SLOVENSKI STANDARD
SIST-TS CEN/TS 16817-1:2016

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Zunanji zrak - Monitoring učinkov gensko spremenjenih organizmov (GSO) - Monitoring peloda - 1. del: Tehnično vzorčenje peloda z uporabo masnega filtra peloda in vzorčevalnika Sigma-2

Ambient air - Monitoring the effects of genetically modified organisms (GMO) - Pollen monitoring - Part 1: Technical pollen sampling using pollen mass filter (PMF) and Sigma-2-sampler

Außenluft - Monitoring der Wirkungen gentechnisch veränderter Organismen (GVO) - Pollenmonitoring - Teil 1: Technische Pollensammlung mit Pollenmassenfilter (PMF) und Sigma-2-Sammler

Air ambiant - Surveillance des effets d'organismes génétiquement modifiés (OGM) - Surveillance du pollen - Partie 1 : Échantillonnage technique du pollen à l'aide d'un filtre de masse à pollen (FMP) et d'un échantillonneur Sigma-2

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Ambient air - Monitoring the effects of genetically modified organisms (GMO) - Pollen monitoring - Part 1: Technical pollen sampling using pollen mass filter (PMF) and Sigma-2-sampler

Air ambiant - Surveillance des effets d'organismes génétiquement modifiés (OGM) - Surveillance du pollen - Partie 1 : Échantillonnage technique du pollen à l'aide d'un filtre de masse à pollen (PMF) et d'un échantillonneur Sigma-2

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (CEN/TS 16817-1:2015) has been prepared by Technical Committee CEN/TC 264 “Air quality”, the secretariat of which is held by DIN.

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CEN/TS 16817, *Ambient air — Monitoring the effects of genetically modified organisms (GMO) — Pollen monitoring,* is composed of the following parts:

— *Part 1: Technical pollen sampling using pollen mass filter (PMF) and Sigma-2-sampler* [the present document];

— Part 2: Biological pollen sampling using bee colonies.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

The European Parliament and the European Council require an environmental risk assessment and a post-marketing monitoring for any GMO released to the environment [5; 6]. This had to be implied in national law in any member state of the EC by date.

Pollen dispersal plays a significant role in the dissemination of genetically modified organisms (GMO). A procedure is described for GMO monitoring that enables quantification and documentation of GMO input and spread through pollen in a nationwide monitoring network which represents natural landscapes. Technical and biological pollen sampling (the present Technical Specification and CEN/TS 16817-2) and molecular biological analysis methods (polymerase chain reaction (PCR) for DNA; Enzyme-linked immunosorbent assay (ELISA) for proteins) are used for the detection of GMO input.

It is reasonable to use both technical and biological sampling of pollen, thus they supplement each other in manifold ways. The technical sampling (i.e. the present document) is conducted with stationary point-samplers. They give a record of pollen input at the sample site that correlates with the prevailing wind direction and relative position to the surrounding pollen sources. Bee colonies actively roam an area and are therefore area related samplers. Further, pollen sampling depends here on the collection activity of the bees and the availability of pollen sources within the roaming area according to the bees' preferences and supply of melliferous plants [32].

Presently known pollen traps are only partially suited for GMO monitoring, since they can neither be standardized nor is the instrumentation designed for exposure times that are suitable for this purpose. Another limitation of commonly used pollen samplers is the requirement for a power supply, e.g. as for the Hirst type trap. The use of these instruments is therefore restricted to a limited exposure area.

For these reasons, a new type of passive pollen sampler, the pollen mass filter (PMF), was developed. The PMF is used either in combination with the Sigma-2 passive sampler or solely.

The present Technical Specification is largely based on German VDI/Guideline 4330 Part 3 [31].

1 Scope

This Technical Specification describes a procedure for the use of the passive samplers Sigma-2 and PMF to sample airborne pollen. Both are designed to sample coarse aerosol particles. Collected samples are used to analyse pollen input with regard to pollen type and amount, and input of transgenic pollen. The Sigma-2 passive sampler here provides a standardized sampling method for direct microscopic pollen analysis and quantifying the input of airborne pollen at the site. The PMF yields sufficient amounts of pollen to additionally carry out molecular-biological diagnostics for detection of GMO.

Essential background information on performing GMO monitoring is given in VDI/Guideline 4330 Part 1 [4], which is based on an integrated assessment of temporal and spatial variation of GMO cultivation (sources of GMO), the exposure in the environment and biological/ecological effects. Ideally, the pollen sampling using technical samplers for GMO monitoring should be undertaken in combination with the biological collection of pollen by bees (CEN/TS 16817-2).

The application of technical passive samplers and the use of honey bee colonies as active biological collectors complement each other in a manifold way when monitoring the exposure to GMO pollen. Technical samplers provide results regarding the pollen input at the sampling site in a representative way, whereas with biological sampling by honey bee colonies, pollen from flowering plants in the area is collected according to the bees' collection activity. Thus, this method represents GMO exposure to roaming insects. By combining the two sampling methods these two main principles of exposure are represented. Furthermore, a broad range of pollen species is covered.

The sample design depends on the intended sampling objective. Some examples are given in 6.2.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

VDI 2119:2013-06[1)], *Ambient air measurements — Sampling of atmospheric particles > 2,5 µm on an acceptor surface using the Sigma-2 passive sampler — Characterisation by optical microscopy and calculation of number settling rate and mass concentration*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
acceptor surface
natural or manmade collection surface for airborne particles

3.2
concentration
number concentration
number of particles per unit air volume; here number of pollen per m^3 air

3.3
deposition
pollen deposition
deposition of atmospheric particles; here pollen on an acceptor surface

1) For application of the Sigma-2.

3.4
dispersal
pollen dispersal
spread of pollen from the flower/field into the surrounding environment by wind drift

3.5
event
<genetics> unique DNA recombination event that took place in one plant cell, which was then used to generate entire transgenic plants

3.6
flux
horizontal flux
number of particles (here pollen) that are drifted horizontally per wind

3.7
genetically modified organism
GMO
organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination

[SOURCE: Directive 2001/18/EC [5], modified — The content of the definition was changed.]

3.8
monitoring
environmental monitoring
characterizing the state and quality of the environment and its changes by measurements/observations in regard to defined objectives

3.9
pollen
male gametophyte of the flowering plant

3.10
pollen type
species
class of pollen being distinguished by microscopic means on species, family or other order level

3.11
sampler
device for sampling here of pollen

3.12
sampling
pollen sampling
collection of particles, here pollen by technical or biological means

3.13
sedimentation
directed particle movement by gravity (here pollen in the air), which consists in a vertical flux towards the ground

4 Basic principle of the procedure

For the technical pollen sampling, two passive samplers are used, the PMF and Sigma-2 passive sampler, either in combination or the PMF solely:

The Sigma-2 passive sampler is designed for determining the pollen deposition rate (dry deposition by sedimentation). Wind-dispersed pollen grains enter the interior through the laterally shifted slits of the sampler. The pollen are deposited on an adhesive tray as acceptor (tape, foil, slide) at the bottom of the sampler. Thus, the deposition takes place in the turbulence-depleted interior of the sampler which provides protection from wind and rain. Pollen adhering to the adhesive tray are directly analysed with regard to pollen type and counts by means of light microscopy. For the purpose of GMO monitoring, an exposure time in the range of four weeks is recommended to be able to cover the main flowering period of the target plant species with as few sampling periods as possible (the rationale for this is given in 6.5). The microscopic single-particle analysis yields an average pollen deposition rate for the respective pollen species and time period. Summing up the deposition rates of all sampling periods in the season yields the total pollen deposition per season/year as target parameter.

The pollen mass filter (PMF) exhibits a 10 times to 100 times higher sampling efficiency, so that pollen samples can be analysed both microscopically to quantify pollen input and further on, with regard to possible GMO input by using molecular-biological based methods (e.g. PCR for DNA, ELISA for proteins/toxins). The PMF consists of a layered hollow filter that is constructed in such a way as to let the air pass through nearly unopposed. However, coarse aerosol particles bigger than 10 µm, such as pollen, are retained. A laterally mounted collection flask is used for collecting rainwater. For the PMF, an exposure time of four weeks is recommended (see 6.5) so that only a few samples are needed to cover the relevant flowering period. In order to cover a complete blooming period of one or more target plant species a respective number of exposure (sampling) periods lasting four weeks each can be carried out.

The Sigma-2 passive sampler collects aerosol particles bigger than 1 µm covering the size range of most pollen and fungal spores. Its sampling efficiency reaches its limitation towards bigger and heavier aerosol particles over 60 µm diameter, like e.g. maize pollen. In such cases, the evaluation of pollen deposition shall be based on the PMF solely.

Field experiments have shown that the method is well suited for environmental monitoring of GMO [14; 17; 20].

5 Sampling

5.1 Instruments and materials

5.1.1 General

The combined sampling equipment consisting of a Sigma-2 passive sampler and PMF is described in Figure 1. For some tasks the PMF sampler is used solely, e.g. for maize pollen, and/or when it is necessary to increase the amount of sampled pollen at a site within a certain period (see 10.5.2, e.g. for keeping detection limits for PCR-analysis of pollen DNA). For such tasks, stacked versions of the PMF sampler with more than one PMF-unit per sampler are additionally available as shown in Figure 2. The complete sampling equipment is available.[2) 3)]

2) TIEM technic GbR, Hohenzollernstr. 20, 44135 Dortmund. Samplers are manufactured by the supplier mentioned above. It is an example of a suitable product. This information is given for the convenience of users of this European Technical Specification and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

3) Breitfuß Messtechnik GmbH, Danziger Str. 20, 27243 Harpstedt. Samplers are manufactured by the supplier mentioned above. It is an example of a suitable product. This information is given for the convenience of users of this European Technical

5.1.2 Sigma-2 passive sampler

The Sigma-2 device is a passive sampler for coarse atmospheric particles as described in VDI/Guideline 2119. It consists of a cylindrical sedimentation chamber with a protective hood on top as inlet.

As acceptor surface for particle deposition, conventional microscopic slides are recommended (76 mm × 26 mm) with two quadratic acceptor fields (18 mm × 18 mm) coated with weather-proof adhesive, such as polymeric acrylic ester. The slides are attached to the base with an adapter. Alternatively adhesive acrylic foil (60 mm × 60 mm), also coated with weather-proof adhesive, on special adapters can be used, too.

Prepared adhesive foils and slides for sample handling and suitable shipping cans are available from the supplier mentioned above. It is recommended to use slides with marked acceptor fields (frames with plotted scales, dots or lines) for facilitating the microscopic pollen analysis. In this TS the handling is described for slides only.[4)]

5.1.3 Pollen mass filter PMF

The PMF consists of:

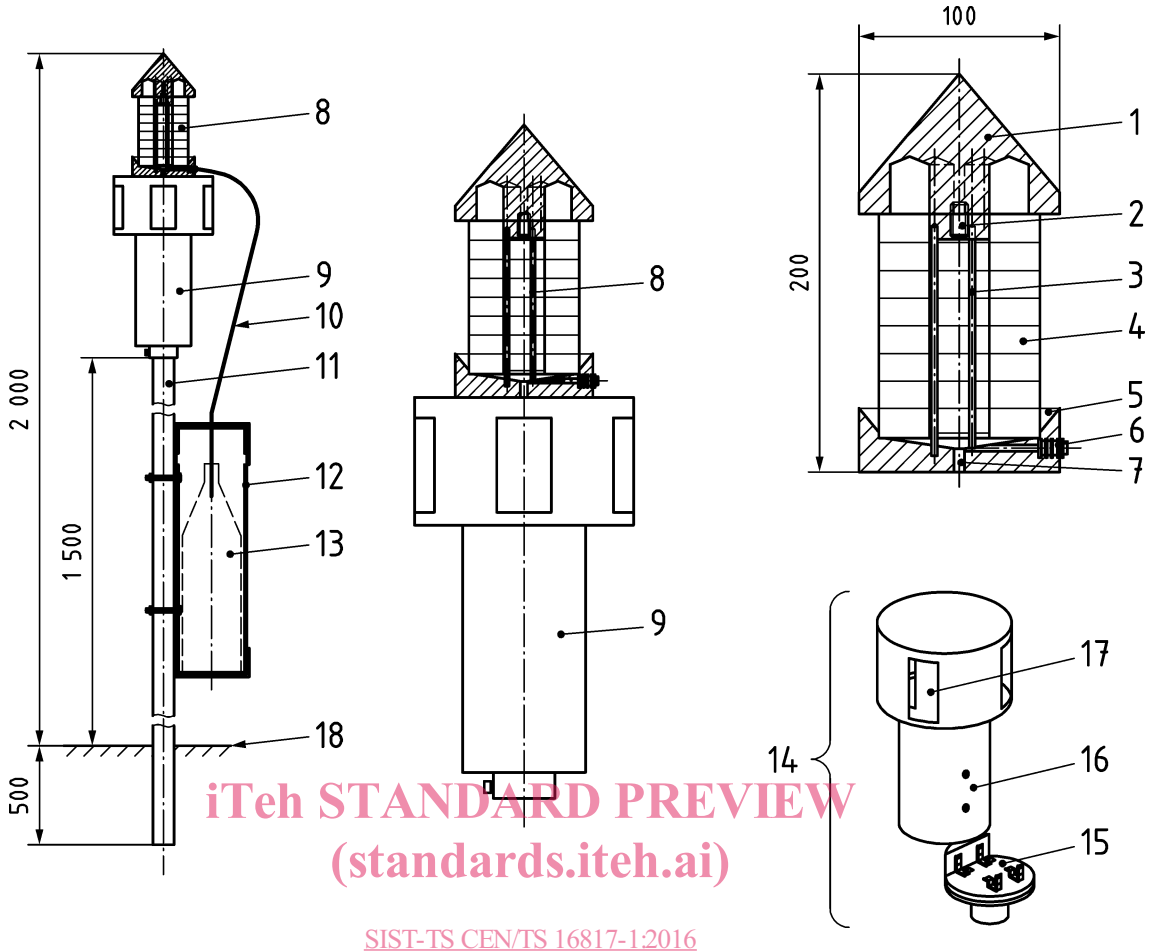
— filter cladding (depth filter) consisting of a stack of eight filtering discs;

— filter holder conical top, base with distance rods and quick-connect tube coupling;

— collection flask, sheath, connecting tube, floor stand pole, length 2 m, diameter e.g. 34 mm.

Specification and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

4) For the handling of foil as acceptor, see Guideline VDI 2119.



Key

1 conical top
2 screwed joint for removing filter discs
3 distance rods
4 filter cladding (consisting of eight stacked filter discs)
5 base with conical outlet
6 quick-connect tube coupling'
7 screw joint to Simga-2 passive sampler
8 complete PMF sampling unit
9 Sigma-2 passive sampler
10 connecting sample tube
11 pole, length 2 m
12 sheath for collection flask
13 collection flask
14 Sigma-2 passive sampler opened
15 base with acceptor surface and adapter
16 sedimentation cylinder
17 inlet (top cover with slits)
18 ground

Figure 1 — Detailed views of the complete sampling equipment [[17]; VDI 2119:2013-06]

a) PMF/Sigma-2 passive sampler **b) PMF-mono** **c) PMF-duo** **d) PMF-trio** **e) PMF-quattro**

Figure 2 — Types of technical sampler [Source: TIEM technic GbR]

5.2 Technical implementation

The Sigma-2 passive sampler provides a suitable method for sampling airborne particles including pollen. This instrument consists of a cylindrical sedimentation chamber which is covered by a cylindrical hood. Both parts have notches enabling air exchange between the interior of the sedimentation chamber and ambient air. Inside the chamber with a volume of calmed air particles suspended in the air sediment onto a transparent adhesive foil or slide that can be removed for subsequent microscopic analysis. Under the microscope, particles including pollen can be identified and counted. The average pollen deposition rate for the exposure time is calculated from the pollen count determined on a defined area on the adhesive carrier exposed over a defined time (see 9.2.1). However, within the conventional exposure time the amount of pollen collected by the Sigma-2 passive sampler is insufficient to carry out unambiguous pollen DNA analyses using PCR techniques for the detection of specific gene sequences such as transgenes.

In order to collect sufficient amounts of pollen for molecular-biological analyses the passive sampler PMF was developed.

The PMF consists of a filter holder with filter cladding as sampling unit, collection flask, and connecting tube. The PMF sampling unit is screwed on top of the Sigma-2 passive sampler or used solely with an adapter. The PMF sampling units can be stacked up to four units per pole.

The PMF filter cladding provides a low aerodynamic resistance and flux of ambient air through the filter. In addition, the characteristics of the filter material ensure that pollen and other particles larger than 10 µm in diameter can attach and adhere to the surface of the fibres. If it rains, some of the pollen and other particles can be washed off the surface of the fibres. Therefore, the rainwater is collected in the collection flask. The samples for downstream analyses are extracted from the filter material and from the rainwater in the collection flask using a sample preparation procedure.

— **Filter holder** (Figure 1, parts 1, 2, 3, 5, 6, and 7):

The filter holder consists of the conical top and the base part.

The conical shape of the top prevents birds from landing on it.

Three distance rods in the base part form a track for the filtering discs. The rods’ length determines the height of the filter cladding and the pressure between the filtering discs.

Rain water is collected in the flanged collar of the base part, the bottom of which is conical – thus, water is drained off through the quick-connect coupling and connecting tube into the collection flask.

The conical top of the filter holder can be screwed off in order to insert the filter cladding.

Components are made up of inert materials (e.g. aluminium with an anodized surface, stainless steel).

— **Filter cladding** (Figure 1, part 4):

The filter cladding consists of eight filtering discs layered on top of each other.

The annulate filtering discs have an outer diameter of 80 mm, while the centre-hole is 30 mm in diameter. Discs are punched out from a flat 20 mm thick depth filter fleece. The fleece is made of thermally bound and progressively layered polypropylene fibres.

For the horizontal wind component the effective flux cross-section (height × width) of the filter cladding is 100 mm × 80 mm = 0,008 m^2 for all wind directions. Filter dimension and material are characterized by a low flux drag and back pressure.

— **Connecting tube** (Figure 1, part 10):

Polyamide tubes with an outer diameter of 6 mm and a wall thickness of 1 mm have proven to be effective as inert connecting tubes.

— **Collection flask** (Figure 1, part 13):

Clean 1,5-l PET collection flasks with flat, round bottoms are suitable for the average amount of rain-fall in Germany and sampling times of four weeks. In cases of extreme high rainfall during the sampling period (approx. more than 120 l/m^2), the overflow of the bottle will start. This will not affect the sampled amount of pollen though because they sediment to the bottle base. For record of the complete amount of rainfall in regions with higher precipitation, bigger flasks and container might be used or the flask might be changed intermediately. For visual inspection the collection flask should be transparent, but during sampling time it shall be protected from daylight by aluminium foil and placed into an opaque sheath (Figure 1, part 12).

— **Floor stand** (Figure 1, part 11):

All components of the pollen sampling equipment are mounted onto a floor stand: the Sigma-2 passive sampler with the PMF screwed on top of it, and the collection flask underneath, so that sample liquid can drain off easily.

A pole of 2 m in length with an outer diameter of 34 mm is suitable. It is helpful to drill an 11-mm wide hole through the pole 0,5 m from the bottom end, through which a stabilizing rod can be inserted. This facilitates sinking the floor stand 0,5 m into the ground.

6 Sampling procedure

6.1 General

For documentation purpose, a protocol about sampling and site conditions needs to be prepared (see 11.2).

6.2 Sampling design

6.2.1 General

The sample design depends on the intended monitoring objective. Some examples are given here: