet have marketing authorisation according to the Regulation, their detailed compositions were not provided to ANSES.

Two main product types were tested: solid products in powder form (WP: wettable powder and DP: dustable powder), and liquid products in suspension form (SC: suspension concentrate and CS: capsule suspension). Regarding the products in capsule suspension form, it should be noted that the capsules consist of a polymer "shell" containing a more or less viscous solution made up of the active substance(s) and co-formulant(s). Once applied, the active substance(s) diffuse(s) through the shell.

More specifically, the following products were tested:

- WP plant protection products: four products based on copper compounds, four products based on micro-organisms, two products containing several active substances (mancozeb, fosetyl, cymoxanil and copper compounds) and one product based on potassium hydrogen carbonate;
- DP plant protection products: four products based on sulphur;
- CS plant protection products: five products based on several active substances (lambda-cyhalothrin, clomazone, geraniol, thymol, eugenol, clomazone, metazachlor and flurochloridone);
- SC plant protection products: three products based on several active substances (chlorothalonil, cymoxanil, chlorotoluron and isoxaben);
- Biocidal products: 10 products based on several active ingredients (silica, geraniol, pyrethrum extract and margosa extract).

The analytical techniques used were selected on the basis of their applicability in terms of the range of particle sizes measured and the types of samples they were able to analyse.

For solid products, the scanning mobility particle sizer (SMPS) technique coupled with the aerodynamic particle sizer (APS) was chosen to measure particle number size distributions

(PNSDs) ranging from 20 to 570 nm in terms of electrical mobility diameter and from 1 to 20 µm in terms of aerodynamic diameter. The PNSDs were obtained using a condensation particle counter (CPC). The PNSDs were normalised with regard to the total number of particles measured by SMPS and APS in order to obtain a comparable number scale.

These techniques required samples to be suspended in the air in aerosol form for analysis. This experimental suspension could induce the formation of aggregates[7] and agglomerates[8], or break up those already present.

As the liquid products tested were highly concentrated, the dynamic light scattering (DLS) technique initially selected could not be used. This is because the high particle concentration of the products meant that they were opaque, making DLS analysis impossible. The scanning electron microscopy (SEM) technique coupled with an energy-dispersive X-ray (EDX) detector was proposed by the laboratory as an alternative technique better suited to this type of sample. Preliminary preparation was needed for these highly concentrated samples.
For the liquid products, only particles smaller than 100 nm were searched for and identified. A particle size distribution was obtained for each particle identified in the entire sample tested.
The laboratory carried out all the analyses without knowing the detailed compositions of the tested products.

3.1.2.Results

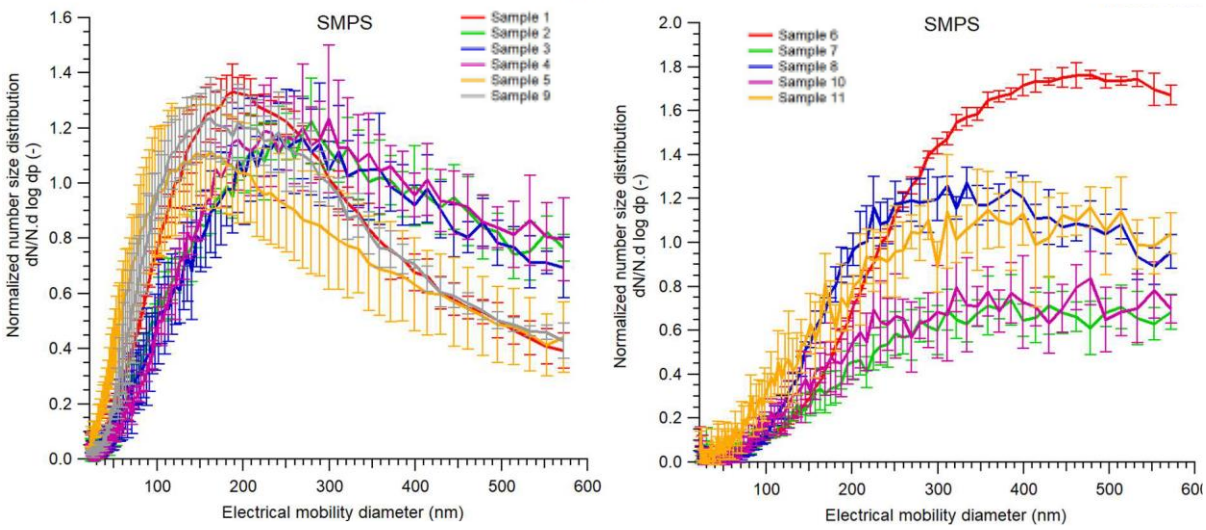
Plant protection products: wettable powders (WPs)

Products in WP form need to be diluted with water before use. These diluted products are then applied to crops by spraying. Use concentrations (concentrations of the diluted product ready for use) depend on the target crops/uses and the composition of the products. Therefore, in order to be able to compare the different products, the analyses were carried out on undiluted products, as sold.

The particle size distributions obtained in the 20 to 570 nm size range revealed two groups of products. The particle distributions obtained by SMPS and APS are shown below (Figures 1 and 2).

[7] Aggregate: a set of particles comprising strongly bound or fused particles whose resulting external surface area may be significantly smaller than the sum of the calculated surface areas of each of the components (ISO/TS 80004-2:2015).

[8] Agglomerate: a collection of weakly bound particles, aggregates or a mixture of the two whose resulting outer surface area is similar to the sum of the surface areas of each of the components (ISO/TS 80004-2:2015).



Group 1 Group 2

Figure 1: Particle size distributions obtained by SMPS for wettable powders

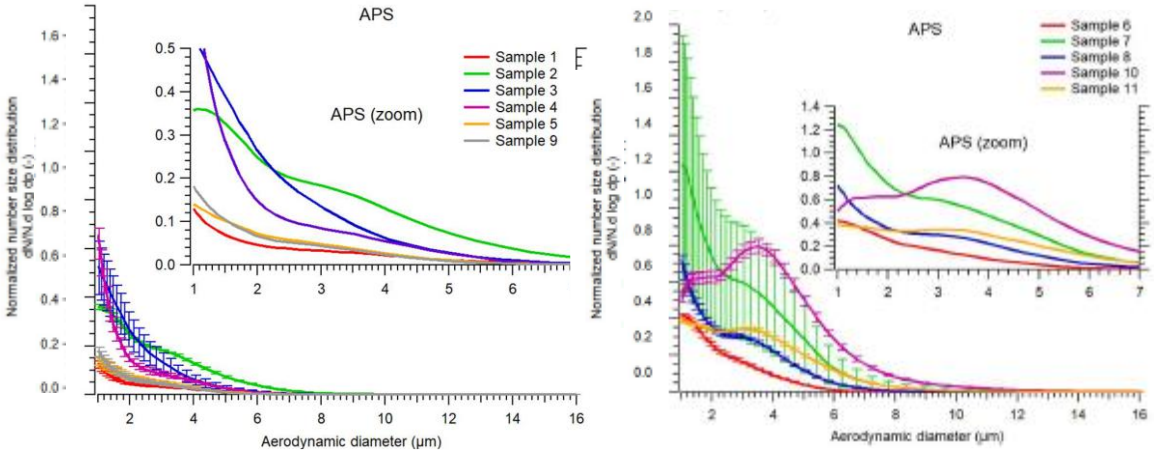


Figure 2: Particle size distributions obtained by APS for wettable powders

Detailed results for each product sample are shown in Table 1 below.

Table 1. Particle distributions obtained by SPMS and APS for WP products

Group number	Sample number	Median diameter SMPS (20 nm–570 nm)	Median diameter APS (1 µm–20 µm)
1	1	183 nm	1.5 µm
	2	227 nm	1.7 µm
	3	219 nm	1.4 µm
	4	227 nm	1.3 µm

	5	151 nm	1.5 µm
	9	172 nm	1.4 µm
2	6	336 nm	1.5 µm
	7	289 nm	1.7 µm
	8	283 nm	1.7 µm
	10	276 nm	2.6 µm
	11	265 nm	2.2 µm

In Group 1, the six products had a comparable particle distribution in the 20 nm to 570 nm size range. All the products contained clay or kaolin as a bulking agent and copper as the active substance, with the exception of one product that contained no copper (Sample 5). The particles could correspond to these constituents, but it was impossible to be certain given the methods used (ANSES hypothesis).

In Group 2, the five products had a comparable particle distribution for the 20 nm to 570 nm size range, although Sample 6 had slightly more particles larger than 300 nm than the other products in the group. Nevertheless, the overall shape of the distribution was similar to the other samples.

All the products contained clay or kaolin as a bulking agent and micro-organisms as the active substances, with the exception of one product that contained potassium hydrogen carbonate (Sample 11). The bacteria or spores were typically larger than 500 nm. As with Group 1, the measured particles may have come from the bulking agents in the products and/or from the potassium hydrogen carbonate, for one of the products.

The results (Figure 1) found particles smaller than 100 nm in both groups.

Plant protection products: dustable powders (DPs)

Products in DP form can be used without prior dilution. These products are dusted onto crops using an air stream. In the study, the analyses were carried out on the products as sold, in order to be able to compare them with each other. The particle size distributions obtained in the 20–570 nm size range revealed two groups of products. The particle distributions obtained by SMPS and APS are shown below. It should be stressed that the groups shown here are different to those presented in the LNE report, because Sample 12 (Group 2) was not considered to have the same distribution shape as samples in Group 1.

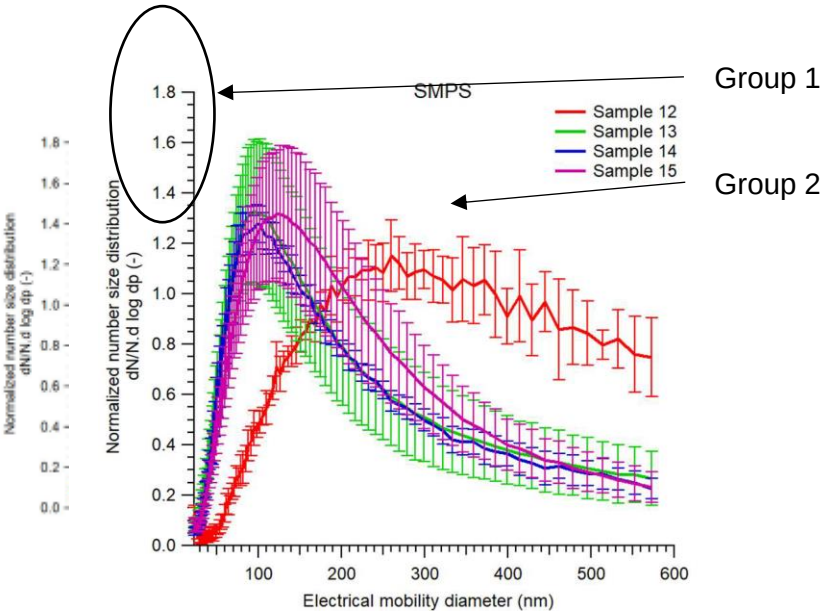


Figure 3: Particle size distributions obtained by SMPS for DP products

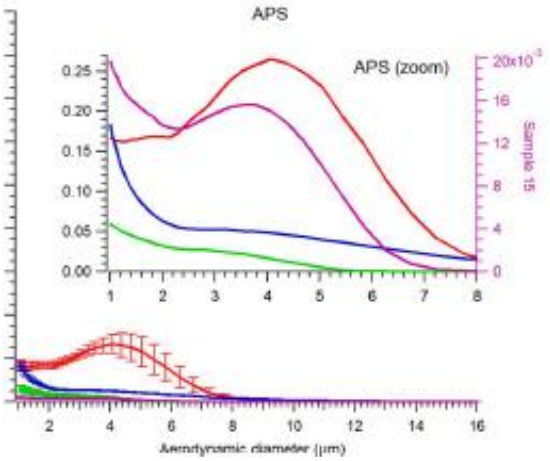


Figure 4: Particle size distributions obtained by APS for DP products

Detailed results for each product sample are shown in Table 2 below.

Table 2. Particle distributions obtained by SPMS and APS for DP products

Group number	Sample number	Median diameter SMPS (20 nm–570 nm)	Median diameter APS (1 µm–20 µm)
1	13	111 nm	1.7 µm
	14	113 nm	1.8 µm
	15	127 nm	2.1 µm
2	12	221 nm	2.7 µm

The products in Group 1 had a comparable distribution for particles between 20 nm and 570 nm. The product in Group 2 had a different distribution, with fewer particles below 100 nm than Group 1 and more particles above 1 µm.

The DP products tested contained only a few co-formulants, unlike the other product types. It is therefore reasonable to assume that the vast majority of the measured particles were of the active substance, sulphur. The difference in distribution between Groups 1 and 2 was therefore probably associated with the active substance manufacturing process.

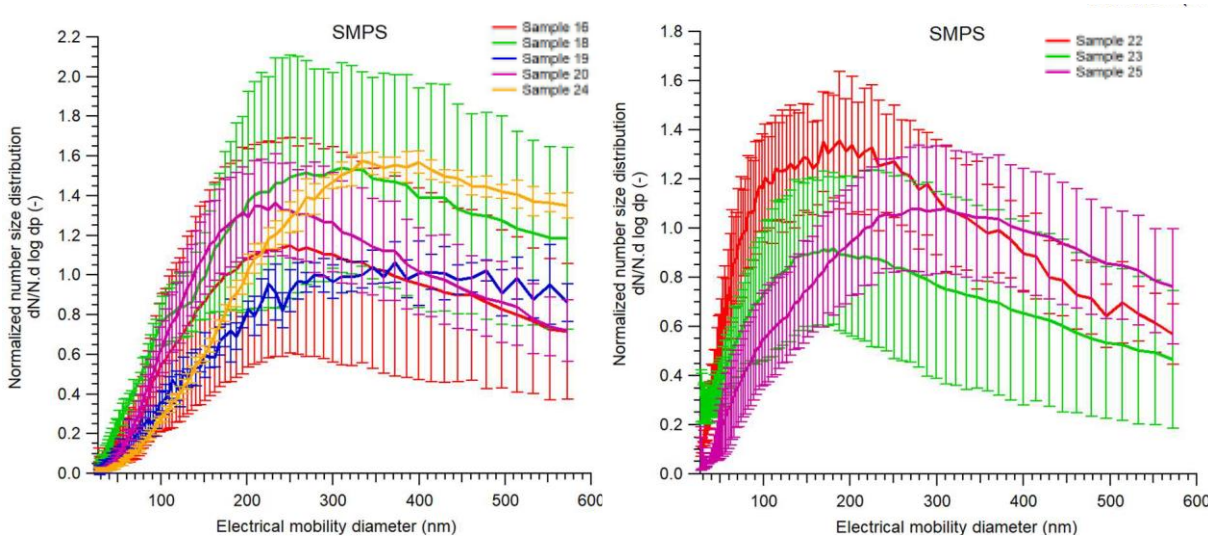
All the products contained particles smaller than 100 nm.

<u>Biocidal products: dustable powders or aerosolised powders</u>

Most of the biocidal products tested were "ready-to-use" products in aerosolised or dustable powder form. The analyses were therefore carried out on the products as applied, without dilution or the use of any equipment.

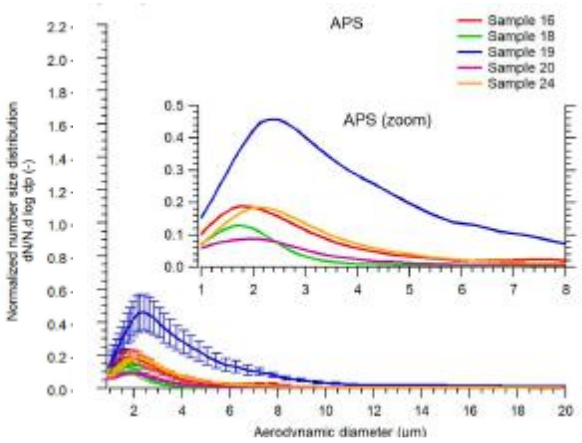
The results of the particle size distributions obtained by SMPS revealed four groups of products. The particle distributions are shown below (Figure 5). The groups shown here are different to those presented in the LNE report, because the two products identified in Group 2 by the LNE were not considered here to have a similar particle distribution.

For Groups 2 and 2b, the particle distributions obtained by SMPS and APS are shown below.

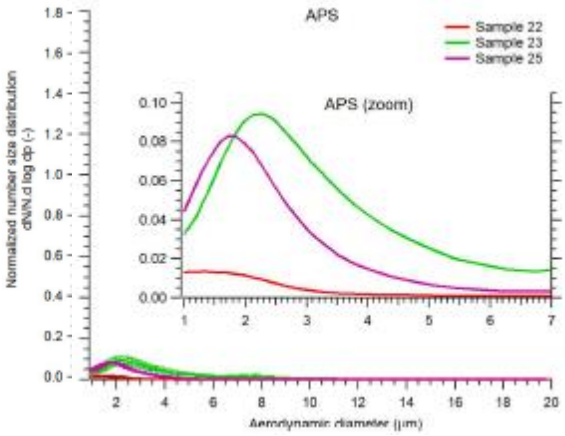


Group 1 Group 3

Figure 5: Particle size distributions obtained by SMPS for Groups 1 and 3 biocidal products

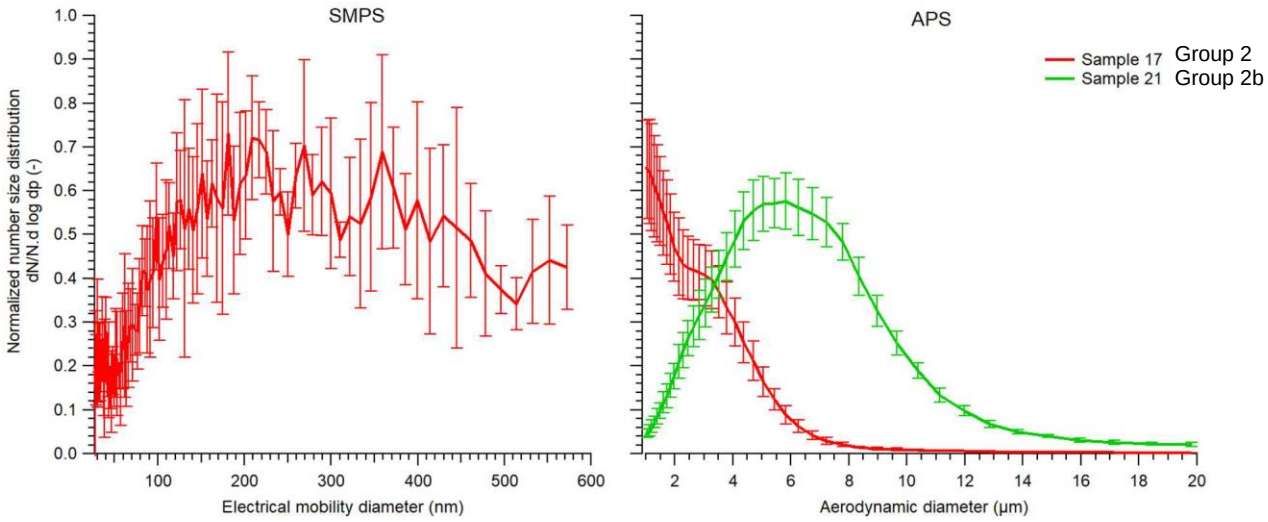


Group 1



Group 3

Figure 6: Particle size distributions obtained by APS for Groups 1 and 3 biocidal products



Group 2 and 2b

Figure 7: Particle size distributions obtained by SMPS and APS for Groups 2 and 2b biocidal products

The results are shown in Table 3 below.

Table 3. Particle distributions obtained by SPMS and APS for biocides

Group number	Sample number	Median diameter SMPS (20 nm–570 nm)	Median diameter APS (1 µm–20 µm)
1	16	208 nm	1.9 µm
	18	218 nm	1.7 µm
	19	251 nm	2.6 µm
	20	215 nm	1.9 µm
	24	289 nm	2.1 µm

2	17	157 nm	1.8 µm
2b	21	/	4.5 µm
3	22	152 nm	11.5 µm
	23	135 nm	2.2 µm
	25	189 nm	1.8 µm

The products in Group 1 had a comparable particle size distribution for particles smaller than 570 nm. All the products in this group consisted of silica or diatomaceous earth made up of silica exoskeletons.

The product in Group 2 had a lower particle size distribution for particles smaller than 570 nm, compared with Group 1. This Group 2 product was based on pyrethrum extract. The presence of particles smaller than 100 nm can probably be explained by the use of a bulking agent such as clay in this product, but this hypothesis cannot be verified since the detailed composition of the product was unknown.

The Group 2b product did not contain any particles smaller than 570 nm.

Lastly, the products in Group 3 had a comparable particle size distribution for particles smaller than 570 nm. Two of the three products were essentially made up of diatomaceous earth, while one contained geraniol as its active substance. The presence of particles smaller than 100 nm in the latter is probably due to the use of a bulking agent.

All but one of the biocidal products tested contained particles smaller than 100 nm.

Plant protection products: suspension concentrate (SC)

The tested products need to be diluted before use. Once diluted, the products are sprayed. The particle distribution analysis was carried out on the products as sold in order to be able to compare them with each other. These products had high concentrations of particles, so the samples had to be prepared before each analysis. This sample preparation involved diluting and/or washing the samples before analysis. This could lead to the formation of aggregates/agglomerates or break up those already present. The sample preparation protocols were standardised.

The analytical technique used was SEM coupled with an EDX probe. This technique is able to measure the distribution of particles with the same elemental chemical composition. The measured distributions do not therefore cover all the particles present in the sample.

Three products were analysed, each containing one or two active substances. For these three products, the analyses revealed the presence of agglomerates/aggregates with at least one nanoscale dimension. EDX analysis of these particles found mainly carbon and oxygen. The median particle sizes analysed for the three products were, respectively, 22.7, 23.4 and 33.3 nm per 100 particles measured.

Chlorine and nitrogen were also detected in small quantities in some agglomerates/aggregates, indicating the presence of certain active substances.

Nanoscale fibres were detected in two of the tested products. EDX analysis of these fibres found magnesium and aluminium silicate in the form of sepiolite and halloysite.

The detailed compositions provided in the marketing authorisation dossiers indicate that only the active substances and their impurities are in solid form in the three products. The other co-formulants are water-soluble. The presence of agglomerates/aggregates containing mainly carbon and oxygen and of sepiolite/halloysite fibres was not identified in the detailed compositions of the products.

Based on the results, all three products contained particles smaller than 100 nm.

Plant protection products: capsule suspension (CS)

The tested products need to be diluted before use. Once diluted, the products are sprayed. The particle distribution analysis was carried out on the products as sold, before dilution, in order to be able to compare them with each other. These products had high concentrations of particles, so the samples had to be prepared before each analysis. SEM analysis of the samples also required them to be placed in a vacuum. In the case of products in capsule form, this step appeared to be critical: the products are made up of polymer capsules containing the active ingredients diluted in a solvent. When the samples are placed in the vacuum chamber, the polymer capsules can burst and the solvents evaporate, thus altering the physical state of the compounds in the capsules.

Five products were analysed, each containing one to three encapsulated active substances. For all the products, the particles observed corresponded mainly to those in the capsules. Therefore, without precise identification of the particles, it is difficult to say whether these compounds were actually present in solid particle form in the product before analysis. It is possible that placing the samples in the vacuum led to the crystallisation of these compounds, which were initially diluted in a solvent.

Nevertheless, iron and titanium dioxide (TiO_2) particles were detected in one product. No particle size distributions could be obtained because there were too few particles present. However, all the particles observed were smaller than 100 nm.

The presence of titanium dioxide was indicated in the full composition of one of the tested products. Titanium dioxide is added to the product as a colourant at concentrations of more than 1 g/L. As the number of TiO_2 particles in nanoscale form present in the tested sample was not sufficient to obtain a distribution (fewer than 150 particles), it can be assumed that the majority of the TiO_2 particles in the product are not nanoscale in size (i.e. do not have at least one dimension smaller than 100 nm). On the other hand, the presence of small quantities of iron was not mentioned in the full composition. The iron may be an impurity in the co-formulants in the product.

Moreover, in some products, sepiolite/halloysite fibres were measured with median sizes of 19.1 and 22.1 nm. The presence of these particles was confirmed in the detailed compositions of the products, which contained bulking agents of this type.

3.1.3.Conclusion

The plant protection products (both the solid products and the liquids in suspension concentrate form), as well as all but one of the solid biocidal products tested, contained particles smaller than 100 nm. The proportion of the particles varied widely according to product type and composition.

For the plant protection products in capsule suspension form, it was difficult to reach a general conclusion on the presence of particles smaller than 100 nm, because the conditions under which the samples were prepared probably modified the physical state of the capsules in the tested products. However, particles smaller than 100 nm were detected in some products, albeit in proportions too low to determine a size distribution for these particles observed in the tested samples.

It should be noted that, for the solid products, the techniques used to determine particle distributions were unable to measure the primary particles and do not cover all the particle sizes present in the products (limited to 20–570 nm and 1–20 µm). Therefore, the proportions given in the LNE report annexed to this opinion were not taken into account for a quantitative analysis of the results. For the liquid products, it was not possible to obtain the distributions for all the particles because the technique used is only able to measure the distribution for particles of the same chemical nature.

3.2. Extrapolation of the analysis results from products as sold (undiluted form) to products as used (generally in diluted form) or to their residues after application

Among the plant protection products tested, WP, SC and CS products all need to be diluted before use. Their concentrations diluted in water range from 0.005–5% mass/volume, depending on the target crops and uses. The products must be diluted while stirring constantly, in accordance with good agricultural practice. Once the solution has been obtained, it is sprayed under pressure using suitable equipment. During spraying and then once on the crops, some of the substances in the droplets may evaporate, while the others remain as residues on the plants and soil.

Therefore, the characteristics of the particles measured in the products before dilution may be modified during these different steps. In the tested products, the particles were in the form of primary particles, agglomerates of primary particles/aggregates or aggregates of primary particles. When these products are used, the primary particles may agglomerate/aggregate, and the agglomerates may disintegrate. Since there are stronger bonds between particles in aggregates, there is a low probability of the particles contained in them separating. These phenomena are associated with the movement of fluids, Brownian diffusion of particles and chemistry of the solutions.

Therefore, the results obtained for the products as sold cannot be extrapolated to the residues of these products during and after application.

DP plant protection products are not diluted before use. However, they are applied using dusting equipment fitted with an air pump. As with the products to be diluted, the form of the particles present may be modified during application. Therefore, the results obtained for the products as sold cannot be directly extrapolated to the residues of these products during and after application.

Lastly, the biocidal products tested were ready-to-use products. Nevertheless, some products can be applied by manual dusting, while others are in the form of aerosol sprays. In these cases, the tested products corresponded to the products once sprayed, so the analyses were carried out on the products as applied. Therefore, the results obtained for these products (aerosols and powders) can be extrapolated to the residues of these products after application. However, as the particles can interact with the matrices to which they are applied, a change in the form of these particles cannot be ruled out. In addition, the preparation of samples for analysis may also modify the particle size distribution.

3.3. Management measures that could be implemented for potentially exposed people and the environment, in order to reduce exposure to nanoparticles from the use and application of plant protection products and biocides

Given the current state of knowledge on the characterisation of particle size distribution, particularly for plant protection products, and the possible changes in particle form, for example after the product is diluted and depending on the application method, there is considerable uncertainty about the exposure of people and ecosystems, and the hazards associated with these nanoparticles.

With regard to operators/users during product application, the prevention and management measures likely to reduce exposure are presented below. No management measures likely to limit exposure were identified for the other exposure situations.

According to the INRS's dossiers on nanomaterials[9], strategies for preventing the risks associated with nanomaterials, as well as good working practices, need to be developed on a case-by-case basis. Current prevention is mainly based on limiting occupational exposure.

Generally speaking, the main prevention approaches to ensure worker protection are as follows (INRS[9]):

- Remove or reduce exposure;
- In the event of exposure, apply:
    - collective protective measures,
    - personal protective measures, when collective protection is insufficient.

Collective protection is mainly based on ventilation of the premises (extract from the INRS document):

The concentration of nanomaterials in the workplace atmosphere must always be kept as low as possible. To achieve this objective, ventilation should be installed, prioritising local ventilation. Since nano-aerosol transport is largely dominated by air flows, ventilation remains the preferred means of purifying workplace air.

Personal protection may also be recommended (extract from the INRS document):

The choice of personal protective equipment must be based on the best possible compromise between the highest level of safety that can be achieved and the need for the worker to carry out their task in conditions of maximum comfort. All personal protective equipment must be kept in good condition and any non-disposable equipment must be cleaned after each use.

- Respiratory protection (extract from the INRS document):

If local ventilation of the working atmosphere is inadequate, operators/users must wear breathing apparatus, bearing in mind that nanoscale objects may pass through the slightest leak (problem of tightness of the facepiece in contact with the face, perforation, etc.).

- *For short-duration work involving low exposure:*

A full-face free-breathing mask fitted with a P3 filter in accordance with the EN 143 standard or, possibly, a disposable FFP3 filtering mask in accordance with the EN 149 standard.

- *For work involving low exposure lasting more than one hour:*

[9] As specified in the INRS's dossiers (in French):
- Nanomaterials dossier (INRS, 2024): www.inrs.fr/risques/nanomateriaux
- "*Les nanomatériaux manufacturés - Définition, effets sur la santé, caractérisation de l'exposition professionnelle et mesures de prévention*", M. Ricaud and O. Witscheger, ED 6050, (December 2020)
- "*Nanomatériaux – Ventilation et filtration de l'air des lieux de travail*" M. Ricaud, S. Chazelet, E. Belut and D. Bemer (INRS), D. Thomas (CNRS Nancy), ED 6181 (November 2014)

A powered air-purifying respirator, either a half-mask (TM2 P), a full-face mask (TM3 P) or a hood (TH2 P) with power-assisted ventilation complying with the EN 12942 and EN 12941 standards and a minimum air flow of 160 L/min.

- *For work involving exposure:*

Self-contained breathing apparatus (= full-face mask, hood or full suit with compressed airline breathing apparatus).

- *Dermal protection*

The literature on the effectiveness of chemical protective clothing with regard to nanomaterials is still limited. Nevertheless, in view of the initial data, the wearing of type 5 chemical protective clothing (resistant to penetration by airborne solid particles) is recommended. Single-use clothing, in particular disposable hooded coveralls (or a coat) with tightening at the neck, wrists and ankles, without pleats or cuffs, and with flap pockets, is therefore recommended. Initial research indicates that butyl, vinyl or nitrile gloves provide an effective barrier to nanomaterials. In the case of prolonged and repeated dermal exposure or work likely to damage gloves, the wearing of two pairs of gloves or thicker gloves is recommended. Shoe covers are also necessary to prevent contamination of areas outside the work premises.

3.4. Additional tests to be carried out

In Regulation (EU) No 284/2013 setting out the data requirements for plant protection products, the determination of particle size distribution is required for products in DP, SC and CS form. The recommended methods are the CIPAC[10] MT 170 and CIPAC MT 187 methods. The CIPAC MT 170 method is a preliminary method that indicates whether the MT 187 method should be carried out (based on the results of the MT 170 method). It involves gravimetric measurement of particles passing through a sieve of defined size (40–100 µm). If more than 1% of particles (mass/mass) are quantified, the CIPAC MT 187 method must be carried out. It consists in analysing the particles by laser diffraction, enabling particles between 0.01 µm and 4 mm in size to be measured. These methods are unable to determine the distribution of all the nanoscale particles that may be found in the products.

The methods recommended in the regulations applicable to plant protection products therefore need to be revised to bring them into line with current regulations on chemicals: REACH, in particular ECHA's guidance documents Chapter R7a (7.1.14 Granulometry) and Appendix R7-1 specific to nanomaterials, which specify suitable methods for measuring the granulometry of mixtures/solid substances.

[10] CIPAC: Collaborative International Pesticides Analytical Council

In addition, distribution measurements are currently required for solid products and liquid products in the form of suspension concentrates and capsule suspensions. On the other hand, no particle size distribution measurement is required for solid active substances (powders or granules). These requirements should also be added to the regulatory requirements concerning the tests to be submitted.

In contrast, Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products includes the requirement to submit measurements of particle distribution for all products containing particles and for all solid active substances in powder or granule form. The recommended methods correspond to those indicated in ECHA's REACH Regulation guidance document (Chapter R7a, 7.1.14 Granulometry). These methods are suitable for measuring the granulometry of solid products/substances.

3.5. Regulatory background

3.5.1. Nanomaterials in the context of the regulations on plant protection products and biocides

Unlike the Plant Protection Products Regulation, the Biocidal Products Regulation (BPR) contains specific provisions for nanomaterials. These provisions apply to products and substances that meet the criteria defined in the BPR. These definitions are based on the 2011 Commission Recommendation on the definition of nanomaterials:

- 50% or more of the particles have a size of between 1 and 100 nanometres in at least one dimension;
- the particles are in an unbound state or as an aggregate or agglomerate.

However, the Regulation stipulates that the European Commission should regularly review the provisions on nanomaterials in the light of scientific progress. It should be noted that the Commission published a new recommendation on a definition in 2022 (see Section 1. Background and purpose of the request).

According to the BPR, the approval of an active substance shall not cover the nanoform of that active substance, except where explicitly mentioned. However, when nanomaterials are used in products, the risks to human health, animal health and the environment must be examined separately.

A specific risk assessment is needed when the nanoform of both the active substance and the non-active substance is used in a biocidal product. The label of the biocidal product must indicate the name of each nanomaterial followed by the word "nano" in brackets.

3.5.2.EFSA "Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles, including nanoparticles"

The nanospecific assessment of pesticides is addressed in an EFSA document ("Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles[11]), defining the criteria for assessing the presence of a fraction of small particles, and setting out information requirements for applications in the regulated food and feed product areas (e.g. novel foods, food/feed additives, food contact materials and pesticides). These requirements apply to particles requiring specific assessment at the nanoscale in conventional materials that do not meet the definition of engineered nanomaterial as set out in the Novel Food Regulation (Regulation (EU) 2015/2283). The guidance document outlines appraisal criteria to confirm whether or not the conventional risk assessment should be complemented with nanospecific considerations.

This EFSA guidance on particles is applicable to all chemical materials – marketed or to be marketed as substances or mixtures – to be assessed by EFSA, including mixtures and products marketed as liquid formulations unless the information confirms that they are true liquids and do not contain small particles in suspension. The characterisation of the fraction of small particles, including the particle size distribution, is needed in all cases unless the applicant demonstrates that the material will be fully dissolved under the intended use conditions and consumers will not be exposed to particles. For multi-constituent substances and mixtures, the information to be submitted should cover each single constituent or each component in the mixture, as well as the multi-component material.

In the case of (a) botanicals and other chemically complex materials of biological origin with unknown or variable composition, (b) macromolecules of biological origin (e.g. enzymes and other proteins) or (c) other similar cases, the applicant should provide a rationale demonstrating that an assessment of the fraction of small particles including nanoparticles is not needed, or that is already covered in the safety assessment process.

The general principles and the summary of appraisal routes that applicants may follow to confirm that a fraction of small particles is either not present or covered by the conventional risk assessment are presented in this guide.

This guidance document on particles supplements EFSA's "Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: human and animal health"[12].

[11] *EFSA Journal* 2021;19(8):6769, 48 pp. https://doi.org/10.2903/j.efsa.2021.6769

[12] *EFSA Journal* 2021;19(8):6768 – https://doi.org/10.2903/j.efsa.2021.6768

3.5.3.Literature review commissioned by ECHA

A summary of the information available on nano-formulated plant protection products, biocides and fertilisers was commissioned by ECHA, as a contribution to the work of the European Union Observatory for Nanomaterials (EUON). This summary, produced by Innovamol Srl (Italy), was published in 2024[13].

It begins with an exhaustive review of the scientific and grey literature. The vast majority of the documents listed relate to the activity, efficacy and characterisation of nano-formulated substances and products. Few documents on toxicity or ecotoxicity were identified, and none on the placing on the market of such products in Europe.

The second part presents an analysis of the collected data, detailing the results by use and substance or formulation type.

The results of a survey of experts and stakeholders are also presented. The questions asked related to the possible uses of nano-formulated products in agrochemicals, the expected benefits and concerns associated with these uses, as well as the potential developments and advances considered with the implementation of such formulations.

The first part of the discussion concerns the adequacy of current practices and regulatory frameworks with regard to the application of nanomaterials in products intended for agriculture. The discussion focuses in particular on analytical techniques, quantification and sampling methods, and information on toxicity and ecotoxicity.

The second part of the discussion focuses on the possibility of improving the quantity and quality of information on nano-formulated products, from both a technical and a legal perspective, with regard to the regulations on biocidal products (Regulation (EU) No 528/2012), plant protection products (Regulation (EC) No 1107/2009) and fertilisers (Regulation (EU) 2019/1009).

Lastly, a number of recommendations on alternative approaches are proposed as part of this work commissioned by ECHA:

- *Include specific provisions for nano-formulated products in the current regulations, following the example of the Biocidal Products Regulation and the guidance documents published by EFSA in 2021;*
- *Create a framework for standardised instructions for use;*
- *Create a specific database at European level, along the lines of the R-Nano scheme;*
- *Establish a notification system for manufacturers and importers, and a "common entry gate" for submitting information to the regulatory authorities;*
- *Conduct literature searches to justify efficacy claims;*
- *Mandate the submission of toxicity and ecotoxicity tests.*

[13] Urbani D, Evangelisti M, Bebi C, Rovegno C, Parenti MD, Varchi G, Del Rio A. Collection and review of information on nanomaterial-based and nano-enabled plant protection products, biocidal products and fertilising products. 2024. Ref. ECHA/2022/512. https://doi.org/10.5281/zenodo.14546239

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

ANSES issued an internal request to analyse the results of an exploratory study that sought to measure the presence of nanoparticles in plant protection products and biocidal products and to propose, where appropriate, management measures to protect potentially exposed populations and the environment. The aim of this exploratory study was not to conduct a quantitative risk analysis.

In this exploratory study, the plant protection products (both the solid products and the liquid products in the form of suspension concentrates), as well as all but one of the solid biocidal products tested, contained particles smaller than 100 nm. The proportion and probable nature of the particles also varied widely according to product type and composition.

For the **plant protection products in capsule suspension form**, it was difficult to reach a general conclusion on the presence of particles smaller than 100 nm, because the conditions under which the samples were prepared probably modified the physical state of the capsules in the tested products. **Particles smaller than 100 nm were detected in some products, albeit in proportions too low to determine a size distribution for these particles observed in the tested samples.**

It should be noted that, for solid products, the techniques used to determine particle distributions are unable to measure the primary particles and do not cover all the particle sizes present in the products (limited to 20–570 nm and 1–20 µm), and therefore did not allow the proportions to be quantified.

For the liquid products, it was not possible to obtain the distributions for all the particles because the technique used is only able to measure the distribution for particles of the same chemical nature.

The wettable powder (WP), suspension concentrate (SC) and capsule suspension (CS) plant protection products tested all need to be diluted before use. Once the solution has been obtained, it is sprayed under pressure using suitable equipment. During spraying and once on the plants and in the environment, the substances may be subjected to various phenomena (heat, dilution, mainly due to the impact of precipitation, evaporation) that may modify the form of the particles.

Therefore, for the types of formulations mentioned above, the results obtained for the products as sold cannot be extrapolated to the residues of these products during and after application.

DP plant protection products are not diluted before use. However, they are applied using dusting equipment fitted with an air pump. As with the products to be diluted, the form of the particles present may be modified during application. Therefore, **the results obtained for the products as sold cannot be directly extrapolated to the residues of these products during and after application.**

The **biocidal products tested were ready-to-use products. Nevertheless, some products can be applied by manual dusting, while others are in the form of aerosol sprays**. In these cases, since the tested products corresponded to the products once sprayed, the analyses were carried out on the products as applied and not on the products as sold.

Therefore, **the results obtained for these products (aerosols and powder) can be extrapolated to the residues of these products after application. However, as the particles can interact with the matrices to which they are applied, a change in the form of these particles cannot be ruled out.**

Given the current state of knowledge on the possible changes in particle form, for example after the product is diluted and depending on the application method used, there is considerable uncertainty about the exposure of people and organisms in the environment.

The Biocidal Products Regulation contains specific provisions for nanomaterials, unlike the Plant Protection Products Regulation.

In the absence, to date, of any provisions in the plant protection product regulations or any dedicated nanospecific risk assessment methodology, ANSES recommends **reducing as much as possible the nanoparticles that may be found in products**, when exposure cannot be considered negligible for people and ecosystems despite the application of management measures. To achieve this, particular attention should be paid to the specifications of the active substances or co-formulants used. Minimising exposure at source is appropriate given the high uncertainty surrounding nanoparticle exposure levels and the complexity of assessment methodologies, particularly for risks to the environment.

With regard to **particle size characterisation**, ANSES recommends that **the requirements for tests to be submitted under the regulations on plant protection substances and products include the methods recommended in the current regulations on chemicals (REACH Regulation)**, in particular the guidance documents Chapter R7a (7.1.14 Granulometry) and Appendix R7-1 specific to nanomaterials, which specify suitable methods for measuring the granulometry of solid products/substances.

The results of this exploratory study will be brought to the attention of the Member States, EFSA, ECHA and the European Commission in order to define the improvements to be implemented. EFSA is currently working on updating its guidance document on the risk assessment of

nanomaterials in the food chain and on developing a new guidance document[14]. ANSES had also produced its own guide [ANSES, 2021][15] and will contribute to this process with a view to harmonisation.

It should also be noted that research projects, particularly those conducted within the European Partnership for the Assessment of Risks from Chemicals (PARC), may be able to improve knowledge of assessment methodologies and the effects on health and the environment.

Pr Benoit Vallet

[14] Guidance document for risk assessment of nanomaterials and materials containing nanoparticles in the food chain, and a new Guidance document for the risk assessment of feed additives containing nanoparticles
https://open.efsa.europa.eu/questions/EFSA-Q-2024-00439
https://open.efsa.europa.eu/questions/EFSA-Q-2024-00724

[15] Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on a specific health risk assessment guide for nanomaterials in food products. 30 September 2021. https://www.anses.fr/fr/system/files/ERCA2016SA0226.pdf

KEY WORDS

Produits phytopharmaceutiques, nanoparticules, étude exploratoire

Plant protection products, nanoparticles, exploratory study

SUGGESTED CITATION

ANSES. (2025). Analysis of the results of an exploratory study seeking to measure the presence of nanoparticles in plant protection products and biocidal products and proposing, where appropriate, management measures to protect potentially exposed populations and the environment. Request No. 2024-SA-0038. Maisons-Alfort: ANSES, 71 p.

ANNEX 1

Presentation of the participants

PREAMBLE: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity, *intuitu personae*, and do not represent their parent organisation.

EXPERT COMMITTEE

The work that is the subject of this opinion was monitored and adopted by the following Expert Committee:

- **CES on "Plant protection substances and products, biocontrol"** – 14/01/2025

Chair

Mr Jean-Ulrich MULLOT – Military Pharmacist (Military Health Service). Speciality: toxicology, risk assessment, regulations, radionuclides, analytical chemistry

Vice-Chair

Ms Laure MAMY – Research Director (National Research Institute for Agriculture, Food and the Environment – INRAE). Speciality: environmental fate of pesticides, modelling

Members

Mr Marcel AMICHOT – Research Officer (INRAE). Speciality: biocontrol (mode of action), ecotoxicology, resistance

Mr Marc BARDIN – Research Director (INRAE). Speciality: phytopathology, plant protection, microbiology, biocontrol, efficacy, mode of action

Mr Philippe BERNY – Teacher-Researcher (VetAgro Sup). Speciality: pesticides, terrestrial vertebrates, ecotoxicology, rat poisons, insecticides

Mr Romain BONAFOS – Engineer in charge of training on plant protection (Institut Agro Montpellier). Speciality: pests, non-target insects, resistance, plant protection methods, macro-organisms

Mr Bruno CHAUVEL – Research Director (INRAE). Specialities: weed science, integrated management, invasive plants, weed control, herbicide resistance, agroecology

Mr Jean-Pierre CUGIER – Retired from the Ministry of Agriculture, Senior Scientific Officer (European Food Safety Authority) until 30/09/2016. Speciality: pesticide residues, plant and animal metabolism, consumer safety (chronic and acute risk)

Ms Caroline DE CLERCK – Assistant Professor in Agronomy and Microbial Ecotoxicology (University of Liège, Gembloux Agro-Bio Tech). Speciality: biocontrol, phytopathology, weed science, plant nutrition, production systems, microbial ecotoxicology

Mr Georges DE SOUSA – Senior Research Engineer (INRAE). Speciality: toxicology, ADME of xenobiotics, endocrine disruptors, mixture effects, modelling

Mr Marc GALLIEN – Project Officer, Agricultural Mutual Insurance Scheme (MSA). Speciality: application of plant protection products, occupational health and safety, prevention, plant protection

Ms Sonia GRIMBUHLER – Researcher in exposure assessment (INRAE). Speciality: assessment of exposure of farmers and local residents – agricultural machinery – metrology risk assessment

Mr François LAURENT – Research Officer (INRAE). Speciality: metabolism, organic compound residues, environmental contamination, plant physiology

ANNEX 2: TEXT OF THE INTERNAL REQUEST 024-AUTO-0038

RÉPUBLIQUE FRANÇAISE
Liberté
Égalité
Fraternité

anses

Decision No 2024-046

INTERNAL REQUEST

The Director General of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES),

Having regard to the Public Health Code, and in particular its Article L.1313-3 giving ANSES the prerogative to issue an internal request on any question with a view to accomplishing its missions,

Has decided the following:

Article 1: The French Agency for Food, Environmental and Occupational Health & Safety is issuing an internal request to conduct an expert appraisal whose characteristics are listed below.

1.1 Themes and objectives of the expert appraisal

It involves analysing the results of an exploratory study seeking to measure the presence of nanoparticles in plant protection products and biocidal products and proposing, where appropriate, management measures to protect potentially exposed populations and the environment. Proposals for regulatory changes are also called for, with the aim of improving methodologies enabling a better characterisation of the nanoparticles that may be found in these products.

1.2 Background of the internal request

The R-Nano 2016 data regularly show numerous declarations for which the descriptor PC 27 "Plant protection products" has been entered.

In order to clarify these declarations, additional checks were carried out. These found that the reasons given for these declarations in R-Nano concerned almost exclusively the presence of co-formulants in nanoparticle form.

Following this observation, ANSES commissioned an exploratory study to measure the presence of nanoparticles potentially contained in plant protection products and biocides.

1.3 Questions on which the expert appraisal work will focus

1) Analyse the results of the exploratory study carried out on plant protection products and biocides.

2) Based on the results of the product analyses, indicate whether the results obtained on products as sold (undiluted form) can be extrapolated to products as used (generally in diluted form) or to their residues after application.

3) Depending on the results of the analysis conducted for the first point above, specify whether management measures should be taken for potentially exposed populations, in order to reduce exposure to nanoparticles from the use and application of plant protection products and biocides.

4) In order to better characterise the distribution of the particles found in the products, indicate the types of additional tests that could be added to the regulatory requirements. The formulation types should be taken into account.

1.4 Estimated duration of the expert appraisal

12 months

Article 2: An opinion will be issued and published by the Agency following completion of the work.

Signed in Maisons-Alfort on 09 April 2024

Pr Benoit VALLET

Director General

ANNEX 3: REPORT ON THE EXPLORATORY STUDY CONDUCTED BY THE LNE (2021)

TEST REPORT

Demandeur	Anses Direction de l'évaluation des produits réglementés 14, rue Pierre et Marie Curie 94701 Maisons Alfort Cedex
Date et référence de la commande	Marché XFTC000343
Objet	Analyse, quantification, et caractérisation en taille et en nombre des particules présentes dans 33 préparations phytosanitaires/biocides *Analysis, quantification, and characterization in terms of size and number concentrations for 33 phytosanitary and biocidal preparations*

APPROPRIATE QUALITY MANAGEMENT SYSTEM (GLP COMPLIANCE AND QUALITY ASSURANCE STATEMENT)

LNE has a fulfillment of the requirements of ISO/IEC 17025 (2005) which means that LNE meets both the technical competence requirements and management system requirements that are necessary for delivering technically valid test results and calibrations. LNE is also an organization certifying company management system according to ISO 9001, ISO 13485 (medical), ISO 14001 (environmental quality system), ISO 22000 (hygiene of materials and packaging for food products).

ABBREVIATIONS AND SYMBOLS

APS : Aerodynamic Particle Sizer

CaS : Capsules Suspension

CoS : Concentrated Suspension

CPC : Condensation Particle Counter

DMA : Differential Mobility Analyzer

DP : Dry Powder

MA : Marketing Authorization

PNSD : Particle Number Size Distribution

SEM : Scanning Electron Microscopy

SMPS : Scanning Mobility Particle Sizer

WP : Wet Powder

SUMMARY

As part of its mission to assess human health security risks in the areas of food, environment and work, ANSES launched a consultation corresponding to the analysis, quantification and characterization of particles in terms of size and number concentrations for phytosanitary and biocide preparations having marketing authorizations (MA) in France.
For the characterization of powder samples (phytopharmaceutical dry, wet and biocide, i.e. phytopharmaceutical DP, WP and biocide samples) in the aerosol phase, a dust disperser was coupled to two spectrometers to measure average normalized particle number size distribution (PNSD) for 20 nm – 570 nm and 1 µm – 20 µm size ranges respectively, without associated chemical analysis. About suspension samples in aqueous phase (phytopharmaceutical CoS and CaS samples), particle size distribution were characterized by SEM and chemical identification by EDX. This report presents the results of this study for samples given and identified by ANSES.

1. INTRODUCTION

The objective of this study was the characterization of particle number size distribution (PNSD) for a selection of 15 phytosanitary and 10 biocide preparations, and 8 suspension samples having marketing authorizations in France. Studied products were in the form of dry powder (DP), wet powder (WP), liquid in aerosol spray and suspension in aqueous phase (phytopharmaceutical concentrated suspensions (CoS) and capsule suspensions (CaS)). The sections below present for each studied sample, the associated PNSD analytical methods and the results in terms of PNSD measurements.

2. MATERIALS

2.1 Phytopharmaceutical DP, WP, biocide samples and associated references

Table 1 presents the phytopharmaceutical dry (DP) and wet (WP) powder samples (references 1 to 15). These references were studied in the aerosol phase using the analytical method presented in sections 3.1.1 and 3.2.1.

Table 1 : List of phytopharmaceutical dry (DP) and wet (WP) powder samples studied in aerosol phase

	Product name	Supplier	Active substance	Total mass/volume	Type	Packaging
Phytopharmaceutical WP						
1	[redacted]		Copper	1 kg	Fungicide	Bag
2			Copper	100 g	Fungicide	Bottle
3			Copper	55 g	Fungicide	Bottle
4			Copper	100 g	Fungicide, bactéricide	Bottle
5			Mancozeb + fosetyl	100 g	Fungicide	Bag
6			Gliocladium catenulatum J1446	1 kg	Fungicide	Jar
7			Bacillus subtilis QST 713	100 g	Fungicide, bactericide	Bottle
8			Trichoderma asperellum TV1	1 kg	Fungicide	Bag
9			Mancozeb + cymoxanil + cuivre	100 g	Fungicide	Bag
10			Bacillus subtilis QST 713	100 g	-	Bag
11			Hydrogénocarbonate de potassium	100 g	Fungicide	Bag
Phytopharmaceutical DP						
12	[redacted]		Sulfur	200 g	Fungicide	Bag
13			Sulfur	100 cm^3	Fungicide	Bottle
14			Sulfur	200 g	Fungicide	Bottle
15			Sulfur	1.5 kg	Fungicide	Bag

Table 2 presents the biocide samples (references 16 to 25). These references were studied in the aerosol phase using the analytical method presented in sections 3.1.1 and 3.2.1. It is important to mention that sample references 16 and 22 concern liquid samples contained in aerosol spray systems.

Table 2 : List of biocide sample studied in aerosol phase

	Product name	Supplier	Total mass/volume	Packaging
BIOCIDES				
16	[redacted]	[redacted]	500 mL / 321 g	Aerosol spray
17			250 g	Powder
18			100 g	Powder
19			125 g	Powder
20			100 g	Powder
21			150 g	Powder
22			400 mL	Aerosol spray
23			500 g	Powder
24			1 kg	Powder
25			350 g	Powder

2.2 Suspension samples and associated references

Table 3 presents the phytopharmaceutical concentrated (CoS) and capsules (CaS) suspensions (references 26 to 33). These references were studied by scanning electronic microscopy (SEM) using the analytical method presented in sections 3.1.2 and 3.2.2.

Table 3 : List of phytopharmaceutical concentrated (CoS) and capsules (CaS) suspensions studied by scanning electronic microscopy (SEM).

	Product name	Supplier	Active substance	Total volume	Type	Packaging
Phytopharmaceutical Capsules Suspensions (CaS)						
26	[redacted]	[redacted]	Lambda-Cyhalothrin	50 mL	Insecticide	Bottle
27			Clomazone	20 mL	Herbicide	Bottle
28			Geranol, thymol, eugenol	---	Fungicide	Bottle
29			Clomazone, metazachlor	30 mL	Herbicide	Bottle
30			Flurochloridone	30 mL	Herbicide	Bottle
Phytopharmaceutical Concentrated uspensions (CoS)						
31	[redacted]	[redacted]	Chlorothalonil, cymoxanil	20 mL	Fongicide	Bottle
32			Chlorotoluron	100 mL	Herbicide	Jar
33			Chlorotoluron, isoxaben	100 mL	Herbicide	Jar

3. METHODS

3.1 Analytical methods

3.1.1 Phytopharmaceutical DP, WP and biocide samples

For the characterization of phytopharmaceutical DP, WP and biocide samples in the aerosol phase, a dust disperser was coupled to two spectrometers respectively called Scanning Mobility Particle Sizer (SMPS, TSI) and Aerodynamic Particle Sizer (APS, TSI) (Figure 1) to measure PNSD from 20 nm to 20 µm without associated chemical analysis.

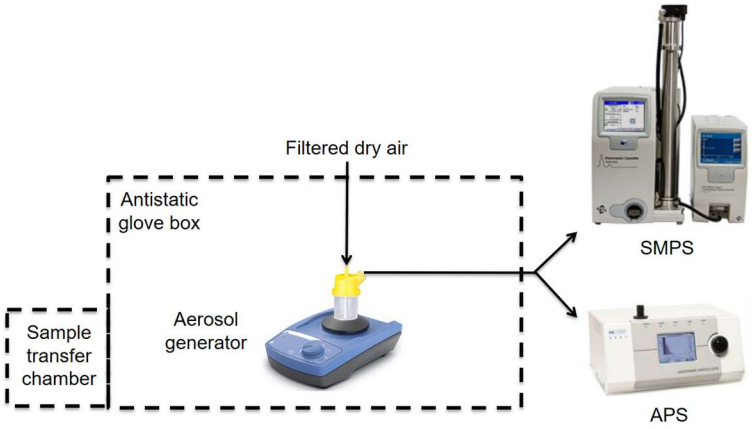


Figure 1 : Schematic bench test used for phytopharmaceutical DP, WP and biocide samples analysis

Concerning the aerosol generator, the dust disperser used for this study was single use venturi nebulizers (RN200, RESPINEB) coupled to a vortex shaker (Top-Mix 3, IKA). The dispersion of 5 to 10 cm^3 of each sample was performed by a nozzle suction from the nebulizer vial and fed into the carrier gas. Shear forces created in such injector disperse and deagglomerate the powder to form an aerosol. However, it is important to mention that agglomerates/aggregates generated in the aerosol phase will not give access to primary particles measurements as requested in the definitions of a nanomaterial at EU (Biocides Regulation n°528/2012) and French (mandatory declaration R-Nano) levels.

Downstream the generator, the scanning mobility particle sizer (SMPS) is an instrument for characterizing PNSD in the aerosol phase with a measurement range from 20 nm to 570 nm for this study. The SMPS is composed of a differential mobility analyzer (DMA), to select a diameter, and a condensation particle counter (CPC) to count the number of selected particles. Therefore, the SMPS allows PNSD measurements in terms of **electrical mobility diameter** SMPS measurements which were carried out in accordance with ISO15900.

The Aerodynamic Particle Sizer (APS) is based on a laser velocimetry principle for characterizing PNSD in the aerosol phase in a size range from 1 µm to 20 µm for this study. In the APS, particles are accelerated through an acceleration nozzle and the particle velocity is measured *via* an optical process. **An equivalent aerodynamic diameter is then deduced from this speed and APS allows PNSD measurements in terms of aerodynamic diameter**.

3.1.2 Phytopharmaceutical CoS and CaS suspensions

For the commercial pesticide products, the nanoparticles (NPs) are embedded in various controlled release systems (CRS) (A. Singh et al., 2020 [1]). The suspensions received were opaque, so the DLS technique could be not used for the study. Consequently, before their SEM characterization, they have to be extracted from the concentrated or nano-capsules suspension. After extraction step, the particles have been deposited on the silicon substrate and observed by SEM. SEM images have been performed with the CARMEN (LNE) platform's Zeiss ULTRA-Plus equipped of a Field Emission Gun (FEG) microscope and an in-Lens SE detector located within the column (Figure 2).

Figure 2 : SEM at LNE (Zeiss ULTRA-Plus equipped of a Field Emission Gun (FEG))

Size measurements of particles have been carried out by using specific semi-automatic routine PLATYPUS™ software developed by Pollen Metrology. A total of 100 up to 150 particles was taken into consideration to build number size distribution. For all measurements the minimum Feret diameter was considered and the average values were determined from the number-based particle size distribution. The minimum Feret diameter (Figure 3) has been chosen because it corresponds to the smallest dimension of the measured object as recommended by the EU biocide regulation [2].

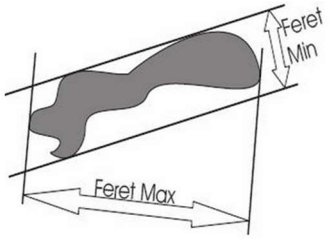


Figure 3 : Illustration of minimum Feret diameter

The chemical analysis has been performed through an EDX detector ULTIM MAX (65 mm^2) in SATW window from Oxford Instruments installed on the SEM which recovers photons from the particles.

[1] Amrita Singh, et al., Advances in controlled release pesticide formulations: Prospects to safer integrated pest management and sustainable agriculture, Journal of Hazardous Materials, Volume 385,2020, 121525, ISSN 0304-3894, https://doi.org/10.1016/j.jhazmat.2019.121525.

[2] According to the EU Biocides Regulation 528/2012 (EU BPR), nanomaterials are defined as a natural or manufactured active substance or non-active substance containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1-100 nm.

3.2 Specimen handling

A “Certiphyto” training was performed for the “operator category” in order to obtain individual certificates for the professional use of phytosanitary products.

3.2.1 Phytopharmaceutical DP, WP and biocide samples

For safety reason, samples were handled inside an antistatic glove box equipped with a transfer chamber. The previously described aerosol generator was also implemented inside the glove box and connections to SMPS and APS were performed in an external way (Figure 1).

3.2.2 Phytopharmaceutical CaS and CoS suspensions

The extraction method depends on the nature of the studied pesticide. Four protocols have been developed to assess to the “nano” aspect of a pesticide substance described below.

(a) Static deposition: the initial suspension was homogenized and a droplet was directly deposited on a silicon wafer substrate.
(b) Initial suspension and spin-coater deposition: the initial suspension was homogenized and then, a droplet of particle suspension was deposited on the center of the silicon wafer substrate and put on the spin coater. The spin coater used is a LabSpin 6 SUSS Microtec.
(c) Diluted suspension and deposition by spin-coater : the initial suspension was diluted (x100) in Milli-Q water and homogenized. Then, a droplet of particle suspension was deposited on the center of the silicon wafer substrate fixed on the spin coater (60 s at 1000 rpm for spreading and 10s at 8000 rpm for drying).
(d) Diluted suspension with step of washing and deposition by spin coater: the suspension was washed five times by following these steps:
    i. Dilution step in Milli-Q® water (X100),
    ii. Centrifugation at 4500 rpm during 20 min,
    iii. Removal of the supernatant and replacement with fresh Milli-Q® water,
    iv. Ultra-sonication was conducted using a Vibracell 75043 Ultrasonifier (750 W, 20 kHz, Fischer Bioblock Scientific, 13 mm horn). The dispersions were sonicated in a cold-water bath maintained at a constant temperature. Suspension has been sonicated with a probe-sonicator (20 min with cycles of 10 s ON with amplitude of 20% and 30 s OFF).
    v. Deposition by using spin coater (60 s at 1000 rpm for spreading and 10s at 8000 rpm for drying).

4. RESULTS

4.1 Phytopharmaceutical DP, WP and biocide samples

In terms of PNSD measurements, average normalized PNSD were calculated from 5 SMPS and 5 APS PNSD measured for each sample involved in this study. The error bars correspond to the standard deviations (STD) calculated on the five measurements performed for each sample (k = 1). PNSD were normalized with regard to the total particle number concentration measured by SMPS and APS for each sample in order to obtain comparable PNSD concentration scales. Therefore, normalized PNSD allow comparison and classification of each studied sample in terms of particle sizes. For each average normalized PNSD, sub-

groups were identified in order to classify PNSD and an analysis of associated statistical diameters is presented.

Figure 4 presents group n°1 of average PNSD for phytopharmaceutical WP samples. SMPS measurements are presented on the left hand side graph with a particle size range of 20 nm - 570 nm. APS measurements are presented on the right hand side graph with a particle size range of 1 µm - 16 µm with a dedicated zoom between 1 µm and 7 µm.

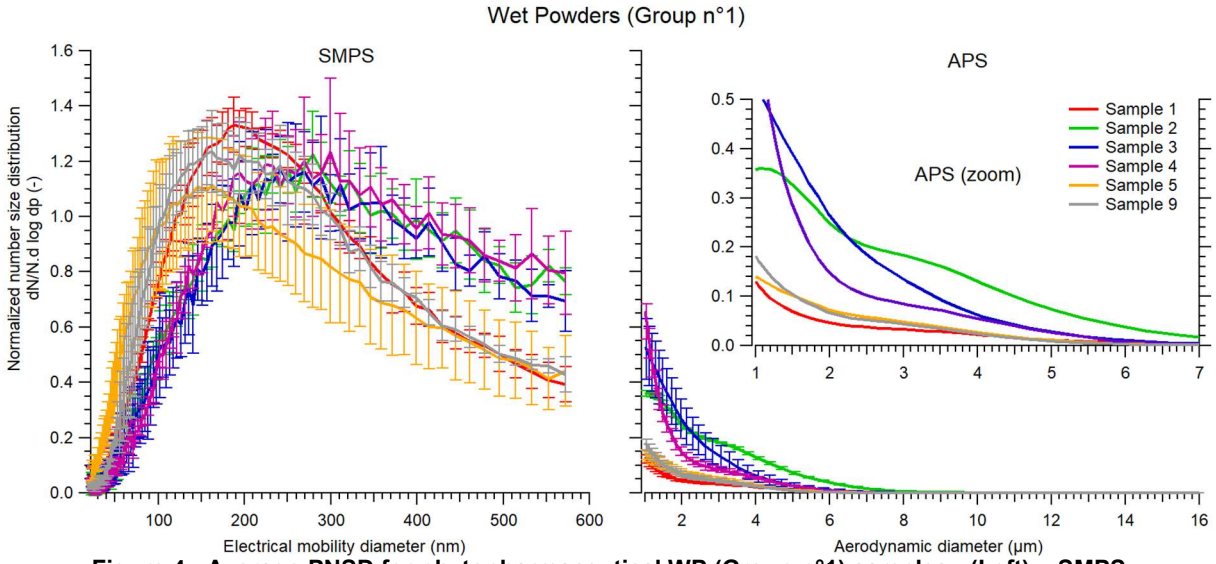


Figure 4 : Average PNSD for phytopharmaceutical WP (Group n°1) samples : (Left) - SMPS measurements between 20 nm and 570 nm ; (Right) - APS measurements between 1 µm and 16 µm. Error bars of each graph represent standard deviation (STD).

Associated statistical diameters are presented in Table 4 in terms of median, mean and modal diameters. It is important to point out that only sample 2 was characterized by a bimodal PNSD with SMPS and APS average modal diameters of 264 nm ± 17 nm and 1.10 µm ± 0.05 µm respectively.

Table 4 : SMPS and APS statistical diameters associated to average PNSD for phytopharmaceutical WP (Group n°1) samples.

WP (Group n°1)		SMPS statistical diameter (nm)			APS statistical diameter (µm)		
		Median	Mean	Modal	Median	Mean	Mode
Sample 1	Average	183	208	200	1.50	2.00	---
	STD	6	7	24	0.05	0.05	---
Sample 2	Average	227	250	264	1.70	2.20	1.10
	STD	2	1	17	0.05	0.05	0.05
Sample 3	Average	219	242	250	1.40	1.90	---
	STD	22	18	47	0.05	0.05	---
Sample 4	Average	227	250	266	1.30	1.80	---
	STD	5	5	71	0.05	0.05	---
Sample 5	Average	151	187	141	1.50	2.00	---
	STD	10	10	19	0.05	0.05	---
Sample 9	Average	172	203	186	1.40	1.90	---
	STD	9	7	50	0.05	0.05	---

From average PNSD for group n°1 of phytopharmaceutical WP samples, references 1, 2, 3, 4, 5 and 9 have particulate contribution < 100 nm of 21%, 15%, 16%, 14%, 32% and 7% respectively in the 17 nm – 570 nm SMPS measurement size range. **It is important to mention that such measurements in aerosol phase are associated to aggregates/agglomerates measurements and not to primary particles.**

Figure 5 presents group n°2 of average PNSD for phytopharmaceutical WP samples. SMPS measurements are presented on the left hand side graph with a particle size range of 20 nm - 570 nm. APS measurements are presented on the right hand side graph with a particle size range of 1 µm - 16 µm with a dedicated zoom between 1 µm and 7 µm.

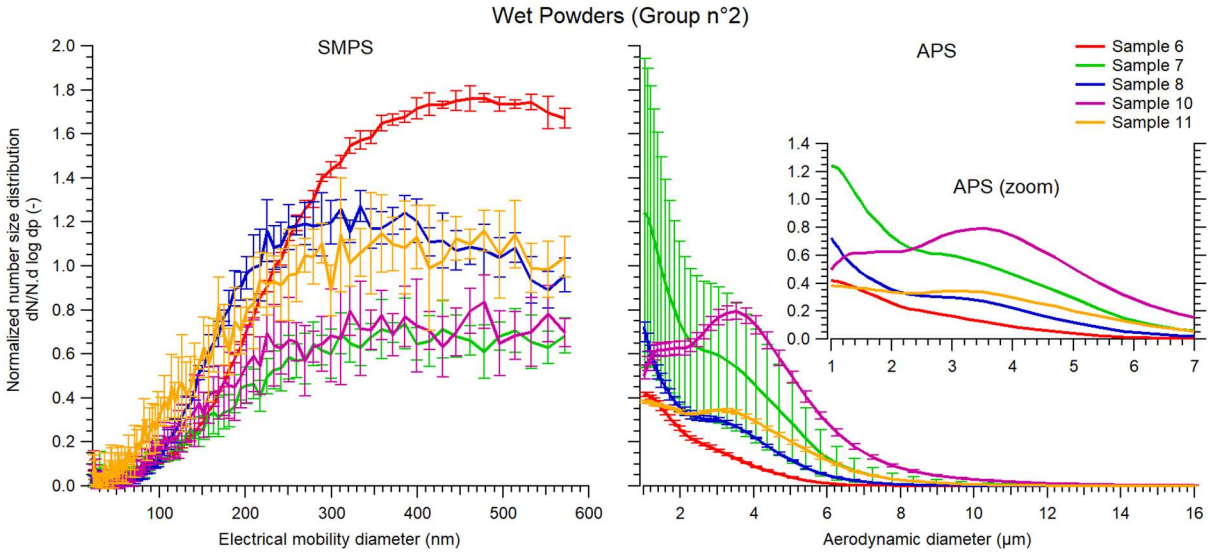


Figure 5 : Average PNSD for phytopharmaceutical WP (Group n°2) samples : (Left) - SMPS measurements between 20 nm and 570 nm ; (Right) - APS measurements between 1 µm and 16 µm. Error bars of each graph represent measurement standard deviation (STD).

Associated statistical diameters are presented in Table 5 in terms of median, mean and modal diameters. It is important to point out that sample 10 and 11 were characterized by bimodal PNSD with SMPS average modal diameters of 382 nm ± 65 nm and 370 nm ± 77 nm respectively, and with APS average modal diameters of 3.5 µm ± 0.1 µm and 3.1 µm ± 0.1 µm respectively.

Table 5 : SMPS and APS statistical diameters associated to average PNSD for phytopharmaceutical WP (Group n°2) samples.

WP (Group n°2)		SMPS statistical diameter (nm)			APS statistical diameter (µm)		
		Median	Mean	Modal	Median	Mean	Mode
Sample 6	Average	336	332	480	1.50	2.00	---
	STD	3	3	42	0.05	0.05	---
Sample 7	Average	289	296	454	1.70	2.30	---
	STD	8	7	46	0.10	0.10	---
Sample 8	Average	283	297	313	1.70	2.20	---
	STD	1	2	60	0.05	0.05	---
Sample 10	Average	276	288	382	2.60	3.00	3.50
	STD	17	13	65	0.05	0.05	0.10
Sample 11	Average	265	277	370	2.20	2.70	3.10
	STD	10	5	77	0.05	0.05	0.10

From average PNSD for group n°2 of phytopharmaceutical WP samples, references 6, 7, 8, 10 and 11 have particulate contribution < 100 nm of 11%, 6%, 23%, 11% and 13% respectively in the 17 nm – 570 nm SMPS measurement size range. **It is important to mention that such measurements in aerosol phase are associated to aggregates/agglomerates measurements and not to primary particles.**

Figure 6 presents average PNSD for phytopharmaceutical DP samples. SMPS measurements are presented on the left hand side graph with a particle size range of 20 nm - 570 nm. APS measurements are presented on the right hand side graph with a particle size range of 1 µm - 16 µm with a dedicated zoom between 1 µm and 8 µm and with a different concentration scale for sample 15 (in purple).

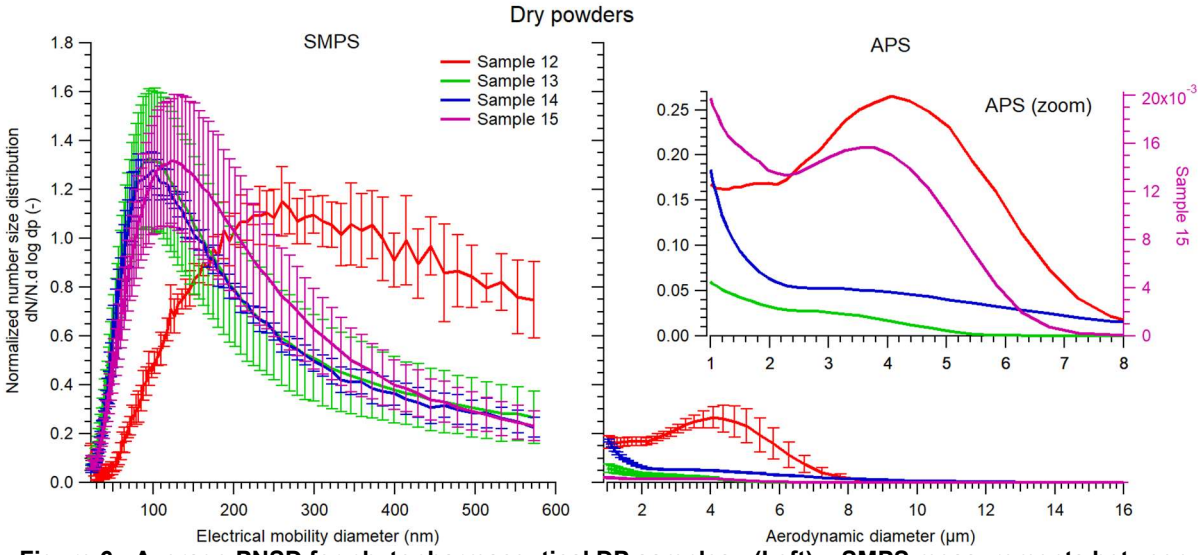


Figure 6 : Average PNSD for phytopharmaceutical DP samples : (Left) - SMPS measurements between 20 nm and 570 nm ; (Right) - APS measurements between 1 µm and 16 µm. Error bars of each graph represent standard deviation.

Associated statistical diameters are presented in Table 6 in terms of median, mean and modal diameters. It is important to point out that sample 12 and 15 were characterized by bimodal PNSD with SMPS average modal diameters of 249 nm ± 19 nm and 123 nm ± 7 nm respectively, and with APS average modal diameters of 4.2 µm ± 0.4 µm and 3.6 µm ± 0.2 µm respectively.

Table 6 : SMPS and APS statistical diameters associated to average PNSD for phytopharmaceutical DP samples.

DP		SMPS statistical diameter (nm)			APS statistical diameter (µm)		
		Median	Mean	Modal	Median	Mean	Mode
Sample 12	Average	221	242	249	2.70	3.20	4.20
	STD	12	10	19	0.20	0.20	0.40
Sample 13	Average	111	148	96	1.70	2.10	---
	STD	5	7	6	0.10	0.10	---
Sample 14	Average	113	150	99	1.80	2.70	---
	STD	3	3	11	0.05	0.05	---
Sample 15	Average	127	159	123	2.10	2.60	3.60
	STD	4	3	7	0.10	0.10	0.20

From average PNSD for phytopharmaceutical DP samples 12, 13, 14 and 15, particulate contribution < 100 nm was characterized to be 18%, 46%, 45% and 38% respectively in the 17 nm – 570 nm SMPS measurement size range. **It is important to mention that such measurements in aerosol phase are associated to aggregates/agglomerates measurements and not to primary particles.**

Figure 7 presents group n°1 of average PNSD for biocide samples. SMPS measurements are presented on the left hand side graph with a particle size range of 20 nm - 570 nm. APS measurements are presented on the right hand side graph with a particle size range of 1 µm - 20 µm with a dedicated zoom between 1 µm and 8 µm.

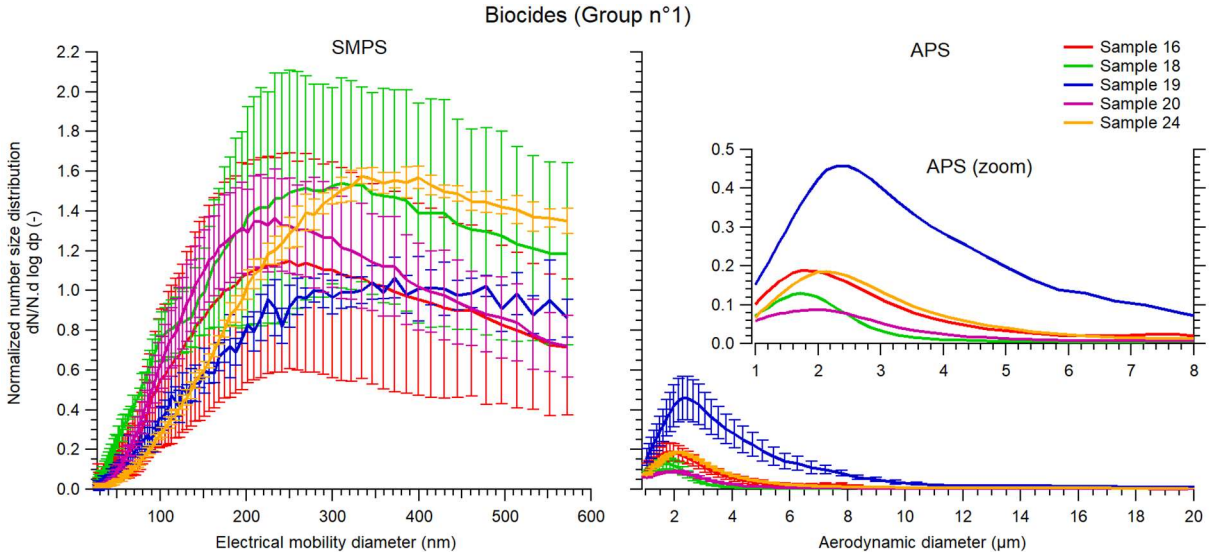


Figure 7 : Average PNSD for biocide (Group n°1) samples : (Left) - SMPS measurements between 20 nm and 570 nm ; (Right) - APS measurements between 1 µm and 20 µm. Error bars of each graph represent standard deviation.

Associated statistical diameters are presented in Table 7 in terms of median, mean and modal diameters. It is important to point out that all the samples of this Biocides – Group n°1 were characterized by bimodal PNSD from SMPS and APS measurements.

Table 7 : SMPS and APS statistical diameters associated to average PNSD for biocide (Group n°1) samples.

Biocides (Group n°1)		SMPS statistical diameter (nm)			APS statistical diameter (µm)		
		Median	Mean	Modal	Median	Mean	Mode
Sample 16	Average	208	229	200	1.90	2.60	1.90
	STD	20	16	107	0.10	0.10	0.10
Sample 18	Average	218	241	288	1.70	2.30	1.70
	STD	23	16	59	0.05	0.05	0.10
Sample 19	Average	251	265	408	2.60	3.40	2.40
	STD	12	8	83	0.10	0.10	0.10
Sample 20	Average	215	240	214	1.90	2.50	2.0
	STD	3	2	19	0.05	0.05	0.1
Sample 24	Average	289	296	373	2.10	2.80	2.20
	STD	6	6	31	0.05	0.10	0.10

From average PNSD for group n°1 of biocides samples, references 16, 18, 19, 20 and 24 have particulate contribution < 100 nm of 22%, 36%, 20%, 16% and 9% respectively in the 17 nm – 570 nm SMPS measurement size range. **It is important to mention that such measurements in aerosol phase are associated to aggregates/agglomerates measurements and not to primary particles.**

Figure 8 presents group n°2 of average PNSD for biocide samples. Average SMPS measurement is presented on the left hand side graph with a particle size range of 20 nm - 570 nm for sample 17 only, since average SMPS PNSD for sample 21 was considered as not significant. For both samples, APS measurements are presented on the right hand side graph with a particle size range of 1 µm - 20 µm.

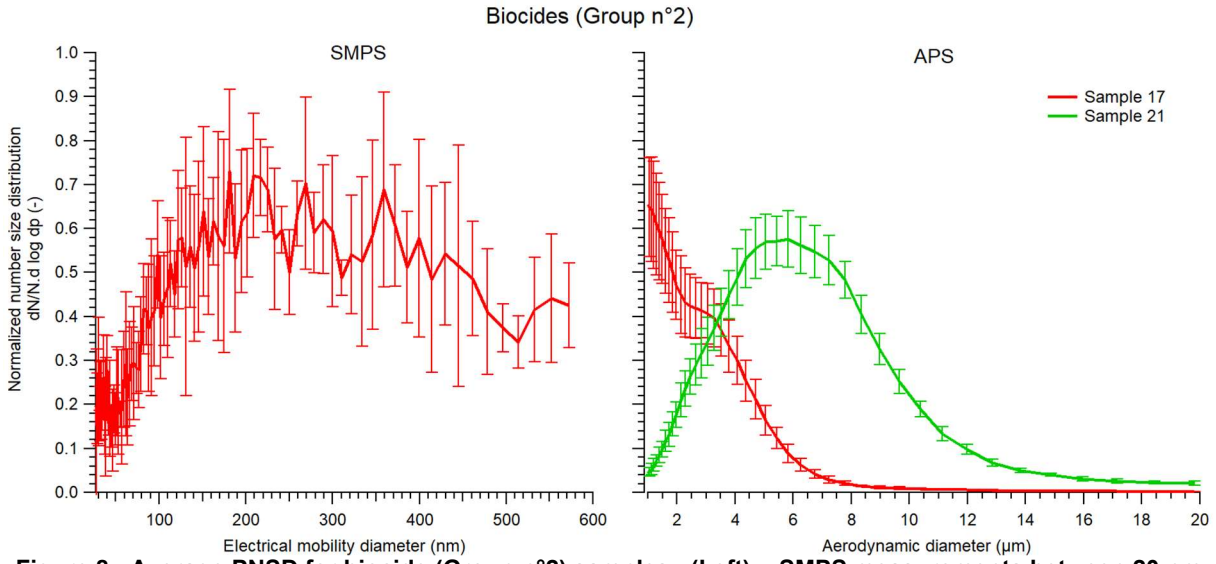


Figure 8 : Average PNSD for biocide (Group n°2) samples : (Left) - SMPS measurements between 20 nm and 570 nm ; (Right) - APS measurements between 1 µm and 20 µm. Error bars of each graph represent standard deviation.

Associated statistical diameters are presented in Table 8 in terms of median, mean and modal diameters. It is important to point out that sample 21 was characterized by an APS PNSD with an average modal diameter of 5.8 µm ± 0.3 µm.

Table 8 : SMPS and APS statistical diameters associated to average PNSD for biocide (Group n°2) samples.

Biocides (Group n°2)		SMPS statistical diameter (nm)			APS statistical diameter (µm)		
		Median	Mean	Modal	Median	Mean	Mode
Sample 17	Average	157	190	90	1.80	2.40	---
	STD	10	9	107	0.05	0.05	---
Sample 21	Average	---	---	---	4.50	5.30	5.80
	STD	---	---	---	0.10	0.10	0.30

From average PNSD for group n°2 of biocides samples, references 17 and 21 have particulate contribution < 100 nm of 15% and 36% respectively in the 17 nm – 570 nm SMPS measurement size range. **It is important to mention that such measurements in aerosol phase are associated to aggregates/agglomerates measurements and not to primary particles.**

Figure 9 presents group n°3 of average PNSD for biocide samples. SMPS measurements are presented on the left hand side graph with a particle size range of 20 nm - 570 nm. APS measurements are presented on the right hand side graph with a particle size range of 1 µm - 20 µm with a dedicated zoom between 1 µm and 7 µm.

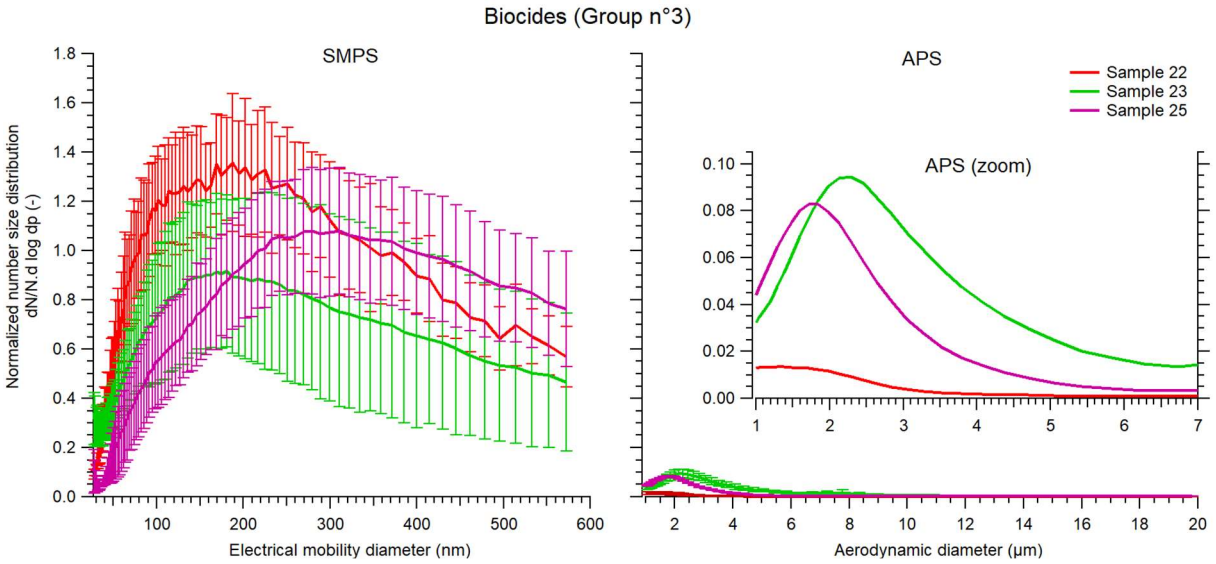


Figure 9 : Average PNSD for biocide (Group n°3) samples : (Left) - SMPS measurements between 20 nm and 570 nm ; (Right) - APS measurements between 1 µm and 20 µm. Error bars of each graph represent standard deviation.

Associated statistical diameters are presented in Table 9 in terms of median, mean and modal diameters. It is important to point out that all the samples of this Biocides – Group n°3 were characterized by bimodal PNSD from SMPS and APS measurements.

Table 9 : SMPS and APS statistical diameters associated to average PNSD for biocide (Group n°3) samples.

Biocides (Group n°3)		SMPS statistical diameter (nm)			APS statistical diameter (µm)		
		Median	Mean	Modal	Median	Mean	Mode
Sample 22	Average	152	188	192	1.50	2.30	1.20
	STD	5	3	18	0.10	0.10	0.20
Sample 23	Average	135	172	145	2.20	3.00	2.20
	STD	20	19	79	0.10	0.10	0.10
Sample 25	Average	189	216	173	1.80	2.40	1.80
	STD	26	22	145	0.05	0.05	0.10

From average PNSD for group n°3 of biocides samples, references 22 , 23 and 25 have particulate contribution < 100 nm of 29%, 39% and 31% respectively in the 17 nm – 570 nm SMPS measurement size range. **It is important to mention that such measurements in aerosol phase are associated to aggregates/agglomerates measurements and not to primary particles.**

4.2 Phytopharmaceutical CoS and CaS samples

All samples have been prepared (dilution, drying and ultrasonication) before measurements, except for static deposition. Note that the phase of sample preparation can generate modifications like opening shell.

It should be remembered that the study consists in only targeting particles smaller than 100 nm, identifying them and then measuring their size distribution.

4.2.1 Sample reference: [redacted] Sample 26

[redacted] The nano-capsules have been developed as insecticide carrier for lambda-cyhalothrine ($C_{23}H_{19}ClF_3NO_3$), as active ingredient (Figure 10).

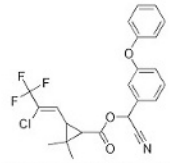


Figure 10 : Chemical formula of the active substance

SEM images of particles of various shapes (fibrillar and spherical structure) are reported on Figure 11, corresponding to the static deposition without ultrasonication. Isolated particles of different shapes and agglomerates (3)/ aggregates (4) are observed.

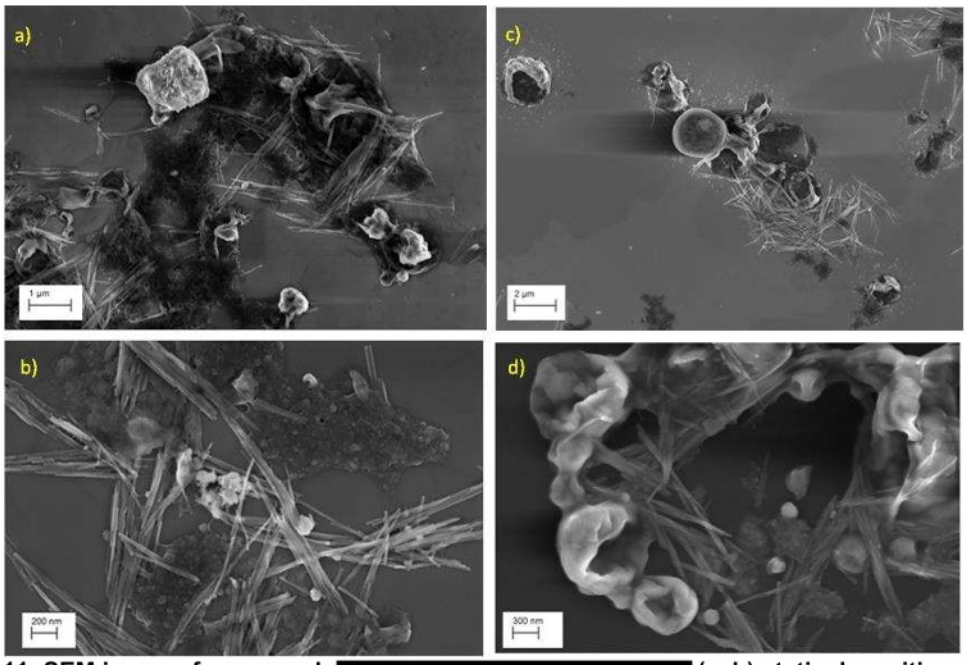


Figure 11: SEM images from sample [redacted] (a, b) static deposition without ultrasonication step and (c, d) diluted suspension with step of washing, ultrasonication and spin coater deposition

[3] ISO/TS 80004-2:2015 - Agglomerate: collection of weakly or medium strongly bound *particles* where the resulting external surface area is similar to the sum of the surface areas of the individual components

[4] ISO/TS 80004-2:2015 - Aggregate: *particle* comprising strongly bonded or fused particles where the resulting external surface area is significantly smaller than the sum of surface areas of the individual components

To confirm the chemical nature of the observed particles (spherical and fibrillary), an elemental analysis was performed on the agglomerates/aggregates using the EDX technique (Figure 12).

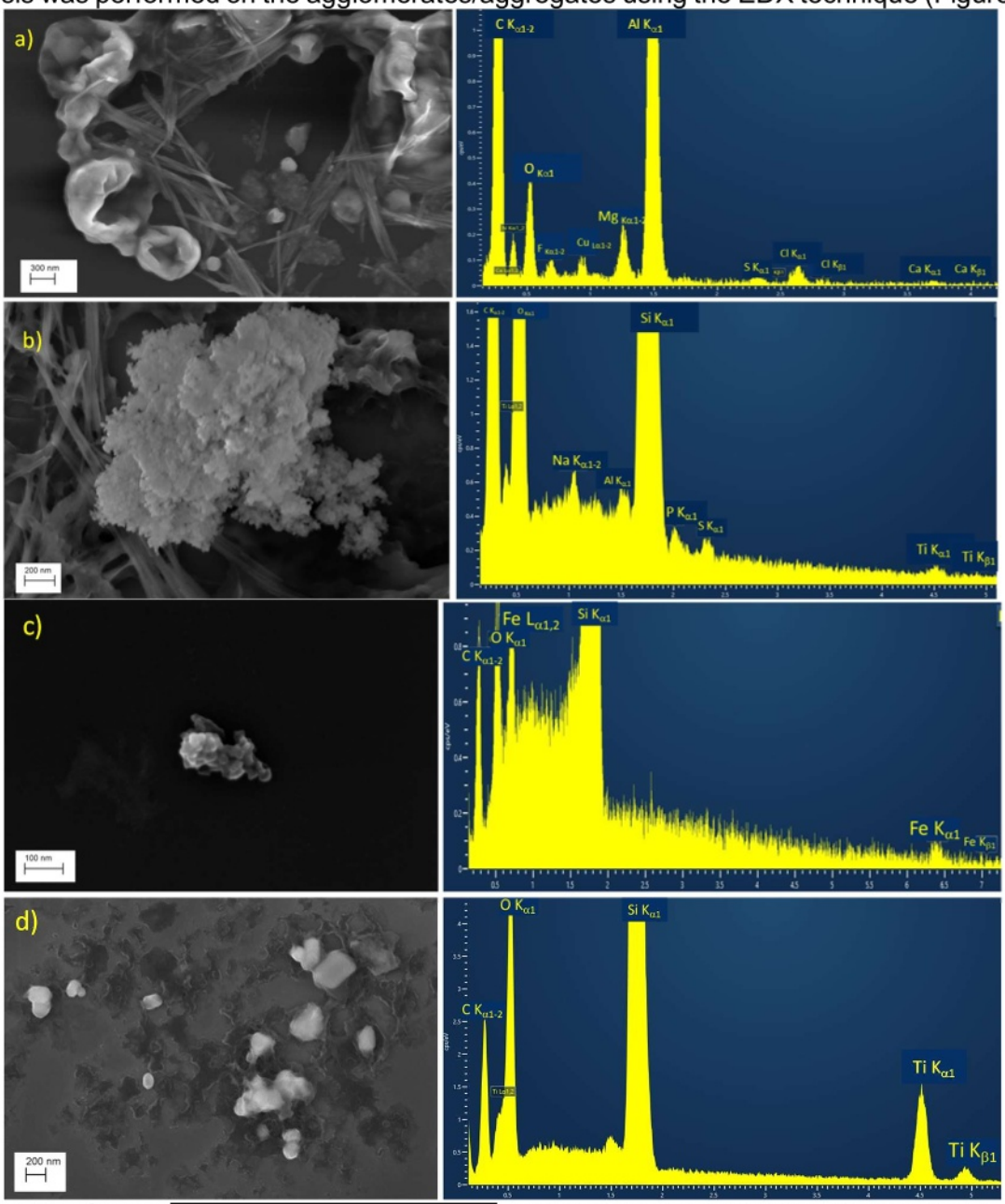


Figure 12: Sample [redacted] corresponding to diluted suspension with step of washing, ultrasonication and spin coater deposition a) SEM image and EDX spectrum performed on agglomerate with fibers and nano-capsules, b) SEM image and EDX spectrum performed particles and fibers, and c), d) SEM images and EDX spectrums performed on particles outside the shell

The peaks relative to the Cl Kα and Kβ, F Kα, N Kα, rays and the oxygen O Kα ray present on the EDX spectrum (Figure 12a) are perfectly visible. Fluor, Chlorine and Nitrogen peaks coming from active ingredient is clearly identified on the EDX spectrum. Fibrillar structure is likely composed of a mixture of sepiolite and halloysite structure confirmed by elementary composition with Mg, Si, Al identified on EDX/SEM fibers images (Figure 12b). The silicon Si Kα peak comes on one hand, from the substrate used for the deposition of the particles and on the other hand, from the fibers. The iron Fe Kα peak and Ti Kα peak are also visible on the

EDX spectrum. A focus is made on particles (Figure 12 c and d) with identification of iron and titanium as macronutrients (5) inside the shell that should be released.

In order to confirm the chemical composition of the particles, EDX-mapping was carried out on the agglomerated particles (Figure 12d). The results are given in Figure 13. For the other particles present in minor quantities with small size, we are at the limit of detection to carry out a cartography.

The presence of titanium and oxygen corresponds exactly to the location of the particles, confirming clearly the presence of titanium dioxide particles.

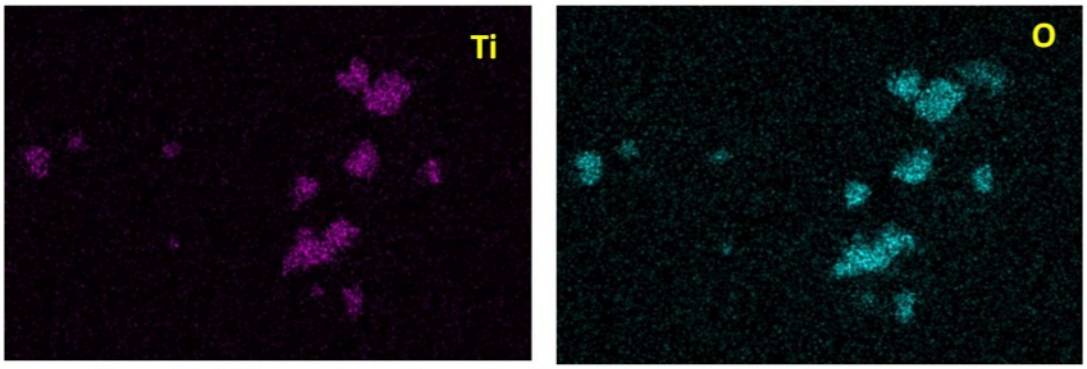


Figure 13: EDX mapping performed on the agglomerated particles in Figure 12d

From the SEM images obtained, a set of fiber was measured in order to construct a histogram of number size distribution (Figure 14). Only the smallest dimension ($D_{Feret\ min}$) of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire population studied, around 150 constituent fibers were analyzed by using Platypus software.

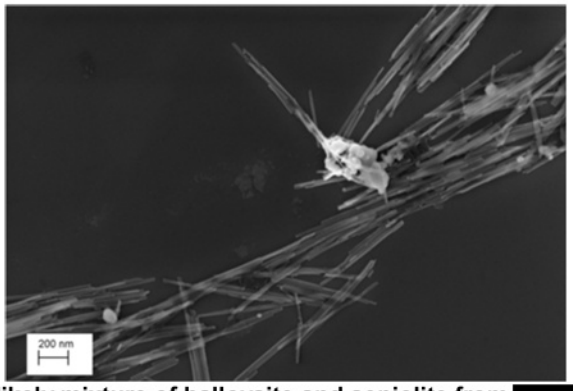


Figure 14: SEM image of likely mixture of halloysite and sepiolite from [redacted]

For each set of data, the number size distribution was constructed (Figure 15) and different parameters were extracted (average size, size distribution, mode of the distribution, median (D50)) (Table 10).

[5] Macronutrient is a chemical element (e.g. potassium, magnesium, calcium) required in large amounts for plant growth. Macronutrient are included such as Nitrogen (N), Phosphorus (P), Potassium (K), Magnesium (Mg), Sulfur (S) and Calcium (Ca) which are essential for plant growth. (Liu, R., Lal, R. Synthetic apatite nanoparticles as a phosphorus fertilizer for soybean (Glycine max). Sci Rep 4, 5686 (2015).

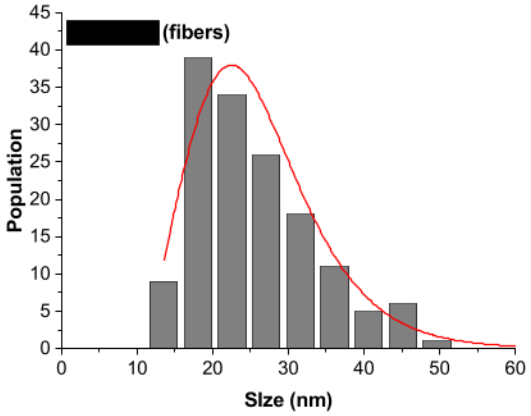


Figure 15: Number size distribution histogram of fibers from [redacted]

The table 10 summarized the various parameters from this measurement.

Table 10 : Parameters from the size distribution of the [redacted] from fibers

Mesurand	Value in nm
Average size (DFeret Min)	23.4
Size distribution (standard deviation)	8.2
Mode	19.6
Median (D50)	22.1

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

From the results of the histogram, **100%** by number **of fibers** population (likely composed by a mixture of halloysite and sepiolite) of [redacted] is **less than 100 nm.**

From the SEM images obtained from initial suspension and after spin-coater deposition (Figure 16), a set of particles containing iron (Fe) (Figure 16a) and titanium dioxide (TiO_2) constituent particles (Figure 16b) are clearly identified.

But, regarding particles containing iron (Fe) the particle concentration is too low, that makes it impossible to count 100 particles with the protocol set up. Nevertheless, the particles present a minimum Feret diameter smaller than 100 nm as observed in Figure 16a.

For titanium dioxide, the particle concentration is too low to obtain a size distribution. Moreover, on SEM images 100% by number of the constituent particle population is greater than 100 nm.

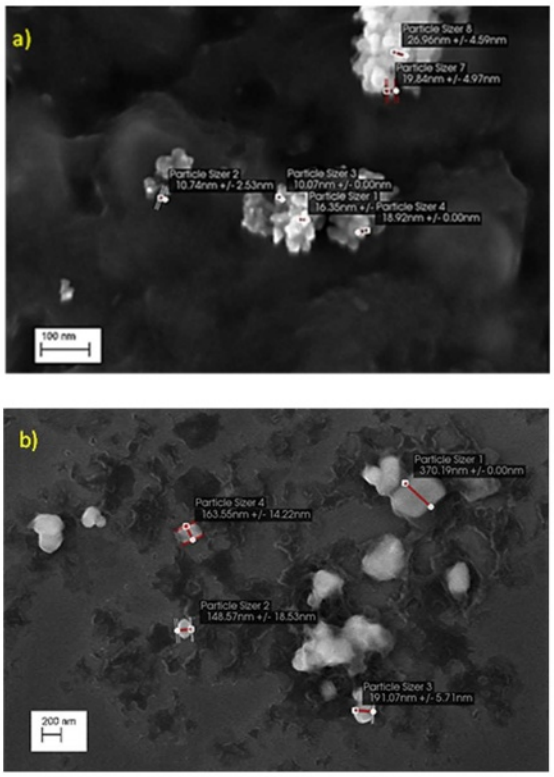


Figure 16: SEM images from [redacted] (a) particles containing iron (Fe) and (b) titanium dioxide (TiO_2) particles

4.2.2 Sample reference: [redacted] Sample 27

[redacted]. The nano-capsules have been developed as herbicide carrier for 2-(2-chlorobenzyl)-4,4-diméthyl-1,2-oxazolidin-3-one ($C_{12}H_{14}ClNO_2$), as active ingredient (Figure 17).

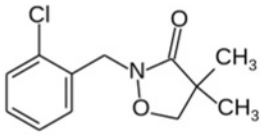


Figure 17: Chemical formula of the active substance

SEM images of nano-capsules are reported on Figure 18(a) corresponding to the static deposition without sonication step. Isolated particles of various shapes and agglomerates/aggregates are observed Figure 18(b and c) after dilution and washing step to extract particles.

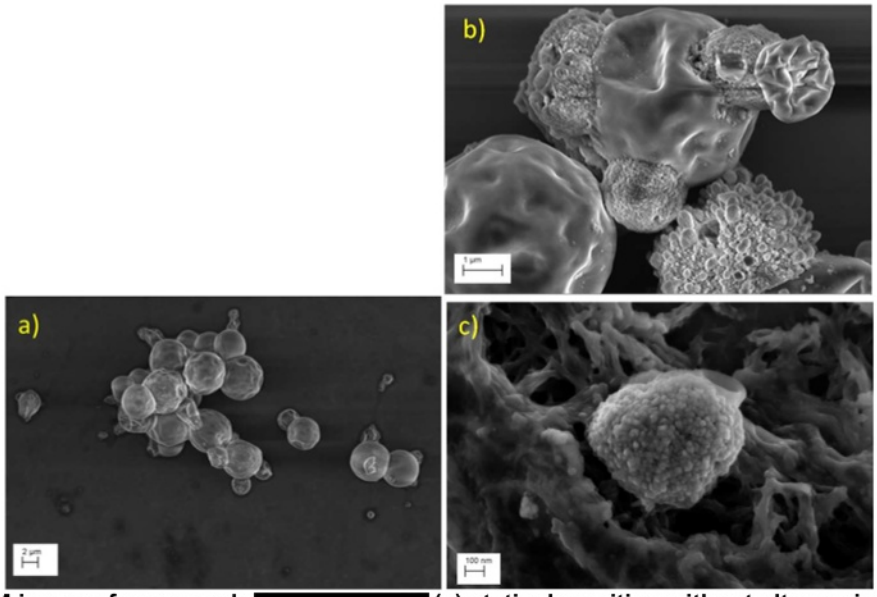


Figure 18: SEM images from sample [redacted] (a) static deposition without ultrasonication step and (b, c) diluted suspension with step of washing, ultrasonication and spin coater deposition

To confirm the chemical nature of the observed particles on (Figure 18 b and c), an elemental analysis was performed using the EDX technique (Figure 19) on the agglomerate/aggregates.

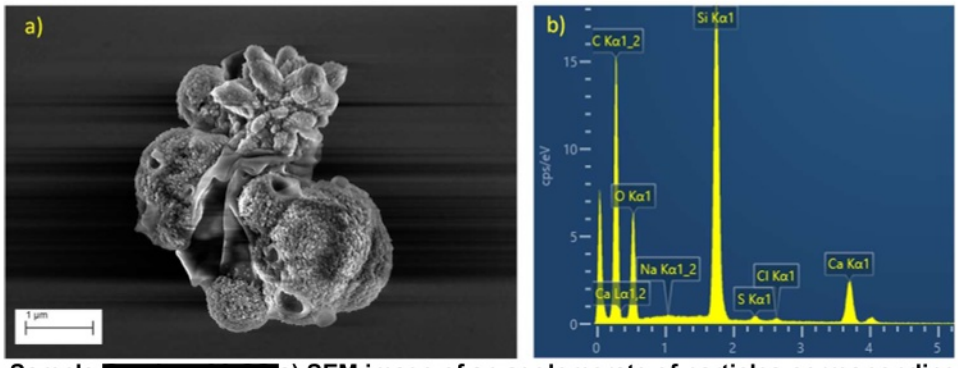


Figure 19: Sample [redacted] a) SEM image of an agglomerate of particles corresponding to diluted suspension with step of washing, ultrasonication and spin coater deposition and b) EDX spectrum performed on the particles agglomerates

The peaks relative to the carbon C Kα ray, the calcium Ca Kα, calcium Ca Lα rays, S Kα ray and the oxygen O Kα ray present on the EDX spectrum (Figure 19) are perfectly visible. The silicon Si Kα peak comes from the substrate used for the deposition of the particles. The presence of the chlorine (Cl Kα) is related to the active ingredient.

In order to confirm the chemical composition of the particles, EDX-mapping was carried out on the same agglomerated particles. The results are given in Figure 20. The presence of calcium, carbon and oxygen corresponds exactly to the location of the particles, confirming that these particles are indeed likely composed of calcium carbonate. According to the literature, calcium carbonate was also studied in terms of acting as a carrier (6).

[6] Qian, K., Shi, T., Tang, T. et al. Preparation and characterization of nano-sized calcium carbonate as controlled release pesticide carrier for validamycin against Rhizoctonia solani . Microchim Acta 173, 51–57 (2011). https://doi.org/10.1007/s00604-010-0523-x

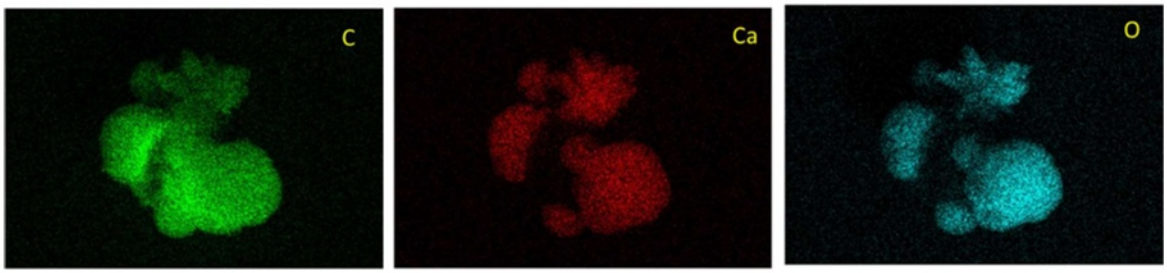


Figure 20: EDX mapping performed on the agglomerated particles in Figure 19

From the obtained SEM images (Figure 21), a set of likely calcium carbonate constituent particles was measured in order to construct a histogram of number size distribution (Figure 22).

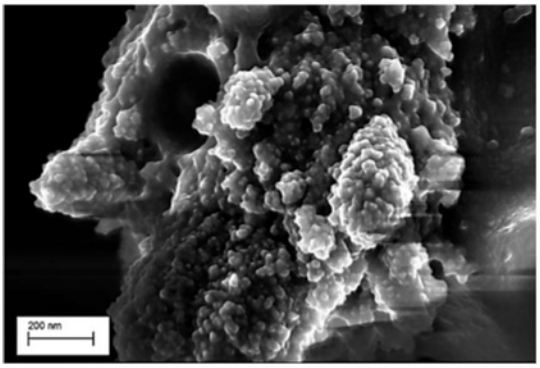


Figure 21: SEM image of likely calcium carbonate constituent particles from [redacted]

Only the smallest dimension of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire population studied, around 100 constituent particles were analyzed by using Platypus software. For each set of data, the number size distribution was obtained (Figure 22) and different parameters were extracted (average size, size distribution, mode of the distribution, median) (Table 11).

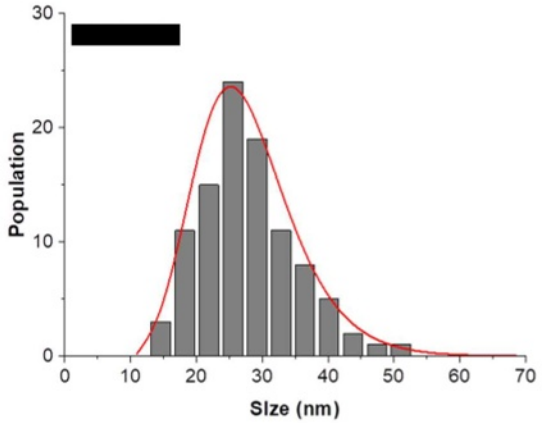


Figure 22: Number size distribution histogram of likely calcium carbonate constituent particles from [redacted]

The table 11 summarized the various parameters from this measurement.

Table 11 : Parameters from the size distribution of the [redacted]

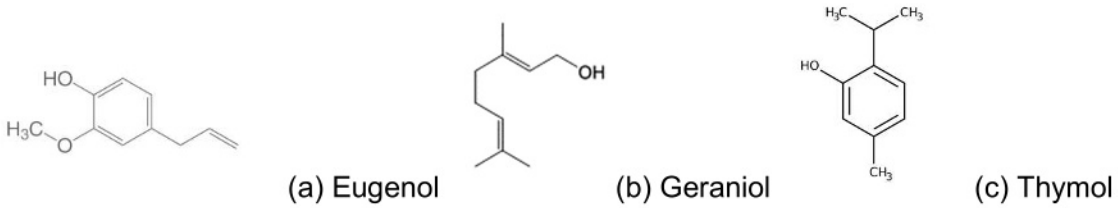
Mesurand	Value in nm
Average size	25.9
Size distribution (standard deviation)	7.3
Mode	25.5
Median (D50)	24.9

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

From the results of the histogram, **100% by number of** likely calcium carbonate **constituent particles** population **of [redacted] is less than 100 nm**.

4.2.3 Sample reference: [redacted] Sample 28

[redacted]. The nano-capsules suspensions have been developed as fongicide carrier for Eugenol (3.3%), Geraniol (6.6%) and Thymol (6.6%), as active ingredients (Figure 23).



(a) Eugenol (b) Geraniol (c) Thymol

Figure 23: Chemical formula of the active substances

SEM images of particles of various shapes are reported on Figure 24, corresponding to the static deposition without ultrasonication (Figure 24 a) and diluted suspension with step of washing, ultrasonication and spin coater deposition (Figure 24 b, Figure 24 c). Isolated particles made of constituent particles are observed.

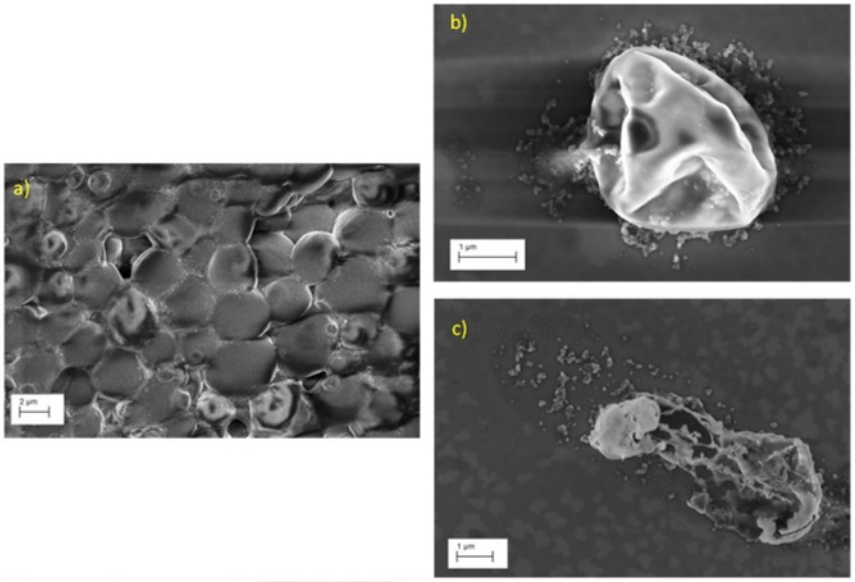


Figure 24: SEM images from sample [redacted] (a) static deposition without ultrasonication and (b, c) diluted suspension with step of washing, ultrasonication and spin coater deposition

To confirm the chemical nature of the observed particles (spherical), an elemental analysis was performed on the agglomerate/aggregate using the EDX technique (Figure 25).

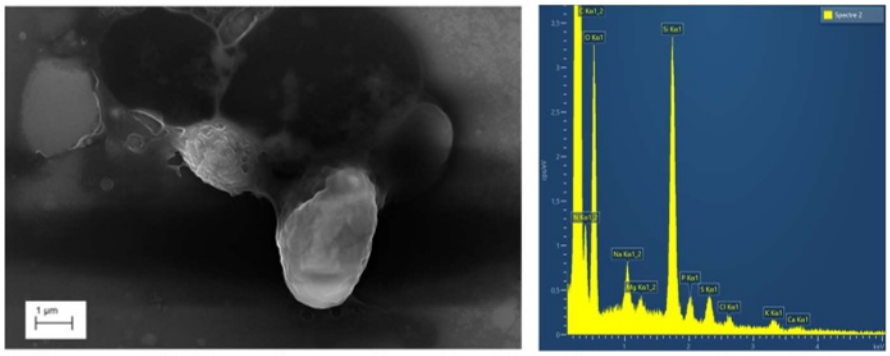


Figure 25: SEM image and EDX spectrum performed on the agglomerates of spherical particles outside the shell

The peaks relative of various element such as Mg Kα, K Kα, S Kα, Na Kα, P Kα, Cl Kα, Ca Kα, Ca Kβ, N Kα rays and the oxygen O Kα ray present on the EDX spectrum (Figure 25) are perfectly visible. The silicon Si Kα peak comes from the substrate used for the deposition of the particles.

In order to confirm the chemical composition of the particles, EDX-mapping was carried out on the agglomerated particles (Figure 26).

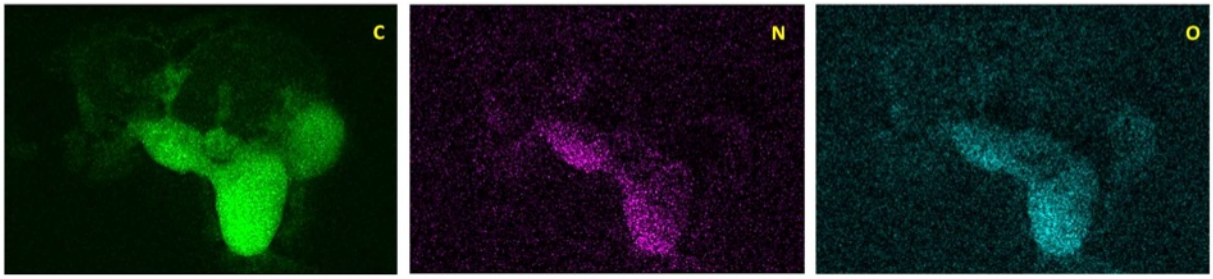


Figure 26: EDX mapping performed on the agglomerated particles in Figure 23

From the SEM images obtained (Figure 27), a set of spherical constituent particles was measured in order to construct a histogram of number size distribution.

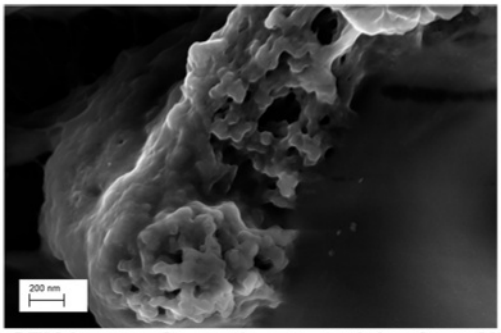


Figure 27: SEM image of constituent particles from [redacted]

Only the smallest dimension (min. Feret Diameter) of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire population studied, 100 constituent particles were analyzed by using Platypus software. For each set of data, the number size distribution was constructed (Figure 28) and different parameters were extracted (average size, size distribution, distribution mode, median) (Table 12).

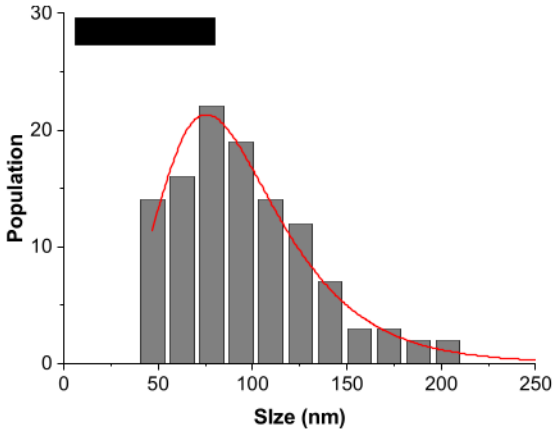


Figure 28: Number size distribution histogram of constituent particles (containing carbon, nitrogen and oxygen) extracted from [redacted]

The table 12 summarized the various parameters from this measurement.

Table 12: Parameters from the size distribution of the [redacted]

Mesurand	Value in nm
Average size	87.5
Size distribution (standard deviation)	40.2
Mode	65.6
Median (D50)	79.5

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

From the results of the histogram, **69%** by number **of the constituent particles** population containing mainly, carbon of [redacted] is **less than 100 nm**.

4.2.4 Sample reference: [redacted] Sample 29

[redacted]. The nano-capsules have been developed as herbicide carrier for 2-(2-chlorobenzyl)-4,4-diméthyl-1,2-oxazolidin-3-one (clomazone) in metazachlor solution, as active ingredient (Figure 29).

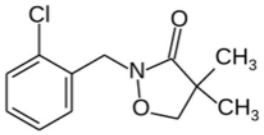


Figure 29: Chemical formula of the active substance

SEM images of particles are reported on Figure 30, corresponding to the static deposition without ultrasonication (Figure 30 a) and diluted suspension with step of washing, ultrasonication and spin coater deposition (Figure 30 b, Figure 30 c).

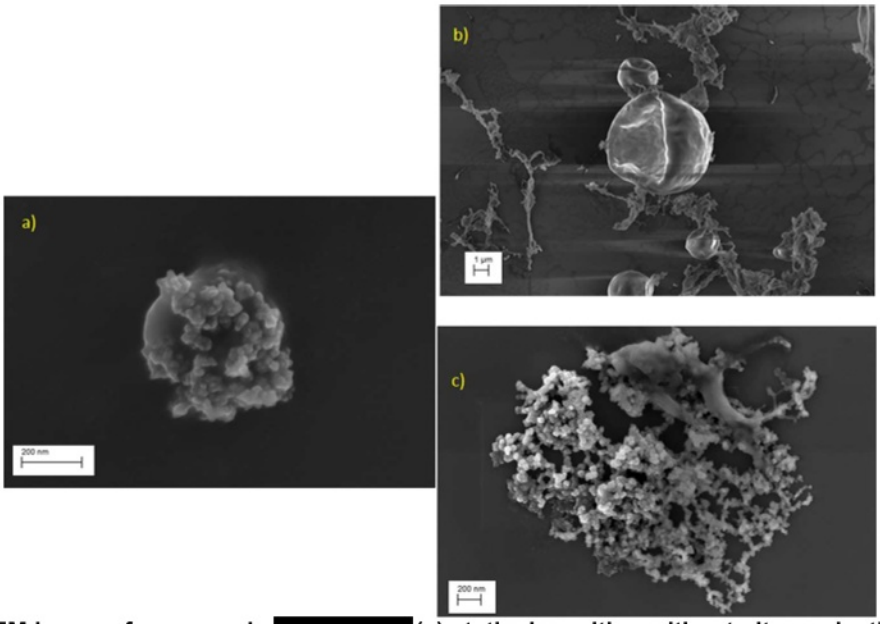


Figure 30: SEM images from sample [redacted] (a) static deposition without ultrasonication and (b, c) diluted suspension with step of washing, ultrasonication and spin coater deposition

To confirm the chemical nature of the observed particles (spherical), an elemental analysis was performed on the agglomerate/aggregate using the EDX technique (Figure 31).

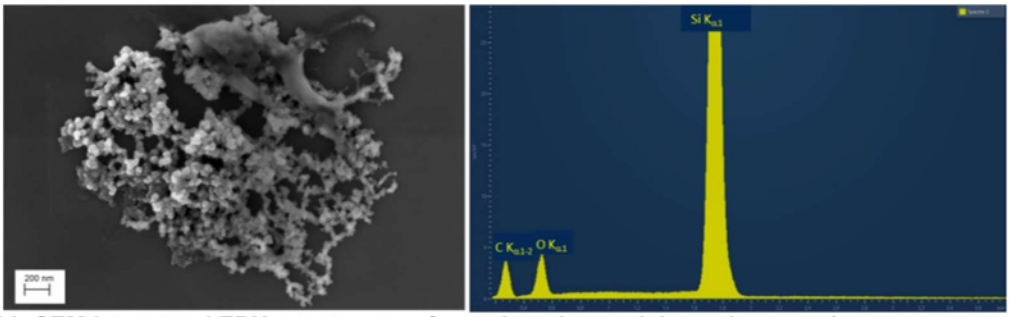


Figure 31: SEM image and EDX spectrum performed on the particle agglomerate/aggregate on spherical particles

The peaks relative to the C Kα ray and the oxygen O Kα ray present on the EDX spectrum above are perfectly visible on particles. The silicon Si Kα peak comes from the substrate used for the deposition of the particles.

In order to confirm the chemical composition of the particles, EDX-mapping was carried out on the agglomerates/aggregates (Figure 32). Spherical particles are mainly made of carbon and oxygen.

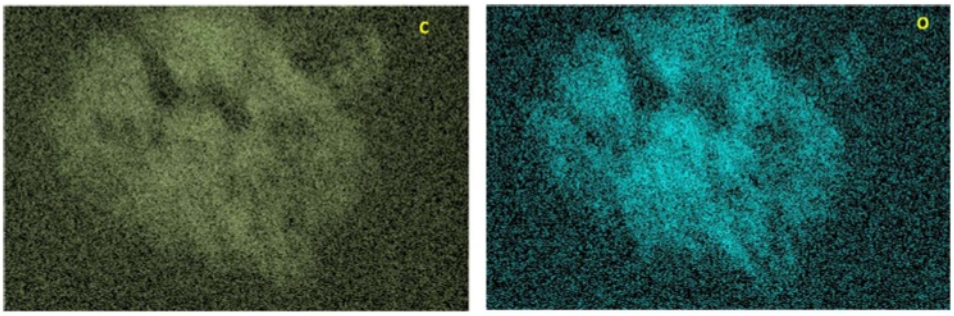


Figure 32: EDX mapping performed on the agglomerated/aggregated particles in Figure 31

From the SEM images obtained (Figure 33), a set of spherical constituent particles was measured in order to construct a histogram of number size distribution.

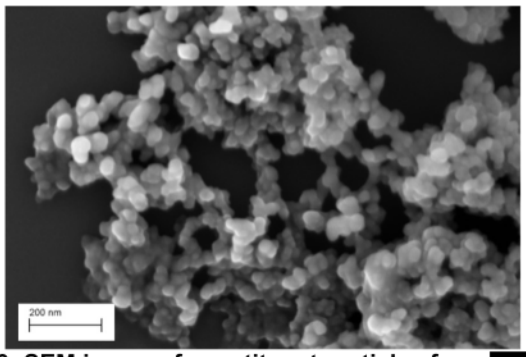


Figure 33: SEM image of constituent particles from [redacted]

Only the smallest dimension (min. Feret Diameter) of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire population studied, 100 constituent particles were analyzed by using Platypus software. For each set of data, the number-based particle size distribution was constructed (Figure 34) and different parameters were extracted (average size, size distribution, mode of the distribution, median) (Table 13).

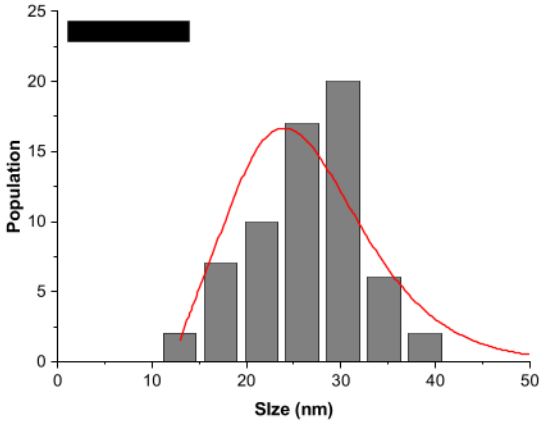


Figure 34: Number size distribution histogram of constituent particles from [redacted]

The table 13 summarized the various parameters from this measurement.

Table 13: Parameters from the size distribution of the [redacted]

Mesurand	Value in nm
Average size	26.4
Size distribution (standard deviation)	7.2
Mode	26.4
Median	26.4

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

From the results of the histogram, **100%** by **number of the constituent particles** population **mainly carbon and oxygen** of [redacted] is **less than 100 nm**.

4.2.5 Sample reference: [redacted] Sample 30

[redacted]. The nano-capsules suspensions have been developed as herbicide carrier for 2-(2-chlorobenzyl)-4,4-diméthyl-1,2-oxazolidin-3-one (Fluorochloridone), as active ingredient (Figure 35).

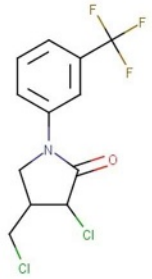


Figure 35: Chemical formula of the active substance

SEM images of particles with various shapes are reported on Figure 36, corresponding to the static deposition without ultrasonication (Figure 36 a) and diluted suspension with step of washing, ultrasonication and deposition by spin-coater (Figure 36 b, Figure 36 c). Isolated particles made of constituent particles are observed.

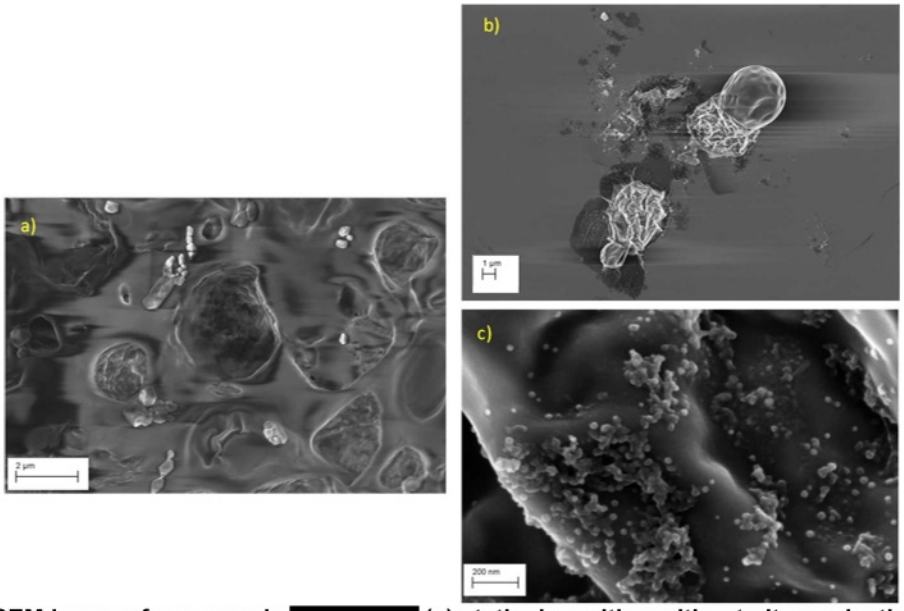


Figure 36: SEM images from sample [redacted] (a) static deposition without ultrasonication and (b, c) diluted suspension with step of washing, ultrasonication and spin coater deposition

To confirm the chemical nature of the observed spherical particles (outside the shell), an elemental analysis was performed on the agglomerate/aggregate using the EDX technique (Figure 37).

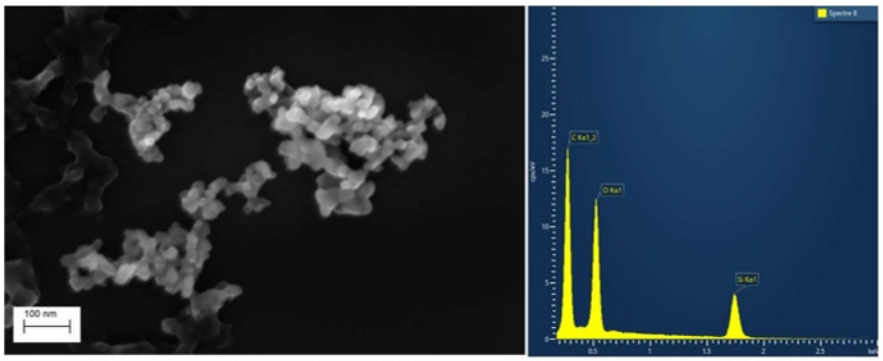


Figure 37: SEM image and EDX spectrum performed on the agglomerate of spherical particles outside the shell

The peaks relative F Kα peak, the carbon C Kα peak and the oxygen O Kα ray present on the EDX spectrum (Figure 37) on spherical particles are perfectly visible and clearly indicate the presence of fluor (chemical element in active substance). The silicon Si Kα peak comes from the substrate used for the deposition of the particles.

In order to confirm the chemical composition of the particles, EDX-mapping was carried out on the agglomerates/aggregates (Figure 38). Spherical particles are mainly made of carbon and oxygen.

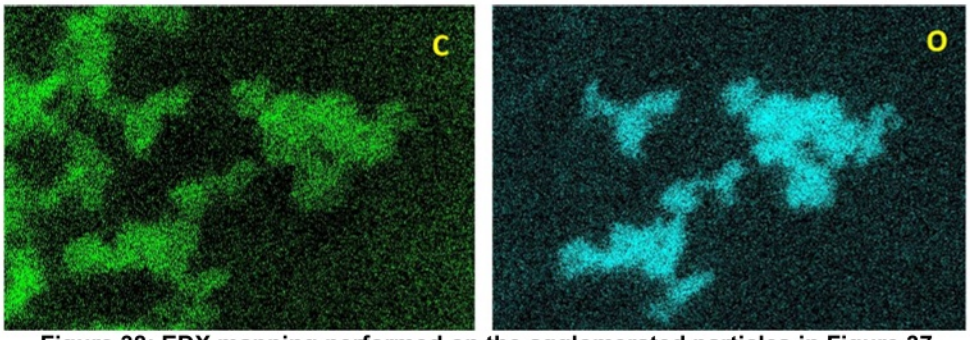


Figure 38: EDX mapping performed on the agglomerated particles in Figure 37

From the SEM images obtained (Figure 39), a set of spherical constituent particles was measured in order to construct a histogram of number size distribution.

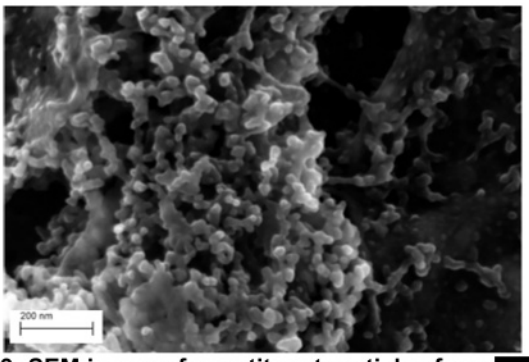


Figure 39: SEM image of constituent particles from [redacted]

Only the smallest dimension (min. Feret Diameter) of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire studied population, 100 constituent particles were analyzed by using Platypus software. For each set of data, the number size distribution

was constructed (Figure 40) and different parameters were extracted (average size, size distribution, mode of the distribution, median) (Table 14).

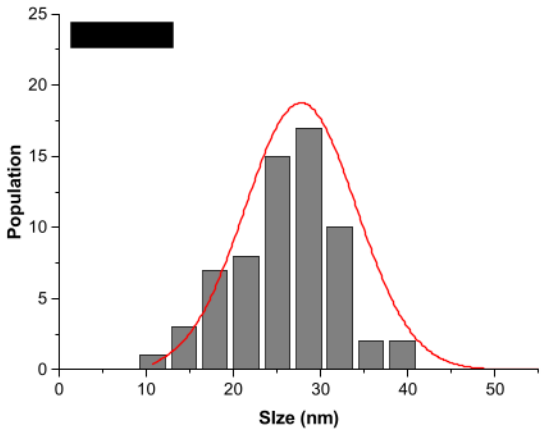


Figure 40: Number size distribution histogram of constituent particles from [redacted]

The table 14 summarized the various parameters from this measurement.

Table 14: Parameters from the size distribution of the [redacted]

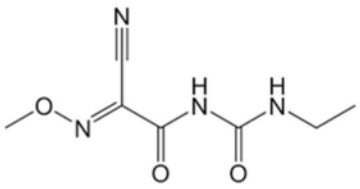
Mesurand	Value in nm
Average size	24.2
Size distribution (standard deviation)	5.9
Mode	24.2
Median (D50)	24.2

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

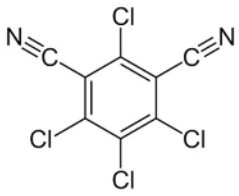
From the results of the histogram, **100%** by number **of the constituent particles** population (mainly composed of carbon and oxygen) of [redacted] is **less than 100 nm.**

4.2.6 Sample reference: [redacted] Sample 31

[redacted]. The suspensions have been developed as fongicide carrier for cymoxanil and chlorothalonil, as active ingredients (Figure 41).



(a) Cymoxanil



(b) Chlorothalonil

Figure 41: Chemical formula of the active substances

SEM images of suspension are reported on Figure 42 (a, b) corresponding to the static deposition without ultrasonication. Isolated particles with various shapes (fibrillar and spherical structure) and agglomerates composed of constituent particles are observed Figure 42 (c) with the diluted suspension and spin-coater deposition without ultrasonication and Figure 42 (d) after dilution, ultrasonication and washing step to extract particles.

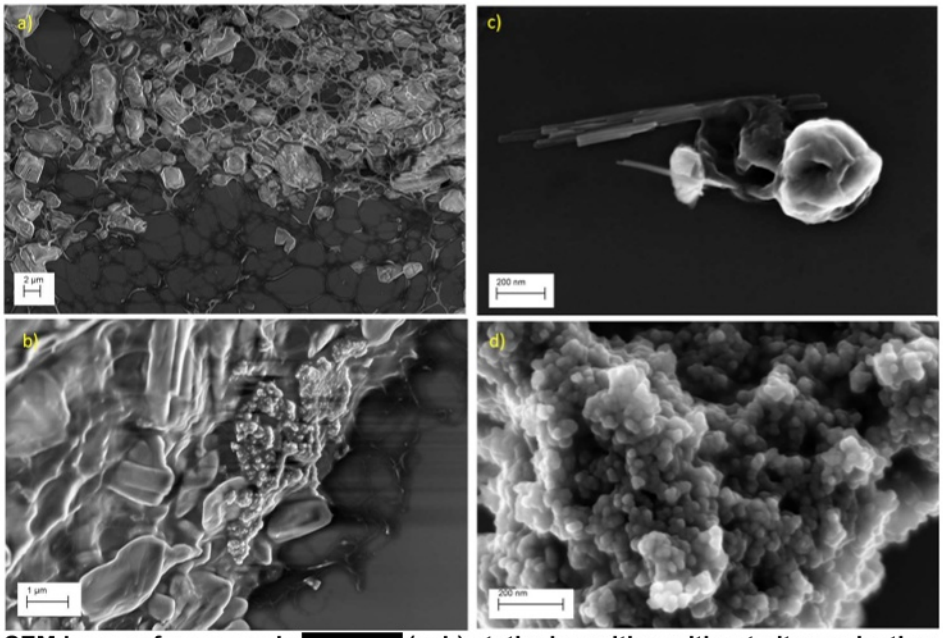


Figure 42: SEM images from sample [redacted] (a, b) static deposition without ultrasonication, (c) diluted suspension without ultrasonication with spin coater deposition and (d) diluted suspension with step of washing, ultrasonication and spin coater deposition

To confirm the chemical nature of the nanoparticles observed outside the shell (spherical and fibrillar structures), an elemental analysis was performed on the agglomerate/aggregate using the EDX technique (Figure 43).

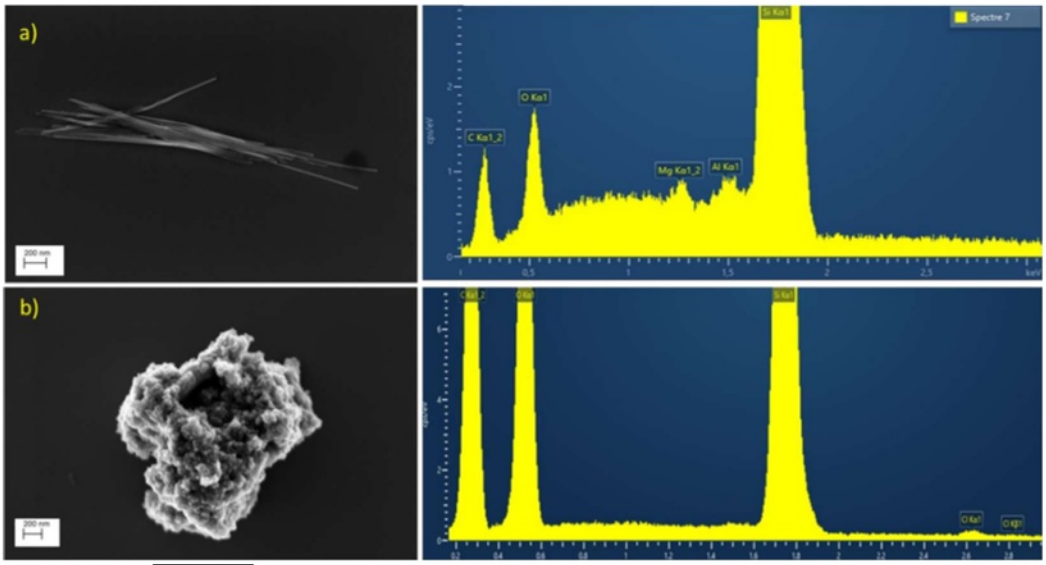


Figure 43: Sample [redacted] corresponding to diluted suspension with step of washing, ultrasonication and spin coater deposition a) SEM image and EDX spectrum performed on fibers observed outside the shell and b) SEM image and EDX spectrum performed on particles outside the shell

The peaks (Figure 43 a) relative to the Mg Kα , Al Lα rays and the oxygen O Kα ray present on the EDX spectrum are perfectly visible and clearly indicate the presence of a mixture of sepiolite and halloysite structure. The silicon Si Kα peak comes on the one hand, from the substrate used for the deposition of the particles and on the other hand from the fibers. The presence of the chlorine (Cl Kα, Figure 43 b) is related to the active ingredient.

In order to confirm the chemical composition of the particles, EDX-mapping was carried out on the agglomerates/aggregates (Figure 44). Spherical particles are mainly made of carbon and oxygen.

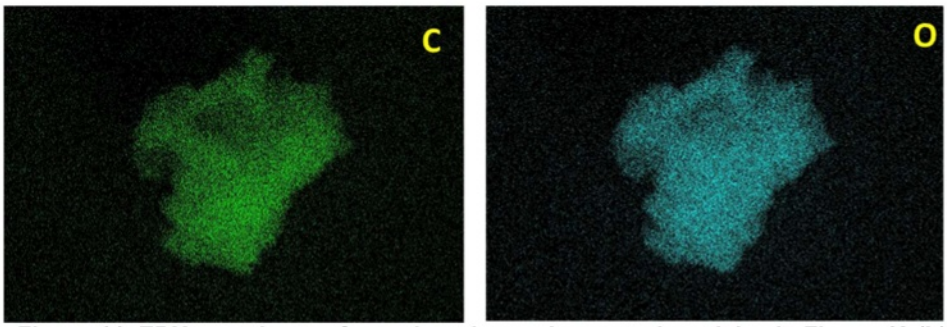


Figure 44: EDX mapping performed on the agglomerated particles in Figure 43 (b)

From the SEM images obtained (Figure 45), a set of spherical constituent particles was measured in order to construct a histogram of number size distribution.

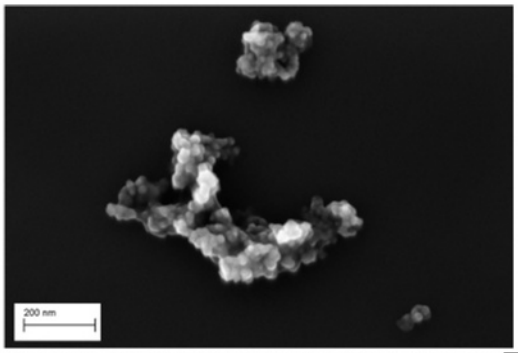


Figure 45: SEM image of constituent particles from [redacted]

Only the smallest dimension (min. Feret Diameter) of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire population studied, 100 constituent particles were analyzed by using Platypus software. For each set of data, the number size distribution was constructed (Figure 46) and different parameters were extracted (mean size, size distribution, mode of the distribution, median) (Table 15).

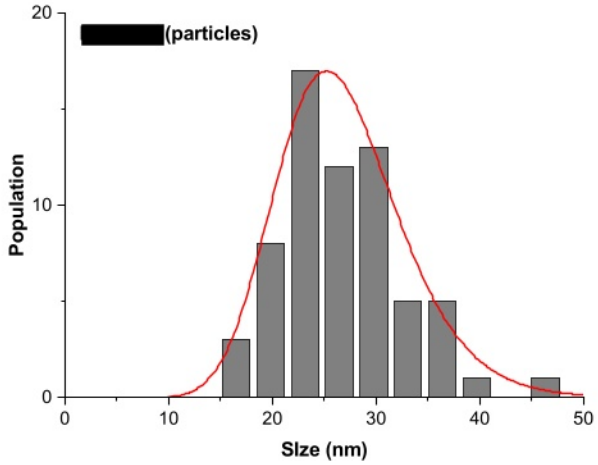


Figure 46: Number size distribution histogram of constituent particles extracted from [redacted]

The table 15 summarized the various parameters from this measurement.

Table 15: Parameters of the particle size distribution extracted from [redacted]

Mesurand	Value in nm
Average size	23.5
Size distribution (standard deviation)	6.5
Mode	21.1
Median (D50)	22.7

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

From the results of the histogram, **100%** by number **of the constituent** particles population (mainly composed of carbon and oxygen) of [redacted] is **less than 100 nm**.

From the SEM images obtained (Figure 47), a set of fibers was measured in order to construct a histogram of number size distribution.

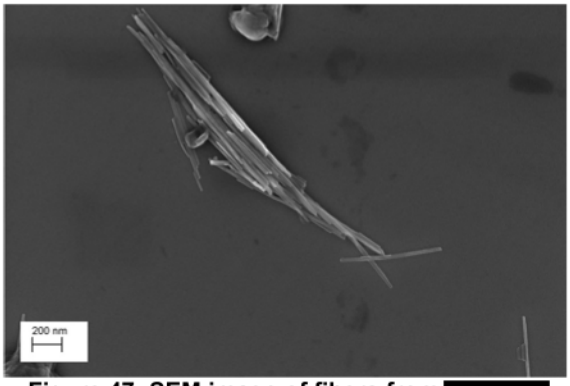


Figure 47: SEM image of fibers from [redacted]

Only the smallest dimension (min. Feret Diameter) of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire population studied, around 150 constituent fibers were analyzed by using Platypus software. For each set of data, the number size distribution was constructed (Figure 48) and different parameters were extracted (mean size, size distribution, mode of the distribution, median) (Table 16).

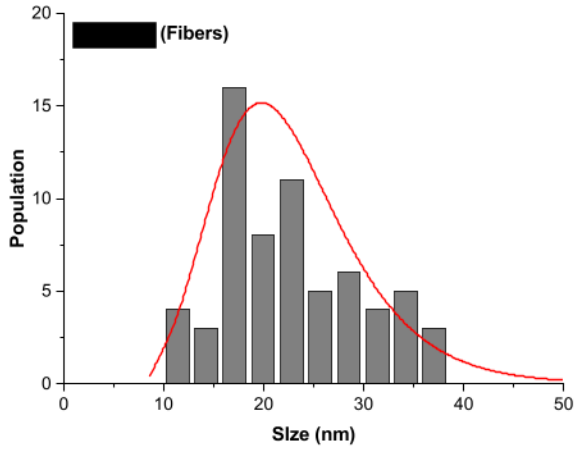


Figure 48: Number size distribution histogram of fibers from [redacted]

The table 16 summarized the various parameters from this measurement.

Table 16: Parameters of the fiber size distribution extracted from [redacted]

Mesurand	Value in nm
Average size (D Feret Min)	20.4
Size distribution (standard deviation)	7.8
Mode	16.7
Median	19.1

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

From the results of the histogram, **100%** by number **of the Min Feret Diameter** from fibers (likely composed of halloysite and sepiolite) of [redacted] is **less than 100 nm**.

4.2.7 Sample reference: [redacted] Sample 32

[redacted]. The suspensions have been developed as herbicide carrier for chlortoluron, as active ingredients (Figure 49).

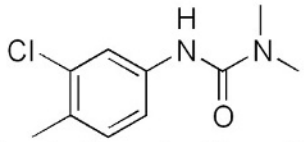


Figure 49: Chemical formula of the active substance

SEM images of suspension are reported on Figure 50 (a, b) corresponding to the static deposition. Isolated particles of various shapes (fibrillar and spherical structure) and agglomerates/aggregates are observed Figure 50 (d) with the diluted suspension and spin-coater deposition and Figure 50 (c) after dilution and washing step to extract particles.

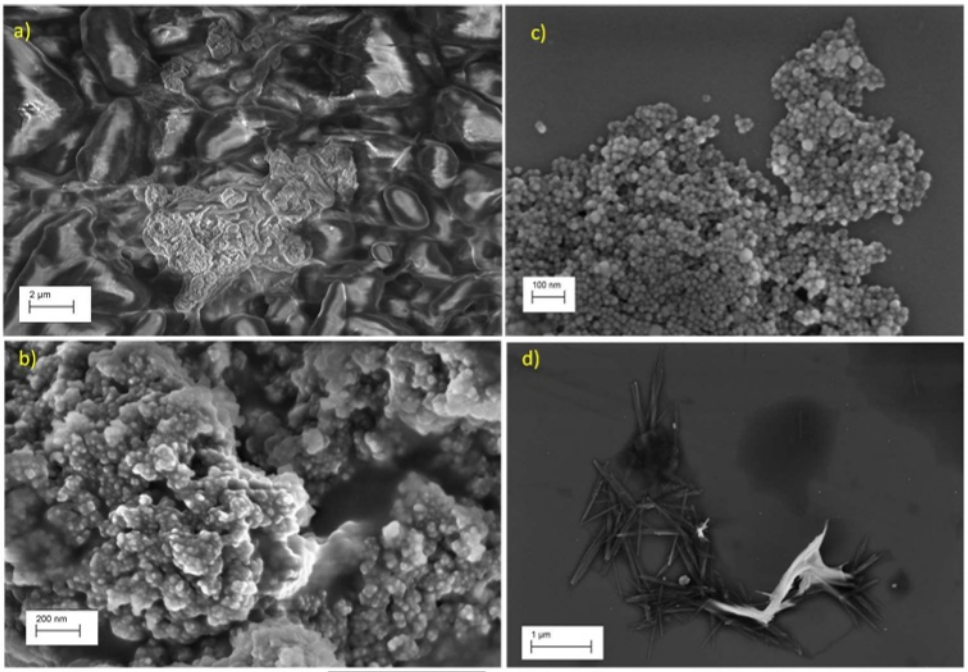


Figure 50: SEM images from sample [redacted] (a, b) static deposition without ultrasonication, (c) diluted suspension with step of washing, ultrasonication and spin coater deposition and (d) diluted suspension with spin coater deposition

To confirm the chemical nature of the observed particles (spherical and fibrillar structure), an elemental analysis was performed on the agglomerate/aggregate using the EDX technique (Figure 51).

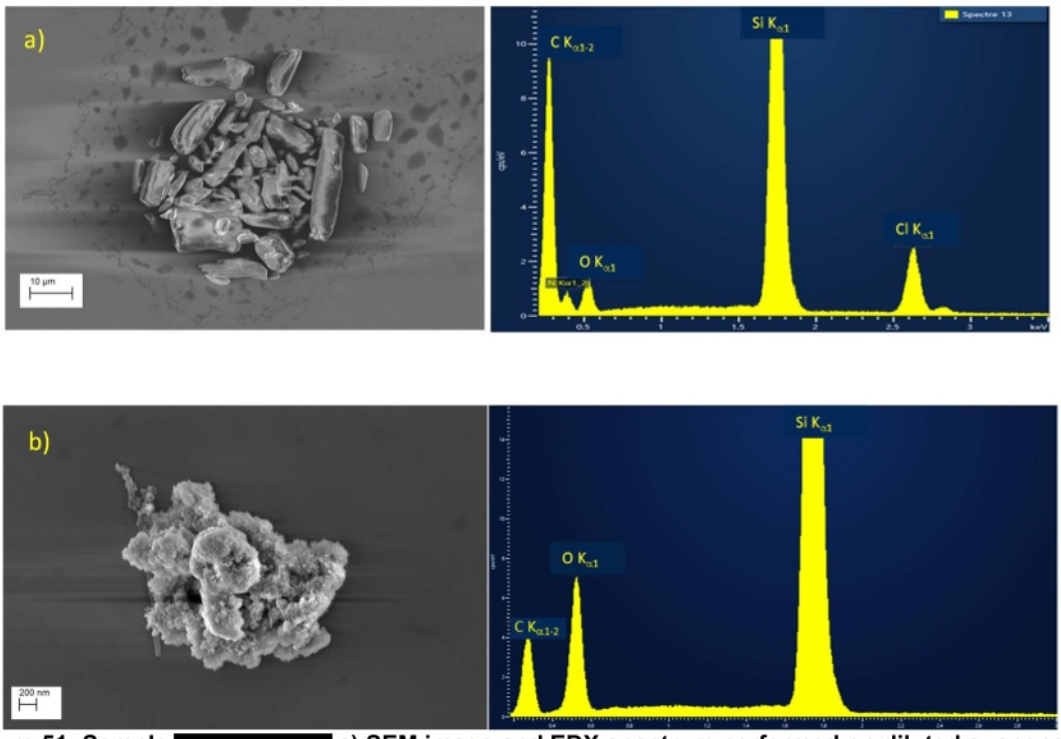


Figure 51: Sample [redacted] a) SEM image and EDX spectrum performed on diluted suspension without ultrasonication and spin coater deposition and b) SEM image and EDX spectrum performed on particles on diluted suspension with step of washing, ultrasonication and spin coater deposition

The peaks (Figure 51 a) relative to the Cl Kα , N Lα rays and the oxygen O Kα ray present on the EDX spectrum are perfectly visible and clearly related to the active ingredient structure. The silicon Si Kα peak comes from the substrate used for the deposition of the particles. The carbon C Kα peak and the oxygen O Kα ray (Figure 51 b) are related to spherical nanoparticles.

The particle from Figure 51a show microscale size and are not analyzed.

In order to confirm the chemical composition of the particles (Figure 51 b), EDX-mapping was carried out on the agglomerate/aggregate (Figure 52). Spherical particles are mainly made of carbon and oxygen.

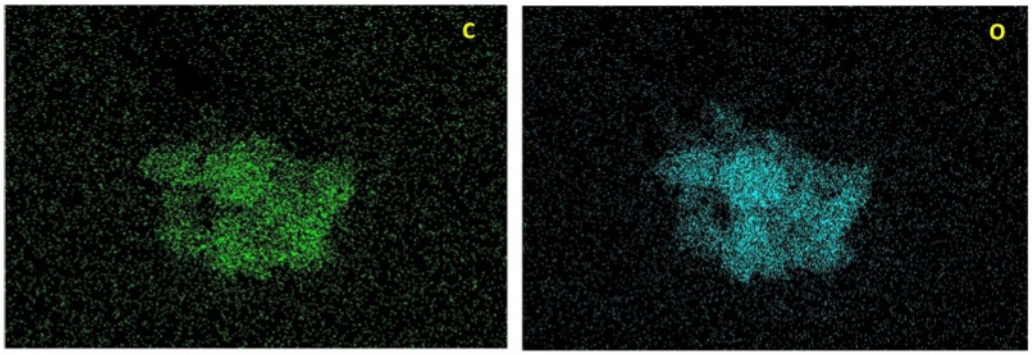


Figure 52: EDX mapping performed on the agglomerated particles in Figure 51 (b)

From the SEM images obtained (Figure 53), a set of spherical constituent particles was measured in order to construct a histogram of number size distribution.

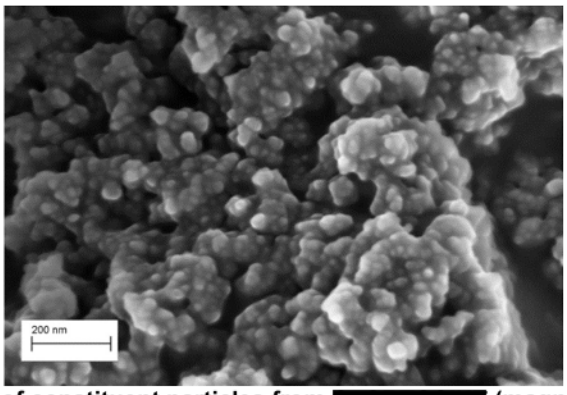


Figure 53: SEM image of constituent particles from [redacted] (magnification of figure 51 b)

Only the smallest dimension (Min. Feret Diameter) of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire population studied, 100 constituent particles were analyzed by using Platypus software. For each set of data, the number size distribution was constructed (Figure 54) and different parameters were extracted (mean size, size distribution, mode of the distribution, median) (Table 17).

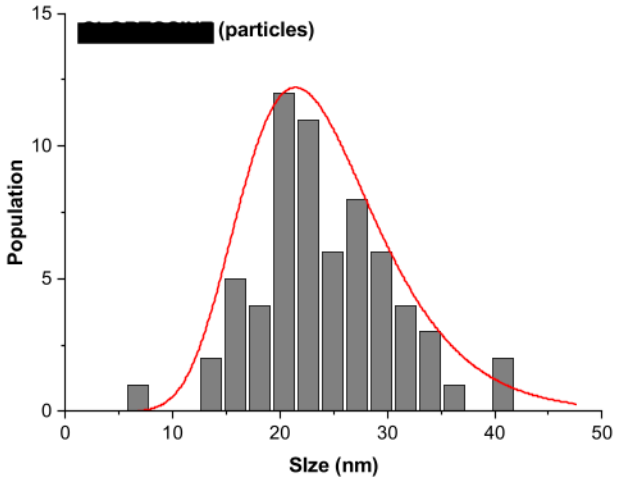


Figure 54: Number size distribution histogram of constituent particles observed in Figure 53 (mainly composed of carbon and oxygen) extracted from sample [redacted]

The table 17 summarized the various parameters from this measurement.

Table 17: Parameters of the particle size distribution extracted from [redacted]

Mesurand	Value in nm
Average size	19.0
Size distribution (standard deviation)	6.2
Mode	16.4
Median	18.1

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

From the results of the histogram, **100%** by number **of the constituent particles** (mainly composed of carbon and oxygen) population of [redacted] is **less than 100 nm.**

From the SEM images obtained (Figure 55), a set of fibers was measured in order to construct a histogram of number size distribution.

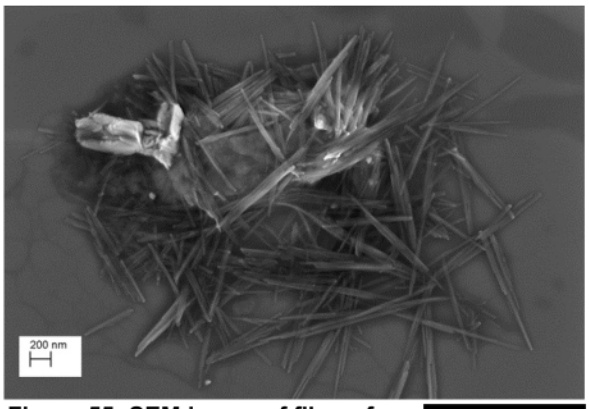


Figure 55: SEM image of fibers from [redacted]

Only the smallest dimension (min. Feret Diameter) of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire population studied, around 150 constituent fibers were analyzed by using Platypus software (Figure 56).

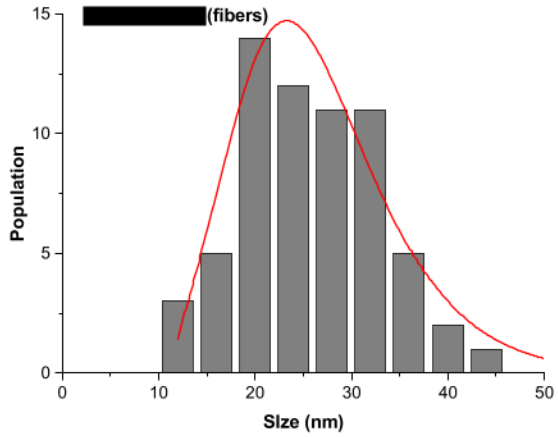


Figure 56: Number size distribution histogram of fibers from [redacted]

The table 18 summarized the various parameters from this measurement.

Table 18: Parameters of the fiber size distribution extracted from [redacted]

Mesurand	Value in nm
Average size (D Feret Min)	24.7
Size distribution (standard deviation)	8.2
Mode	21.1
Median (D50)	23.4

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

From the results of the histogram, **100%** by number **of the D Feret Min** from fibers (no identified) of [redacted] is **less than 100 nm**.

4.2.8 Sample reference: [redacted] Sample 33

[redacted]. The suspensions have been developed as herbicide carrier for chlorotoluron and isoxaben, as active ingredients (Figure 57).

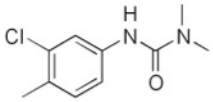
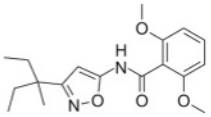
(a) Chlorotoluron  (b) Isoxaben 

Figure 57: Chemical formula of the active substances

SEM images of suspension are reported on Figure 58 (a) corresponding to the static deposition. Isolated particles of spherical shape and agglomerates/aggregates are observed Figure 58 (b) with the diluted suspension and deposition by spin-coater.

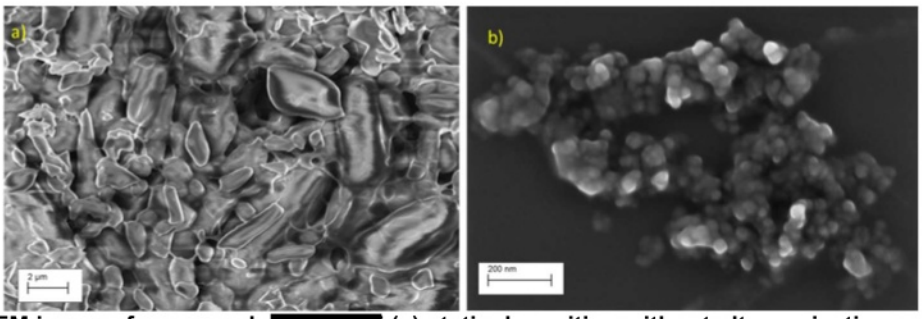


Figure 58: SEM images from sample [redacted] (a) static deposition without ultrasonication and (b) diluted suspension with spin coater deposition without ultrasonication

To confirm the chemical nature of the observed particles with spherical structure, an elemental analysis was performed on the agglomerate/aggregate using the EDX technique (Figure 59).

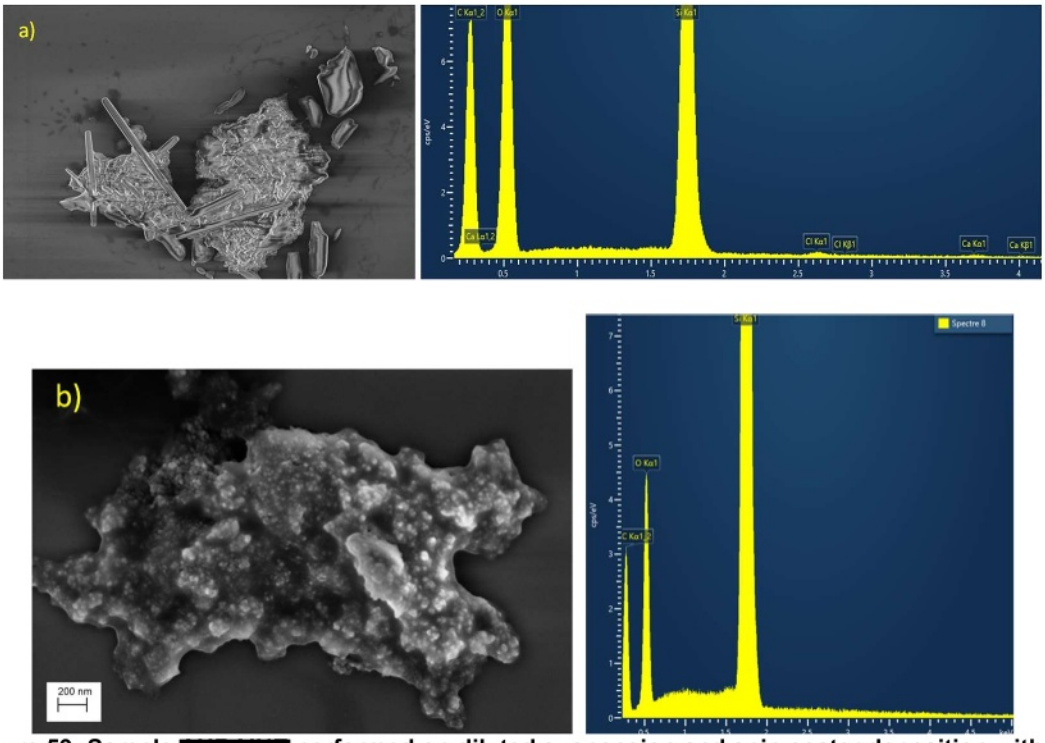


Figure 59: Sample [redacted] performed on diluted suspension and spin coater deposition without ultrasonication a) SEM image and EDX spectrum on active substance and b) SEM image and EDX spectrum performed on particles

The peaks relative to the oxygen O Kα ray and the carbon C Kα peak present on the EDX spectrum from particles are perfectly visible (Figure 59 a). The silicon Si Kα peak comes from the substrate used for the deposition of the particles. The presence of the chlorine (Cl Kα) (Figure 59 b) is related to the active ingredient. The Ca Kα peak from Figure 59 b should be macronutrient included in the suspension.

The particles from Figure 59 a show microscale size and are not analyzed.

In order to confirm the chemical composition of the particles (Figure 59 b), EDX-mapping was carried out on the agglomerates/aggregates (Figure 60). Spherical particles are mainly made of carbon and oxygen.

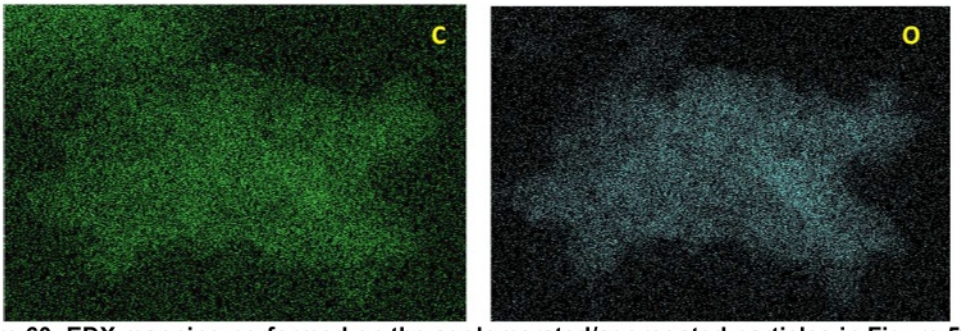


Figure 60: EDX mapping performed on the agglomerated/aggregated particles in Figure 59 (b)

From the SEM images obtained (Figure 61), a set of spherical constituent particles was measured in order to construct a histogram of number size distribution.

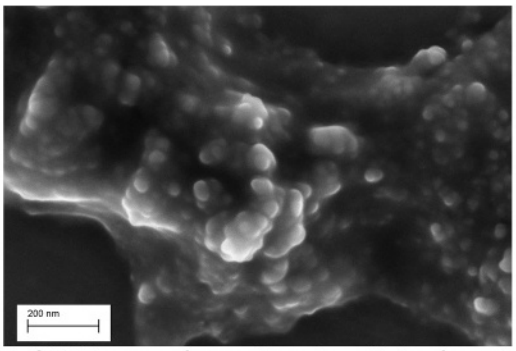


Figure 61: SEM image of constituent particles from [redacted]

Only the smallest dimension (min. Feret Diameter) of each constituent particle was measured as recommended by the European authorities. In order to ensure that the dimensional measurements are representative of the entire population studied, 100 constituent particles were analyzed by using Platypus software. For each set of data, the number size distribution was constructed (Figure 62) and different parameters were extracted (average size, size distribution, mode of the distribution, median) (Table 19).

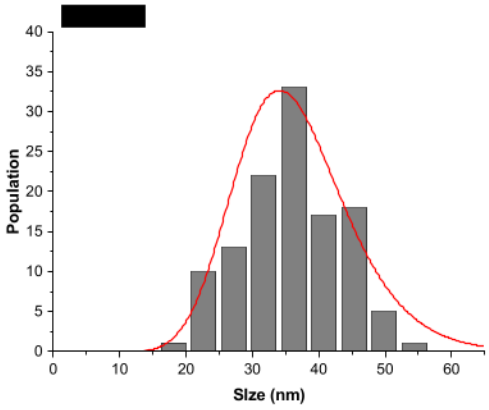


Figure 62: Number size distribution histogram of constituent particles extracted from sample [redacted]

The table 19 summarized the various parameters from this measurement.

Table 19: Parameters from the size distribution of [redacted]

Mesurand	Value in nm
Average size	34.3
Size distribution (standard deviation)	8.3
Mode	31.5
Median	33.3

Mode: The mode of the distribution is the average size of the most common class.
D50 (Median size): the size which divides the distribution into two parts of equal area.

From the results of the histogram, **100%** by number **of the constituent particles** population (mainly composed of carbon and oxygen) of [redacted] is **less than 100 nm**.

5. CONCLUSIONS

For the characterization of phytopharmaceutical DP, WP and biocide samples in the aerosol phase, a dust disperser was coupled to two spectrometers respectively called Scanning Mobility Particle Sizer (SMPS) and Aerodynamic Particle Sizer (APS) to measure average normalized particle number size distribution (PNSD) for 20 nm – 570 nm and 1 µm – 20 µm size ranges respectively without associated chemical analysis.

Sub-groups were identified in order to classify PNSD and to reach understandable comparisons. For each average normalized PNSD, an analysis of associated statistical diameters was presented :

- For phytopharmaceutical WP samples (references 1 to 11), for which two groups were identified and characterized by average SMPS median diameters (d_{median}) of 197 nm ± 32 nm and 290 nm ± 27 nm, only three samples (n°2, 10 and 11) were characterized by bimodal PNSD with an APS d_{median} of 1.70 µm ± 0.05 µm, 2.60 µm ± 0.05 µm and 2.20 µm ± 0.05 µm respectively ;
- For phytopharmaceutical DP samples (references 12 to 15), one group of sample was identified and characterized by an average SMPS d_{median} of 143 nm ± 52 nm. Two samples (n°12 and 15) were characterized by APS d_{median} of 2.7 µm ± 0.2 µm and 2.1 µm ± 0.1 µm respectively ;
- For biocide samples (references 16 to 25), three groups were identified and characterized by average SMPS d_{median} of 236 nm ± 34 nm, 157 nm ± 10 nm and 159 nm ± 28 nm respectively. For group n°1, 2 and 3 average APS d_{median} of 2.0 µm ± 0.3 µm ; 3.2 µm ± 1.9 µm and 1.8 µm ± 0.4 µm were characterized for all the samples of each group.

In terms of particulate contribution, all the sample references were characterized in the aerosol phase by aggregates and agglomerates contributions < 100 nm below 50% in the 17 nm – 570 nm SMPS measurement size range.

Highlight:

For phytopharmaceutical DP, WP and biocides samples, it is important to mention that complementary measurements by SEM are needed in order to better understand the PNSD representativity of the generated aerosols using the involved dust disperser for this study. Indeed, such PNSD measurements in aerosol phase are associated to aggregates/agglomerates measurements, and not to primary particles.

About phytopharmaceutical CoS and CaS samples, results for particle size distribution characterization by SEM and chemical identification by EDX are summarized below.

a) <u>Phytopharmaceutical capsules suspensions (CaS) samples:</u>

All samples have been prepared (dilution, drying and ultrasonication) before measurements, except for static deposition. Note that the phase of sample preparation can generate modifications like opening shell.

It should be remembered that the study consists in only targeting particles smaller than 100 nm, identifying them and then measuring their size distribution.

- [redacted]: **Sample 26 :**
  - The **presence** of **particles** and **fibers is confirmed**.
  - Agglomerates/aggregates made of constituent particles with an **isotropic shape** are observed. Titanium dioxide (TiO_2) and particles containing Iron (Fe) are identified.
    - *i. Particles containing iron and titanium dioxide are observed.*
      - Particles containing Iron present a $D_{Feret\ min}$ smaller than 100 nm, even if concentration is to low to obtain a size distribution.
      - Titanium dioxide particles present 100% by number of the constituent particle population greater than 100 nm.
    - *ii. Fibers likely corresponding to sepiolite and halloysite structure are observed.*
      - The diameter ($D_{Feret\ Min}$) of the fibers is **23.4 nm**.
      - The median size (D50) of the fibers is 22.1 nm.
      - **100 %** by number of fibers have $D_{MinFeret}$ lower than 100 nm. (*i.e.* 100 % of fibers are nanofibers).

- [redacted]: **Sample 27 :**
  - The **presence** of **particles** is **confirmed**.
  - Agglomerates/aggregates made of constituent particles with an **isotropic shape** are observed.
    - *Particles likely corresponding to $CaCO_3$ are observed.*
      - The average size of the particles is **25.9 nm**.
      - The median size (D50) of the particles is 24.9 nm.
      - **100 %** by number of constituent particles is less than 100 nm. (*i.e.* 100 % of particles are nanoparticles).

- [redacted]: **Sample 28 :**
  - The **presence** of **particles** is **confirmed**.
  - Agglomerates/aggregates made of constituent particles with an **isotropic shape** are observed. Carbon, nitrogen and oxygen are identified.
    - *Particles containing carbon,* nitrogen *and oxygen are observed.*
      - The average size of the particles is **87.5 nm**.
      - The median size (D50) of the particles is 79.5 nm.
      - **69 %** by number of constituent particles is smaller than 100 nm. (*i.e.* 69 % of particles are nanoparticles).

- [redacted]: **Sample 29 :**
  - The **presence** of **particles** is **confirmed**.
  - Agglomerates/aggregates made of constituent particles with an **isotropic shape** are observed. Carbon and oxygen are identified.
    - *Particles containing carbon and oxygen are observed.*
      - The average size of the particles is **26.4 nm**.
      - The median size (D50) of the particles is 26.4 nm.
      - **100 %** of constituent particles are lower than 100 nm. (*i.e.* 100 % of particles are nanoparticles).

- [redacted]: **Sample 30 :**
  - The **presence** of **particles** is **confirmed**.
  - Agglomerates/aggregates made of constituent particles with an **isotropic shape** are observed.
    - *Particles containing carbon and oxygen are observed.*
      - The average size of the particles is **24.2 nm**.
      - The median size (D50) of the particles is 24.2 nm.
      - **100 %** by number of constituent particles is smaller than 100 nm. (*i.e.* 100 % of particles are nanoparticles).

b) <u>Phytopharmaceutical concentrated suspensions (CoS) samples:</u>

- [redacted]: Sample 31 :

- The **presence** of **particles** and **fibers** is **confirmed**.
- Agglomerates/aggregates made of constituent particles with an **isotropic shape** are observed.
    i. *Particles containing carbon and oxygen are observed.*
        - The average size of the particles is **23.5 nm**.
        - The median size (D50) of the particles is 22.7 nm.
        - **100 %** by number of constituent particles by number is less than 100 nm. (*i.e. 100* % of particles are nanoparticles).
    ii. *Fibers likely corresponding to sepiolite and halloysite structure are observed.*
        - The diameter ($D_{Feret\ Min}$) of the fibers is **20.4 nm**.
        - The median size (D50) of the fibers is 19.1 nm.
        - **100 %** by number of fibers have $D_{Min\ Feret}$ lower than 100 nm. (*i.e.* 100 % of fibers are nanofibers).

- [redacted]: Sample 32 :

- The **presence** of **particles** and **fibers** is **confirmed**.
- Agglomerates/aggregates made of constituent particles with an **isotropic shape** are observed.
    i. *Particles containing carbon and oxygen are observed.*
        - The average size of the particles is **19 nm**.
        - The median size (D50) of the particles is 18.1 nm.
        - **100 %** by number of constituent particles is less than 100 nm. (*i.e. 100* % of particles are nanoparticles).
    ii. *Fibers are observed and structure is not identified.*
        - The diameter ($D_{Feret\ Min}$) of the fibers is **24.7 nm**.
        - The median size (D50) of the fibers is 23.4 nm.
        - **100 %** by number of fibers have $D_{Min\ Feret}$ lower than 100 nm. (*i.e.* 100 % of fibers diameter are nanofibers).

- [redacted]: Sample 33 :

- The **presence** of **particles** is **confirmed**.
- Agglomerates/aggregates made of constituent particles with an **isotropic shape** are observed.
    - *Particles containing carbon and oxygen are observed.*
        - The average size of the particles is **34.3 nm**.
        - The median size (D50) of the particles is 33.3 nm.
        - **100 %** by number of constituent particles is smaller than 100 nm. (*i.e.* 100 % of particles are nanoparticles).

Paris, January 26, 2021

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