



SLOVENSKI STANDARD
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Izpostavljenost na delovnem mestu - Določanje in karakterizacija lebdečih nanopredmetov ter njihovih agregatov in aglomeratov (NOAA) z elektronsko mikroskopijo - Pravila za vzorčenje in analizo

Workplace exposure - Detection and characterization of airborne NOAA using electron microscopy - Rules for sampling and analysis

Exposition am Arbeitsplatz - Nachweis und Charakterisierung von luftgetragenen NOAA durch Elektronenmikroskopie - Regeln für Probenahme und Analyse

Exposition sur le lieu de travail - Détection et caractérisation des NOAA en suspension dans l'air par microscopie électronique - Règles d'échantillonnage et d'analyse

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**Workplace exposure - Detection and characterization of
airborne NOAA using electron microscopy - Rules for
sampling and analysis**

Exposition sur le lieu de travail - Détection et
caractérisation des NOAA en suspension dans l'air par
microscopie électronique - Règles d'échantillonnage et
d'analyse

Exposition am Arbeitsplatz - Nachweis und
Charakterisierung von luftgetragenen NOAA durch
Elektronenmikroskopie - Regeln für Probenahme und
Analyse

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European foreword

This document (CEN/TS 18117:2025) has been prepared by Technical Committee CEN/TC 137 “Assessment of workplace exposure to chemical and biological agents”, the secretariat of which is held by DIN.

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Introduction

Methods to assess exposure to nano-objects and their agglomerates and aggregates (NOAA) in the workplace are only partially standardized, for example the metrics to be used for exposure assessment, the tiered approach to collect exposure measurements, the way to operate the condensation particle counter (CPC), approach to assess dermal exposure to nanomaterials. In addition, many guidelines are rather outdated by not outlining the requirements to identify nanoforms, as the technical standards by the time of their formulation were not sufficient to detect nanoscale dimensions or nanoforms were not regarded of special interest. To determine workplace exposure to particulate matter, gravimetric methods were established that weigh the collected dust to determine mass load and a respective mass concentration for a compliance test against occupational exposure limit values (OELV). However, those methods are insensitive towards the particle size and in case only the fraction of NOAA needs to be determined, electron microscopy (EM) is a necessary supplemental technique for the identification and physical characterization of NOAA, including nanofibres (e.g. carbon nanotubes (CNTs)), nanoplates (e.g. graphene) and nanoparticles. With the help of energy dispersive X-ray spectroscopy (EDS/EDX) as an add-on to EM, also chemical identification of NOAA can be accomplished. EM methods referred to in this document include scanning electron microscopy (SEM) and transmission electron microscopy (TEM), which are two different techniques with different capabilities.

Fibres are of special interest, since their potential hazard can arise from their length and diameter, calling for geometrical characterization of high aspect ratio particles found in the collected workplace aerosol with EM techniques. In fact, the WHO established a fibre counting convention that identifies fibres in case length $> 5 \mu\text{m}$, diameter $< 3 \mu\text{m}$ and aspect ratio > 3 (WHO-fibres). Legal OELV for fibres are based on this counting convention. However, the diameter had also a lower limit of 200 nm, accounting for the resolution limit of phase contrast microscopy commonly used in the time when these Standards for asbestos exposure assessment were written. Consequently, nanofibres would not be detected and counted. However, the past 20 years of nanotoxicological research attributed asbestos-like hazard potential to some nanofibres like carbon nanotubes, which in parallel became increasingly used at workplaces. Therefore, newer guidelines and standards for fibre workplace exposure assessment decreased or cut the lower diameter limit and implemented the application of EM methods. Nanoparticles of non-fibrous materials indicate a higher toxicity in comparison to micro sized particles due to the higher chemically active surface to mass ratio.

Even though no NOAA-specific legal OELV exist by the time of the formulation of this document, so-called health-based nano reference values (HNRVs) for occupational exposure have been proposed for some engineered nanomaterials (ENM). Especially in mixed dust environments, a quantitative analysis with EM would have great added value for respective compliance tests, because of the possibility to distinguish different morphological particle types, i.e. nanofibres versus other shapes. Chemical analysis included in the EM methodology would allow for a complete identification of ENM against a complex particle background.

Thus, the application of EM analyses for assessing the actual type of exposure can be envisaged for all workplaces dealing with nano-objects, e.g. producers of nanoparticles and also companies subsequently employing nanoparticles in different products. Furthermore, due to the production of unwanted nano-objects during processes including heat or mechanical treatments an assessment of the possible exposure might be favourable. The EM analysis can unambiguously identify the nature and chemical identity of the nano-objects and thus contribute to a complete workplace exposure assessment.

EM analysis relies on aerosol collection methods to produce samples that comprise particles that can firstly be visualized and secondly characterized. Both prerequisites call for aerosol collection protocols specially adjusted for the technical boundary conditions of the used EM as well as the analytical procedure that includes image acquisition and subsequent particle counting as well as the measurement of selected particle parameters.

For aerosol collection, several types of samplers exist, based on different collection principles (e.g. filtration, impaction, electrostatic or thermophoretic precipitation) and collecting different size ranges of particles. However, the analysis of NOAA by EM has strict requirements for samples, which leads to the use of specific methods of sample collection and preparation. Despite the different requirements of SEM and TEM that affect

the way samples are collected and prepared for analysis, it is necessary for both techniques to collect homogeneous deposits, with a minimum of overlapping of the particles. Furthermore, the particles on the collection medium should be in the same particle size range to which workers are potentially exposed to, from single nano-objects to micron sized agglomerates and aggregates.

EM analysis also comes with strict requirements in particular in the context of testing the workplace aerosol against a specific OELV, which are specific to particle types. Hence the technicalities of the analysis would be adjusted to be optimal to identify, count and characterize the chosen particle type. In addition, the statistical requirements in order to be confident towards the compliance test result call for strict rules. In turn, general characterization of the workplace atmosphere to “get an idea” of its particle type composition would call for boundary conditions allowing to visualize, count and analyse all kinds of nano-objects on the sample but without being bound to strict statistical requirements. Generally, applicable protocols for EM analysis of workplace samples should therefore set the rules for image acquisition, particle identification, counting and characterization based on the analytical aim. A modular formulation of an EM protocol might enable this strategy.

A set of validated sampling devices and collection media with experimentally determined collection and sampling efficiencies is presented in this document, together with a set of validated protocols and guidelines for the characterization and quantification of airborne nanoparticles, nanofibres and nanoplatelets and their agglomerates and aggregates using EM methods.

The methodology could be implemented as a higher tier step in an occupational exposure assessment strategy for NOAA. Results from this analysis can be used to compare to health-based limit values, as they become available and to understand potential health risks of workers.

NOTE Examples of direct reading instruments are CPC, SMPS, ELPI; these instruments are listed in the OECD guideline [1].

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1 Scope

This document provides rules for workplace sampling and the sample analysis for the determination and characterization of airborne NOAA for electron microscopy and includes:

- the choice of appropriate samplers and their use for the determination and characterization (e.g. classification of structures and morphology) of airborne NOAA using electron microscopic methods (SEM and (S)TEM);
- counting rules and criteria for the determination and characterization (e.g. classification of structures, chemical composition and morphology) of airborne NOAA using electron microscopic methods (SEM and (S)TEM), especially for nanofibres and platelets.

This document is based on extensive laboratory tests for airborne NOAA, in particular those released during the handling of engineered nanomaterials.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 482, *Workplace exposure - Procedures for the determination of the concentration of chemical agents - Basic performance requirements*

EN 689, *Workplace exposure — Measurement of exposure by inhalation to chemical agents — Strategy for testing compliance with occupational exposure limit values*

EN 1540, *Workplace exposure — Terminology*

EN 17058, *Workplace exposure - Assessment of exposure by inhalation of nano-objects and their aggregates and agglomerates*

EN ISO 13137, *Workplace atmospheres - Pumps for personal sampling of chemical and biological agents - Requirements and test methods (ISO 13137)*

ISO 9276-2, *Representation of results of particle size analysis — Part 2: Calculation of average particle sizes/diameters and moments from particle size distributions*

ISO 9276-4, *Representation of results of particle size analysis — Part 4: Characterization of a classification process*

ISO 9276-6, *Representation of results of particle size analysis — Part 6: Descriptive and quantitative representation of particle shape and morphology*

ISO 13322-1, *Particle size analysis — Image analysis methods — Part 1: Static image analysis methods*

ISO/TS 21361, *Nanotechnologies — Method to quantify air concentrations of carbon black and amorphous silica in the nanoparticle size range in a mixed dust manufacturing environment*

ISO/TS 21383 *Microbeam analysis — Scanning electron microscopy — Qualification of the scanning electron microscope for quantitative measurements*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 1540 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <https://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

3.1

aspect ratio

ratio of length of a particle to its width

[SOURCE: ISO 14966:2002, 3.7] [2]

3.2

blank

NOAA count made on a sample prepared from an unused filter or substrate to determine the background measurement

Note 1 to entry: Background in this context means the clean substrate structure.

3.3

cluster

structures on which four or more particles or bundles, are randomly oriented in a connected grouping

[SOURCE: ISO 14966:2002, 3.10] [2]

3.4

collection substrate

flat substrate used for collecting particles and subsequent EM analysis

Note 1 to entry: Examples of substrates: a filter, TEM grid or silicon wafer.

3.5

contrast (of an image)

difference between the intensity of the particle image with respect to the background near to the particle

[SOURCE EN 13322-1:2024, 3.1.3]

3.6

energy-dispersive X-ray analysis

measurement of the energies and intensities of X-rays by use of a solid-state detector and multi-channel analyser system

[SOURCE: ISO 14966:2002, 3.12] [2]

3.7

Feret diameter

distance between two parallel lines which are tangent to the perimeter of a particle

Note 1 to entry: The Feret diameter is a measure of an object size along a specified direction; it is applied to projections of a three-dimensional object on a two-dimensional plane.

CEN/TS 18117:2025 (E)**3.8****fibre bundle**

structure composed of apparently attached, parallel fibres

[SOURCE: ISO 14966:2002, 3.14] [2]

Note 1 to entry: A fibre bundle may exhibit diverging fibres at one or both ends. The length is defined as equal to the maximum length of the structure, and the diameter is defined as equal to the maximum width of the compact region.

Note 2 to entry: "Parallel" shall not be a requirement, since very flexible CNTs can entangle without actually aligning.

3.9**high aspect ratio object**

object (hybrid aggregate, fibre aggregate, fibre cluster) with an aspect ratio > 3

3.10**image analysis**

processing and data reduction operation which yields a numerical or logical result from an image

[SOURCE: ISO 13322-1:2014, 3.1.8]

3.11**image field**

surface area of the sample substrate (e.g. filter, grid) which is shown on the EM display

[SOURCE: ISO 14966:2002, 3.18] [2]

3.12**limit of detection**

calculated airborne particle concentration, equivalent to the upper 95 % confidence limit of 2,99 structures predicted by the Poisson distribution for a count of zero particles

[SOURCE: ISO 14966:2002, 3.19] [2]

3.13**low aspect ratio object**

object (hybrid aggregate, fibre aggregate, fibre cluster) with a length < 5 µm and an aspect ratio > 3

3.14**maximum Feret diameter**

maximum length of an object whatever its orientation

[SOURCE: ISO/TR 945-2:2011, 2.1] [3]

3.15**measurement frame**

selected area from the field of view in which particles are sized and counted for image analysis

[SOURCE: ISO 13322-1:2014, 3.1.10]

3.16**minimum Feret diameter**

minimum length of an object whatever its orientation

[SOURCE: EN ISO 21363:2022, 3.4.5]

3.17

particle diameter

geometric diameter of a particle

Note 1 to entry: Particle diameter is often referred to simply as 'particle size'.

3.18

pixel

smallest element of an image that can be uniquely processed, and is defined by its spatial coordinates and encoded with colour values

[SOURCE: ISO 12640-2:2004, 3.6] [4]

Note 1 to entry: EM images always come in greyscale.

3.19

primary particle

original source particle of agglomerates or aggregates or mixtures of the two

[SOURCE: ISO 26824:2022, 1.4] [5]

3.20

projected area equivalent diameter

diameter of a circle having the same area as the projected image of the particle

[SOURCE: ISO 13322-1:2014, 3.1.1]

3.21

threshold

grey level value which is set to discriminate objects of interest from background

[SOURCE: ISO 13322-1:2014, 3.1.14]

3.22

WHO object

originally describing elongated particles with minimum length of 5 μm , a diameter between 0,2 μm and 3 μm and a minimum aspect ratio of 3:1

Note 1 to entry: In this document, the lower diameter limit is determined by the pixel size of the EM images.

4 Abbreviations

APS	Aerodynamic Particle Sizer
CP	Coarse Particle
EDX	Energy-Dispersive X-ray Analysis
EM	Electron Microscopy
EPC	Environmental Particle Counter
ESZ	Electrical Sensing Zone

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FEG	Field Emission Gun
HAR	High Aspect Ratio
HARFB	High Aspect Ratio Fibre Bundle
HARFC	High Aspect Ratio Fibre Cluster
HARFO	High Aspect Ratio Fibre Object
LAR	Low Aspect Ratio
LARFB	Low Aspect Ratio Fibre Bundle
LARFC	Low Aspect Ratio Fibre Cluster
LARPA	Low Aspect Ratio Particle Agglomerate/Aggregate
LARPO	Low Aspect Ratio Particle Object
MPPS	Most Penetrating Particle Size
NOAA	Nano-Objects and their Aggregates and Agglomerates > 100 nm
OELV	Occupational exposure limit value
PSD	Particle Size Distribution
SEM	Scanning Electron Microscope
SMPS	Scanning Mobility Particle Sizer
STEM	Scanning Transmission Electron Microscope
TEM	Transmission Electron Microscopy
WHO	World Health Organization

NOTE EDX is sometimes called EDS

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5 Procedure**5.1 General**

Airborne nano-objects, aggregates and agglomerates (NOAA) consists of objects in the nano to micron size range. EM techniques shall be used to identify the nature and chemical identity of the full size range of airborne NOAA and thus contributing to a complete workplace exposure assessment.

To be able to use EM techniques, first airborne NOAA shall be sampled by collecting the objects in a certain volume of air on a collection medium. To identify NOAA and analyse them by EM, specific methods of sample collection, preparation and analysis shall be used. Sampler devices and sampling procedures are described in detail in Clause 6.

NOTE Despite the different requirements of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) that affect the way samples are collected and prepared for analysis, it is necessary for both techniques to collect uniform deposits, with minimal probability of overlapping of the objects. This is especially valid in the framework of an exposure assessment for compliance testing against occupational exposure limits (OELV).

The objects on the collection medium shall be in the same object size range which workers are potentially exposed to, from single nano-objects to micron sized agglomerates and aggregates.

EM analyses are used to determine object sizes, their particle size distributions (PSD) and morphology (such as nanofibres, platelets or spherical particles). Electron microscopes are often equipped with detectors for chemical identification, such as an energy-dispersive X-ray system (EDX), allowing the determination of the

elemental composition of the particles. Detailed analysis procedures and counting rules for airborne NOAA are described in Clause 7.

5.2 Basic approach

For the determination and characterization of airborne NOAA in the workplace, the following stages shall be considered:

- 1) Determine measurement objectives, such as NOAA characterization, indication particle number concentration and/or compliance testing (< > OELV, according to EN 689 and EN 482);
- 2) Determine workplace characteristics, such as activities, nanomaterials, exposure estimates;
- 3) Determine sampling strategy: selection of sampling device and collection media, specification of measurement procedure; i.e. amount of samplers, duration of sampling, position of samplers.

NOTE Additional guidelines and strategies of workplace exposure measurements are described in EN 17058 and ISO/TR 27628.

- 4) Determine the analysis strategy: selection of the microscope and settings (according to ISO/TS 21383, ISO/TS 21361), determination of the analysis procedure (see EN ISO 19749, EN ISO 21363), i.e. off-line vs online, manual vs automatic, with/without EDX, measurands and desired results.
- 5) Perform measurements, including sampling, sample preparation, EM image acquisition and object characterization and counting;
- 6) Process the obtained EM data: calculate the 95 % confidence interval of the number concentration and/or particle size distribution (according to ISO 9276-2, -4, -6 and ISO 13322-1), data evaluation and quality control, data presentation and reporting (Clauses 8 and 9).

5.3 EM within the exposure assessment framework

In general, the objectives of an exposure assessment can vary widely and can include exposure exploration and determination, evaluation of the effectiveness of exposure control measures, check for compliance with occupational exposure limits (OELV) or other benchmark level, and can contribute to risk assessment and epidemiological studies. The measurement strategy depends amongst other factors (e.g. workplace situation, type of NOAA, emission sources/concentrations), on the objective of the assessment. EN 17058 and ISO/TR 27628:2007 [6] give general guidance on workplace exposure assessment by inhalation of NOAA and describes measuring devices and measurement methods for the characterization of NOAA exposure. In addition, ISO/TR 27628 provides background information on the mechanisms of nanoparticle aerosol formation and transportation within an occupational setting and on industrial processes associated with nanoparticle aerosol exposure.

Several measuring methods of characterizing exposures are covered and specific information is provided on single particle analysis with EM techniques. Each individual measurement method has its drawbacks, but when used in combination, they give an initial insight into the presence of NOAA in the workplace. More specific, a combination of direct-reading instruments and NOAA sampling techniques with subsequent off-line analysis is in most workplace situations the best approach to gain insight into the occupational exposure of NOAA. EM techniques can be used to get in depth information on size and morphology of objects and are indispensable for the determination of number concentrations in mixed dust situations and for non-spherical nanoparticles (nanofibres and nanoplatelets).

EN 17058 gives guidance on different levels of exposure assessment in a tiered-approach framework.

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5.4 Apparatus and materials

5.4.1 General

To perform an exposure assessment to airborne NOAA, various apparatuses and materials are necessary.

5.4.2 Air sampling

- **Sampler:** for air sampling a suitable sampler shall be used depending on the measurement objective. Various samplers are available commercially. An overview is shown in A.1. Annexes B to D provide examples of sampling efficiencies and collection efficiencies of selected samplers.
- **Collection medium:** the sampler is equipped with a collection medium (A.2). Collection media used for collecting airborne NOAA and their subsequent EM analysis have to fulfil some prerequisites. They shall be flat with a uniform surface and preferably electrically conductive to avoid local charging during EM imaging. In addition, during imaging with EM they have to provide a uniform background signal. For SEM different filter membranes, solid wafer pieces, metallic substrates as well as TEM-grids are possible collection substrates. For TEM only TEM grids are possible substrates.
- **Pump:** the pump shall be chosen according to the necessary volumetric airflow of the sampler and the characteristics of the collection substrate used (e.g. pressure drop created by the pore size of the filter), (see A.3).

5.4.3 Sample pre-treatment

5.4.3.1 Plasma cleaner and/or coater: The choice of collection medium will be dictated by the type of sampler and the analytical considerations (e.g. SEM or (S)TEM). For filter samples in special cases (e.g. charging effects, contamination) plasma cleaning and/or coating shall be applied (A.4).

NOTE 1 For samples collected on TEM grids, no coating is necessary and plasma cleaning is not recommended.

NOTE 2 Make sure to perform the plasma cleaning prior to (sputter) coating.

5.4.4 Analysis

- **Electron microscope:** object analysis can be conducted in a scanning electron microscope (SEM) or (scanning) transmission electron microscope ((S)TEM). Both types of instruments can be fitted with various detectors to record multiple signals, electrons and radiation, emitted from the interaction of the electron beam with the sample. Those detectors can determine the (elemental) composition and chemically identify particles. More information on EM selection and qualification and instrument settings and conditions are given in A.5. More specific information on the use of electron microscopes for particle analysis can be found in EN ISO 21363 and EN ISO 19749. Detailed technical information on the qualification of SEMs for quantitative analysis can be found in ISO/TS 21383.

The resolution of the microscope shall be at least four times higher than the minimum diameter of the smallest nano-objects present. For imaging NOAA, with diameters of individual nano-objects below 10 nm, a TEM or high resolution SEM with field emission source is required.

For objects sizes below 5 nm (i.e. nanorods) a TEM is highly recommended.

- **Image analysis software:** the purpose of image analysis is to measure size and morphological features, such as shape, of the present objects on the sample surface. Conventional image analysis is based on algorithms, set to recognize and detect particles upon their grayscale level (thresholding) and can be performed in-operando and offline. More information is given in A.6.