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

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A list of organizations represented on this committee can be obtained on request to its committee manager.

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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 In addition, many guidelines are rather outdated by not outlining the requirements to identify nanoforms, as the technical standards by the time of their formulation were not sufficient to detect nanoscale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 µm, diameter < 3 µm 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

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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*

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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.

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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

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[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

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

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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.

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NOTE Be aware that not all image analysis software has been validated.

6 Sampling

6.1 Sampling strategy

Electron microscopic techniques are powerful in-depth techniques, however, the analysis of EM samples is very sensitive to the level of sample load, and thus require careful consideration of the sampling strategy. Sampling of NOAA starts with setting up a sampling strategy, which is based on the objectives of the measurements. When determining the objectives, it is important to take into account special aspects of the workplace situation.

The optimum position for collection of air samples shall be determined after a complete survey of the location (initial assessment phase). Detailed information on the process conditions, object types (nanofibres, nanoplatelets or nanoparticles) and, if known, size ranges of the airborne NOAA shall be acquired prior to sampling. In addition, if the workplace is (partly) outdoors, the outdoor environment shall be taken into account as well, such as weather conditions. All available information such as local topography shall be recorded. Furthermore, the position of the samplers shall be carefully recorded. Also, timing of sampling shall be taken into account.

It is essential to define the objective of the measurements, before samples are collected, such as the level of exposure assessment and the desired information/results. Available information on emission sources and local situations shall be taken into account, as stated in the initial assessment of EN 17058. The best sampler and sample substrate shall be selected based on the objectives of the measurements.

A sufficient number of samples shall be collected and depending on the objectives and the level of the workplace, assessment by sequential multipoint sampling might be necessary to provide adequate characterization. The collection medium needs to be appropriately loaded for EM analysis, resulting in a uniform and homogeneous deposit, with a minimum of overlapping of the objects. Therefore, substrate material, pore sizes, sampling flows and sampling time play an important role.

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

If the measurement objectives and workplace characteristics are determined and the sampling and analysis strategies are in place, the sampling procedure shall be started.

6.2 Collection of air samples

6.2.1 General

For sampling collection of airborne NOAA, suitable samplers shall be used that contribute to the scope of measurements. Before use, samplers shall be pre-cleaned and checked for defects. The samplers shall be operated according to the manufacturer's instructions.

The sampling (vacuum) pump shall be connected to the EM sampler with a flexible tubing and the flow rate of the sampler needs to be adjusted. Sampling pumps shall meet the requirements of EN ISO 13137 and shall be operated according to the manufacturer's instructions. Test conditions, test methods and instructions for use shall be followed according to EN ISO 13137.

NOTE At the end of sampling, cover the sampler, to avoid contamination and preferably hold the samplers upright (sampled collection medium surfaces face-upwards). Transport the sampler to the EM laboratory for analyses.

If deposits are observed around the edge of the substrate or on unexposed edges of the sampling holder, a leak around the substrate has occurred and the sample shall be rejected.

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6.2.2 Personal and area sampling

For a basic exposure assessment area (fixed-point) sampling can be performed, but several considerations shall be made when choosing a proper location, e.g. distance to the source, ventilation/airflows, movement of the workers. The sampler shall be oriented according to the manufacturer's requirements. For area sampling, there are basically no restrictions for EM sampling techniques and pumping devices.

For a comprehensive assessment of the exposure by inhalation, measurements in the breathing zone are necessary. To allow free movement of the workers, small wearable and battery-based sampling pumps need to be worn by the worker with the inlet of the EM sampler placed within a hemisphere of 30 cm within the breathing zone of the person. There are certain restrictions for personal air sampling. The EM sampler shall be physically placed in the breathing zone and the worker shall not be inconvenienced by it, in a way that his movements are hindered. Additionally, the capacity of the personal air pump needs to be considered when choosing the sampler and collection substrate. If personal breathing zone measurements are not possible, a comprehensive assessment of the exposure by inhalation cannot be performed.

6.2.3 Time, duration and frequency of sampling

Depending on the sampling techniques, the sampling time can vary. For compliance testing, the sampling time (sampling period) shall be representative of the reference period of the OELV.

NOTE For example, a full shift average concentration, typically the 8 h time-weighted concentration, gives a representative description of the occupational exposure situation. If possible, the sampling period is equal or close to 8 h with a minimum of 4 h.

For objects (size) counts, the sample substrate needs to be appropriately loaded to allow efficient analysis, while avoiding object agglomeration and overloading. In A.4 of ISO/TR 27628:2007 [6], details can be found of the calculation of sample collection times.

6.3 Collection and sampling efficiencies

Sampling and collection efficiencies play an important role in the sampler selection. As well as the ability of the sampler to collect uniform and homogeneous deposits, with a minimum of overlapping objects. In this document samplers were validated during three Round Robins. Table B.1 to B.7 in Annex B present a summary of the collection efficiencies of 8 tested samplers for different types of NOAA, Annex C shows results of collection efficiencies specific for fibrous objects. Annex D gives examples of complete overall sampling efficiency curves of 4 selected filter-based samplers for NOAA from around 30 nm to around 30 µm and provides information on the sampling of both submicronic nano-objects and micronic aggregates and agglomerates. The results presented in Annexes B to D shall be used as guideline to select the best possible sampler.

6.4 Transport, handling and storage

Overall, the samplers, and collected samples, shall be handled carefully. During transport and storage, the samples shall be stored in labelled anti-static containers and shall be stored upright in dust-tight sample containers. The sampler holder needs to be covered at all times during transport and storage to prevent contamination of the collection medium. The cover shall be anti-static and only be removed in the laboratory for sample preparation and for insertion into the EM instrument. In the laboratory, the transported containers shall be visually checked for signs of disturbance.

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7 Analysis

7.1 Analysis strategy

7.1.1 General

There are multiple procedures to analyse a collection medium surface using EM techniques. Procedures shall be performed manually or (semi-) automatically, online or via micrographs (offline) (see A.6).

7.1.2 Differentiation of particles

If a variety of objects are present (mixed dust) at the collection medium surface, object differentiation shall be performed based on morphology and/or shape if particles have clearly differing morphologies (e.g. fibres vs. spherical particles), or their z-contrast visible when using BSE electrons for imaging. If the objects cannot be distinguished via the above, chemical identification (e.g. by EDX detector, see A.5.2) is necessary for an unambiguous identification of particles with the same morphology and contrast.

7.1.3 Image resolution and magnifications

Micrographs shall be taken with a physical resolution high enough to visualise all relevant particles and allow geometric characterization. Ideally, one fixed magnification is used to analyse both primary nano-objects as well as agglomerates and aggregates. If at the chosen image size (to be able to observe the larger agglomerates and aggregates) the resolution (pixel size) is not sufficient to analyse the primary nano-objects, multiple fixed magnifications can be used to obtain a complete particle size distribution (see Clause 8). In this case, multiple subsets of images are acquired, respective for the selected pixel sizes. Each subset requires its own particle analysis and counting. A mixture of images with different pixel sizes for this purpose is not valid.

7.2 Preparation of samples

Samples arriving at the laboratory shall be examined visually. Before sample preparation and analysis, the collection medium needs to be examined on uniformity of deposition. If the deposition shows evidence of non-uniformity, the sample shall be rejected.

For EM analyses, the medium shall be mounted onto a stub (a common mounting method is to use double-sided adhesive conducting carbon pads, augmented with spot-application of colloidal silver or carbon to ensure a clear conducting pathway between the top of the sample and the specimen support), or media could be placed completely in sample holders, or could be placed directly into the EM chamber (e.g. TEM grids). Mounting depends on sample and instrument type and is the responsibility of the operator.

For SEM there needs to be a conductive pathway between the point at which the electron beam hits the substrate surfaces. Localized charging of the substrate during SEM measurements and poor imaging needs to be minimized. Additional preparation techniques such as vacuum evaporator, plasma asher and (sputter) coater could be used to improve the image.

7.3 Sample Quality check

The first sample quality check using EM, after collection of airborne NOAA, consists of observing the sample substrate at low to high resolution (magnification e.g. 100 – 100 000x). An image series with stepwise increase of magnification is acquired containing the information necessary to assess the sample quality based on the following criteria:

— **No impairment of sample physical integrity:** low resolution (overview image). The physical integrity of the sample collection medium is unaffected after sampling. No visible damage apart from peripheral marks by e.g. tweezers originating from sample transfer from the sampler to the EM table is observed.

— **No contamination:** low resolution. The sample shall be free from coarse objects (size > 100 µm) that do not stem from the aerosol sampling. Presence of large particles indicate sample contamination.

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— **Continuous particle load:** medium resolution (appropriate pixel size to visualize objects). The objects shall be distributed homogeneously on the collection medium surface. As a first analysis, the sample shall be screened for sections devoid of or overly loaded with objects compared to the majority of the sample. For a statistical analysis as a prerequisite for extrapolating particle number concentration from particle number density on the sample.

— **Fibres lay flat:** working resolution (appropriate pixel size for object characterization). High aspect ratio (HAR)-objects shall be deposited on the collection medium in a planar manner ideally without them pointing in a vertical direction.

In case the sample does not match quality criteria, the sample shall not be used to determine airborne NOAA concentrations with the objective for compliance testing. However, the sample could still be used for indicative measurements e.g. for particle size distribution, even if samples are locally disturbed. In both cases the quality criteria needs to be reported and judged.

For many collection media, surface features can resemble objects. In particular for track-edge membrane, irregularities in the gold coating might lead to local charge spikes that result in brighter artefacts in the otherwise dark background. Prior to the sampling campaign, one unused representative of the used badge or LOT of sample substrates shall be checked for the regular occurrence of such features. Occurrence of artefacts shall be documented and regarded in the EM analytic procedure applied after sampling.

7.4 Data collection

7.4.1 General

Collective samples of airborne NOAA will comprise objects of different sizes, chemical compositions and morphology (i.e. form, shape, structure). Analysis is performed by electron microscopy (EM) with the goal of characterizing the workplace airborne NOAA regarding its object class composition and estimate average object class concentrations based on object counting inside a representative area section of the sample collection medium.

The analysis procedure comprises two routines:

— Routine 1: Image acquisition using a scanning or transmission electron microscope w/wo chemical identification;

— Routine 2: Morphological analysis, counting and measurement of objects on EM images.

7.4.2 Parameters determining image acquisition routine

The first step in data collection is to gather all information which is necessary to perform analysis of objects and evaluation of data. Such as the sampling conditions documented during the workplace airborne NOAA air sampling:

— Sampling standard volume flow rate Q (l/min);

— Sampling duration τ (min);

— Sampling collection medium diameter d_o (mm);

NOTE 1 The sampling collection medium diameter d_o is the diameter of a circle that has an equivalent area as the sampling medium. Some samplers clamp the collection medium, so that the effective collection medium diameter that is actually exposed to the air flow is d_o minus the clamping width.

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— Sampler deposition efficiency η_c (%).

NOTE 2 Regarding η_c, a non-uniform sampling efficiency needs to be considered. Such is the case when the applied sampler-collection medium combination yields different sampling efficiencies for different particle properties. Taking filtration as an example, particle size can influence the collection efficiency as smaller objects more probably slip through the filter holes. In addition, flexible fibres are known to bend into and ultimately enter filter holes whereas their rigid counterparts resist bending.

The second step is to calculate the following parameters prior to data and object analysis.

The open sampling area A_o is calculated with Formula (1):

$$A_o = \pi / 4 \cdot d_o^2 \quad (1)$$

where

A_o is the open sampling area in mm^2;

d_o^2 is the sampling collection medium diameter in mm.

The total sampled air volume V_o is calculated with Formula (2):

$$V_o = Q \cdot \tau \quad (2)$$

where

V_o is the sampled air volume in l;

Q is the sampling volume flow rate in l/min;

τ is the sampling duration in min.

The specific sampled air volume ϱ_V, is calculated with Formula (3):

$$\varrho_V = V_o / A_o \quad (3)$$

where

ϱ_V is the specific sampled air volume in l/mm^2;

V_o is the sampled air volume in l;

A_o is the open sampling area in mm^2.

The EM analysis procedure yields a lower detection limit (LOD) determined by the size of the sample area subject to particle counting and characterization amongst others. One important application of this document is a test whether the workplace concentration is compliant with one or more OELVs for which it is mandatory that the LOD of the analytical routine is smaller or at least equivalent to the OELV. Therefore, the parameters for EM acquisition (pixel size, number of images) are chosen according to a chosen OELV, i.e. the concentration C_{EL}.

NOTE 3 At the time this document is written, only one legal number-concentration based exist, the fibre OELV corresponding to WHO-objects. Accordingly, C_{EL} = 10 000/m^3 (acceptance level) or C_{EL} = 100 000 /m^3 (tolerance level) is often a sensible choice.

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7.4.3 Image acquisition

7.4.3.1 General

The samples shall be placed inside the electron microscope. The process of image capturing starts. There are certain rules for image acquisition that only apply for one specific instrument, mainly because of the different sample collection media.

7.4.3.2 Image resolution

Micrographs shall be taken with a physical resolution high enough to visualize all relevant particles and allow geometric characterization. This resolution shall first be selected in terms of the dimension one pixel visualize one-pixel edge represents: pixel size. Most modern EMs allow for the setting of pixel size. In case, only the instrument magnification can be adjusted, the respective pixel size s can be calculated. The size s of square pixels can be calculated in nanometers for a microscopy image of $P_x \cdot P_y$ pixels with Formula (4):

$$s = \frac{127 \times 10^6 \text{ nm}}{M \cdot P_x} \text{ and } P_x \geq P_y, \tag{4}$$

where

s is the size of square pixels in nm;

M is the 5x4-inch-Polaroid-image-related magnification factor;

P_x is pixels at x-axes;

P_y is pixels at y-axes.

For reliable visualization of geometrical features of an object, a pixel size is required for which the smallest geometric dimension of the object of interest found on the sample is displayed by four pixels. For example, if the sample contains fibres with minimal diameter of 20 nm, pixel size shall be below 5 nm.

The images shall be obtained in an uncompressed format (e.g. TIFF or BMP). The meta-data of the images (depending on the manufacturer either as part of the image header or as a separate file) has to be saved alongside the images.

7.4.3.3 Position of image acquisition

Images shall be captured at random locations over the whole sample. Randomization can be accomplished by either making use of table navigation software that automatically generates random (x,y)-coordinates on the sample and navigating towards these locations for image capture or by blindly steering towards different positions for image capture. Doubled and overlapping images shall be avoided. Resolution shall not be changed and chosen so that the smallest particles are still visible on the one hand and on the other hand the EM image covers a maximum area.

The position of image acquisition depends on the kind of collection medium analysed. Images captured from collection media of track etched membranes are acquired at random locations over the whole membrane. Images are acquired at random locations over the whole TEM grid and images are only captured at grid holes, without a grid line being visible. And images captured of a wafer surface shall be taken at random locations within the sampler specific defined area suitable for analysis.

Fibres that reach out of the image field need subsequent additional analysis. Routines to positionally calibrate the sample prior to EM image acquisition allow for the recapture of previously analysed positions for taking additional images.

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7.4.3.4 Determination of evaluation area – general remarks

The evaluated collection medium area is the total visualized and analysed area on the sample, which most often is much smaller than the sample area. As mentioned above, it determines the achievable limit of detection (LOD). More precisely, the LOD is determined by the air volume, which correlates to the evaluation area, together with constraints of Poisson statistics arising from the fact that individual objects must be microscopically detected. In practice, an evaluation area can be pre-selected and EM acquisition performed so that the total area represented in the micrographs/EM images represents this area.

Two approaches can be followed to determine the evaluation area prior to image acquisition, which is necessary in case of automated image acquisition:

1) Minimal number of EM images representing a minimal evaluation area that yields a LOD equivalent to the selected OELV C_{EL} under the assumption that no particles of interest are found in the set of EM images (Clause 7.4.3.5);

2) Number of EM images representing an evaluation area in case the presence of particles of interest in the EM image set is expected (Clause 7.4.3.8).

In the first approach, the LOD is chosen and accordingly the number of EM images. In the second, the LOD is not important, since extrapolated concentrations typically yield concentrations much higher than such OELV.

In practice, the first approach is sensible for compliance tests against the fibre OELV, which is several orders of magnitudes smaller than the typical workplace concentration also comprising urban aerosols such as soot particles. Already if one fibre of interest is found in the minimal evaluation area, the procedure yields a high confidence is the conclusion that the true fibre workplace concentration was higher than the OELV. Another example for which the first approach can be used is the test of cleanroom atmospheres where one would expect very low concentrations. The second approach is selected in case non-zero particles of interest are expected to be present in the evaluation area, which is most probable for ubiquitous aerosol particles such as soot or general dust. In case this approach is used for compliance testing, the outcome is determined by the number of found particles of interest and the evaluation area size.

In case, presence of particles of interest is unknown, the sample shall be first surveyed with the help of the EM without image acquisition.

7.4.3.5 Minimal evaluation area for compliance testing

The LOD is defined via $H_S\left(\hat{N}=0,\alpha\right)$, the upper limit of the single-sided probability interval limit of a Poisson probability density function for expecting $\hat{N}=0$ objects at significance α, with Formula (5A).

$$H_S\left(0,\alpha\right)=\frac{1}{2}F^{-1}_{\chi^2}\left(1-\alpha,2\right) \tag{5A}$$

where

$H_S\left(0,\alpha\right)$ is the upper limit of the single sided probability interval limit of a Poisson probability density function for expecting $\hat{N}=0$ objects at significance α;

$F^{-1}_{\chi^2}\left(p,ndf\right)$ is the *p* quantile function of the Chi squared distribution. Mathematically, it corresponds to the inverse of the generalized regularized incomplete gamma function.

And compliance testing against an OELV concentration can be performed with minimum effort by adjusting the LOD to the OELV with Formula (6):

$$A^{S}_{\min}=\frac{H_s\left(0,\alpha\right)}{C_{EL}\cdot\eta_c\cdot\varrho_V} \tag{6}$$

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where

$A_{\min}^{S}$ is the minimum collection medium area in mm^2;

$H_S\left(0,\alpha\right)$ is the upper limit of the single-sided probability interval limit of a Poisson probability density function for expecting $\hat{N}=0$ objects at significance α;

NOTE 1 For a significance of $\alpha=0{,}05$, which corresponds to a confidence level of $p=95\ \%$, $H_S\left(0,0{,}05\right)=2{,}996$.

C_{EL} is the selected OELV for compliance testing (LOD of the analytical routine);

η_c is a known or estimated samples efficiency;

ϱ_V is a sampled specific volume in l/mm^2.

NOTE 2 The hypothesis that an OEL was complied with is accepted with significance α for the non-observation of *one* object when $H_S\left(0,\alpha\right)$ objects are statistically expected for an atmosphere of OELV concentration.

NOTE 3 Such an interpretation of object counts by Poisson statistics assumes randomly, i.e. homogenously, distributed objects on the collection medium surface.

7.4.3.6 Compliance testing with larger evaluation area

If areas are evaluated that let statistically expect to observe more than $H_S\left(0,\alpha\right)$ objects, a more general strategy of OEL compliance testing with $\hat{N}>0$ can be followed. Now, the hypothesis that an OEL was complied with is accepted with significance α if *not more* than $\hat{N}$ objects were observed when $H_T\left(\hat{N},\alpha\right)$ objects were statistically expected for an atmosphere of OELV concentration. Here, $H_T\left(\hat{N},\alpha\right)$ is the upper limit of the two-sided probability interval of a Poisson probability density function for expecting $\hat{N}$ objects at significance α, see Formula (5B):

$$H_T\left(\hat{N},\alpha\right)=\frac{1}{2}F_{\chi^2}^{-1}\left(1-\frac{\alpha}{2},2\left(\hat{N}+1\right)\right) \tag{5B}$$

where

$H_T\left(\hat{N},\alpha\right)$ is the upper limit of the two-sided probability interval of a Poisson probability density function for expecting $\hat{N}$ objects at significance α;

$F_{\chi^2}^{-1}\left(p,ndf\right)$ is the p-quantile function of the Chi-squared distribution.

7.4.3.7 Accuracy of concentration estimation

As the expectation value and the variance of the Poisson distribution are equivalent, small expectation values bear larger statistical uncertainty. For small counts of less than typically 30 objects, the span of the accompanying confidence intervals may exceed 100 % relative error and be so large that the object count may be unsuited for meaningful concentration estimation, see Table 1. In such a case, compliance testing shall be preferred.

Refer to Clause 8.1 for the calculation of the particle of interest number concentration on the basis of $H_S\left(\hat{N}=0,\alpha\right)$ or $H_T\left(\hat{N}\neq 0,\alpha\right)$

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Based on the given evaluation area, the resulting LOD can be determined analogous to Clause 7.4.3.5, i.e. by assuming zero found particles of interest. Solve Formula (6) for C_{EL}.

Table 1 — Relative size of the upper and lower confidence intervals for $\hat{N}$ observed objects and α = 5 % significance

δH_T (%)	H_T	$\hat{N}$	L_T	δL_T (%)
460	5,6	1	0,03	97
190	8,8	3	0,6	79
130	11,7	5	1,6	68
84	18,4	10	4,8	52
65	24,7	15	8,4	44
43	42,8	30	20,2	33
32	65,9	50	37,4	26
22	122	100	81,4	19
17	176	150	127	15

The lower limit of the confidence interval is given by Formula (5C):

$$L_T\left(\hat{N},\alpha\right)=\frac{1}{2}F^{-1}_{\chi^2}\left(\frac{\alpha}{2},2\hat{N}\right) \tag{5C}$$

where

$L_T\left(\hat{N},\alpha\right)$ is the lower limit of the two-sided probability interval of a Poisson probability density function for expecting $\hat{N}$ objects at significance α;

$F^{-1}_{\chi^2}\left(p,ndf\right)$ is the *p*-quantile function of the Chi-squared distribution.

For concentration estimation, more than 30 objects must have been found. The larger the evaluated filter area, the more accurate are extrapolated values.

7.4.3.8 Determination of the number of images

The more common practice is to first take images at random locations on the sample and to analyse the images afterwards using particle analysis software. A set of EM images is acquired, for example by making use of automated image acquisition. The operator considers how many particles shall be visualized, for example in order to determine the size distribution. The size of the set, i.e. number of images, shall not be predetermined without the knowledge of the density of the particles on the sample, the chosen OELV C_{EL} is referring to. The measurement of the average particle number concentration $\hat{C}$ during sampling by direct reading instrument such as a CPC can be used to estimate the average object count $\hat{N}$ per image area A_{image}, using Formula (7):

$$\hat{N}=\eta_c\,\hat{C}\,\varrho_V\,A_{\text{image}} \tag{7}$$

where

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$\hat{N}$ is the average object count;

η_c is a known or estimated samples efficiency;

C is the average number concentration in objects/cm^3;

ϱ_V is a sampled specific volume in l/mm^2;

A_{image} is the image area in mm^2.

The number of images K is then given by the ratio of a predetermined minimum number of objects to be examined N_{total} and $\hat{N}$, in Formula (8):

$$K = \frac{N_{\text{total}}}{\hat{N}} \tag{8}$$

where

K is the number of images;

N_{total} is the minimum number of objects to be examined;

$\hat{N}$ is the average object count.

EXAMPLE 1 The aim of the study is to determine the size distribution of the workplace airborne NOAA. After air sampling with a flow rate of 0,3 l/min for one hour using the MPS with a 3 mm TEM grid with 400 mesh size, a specific volume of $\varrho_V = 2{,}546 l/\text{mm}^2$ has been collected. A rather low collection efficiency of $\eta_c = 0{,}1$ is considered for the MPS. In parallel, a CPC measured an average concentration of $\hat{C} = 5000 / \text{cm}^3$. A TEM takes 12 Megapixel images with a pixel size of 5 nm, yielding $A_{\text{image}} = 0{,}0003\,\text{mm}^2$. Then, a particle count per image of $\hat{N} = 0{,}1 \cdot \frac{5000}{\text{cm}^3} \cdot 2{,}546\text{l/mm}^2 \cdot 0{,}0003\text{mm}^2 = 38{,}2 \approx 38$ can be estimated. The prerequisite to determine particle size distributions given by this document is the measurement of at least 300 particles, thus the examiner shall take 8 images to cover this number.

In case of low measured particle number concentrations, for example inside cleanrooms, and in the context of compliance testing, it is reasonable to use the method described in Formula (6) to derive the minimum evaluation area A^S_{min} and determine the number of images K to take to cover this area with Formula (9):

$$K = \frac{A^S_{\text{min}}}{A_{\text{image}}} \tag{9}$$

where

K is the number of images;

A^S_{min} is the minimal evaluation area in mm^2;

A_{image} is the image area in mm^2.

EXAMPLE 2: A_{image} is specific for the chosen pixel size and the image size. Taking cleanrooms as an example, the specification for cleanrooms requires less than 1000/m^3 objects larger than $d_p = 0{,}1\ \mu$m. Assuming that a FAP sampler

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sampled with 4 l/min for 1 h with filters exhibiting an effective filter area diameter of 23 mm, the specific volume was $\varrho_V = 1{,}1553\,\mathrm{l/mm^2}$. It is further assumed that the FAP sampler exhibits a collection efficiency of $\eta_{dep} = 1$ for particles larger than 0,1 µm since the filters exhibited a pore size of 0,1 µm. The EM takes 20 Megapixel images using a pixel size of 10 nm yielding $A_{\mathrm{image}} = 0{,}012336\,\mathrm{mm^2}$. Using a LOD of $C_{EL} = 1000\ /\ \mathrm{m^3}$, a minimum evaluation area of 0,435 mm² needs to be covered so that in case, the upper limit of the CI would be smaller or equivalent to C_{EL}. This yields that $K \approx 221$ images need to be taken, rounded up. A compliance test would be passed in case no particle larger than 0,1 µm were found. This amount can be lowered by using longer sampling times. Sampling for 8 h would yield $K \approx 28$, rounded up.

EXAMPLE 3: Currently, the lowest European number-based OELV is the German WHO-fibre acceptance level at 10000/cm³, which the following calculation of the minimum area to be analysed is based on. It is assumed that this routine shall be capable of confidently determining this concentration level in case no WHO-fibres are found. Ergo, the concentration $C_{EL} = 10000\ /\ \mathrm{m^3}$ is the LOD of this procedure. Also, a minimum of $\varrho_V = 1\,L/\mathrm{mm^2}$ shall be sampled, according to the VDI 3492 guideline. A minimal evaluation area A^S_{min} can be determined, considering zero WHO-fibre count, yielding 0,3 mm². To cover this area, at a resolution of 10 nm pixel size, 150 images would need to be taken, if the image contains 20 Megapixel, 250 for 12 Megapixels.

7.4.4 Chemical identification

In some cases, e.g. mixed dust airborne NOAA, chemical identification by detectors of objects is necessary to identify objects: two or more object types are present at the collection medium surface which could not be distinguished by morphology nor z-contrast, see A.5.2.

7.5 Object classification, characterization and counting

7.5.1 General

This section defines rules for object characterization and classification for nano-scale objects known to appear on EM images of workplace air sampled samples. Thereby, micro-scale objects without nanoscale substructures are not covered. Still, all objects on the images are counted and classified based on morphological features first.

7.5.2 Object classification rules

Objects present on the collection medium surface shall be classified based on morphology and/or size. A summary of object classes is presented in Table 2. Additional information and examples are shown in Annex E. Geometric particle/fibre properties are shown in Table 3.

The decision process for the evaluator that leads to classification of an object and its full or partial capture in the particle count is a multiple step process. For classification, the first decision is made after assessing the substructure of the objects, whether it is an agglomerate/aggregate or a singular object. The second decision is based on visual impression of the shape of the object to differentiate between particles, fibres and platelets. Due to the complexity of structures found on aerosol samples containing fibres, more detailed counting rules would be needed when classifying and counting high aspect ratio objects. The fibre-based object classes HARFO, HARFB and HARFC are also the only object type with a special subclass requiring the measurement of geometrical properties, WHO-objects. This type of high aspect ratio object is the only one with legally binding number based occupational exposure limit. Therefore, the counting rules are formulated in such way that decisions leading for an object to be classified as a WHO-object are unequivocal.

Table 2 — Objects classes

Morphological Objects Classes	Abbreviation	Annex
Particles		

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Morphological Objects Classes	Abbreviation	Annex
Low Aspect Ratio Particle Object	LARPO	E.1.1
Low Aspect Ratio Particle Agglomerate/Aggregate	LARPA	E.1.2
Fibres[a]		
High Aspect Ratio Fibre Object	HARFO	E 2.1
Low Aspect Ratio Fibre Cluster	LARFC	E 2.6
High Aspect Ratio Fibre Cluster	HARFC	E 2.6
Low Aspect Ratio Fibre Bundle	LARFB	E 2.7
High Aspect Ratio Fibre Bundle	HARFB	E 2.8
Platelets[b]	PL	
Single Platelet	SPL	
Platelet Agglomerate	PLA	
Hybrid Objects		
Hybrid Agglomerate	HA	

[a] HARFO, HARFB and HARFC objects are WHO-Fibres in case they fulfil criteria.

[b] Identification of platelets based on their two-dimensional projection in EM images is often difficult due to the fact that they often lie flat on the substrate. If platelets are expected on the sample and it is technically feasible, the operator of the EM might need to capture images with the sample slightly tilted in order to visualize the width of platelets.

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Table 3 — Geometric particle/fibre properties

Measurand	Symbol	Descriptors determined in case class cardinality > 300
LARPO, LARPA, LARFC, LARFC, PLA, HA		
Minimum Feret diameter	FD_{min}	Statistics: Mean, Median, 10th Quantile, 90th Quantile Cumulative Distribution: Mode(s) and Geometric Standard Deviation of lognormal peak function fitted to the data
Maximum Feret diameter	FD_{max}	
Project area equivalent diameter	D_{proj}	
HARFO		
Arc length closely following the fibre longitudinal axis	L	Statistics: Mean, Median, 10th Quantile, 90th Quantile Cumulative Distribution: Mode(s) and Geometric Standard Deviation of lognormal peak function fitted to the data
Average of at least three diameter measurements distributed over different fibre sections	D_f	
Aspect Ratio	L/D_f	
HARFC, HARFB		
Length of shortest path over the object from one tip to the other	L	Statistics: Mean, Median, 10th Quantile, 90th Quantile Cumulative Distribution: Mode(s) and Geometric Standard Deviation of lognormal peak function fitted to the data
Average of at least three diameter measurements distributed over different object sections	D_f	
SPL		
Diameter of the equivalent lateral surface circle		Statistics: Mean, Median, 10th Quantile, 90th Quantile Cumulative Distribution: Mode(s) and Geometric Standard Deviation of lognormal peak function fitted to the data
Width		
Primary particle/fibres		
Primary particle projected area equivalent diameter	Dpp_{proj}	Statistics: Mean, Median, 10th Quantile, 90th Quantile Cumulative Distribution: Mode(s) and Geometric Standard Deviation of lognormal peak function fitted to the data
Primary fibre diameter	Dp_f	

Figure 1 shows the decision process described in the next sections as a flow scheme. In the following, rules for the individual decision nodes are further explained.

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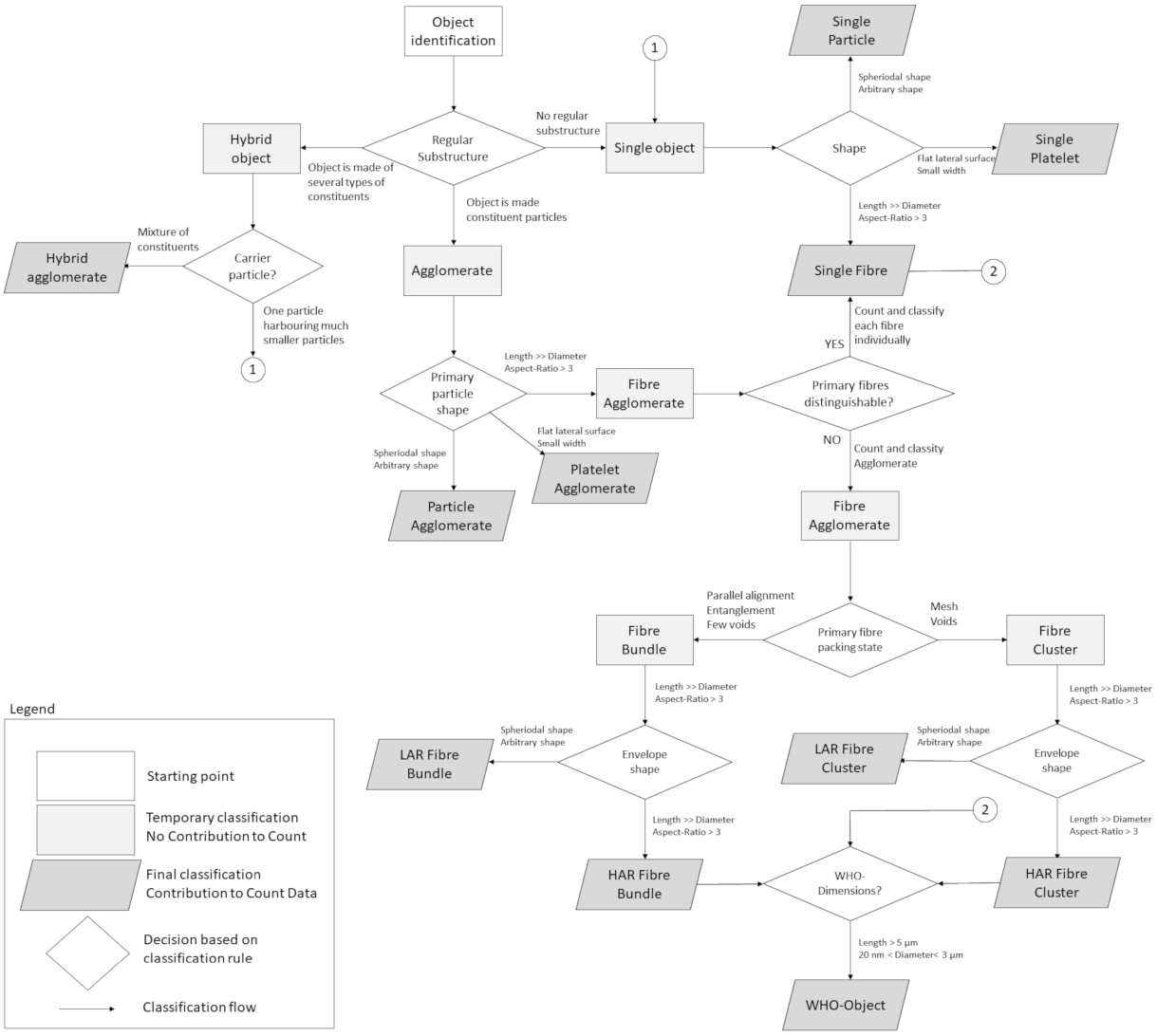


Figure 1 — Object classification flow scheme

A collection of objects typically appearing on SEM images of workplace aerosol samples is shown in Annex E.

Object recognition	(1)	a) Collected objects are recognized by their definitive particular nature. b) Objects on EM images that are not artefacts of the substrate usually yield different brightness compared to their surroundings. c) Substrate features (e.g. holes, grid lines) shall be excluded.

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Regular substructure	(2)	a) Objects that have no regular substructure are single particles. b) Objects appearing to be built from constituent particles are agglomerates. c) Objects that are made from several constituent particles clearly distinguishable by shape or size are hybrid objects.
Single object shape	(3)	a) LARPOs appear spheroidal or with arbitrary shape. Spheroids can be elongated but with aspect ratio < 3 and are continuously curved bodies. b) HARFO have a high aspect ratio and appear isotropic along its longitudinal axis in diameter and material properties. Fibres require further treatment making use of geometrical characterization → go to (8). c) SPL appear with a flat lateral surface and a width much smaller compared to the lateral dimensions.
Primary particle shape	(4)	a) Rules for shape assessment defined in (3) are applicable for primary particles of agglomerates. b) Agglomerates with primary particles of spheroidal or arbitrary shape are LARPA. c) Agglomerates of fibres are fibre agglomerates and require a further classification. d) Agglomerates of platelets are PLA.
Primary fibres distinguish-able	(5)	a) In case several objects have been identified as HARFO and they occur connected to each other, the evaluator needs to make the decision whether the individual HARFO can be distinguished from each other. b) Only in case the evaluator can clearly allocate two tips to one fibre that is part of the object, it can be regarded as an HARFO. c) Only if all participating fibres are identified as individual fibres, those shall be counted and treated as such. d) Otherwise, the object is identified and shall be counted as a fibre agglomerate
Primary fibre packing state	(6)	a) The packing state is a measure of alignment of primary fibres within a fibre agglomerate. b) In case fibres stick to each other in parallel alignment, the agglomerate is considered a fibre bundle. Void parts within the object shall be sparse. c) Arbitrary alignment of primary fibres identifies the agglomerate as a fibre cluster. Another attribute of a cluster is the presence of many visible void parts.

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Enveloping shape	(7)	a) Both fibre bundles and clusters require the assessment of the enveloping shape to determine whether the object is of high aspect ratio. b) The circumference of the object confines the envelope of a fibre bundle. The ratio of the maximum to the minimum Feret diameter of the envelope is the analogue to the fibre aspect ratio to identify HARFB versus LARFB. c) For clusters, the outward tips of protruding fibres serve as anchor points to draw lines similar to a "connect the dots" figure. Lines are drawn from one anchor point to the nearest next, forming an ellipsoid-like area. The ratio of the maximum to the minimum Feret diameter of the area is the analogue to the fibre aspect ratio to identify HARFC versus LARFC.
WHO-dimensions?	(8)	a) The particle class of WHO-objects is attributed to HARFO, HARFB and HARFC. b) A particle allocated to the WHO-object bin is still counted in the bin of the previously identified class. c) The definition of the WHO-dimensions is length > 5 µm, diameter < 3 µm and aspect ratio > 3 for HARFO and maximum Feret diameter > 5 µm, minimum Feret diameter < 3µm and ratio between maximum and minimum Feret diameter > 3 in case of HARFB and HARFC.
Carrier particle	(9)	a) Objects identified as hybrid objects require assessment whether one substructure-particle dominates in size - which would be a carrier particle hosting other much smaller particles. The decision shall be made based on the visual impression. For cases that yield a difficult decision, the host particle shall have size less than 10 % of the carrier particle. b) In case the agglomerate comprises a mixture of particles with diverse shapes and similar dimension, the object is a HA. c) In case a carrier particle is identified, it shall be treated as a single object and the hosted particles are ignored. The carrier particle then requires shape assessment, described in (4). d) Rarely, multiple carrier particles are present. In such case, also the host particles shall be ignored and the object is a HARFC/LARFC in case of fibres or an agglomerate in case of other shapes.

7.5.3 Object counting rules

In each EM micrograph image, a frame is placed in the centre with 5 % of the image width as distance to each edge of the image. Only objects are counted that are inside or touch the frame. For calculating concentrations, the area of the frame and not of the image is to be used.

1) Objects fully confined in the frame shall be counted as 1;

2) Objects partially in the frame shall be counted as 1/2;

3) Objects partially in the frame and partially in the image shall be counted as 1/2, but are not subject to particle characterization;

   This case is more probable for fibres. Additional neighbouring images shall be taken in order to capture the whole fibre to be able to determine whether it matched the WHO-dimensions. Otherwise the number of WHO-objects is underestimated. Given the fact that such objects are usually rare on workplace aerosol samples, underestimation is more impactful compared to typically more numerous spheroidal objects (see rule (3) of Clause 7.4.1).

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4) Objects visible in the image but fully outside the frame shall be counted as 0;

   Fibres can occur with both tips being outside the image. Such fibres shall be counted as 0 and are not subject of further analysis.

5) Objects that apparently entered filter holes but partially visible in the image shall not be counted;

   Exceptions are fibre-objects protruding the filter hole, which shall be counted as 1 in their respective class. If the visible part of the fibre matches the criteria, it can be allocated at the subclass of WHO-objects. There are some additional rules that apply only for objects of connected fibres or particles bound to fibres for which the individual fibres and objects can be clearly distinguished from each other. In case they cannot be, the object is an agglomerate (HARFB/LARFB, HARFC/LARFC, HA).

6) Objects sticking to fibres shall be counted as 1. The fibre is also counted as 1;

7) For fibres with attached fibres, two cases are differentiated:

   a) Attaching fibres with both tips being visible shall be counted as 1;

   b) Attaching fibres with only one tip being visible shall be counted as 0. Typically, this case occurs with fibres that stand up.

   The fibre that carries the other shall be counted as 1.

   NOTE There are cases for which the decision is difficult and highly subjective, whether all fibres of such a network-like structure are treated individually or the whole interconnected network of fibres is a HARFC/LARFC. These cases often occur on samples that are strongly loaded, since fibre could have deposited on top of each other, creating a cluster formed on the substrate. In contrast, fibre clusters are deposited as a whole. Therefore, in case the aerosol sample has low particle density, a network-structure of fibres is more likely a HARFC/LARFC.

There is no contradiction in the fact that fibres with only one tip visible shall not be counted, but with two tips are and the fact that the classification rules (5) only allow identification of single fibres within an object in case all constituents are clearly distinguishable.

Examples for objects and the application of the counting rules can be found in Annex E. In particular, more complex objects like clusters require experience for their treatment. Annex E demonstrates classification, counting and characterization on a library of NOAA objects found in EM images of workplace aerosol samples.

7.5.4 Object characterization rules

Geometrical characterization of identified and classified objects allows further classification in case of fibres, bundles and clusters, which can be WHO-objects in case geometric properties match the WHO-criteria. This is of particular importance, since WHO-objects are the only special object class with its own legally binding occupational exposure limit in many areas such as the EU. Other objects can be subject to geometrical characterization to obtain information about the particle size distribution.

1) Geometric properties of spheroidal particles shall be determined as follows:

   a) Determination of geometrical properties of LAR-objects shall be based on measurement of area of the 2D-projection of the 3D-object to determine an equivalent diameter and/or by measuring the maximum and minimum Feret diameter;

   b) The projected area enveloping the whole object shall be measured. The projected area is converted to an equivalent circle area. The diameter of the circle is determined;

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c) The maximum and minimum Feret diameters are the maximum and minimum distances between two parallel planes restricting the object perpendicular to a specific direction.

2) Geometrical properties of particle agglomerates shall be determined analogous to single particles;

3) Geometrical properties of fibres shall be determined as follows:

a) Rectified length shall be measured. The rectified length is the 2D-projected length that can be based on the length of a spline closely following the longitudinal axis of a fibre as it is displayed in the micrograph;

b) The mean diameter shall be measured as the result from three points of diameter measurement along the longitudinal axis of a fibre. The three points of diameter measurement shall be located in the first, second and last thirds over the fibre length, approximately at the respective centres;

c) Properties shall be determined pairwise, i.e. all properties of one fibre shall be measured before moving on to the next;

In case a fibre is not fully displayed in one image frame, the length shall be measured up to the image edge. Length measurement is continued in the neighbouring image frames. The total length is the sum of all parts.

4) Geometrical properties of fibre clusters shall be determined as follows:

a) For fibre cluster an envelope are probably the hardest objects to measure. An envelope is not applicable since fibre clusters circumference have very high curvature due to protruding fibres. Tracing the circumference of the cluster to fully encapsulate it is often error-prone. An easier method is to mark the tips of all protruding fibres and connect the dots in a circumferential manner. This generates a polygon which marks the projected area of the cluster. The projected area equivalent diameter shall be determined;

b) Minimum and Maximum Feret diameter of the polygon shall be measured. Their ratio is the aspect ratio, identifying HARFC and LARFC;

c) For HARFC, the maximum Feret diameter and minimum Feret diameter replace length and diameter, respective, for the identification of WHO-objects. I.e. maximum Feret diameter > 5 µm, minimum Feret diameter < 3 µm, aspect ratio > 3.

(5) Geometrical properties of fibre bundles shall be determined as follows:

a) A circumference, yielding an enveloping area, traces the outline of the bundle. Protruding fibres are ignored in case their length is small compared to the size of the whole bundle. Minimum and maximum Feret diameter of the envelope area is measured. Their ratio is the aspect ratio, identifying HARFB and LARFB;

b) For HARFB, the maximum Feret diameter and minimum Feret diameter replace length and diameter, respectively, for the identification of WHO-objects. I.e. maximum Feret diameter > 5 µm, minimum Feret diameter < 3 µm, aspect ratio > 3.

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8 Calculation of results

8.1 Concentration

8.1.1 Upper confidence limit for compliance testing

Analysing an area of a collection medium with given collection efficiency for objects of a specific class will result in observing *zero* or a *positive number* of N objects. Half integer values of N are also possible.

For $N = 0$ observed objects, the upper limit of the single-sided confidence interval $\hat{C}_H^S$ of the estimated concentration is calculated with Formula (10)A):

$$\hat{C}_H^S = \frac{H_S\left(\hat{N} = 0, \alpha\right)}{A_{\text{image}} \cdot \varrho_V \cdot \eta_C} \tag{10A}$$

where

$\hat{C}_H^S$ is the single-sided confidence interval;

$H_S\left(\hat{N} = 0, \alpha\right)$ is the upper limit of the single-sided probability interval limit of a Poisson probability density function for expecting $\hat{N} = 0$ objects at significance α;

A_{image} is the analysing an area of the collection medium surface;

$\varrho_V\left(Q, \tau, d_0\right)$ is specific sampled air volume;

η_C is collection efficiency.

For $N > 0$ observed objects, the upper limit of the two-sided confidence interval $\hat{C}_H^T$ of the estimated concentration is calculated with Formula (10)B):

$$\hat{C}_H^T = \frac{H_T\left(N, \alpha\right)}{A_{\text{image}} \cdot \varrho_V \cdot \eta_C} \tag{10B}$$

where

$\hat{C}_H^T$ is the two-sided confidence interval;

$H_T\left(N, \alpha\right)$ is the upper limit of the two-sided probability interval of a Poisson probability density function for expecting N̂ objects at significance α;

A_{image} is the analysed area of the collection medium surface;

ϱ_V is specific sampled air volume;

η_C is collection efficiency.

8.1.2 Object concentration and confidence interval limits for concentration estimation

The estimated object concentration is calculated with Formula (11):

$$\hat{C} = \frac{1}{A_{\text{image}} \cdot \varrho_V} \cdot \frac{N}{\eta_C} = \frac{N}{V_N \cdot \eta_C} \tag{11}$$

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where

C is the estimated object concentration;

N is the amount of observed objects (N > 0);

A_{image} is the analysedarea of the collection medium surface;

ϱ_V is specific sampled air volume;

V_N is the sampling volume;

η_C is collection efficiency.

And for $N > 0$ observed objects, the lower limit of the two-sided confidence interval $\hat{C}_L^T$ of the extrapolated concentration is calculated with Formula (10)C):

$$\hat{C}_L^T = \frac{L_T\left(N,\alpha\right)}{A_{\text{image}} \cdot \varrho_V \cdot \eta_C} \tag{10C}$$

where

$\hat{C}_L^T$ is the two-sided confidence interval;

$L_T\left(N,\alpha\right)$ is the lower limit of the two-sided probability interval of a Poisson probability density function for expecting $\hat{N}$ objects at significance α ;

A_{image} is the analysedan area of the collection medium surface;

ϱ_V is specific samples air volume;

η_C is collection efficiency.

Whereas the upper limit corresponds to Formula (10)B.

NOTE For less than about 30 counted objects, the large relative error on the estimated concentration will render the estimation practically meaningless, see Table 1.

8.2 Particle size distribution

Size data, e.g. length and diameter, is subject to statistical evaluation, yielding mean, median and mode values as well as the 10^{th} and 90^{th} percentiles.

Data on geometrical properties of objects is allocated to respective bins in order to determine the frequency distribution of the object size bins which is essentially, the particle number size distribution. The scaling of the bin edges (k) depend on the total size range, i.e. from A to B, as well as the number of desired bins I, see Formula (12):

$$k = \left(\frac{B}{A}\right)^{\frac{1}{I-1}} \tag{12}$$

where

k are the bin edges;

B is the maximum of the size range;

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A is the minimum of the size range;

I is the number of desired bins.

The size bin edges shall be calculated recursively by the Formula $x\left[I\right] = x\left[I-1\right] \cdot k$. The lower and upper boundaries A and B are selected based on the size data collected, where A represents the size of the smallest particle and B of the largest.

NOTE 1 From experience, I = 20 gives a smooth lognormal size distribution for ca. 500 objects.

NOTE 2 Example: 10 size bins in the range from 10 nm to 2 500 nm can be defined by the scaling factor k = 1,85. The resulting size bins are (10, 19), (19, 34), (34, 63), (63, 116), (116, 215), (215, 397), (397, 733), (733, 1354), (1354, 2500), rounded.

NOTE 3 When multiple magnifications are used, separate particle size distributions are derived for each magnification (image size) using the same fixed size bin edges. These individual PSDs (in particle numbers per mm^2 image field or per m^3 sampled air) will be merged, making sure that between 2 adjacent magnifications there are at least 2 overlapping and matching size bins. As a rule of thumb, a difference up to 20 % between magnifications for each overlapping size bin is reasonable. For a statistical evaluation of these overlapping size bins, the confidence interval limits (see Clause 8.1.2) can be calculated and compared for each of the two object counts. If the particle numbers of the size bins match, for the total number size distribution, the particle numbers of the 2 magnifications are averaged.

8.3 Data evaluation and quality control

8.3.1 Test for homogeneity of particle number density on the sample

Extrapolation of the total number load of objects or specific object classes on the whole sample based on the determination of the object number density in a number of much smaller sample sections assumes homogeneity of the particle load over the whole sample. “Homogeneity” means that the probability to find a certain number of objects in any section of the sample is described by a Poisson probability density function (short: Poisson distributed). The following steps describe a statistical test for deviation from the Poisson distributed particle load based on count data on a required number of EM images randomly taken on the sample. The null hypothesis is that the count data are Poisson distributed, which the test may reject in which case the particle load is most certainly not Poisson distributed and extrapolation of total particle load or concentrations is not viable.

— Step 1: use 20 randomly chosen images from the set of images captured. Count objects fully displayed in the EM image field. Counting can either be performed directly after (re-)positioning the EM and image capture or subsequently on the images. Online counting shall still be conducted on images taken with a long integration interval to provide the best image quality. All objects shall be counted.

— Step 2: if average object count in the 20 images is less than 20 but more than 10, take another 30 images so that the sample size increases to 50. Otherwise the test results for deviation from homogeneity loses confidence. If less than average 10 objects are counted per image, 20 image clusters are generated with cumulative count data. The number of images in the clusters is equal and chosen so that the average cumulative count result is at least 20. Consequently, underloaded samples might require taking a lot of EM images.

— Step 3: the tuple of count data with N values are put into a spreadsheet. Conduct the following workflow performs the Chi-squared goodness of fit test. For that, count data are put into cells A1-AN in the spreadsheet.

  The spreadsheet function that returns the right-tailed probability of the Chi-squared distribution associated with the count data and certain degrees of freedom. The degrees of freedom are calculated as N-1. In this case, degrees of freedom = 20 – 1 = 19. If the p-value is larger than 0,95 then the null hypothesis

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that the count data is Poisson distributed must not be rejected. A successful homogeneity test allows for extrapolation of total object counts on the sample and from that the average object number concentration during sampling.

In Figure 2, two examples are shown. The left spreadsheet with Poisson distributed count data yields no rejection of the null hypothesis of Poisson distributed count data (p-value: 0,99) whereas the right example with bimodally distributed data yields rejection of this null hypothesis (p-value: 0,003).

	A	B	C	D	E	F	G	H	I	J
1	Count (O)	Expect Value €	(O-E)^2/E			Count (O)	Expect Value	(O-E)^2/E		
2	23	23.45	0.008635394			10	18.75	4.0833333		
3	22	23.45	0.089658849			11	18.75	3.2033333		
4	20	23.45	0.507569296			10	18.75	4.0833333		
5	24	23.45	0.012899787			14	18.75	1.2033333		
6	25	23.45	0.102452026			15	18.75	0.75		
7	24	23.45	0.012899787			14	18.75	1.2033333		
8	21	23.45	0.255970149			11	18.75	3.2033333		
9	22	23.45	0.089658849			12	18.75	2.43		
10	26	23.45	0.277292111			16	18.75	0.4033333		
11	27	23.45	0.537420043			27	18.75	3.63		
12	24	23.45	0.012899787			24	18.75	1.47		
13	28	23.45	0.882835821			28	18.75	4.5633333		
14	19	23.45	0.84445629			19	18.75	0.0033333		
15	23	23.45	0.008635394			23	18.75	0.9633333		
16	26	23.45	0.277292111			26	18.75	2.8033333		
17	25	23.45	0.102452026			25	18.75	2.0833333		
18	27	23.45	0.537420043			27	18.75	3.63		
19	20	23.45	0.507569296			20	18.75	0.0833333		
20	21	23.45	0.255970149			21	18.75	0.27		
21	22	23.45	0.089658849			22	18.75	0.5633333		
22		Test statistic	5.413646055	=SUM(C2:C21)			Test statistic	40.626667	=SUM(H2:H21)	
23		p-Value	0.998990973	=CHISQ.DIST.RT(C22;19)			p-Value	0.0027053	=CHISQ.DIST.RT(H22;19)	

Figure 2 — Examples for the homogeneity test for count data

8.3.2 Particle size statistics

Size data, e.g. length and diameter, is subject to statistical evaluation, yielding mean and median values as well as the 10^{th} and 90^{th} percentiles.

Binned size data (see Clause 8.3) can be can be modelled with a lognormal frequency distribution $y(x)$, see Formula (13):

$$y(x) = y0\ x + \frac{N}{\sqrt{2\pi}\ \sigma_g}\mathrm{Exp}\left[-\frac{\left(\log(x)-\mu\right)^2}{2\ {\sigma_g}^2}\right] \tag{13}$$

where

N is the amount of observed objects;

μ is modal value of the frequency distribution;

σ_g is geometric standard deviation of the frequency distribution.

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9 Test report

The test report shall include at least the following information about the six stages of sampling and analysis of airborne NOAA's in the workplace:

1) Determination of measurement objectives: such as the NOAAs characterization, indication of object number concentration and, if compliance, testing are required;

2) Workplace characteristics: workplace factors (e.g. environmental conditions such as relative humidity, in percent (%), and temperature, in degree Celsius (°C), at the time of testing) and estimation of exposure;

3) Sampling strategy:

   a) name and address of sampling location;

   b) name and address of contractor, if applicable;

   c) date and time of sampling;

   d) a reference to this document;

   e) sketch/description of workplace;

   f) amount and type of samplers;

   g) amount and type of collection media;

   h) duration of sampling;

   i) air volume sampled;

   j) position of sampler;

   k) a reference to additional guidelines and/or strategies, if applicable.

4) Sample analysis:

   l) name of operator;

   m) name and address of analysis laboratory;

   n) date and time of analysis;

   o) detail of EM instrument and additional specification and settings used during analysis;

   p) description of pre-treatment steps, such as coater equipment details, if applicable;

   q) image settings, such as magnification, scan mode, detectors used;

   r) state the method and decisions made by the operator during data collection.

5) Results: overall conclusion of measurements, under which calculations such as concentrations, confidence intervals and particle size distribution. In addition, exemplary images obtained during screening or analytical magnification shall be included together with a general description of observed NOAA.

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Annex A
(informative)

Apparatus and materials

A.1 Air samplers

A.1.1 General

For air sampling of airborne NOAA in the workplace, air should be sampled using a sampler combined with a suitable collection medium.

A.1.2 Selection of samplers

The selection of a sampling setup depends on the type of NOAA to be measured, which EM method will be used and whether stationary (fixed-point) or personal samples are collected. The sampler selection (and sampling strategy) is based on the electron microscope technique, which is available: SEM, TEM or STEM.

The analysis of objects by electron microscopy has strict requirements for samples, which leads to the use of specific methods of sample collection and preparation. Due to the different requirements of SEM and (S)TEM, there are significant differences in the way samples are collected and prepared for analysis between the two different types of instruments. However, in the majority of situations, it is necessary to produce samples with a homogenous object layout, with a minimum of overlapping of the objects. This last point is related to the volume of air sampled by the device during the sampling period, for a given concentration. The issue of uniformity is more related to the principle of collection of the sampling device and its internal design. Homogeneity is generally understood in terms of surface density (e.g. number of particles deposited per unit area). To be exact, the probability that a number of objects are deposited within a certain area on the substrate must follow a Poisson probability density distribution. It has also to be understood in terms of sizes as well as morphology and composition of the NOAA.

A wide range of 'commercial' samplers applicable for EM analyses are available[1]. An overview is given in Table A.1.

Table A.1 — Overview 'commercial' samplers applicable for EM analysis

Sampler type	Sampler	Common collection medium	Object size range	Sampling flowrate (l/min)	Suitable EM type	Comments
Personal	FAP sampler (37mm)	Au-coated polycarbonate membrane	< 20 µm	2 – 4	SEM	Sampling of fibrous dusts according to ISO 10312 [7] Suitable for compliance test against fibre OELV Sampler is tested in Annex B and C
	GSA/FAP sampler (25mm)	Au-coated polycarbonate membrane	< 10–40 µm flow rate dependent	0,5 – 8	SEM	Several versions available (i.e. VDI 3492 sampler, plastic cassette (COWL sampler))

[1] The commercial samplers are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of these products.

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