EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Toxicology and Chemical Substances Unit
European Chemicals Bureau
I-21020 Ispra (VA) Italy

Guidance Document on the Determination of Particle Size Distribution, Fibre Length and Diameter Distribution of Chemical Substances

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Mission

The mission of the Institute for Health and Consumer Protection is to support EU policies for health and consumer protection. The institute carries out research to improve the understanding of the hazards, exposure and risk posed by food contaminants, drugs, chemicals, products, services and systems and to develop, validate and apply advanced methods and strategies of high scientific quality.

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FOREWORD

For full notification of a new substance in the framework of Dir 67/548/EEC[1], Annex VII A establishes that, for those substances which may be marketed in a form which gives rise to the danger of exposure by the inhalatory route, a test should be conducted to determine the particle size distribution of the substance as it will be marketed.[2]

Accordingly, and in close relation to the determination of its inhalation toxicity, the notification dossier for new substances, should include a particle distribution measurement[3].

On the other hand, the determination of the ability of the material to form airborne dust and the nature of the dust produced (e.g. fibrous, non-fibrous) is essential for an appropriate risk assessment both for new and existing substances.[4]

In the Annex V of Dir 67/548/EEC there is not yet a suitable testing method for the determination of the particle size distribution of a substance.

On the other hand, the methods described in OECD TG 110[5] are insufficient because, among other limitations, they cover water insoluble compounds only, while many notified substances are indeed water-soluble.

This Guidance Document was developed from a draft document originally prepared by the Netherlands and discussed in several meetings of the EU National Co-ordinators for Testing Methods and the Competent Authorities.

After integrating comments from experts of Member States, the third revised version of the document was presented as a final draft proposal to the 2nd Meeting of the Member

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[1] Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. O.J. L 196/1 of 16 August 1967. And related Amendments, Adaptations to Technical Progress, Commission Decisions and Commission Communications.

[2] Council Directive 92/32/EEC of 30 April 1992 amending for the seventh time Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. O.J. L154/1 of 5 June 1992.

[3] Notification of New Chemical Substances in accordance with Directive 67/548/EEC on the Classification, Packaging and Labelling of Dangerous Substances. Technical guidance for the completion of a Summary Notification Dossier for a New Chemical Substance Utilising the Structured Notification Interchange Format (SNIF). Base-Set and Levels 1 and 2. (ISBN 92-828-0195-0).

[4] Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation(EC) no 1488/94 on Risk Assessment for Existing Substances (ISBN 92-827-8011-2). Presently under revision.

[5] TG 110: Particle Size Distribution/ Fibre Length and Diameter Distribution. OECD Test Guidelines for the Testing of Chemicals, Paris, 1983.

States on Technical Scientific Issues Associated with Directive 67/548/EEC (Ispra, 25-26 March 1996). It was approved, with a minor amendment, by the technical meeting as a basis for the corresponding testing needs for inhalation toxicity and other studies, as an interim solution until an individual method is developed in relation to the inhalation toxicity. The decision of the Technical Scientific meeting was finally endorsed by the 51st meeting of the Competent Authorities for the implementation of Dir 67/548/EEC (Rome, 5-7 June 1996).

This Guidance Document is presented here in order to facilitate its consultation and use by interested parties.

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1. INTRODUCTION

The requirement for the results from the tests described here is linked closely to the inhalation toxicity strategy (7th Amendment of Dir 67/548/EEC) and the need to decide which route of administration is most appropriate for the acute toxicity and 28-day base set studies. The strategy states that an important argument in favour of the performance of inhalation toxicity studies is the following: "substance as used contains particles in the inhalable size range (i.e. may be deposited anywhere in the respiratory tract; the inhalable size range of particles is important in determining not only if the situation poses an inhalation problem, but also where in the respiratory tract the particle may deposit)" (see footnote 4). Therefore, the particle size distribution can be used as an argument in favour of inhalation testing.

It is, therefore, very important that the methods capable of particle size distribution measurement can determine the appropriate fractions as defined in EN481 (1993)[6], using the aerodynamic diameter as the basis of the measurement.
The "aerodynamic" diameter is the diameter of a sphere of unit density which behaves aerodynamically as the particle of the test substance. It is used to compare particles of different sizes, shapes and densities and to predict where in the respiratory tract such particles may be deposited.
The term is used in contrast to "optical", "measured" or "geometric" diameters which are representations of actual diameters which in themselves cannot be related to deposition within the respiratory tract.

The fractions as defined in EN481 (1993) are:

- inhalable fraction (the mass fraction of particles which can be inhaled by nose or mouth); since there are no experimental data on inhalable fraction of particles with an aerodynamic diameter of > 100 µm, particles > 100 µm are not included in the inhalable convention,

- thoracic fraction (the mass fraction of particles that passes the larynx); the median value of the particle size is 11.64 µm with a geometric standard deviation (GSD) of 1.5 µm. It has been shown that 50 % of the particles in air with an aerodynamic diameter of 10 µm belong to the thoracic fraction,

- respirable fraction (the mass fraction of particles that reaches the alveoli); the median value is 4.25 µm with a GSD of 1.5 µm. It has been shown that 50 % of the particles with an aerodynamic diameter of 4 µm belong to the respirable fraction.

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[6] Workplace atmospheres – Size fraction definitions for measurement of airborne particles. CEN, European Committee for Standardisation. European Standard EN 481:1993.

The problem comes in how to convert physical measurements of the size distribution of a bulk substance into aerodynamic diameter and then to the mass fraction of inhaled or respirable sized particles. Ideally, the size distribution measurements should take place from an airborne dispersion of the material. Several commercially available instruments will give measurement of the aerodynamic size distributions (see section 5). It should be noted that the Rotating Drum Method (see section 5.3) is the only method that uses a standard method to disperse the dust and gives a separation by mass based on the respirable, thoracic and inhalable fractions. The other instruments are adequate to determine the respirable fraction but are less adequate to deal with the full range of sizes in the inhalable convention (< 100 µm).

However, since the requirement for the result from this test is linked closely to the inhalation toxicity strategy (see above), the presence of respirable particles is of particular health concern. Therefore, methods, which are capable to determine the presence of respirable particles, are preferred.

The methods described in section 5 are methods that determine the distribution of respirable particles and (to a lesser extent) the distribution of inhalable particles; for an indication of the particle size the Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) can be calculated. The MMAD is a statistically derived figure for a particle sample: for instance, an MMAD of 5 µm means that 50 % of the total sample mass will be present in particles having aerodynamic diameters less than 5 µm, and that 50 % of the total sample mass will be present in particles having an aerodynamic diameter larger than 5 µm.

The other methods (see section 4) have to calculate the aerodynamic diameter indirectly from other measurements of particle size and density. If applied properly, they represent an estimate of the aerodynamic property and mass fractions present.

The notifier is free to use a method described in section 5 instead of a method described in section 4. Furthermore, the notifier has the possibility to demonstrate that there is no inhalation risk by applying a method with which it is possible to determine the respirable and/or inhalable mass fractions and to calculate the MMAD (these methods are described in section 5).

Appropriate sampling procedures should be selected in order to prepare specimens really representative of the material under test. It is also important to note that the original particle size distribution is highly dependent on the industrial processing methods used and can also be affected by subsequent environmental or human transformations.

2. IMPORTANT CRITERIA IN DETERMINING PARTICLE SIZE DISTRIBUTIONS

1- It is very important to note that the methods currently described in OECD 110 determine the particle size distribution of the material under investigation as it is and do not refer to the airborne dust of the material. Thus these methods do not provide a measure for risk exposure during handling of the chemical. Furthermore, they can only be applied to water-insoluble ($< 10^{-6}$ g/l) powdered type products.

2- Methods that determine the mass median aerodynamic diameter (MMAD) need the generation of representative test atmospheres using suitable generation equipment and correct sampling techniques. These methods can, therefore, be used in case of airborne particles (dusts, smokes, fumes), nebulized particles (wet aerosol) or dispersed particles (dry aerosol).

3- The small quantities used as samples must be representative of product batches comprising many kilograms. Therefore, much care should be taken to avoid changing of the particle size distribution. Sample pre-treatment such as addition of dispersing agents, agitation, or low-level ultrasonic treatment should be avoided in as much as possible.

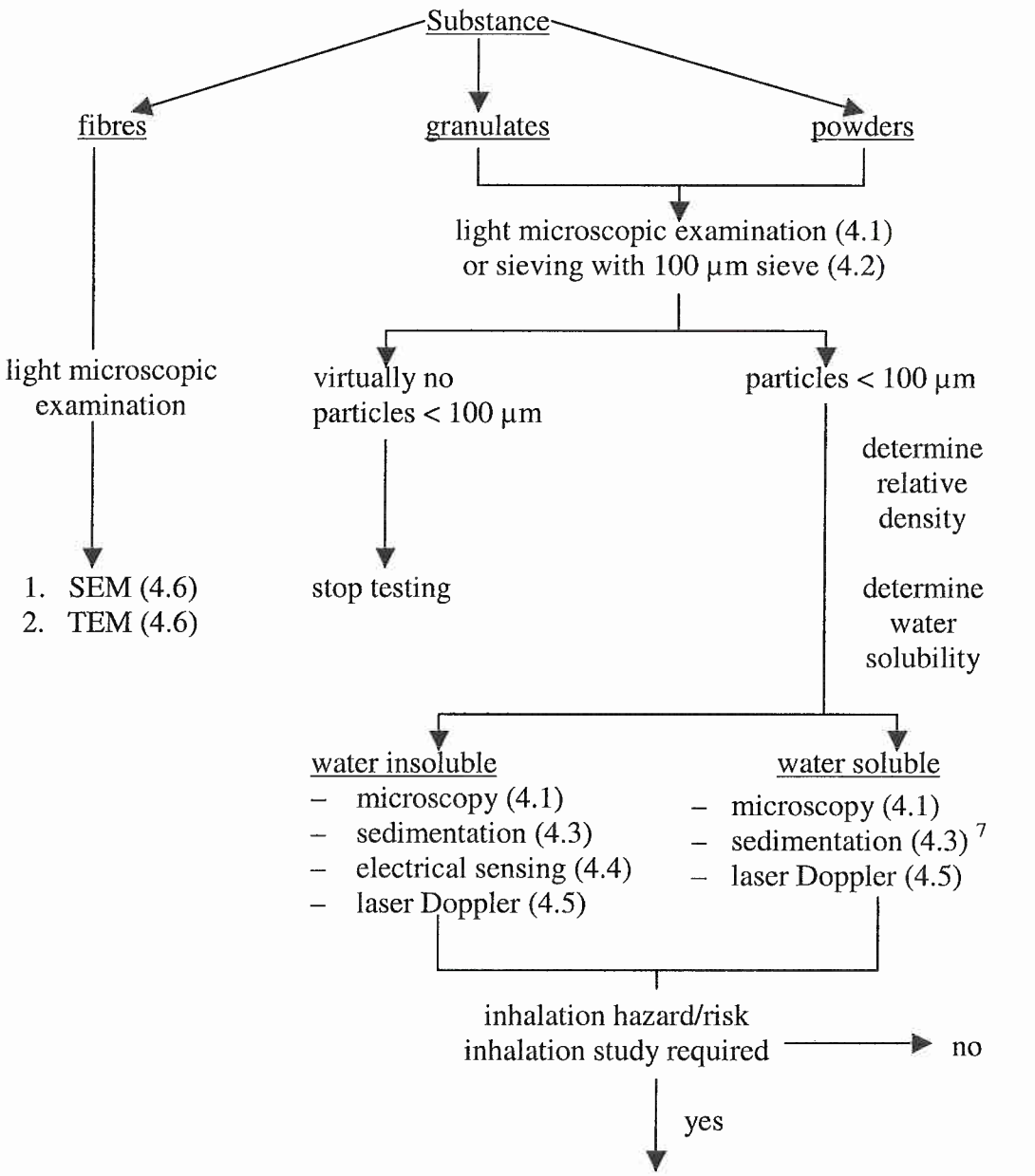
4- Great care should be taken on the fact that non-conducting particles in a non-conducting liquid may be electrically charged resulting in non-representative settling of particles of certain size. In addition, in the process of particle size distribution determination, it is very important to take the electrostatic charge of the particles into account. Electrostatically charged particles behave different and may influence sampling.

5- Samples should be subjected to a simple light microscopy examination to determine the approximate nature of the particles (fibres, plates, spheres, needles, etc.).

6- In order to help to decide which method should be used, it is advised to determine the relative density of the particles in each sample at a certain temperature (void volume) which can be measured by gas centrifuge. In this way an impression of settling time in calm air after manipulation of the material might be obtained.

3. FLOWSHEET OF APPROPRIATE METHODS TO DETERMINE PARTICLE SIZE DISTRIBUTION OF RESPIRABLE/INHALABLE PARTICLES

The methods indicated here are described in section 4 and 5; these methods determine respirable and inhalable fractions. The methods that measure inhalable fractions only or that give no detailed distributions are not included in this flow sheet (see Annex).



MMAD = mass median aerodynamic diameter

[7] appropriate non-aqueous solution

4. METHODS TO DETERMINE PARTICLE SIZE DISTRIBUTION OF THE MATERIAL AS IT IS

Microscopy examination, sedimentation and Electrical Sensing Zone (e.g. Coulter) method are most commonly employed. Less commonly employed is the Laser Doppler Anemometry technique. These methods determine the particle size of the material as it is and do not refer to airborne dust or dispersed or nebulized particles. Therefore, the MMAD cannot be determined. These methods, though can give an indication whether or not respirable particles might be present.

The standard methods for most of these methods are indicated in the current OECD guideline 110.

The currently used elutriation technique is not suitable to determine particles of respirable size (see Annex).

4.1 Microscopy examination (see OECD 110)

Material: particles of all kind
Size range: 0.5 – 5000 microns (light microscope)
0.01 – 10 microns (SEM/TEM)

Samples should be prepared preferably directly in order not to influence shape and size of the particles. Especially for small particles (< 0.1 μm) scanning (SEM) or transmission (TEM) electron microscopy is advised.

Conclusion: this method is suitable to determine the distribution of particles of respirable and inhalable size. The MMAD cannot be determined.

4.2 Sieving (see OECD 110)

Material: dry powders/ granulates
Size range: 100 – 10,000 microns (wire mesh/ metal sieves)
5 – 100 microns (micro mesh)

Sieving using wire-mesh sieves and perforated sheet metal sieves is not suitable to determine the distribution of particles of respirable and inhalable size since their range is only 100–10,000 microns.

Micromesh sieves (range 5–100 microns) may give better results. However, since these sieves are generally operated in combination with mechanical or ultrasonic vibration, modification of median size and form may result.

Conclusion: sieving is not suitable to determine the distribution of particles of respirable size, but might be suitable to determine particles of inhalable size. The MMAD cannot be determined.

4.3 Sedimentation (gravitational settling) (see OECD 110; DIN 66115)[8]

Material: dry powders/ granulates
Size range: 2 – 200 microns

This method is fully based on gravitational settling of particles in liquid and the effective hydrodynamic radius is determined. Settling of the particles in fluid depends on the difference in specific gravity between that of the liquid and the particle material, the specific gravity of the particle material being greater than that of the liquid.

As calibration material binary or ternary mixtures of latex spheres (2–100 microns) are recommended according to OECD 110. The use of latex spheres is, however, under discussion since (a) the surface of latex spheres is not fully smooth, (b) its diameters may change under the influence of humid and (c) their storage is limited due to coagulation and repolymerization.

As mentioned in OECD 110, the effective hydrodynamic radius distribution should be measured three times, with no two values differing by more than 20%. The request size range > 200 µm, 2 – 200 µm, and < 2 µm with a distribution curve in the region 2 – 200 µm may result in a poor estimate of the number of particles < 10 µm (respirable particle) and < 100 µm (inhalable particle). As a better size range is suggested: > 100 µm, 10 –100 µm and < 10 µm, with a distribution curve in the region < 10 µm.
In addition, this method requires that sufficient numbers of radius intervals be used to resolve the radius distribution curve.

A large quantitative of polymer powders will not be measurable in liquid, since their relative density will be too close to that of the liquid in use, resulting in settling times which are far too high.

The size distribution of water soluble particles may also be determined if an appropriate non-aqueous solution can be found in which the particles do not solve or absorb the solution and having a difference in density which is good enough to obtain correct settling times.

Conclusion: this method might be suitable to determine the distribution of particles of respirable and inhalable size. The MMAD cannot be determined but it can be calculated in some cases.

4.4 Electrical Sensing Zone (e.g. Coulter) method (see OECD 110)

Material: dry powders/ granulates
Size range: 1 – 1000 microns

The Coulter Counter technique uses samples suspended in an electrolytic solution. As the particle is drawn through an aperture, the change in conductance gives a measure of particle size. The important parameter is the settling velocity of the particles in the liquid

[8] DIN 66115 Partikelgrößenanalyse; Sedimentationsanalyse im Schwerefeld; Pipette-Verfahren (1983)

phase, which depends on both density and diameter. Particles having a density of several g/cm^3 can be determined with this technique.
However, Coulter counter impedance measurements are only suitable for particles that are complete electrical isolators in the fluid. Moreover, the difference in density between particles and fluid must not be too large; when this difference becomes too large, it is necessary to apply some measures such as increase of liquid density and stirring before/ during analysis.

Conclusion: this method might be suitable to determine the distribution of particle of respirable and inhalable size. The MMAD cannot be determined.

4.5 Phase Doppler Anemometry (PDA)

Material: dry powders/granulates
Size range: 0.5 µm – 80 µm (in air)
0.5 µm – mm (in liquid)

Using this expensive technique, particle size distribution can be measured either in air or in liquid. The method presupposes that the particles are spherical with known refractive index.

Conclusion: this method might be suitable to determine the distribution of particles of respirable and inhalable size. The MMAD cannot be determined.

4.6 Determination of fibre length and diameter distributions (see OECD 110)

Material: fibrous products
Size range: diameters as small as 0.1 µm and as large as 100 µm and lengths as small as 5 µm and as large as 300 µm

A fibre is a water insoluble particle, of length/diameter ratio ≥ 3 and diameter ≤ 100 µm.

Light microscopic examination, similarities to known fibrous or fibre-releasing substances or other data, should indicate the likelihood of fibres present.
The effect of impurities on fibre shape should be considered.

Extreme care must be taken during sample preparation to avoid fibre breaking and clumping. Care should also be taken to avoid contamination by airborne fibres, viz. a hood or a "clean room" should be used.

Samples might be prepared by (a) preparing suspensions in water by gentle hand agitation or vortex mixing. Magnetic stirring is not advised since this procedure may lead to grinding of fibres, or (b) direct transfer of dry material onto copper tape or by spraying of the dry fibres onto the copper tape by using an atomiser or pipette. The direct transfer method is preferred since dry fibre samples are measured directly. The first method uses re-dried samples which might have influenced shape and size of the particles. Spraying might also introduce size differences.

Since small diameter fibres (≥ 0.1 μm) should be detected, scanning (SEM) or transmission (TEM) electron microscopy is required.

As mentioned in OECD 110, length and diameter distributions should be measured independently at least twice and at least 70 fibres should be counted. No two values in a given histogram interval should differ by more than 50 % or 3 fibres, whichever is larger. The presence of long thin fibres, however, would indicate a need for further, more precise measurements.

The optical length/diameter ratio is only valid for straight fibres; care should be taken in case of curved and/or curly fibres.

Conclusion: this method might be suitable to determine the distribution of fibres of respirable and inhalable size.

5. MEASUREMENT OF AIRBONE, DISPERSED OR NEBULIZED PARTICLES

The methods used in this section need the generation of representative test atmospheres using suitable generation equipment and correct sampling techniques. These methods are preferred since the measure particles in air and as such MMAD's can be determined.
The air jet sieve method is not suitable to determine particles of respirable size (see Annex).

5.1 Cascade impaction

Material: particles of all kind
Size range: 0.1 – 20 and 0.5 – 80 microns

Cascade impactors can be used to obtain the size distribution of an aerosol (or a dust cloud). Air samples are withdrawn through a device, which consists of several stages on which particles are deposited on e.g. glass or glass fibre. Particles will impact on a certain stage depending on their size. The cut-off size can be calculated from the jet velocities at each stage by weighing each stage before and after sampling and the MMAD derived from these calculations. Despite the limitations in this method, namely particles bouncing off, overloading and fluctuation in flow rate etc, it is a well established technique to measure the airborne size distribution of an aerosol.

Conclusions: this method is suitable to determine the distribution of particles of respirable or inhalable size. The MMAD can be determined.

5.2 Laser scattering/ diffraction

Material: particles of all kind
Size range: 0.1 – 100 microns

In general, the scattering of the incident light gives distinct patterns which are measured by a detector. This technique is particle property dependent – i.e. material has unique scattering and diffraction properties which are also particle size dependent. Hence it is important that the instrument be calibrated with a similar material (of the same size range) as the material to be measured. Laser scattering techniques are suitable for geometrical particles, viz. spheres, cubes and monocrystals.
Particle size will be established optically. By means of a calculation correction, the MMAD can be calculated.

Conclusion: this method is suitable to determine the distribution of particles of respirable and inhalable size. The MMAD can be calculated.

5.3 Rotating Drum Method

Material: dry powders/ granulates/friable products
Size range: 0.5 – 10,000 microns

This method, currently undergoing inter-laboratory testing, is based on size selective sampling of an airborne dust cloud produced by the repeated lifting and dropping of a material in a rotating drum. Air drawn from the drum passes through a specially designed outlet and a 3-stage fractionating system consisting of two porous polyurethane foams and a membrane filter. The mass of dust collected on each collection stage is determined gravimetrically to give a direct measure of the biologically relevant size fractions (the inhalable, thoracic and respirable fractions) as defined by ISO/CEN [9] conventions.
This method simulates a wide range of material handling processes in industry and determines the biologically relevant size fractions of a material in the airborne state. Full size distribution can be obtained by analysing the contents on the dust collection stages.

Conclusions: this method is suitable to determine the distribution of particles of respirable or inhalable size. The MMAD can be determined.

[9] See footnote 6

6. LITERATURE

Fissan, H. and Schwientek, G. (1987)
Sampling and transport of aerosols
TSI Journal Particle Instrumentation, 2, 10 p.

Marple, V.A. et al. (1976)
Aerodynamic particle size calibration of optical particle counters
J. Aerosol Sc. 7, 425-433

Miller, F.J. et al. (1979)
Size considerations for establishing a standard for inhalable particles
J. Air Poll. Contr. Ass. 29, 610-615

7. ANNEX

These methods are only suitable to determine particles of inhalable size or give only an indication of the presence of respirable and inhalable particles.

7.1 Elutriation (see OECD 110)

Material : dry powders/ granulates
Size range: 15 – 115 microns

Using elutriation techniques, particles are drawn out of a column at varying velocity. The velocity is used to calculate particle size and the weight of the remaining sample at a particular velocity is used to calculate the distribution.

Since the particles need a certain mass to overcome the velocity force and the brownian forces, elutriation is limited to particles greater than 15 microns.

Conclusion: this method is not suitable to determine the distribution of particles of respirable size, but might be suitable to determine the distribution of particles of inhalable size. The MMAD cannot be determined.

7.2 Air jet sieve

Material: particles of all kind
Size range: 10 – 10,000 microns

Air is aspirated through a weighted sample on a fine sieve and the weight loss will be measured. The method is capable of estimation of the non-floatable fraction of the material under investigation.

Aggregation of the particles will result in unreliable values. In addition, since the lower detection limit is only 10 µm, this method is not suitable to determine the distribution of particles of respirable size.

Conclusion: this method is not suitable to determine the distribution of particles of respirable size, but might be suitable to determine the distribution of particles of inhalable size. A MMAD cannot be determined.

7.3 Cyclone

Material: particles of all kind
Size range: 0.1 – 200 microns

A simple method to decide whether respirable and/or inhalable particles are present in the test atmosphere sample is the use of a cyclone. By constructing the cyclone, cut-off

points of 4.25 and 100 µm should be obtained. By measuring the weight of the particles which pass through the cyclone it can be decided whether more sophisticated methods such as described under 5.1 and 5.2 have to be applied to determine the size distribution of the particles smaller than 10 µm.

Conclusion: this method is suitable to determine the fraction of particles of respirable and inhalable size. Since fractions and no distributions are measured a MMAD cannot be determined.

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Abstract

Methods to determine particle size distribution, fibre length and diameter distribution of chemicals in the framework of Directive 67/548 are reviewed and guidance on their applicability and use is given

Mission of the JRC

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

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