

<b>STN</b>	<b>Vonkajšie ovzdušie. Monitorovanie účinkov geneticky modifikovaných organizmov (GMO). Monitorovanie peľov. Časť 1: Technický odber vzoriek peľov pomocou peľového hmotnostného filtra a pomocou vzorkovača Sigma-2.</b>	<b>STN P CEN/TS 16817-1</b>  83 5808
------------	---	--

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

Táto norma obsahuje anglickú verziu európskej normy.  
This standard includes the English version of the European Standard.

Táto norma bola oznámená vo Vestníku ÚNMS SR č. 12/15

Táto predbežná STN je určená na overenie. Pripomienky zasielajte ÚNMS SR najneskôr do októbra 2017.

Obsahuje: CEN/TS 16817-1:2015

**122133**

---

Úrad pre normalizáciu, metrológiu a skúšobníctvo SR, 2016  
Podľa zákona č. 264/1999 Z. z. v znení neskorších predpisov sa môžu slovenské technické normy rozmnožovať a rozširovať iba so súhlasom Úradu pre normalizáciu, metrológiu a skúšobníctvo SR.

---

ICS 07.080; 13.020.99

English Version

## 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

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

This Technical Specification (CEN/TS) was approved by CEN on 16 May 2015 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of 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 United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

**CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels**

<b>Contents</b>	<b>Page</b>
European foreword.....	5
Introduction .....	6
1 Scope.....	7
2 Normative references.....	7
3 Terms and definitions .....	7
4 Basic principle of the procedure .....	9
5 Sampling.....	9
5.1 Instruments and materials.....	9
5.1.1 General.....	9
5.1.2 Sigma-2 passive sampler .....	10
5.1.3 Pollen mass filter PMF.....	10
5.2 Technical implementation.....	12
6 Sampling procedure.....	13
6.1 General.....	13
6.2 Sampling design.....	13
6.2.1 General.....	13
6.2.2 Exposure assessment of pollen input in the vicinity of fields with genetically modified crop (gm-fields) related to a specific GMO and region .....	14
6.2.3 Exposure assessment of pollen input for validating and/or calibrating dispersal models.....	15
6.2.4 General monitoring of pollen exposure at larger scales.....	15
6.2.5 Assessment of standardized and acceptor specific pollen deposition .....	15
6.3 Site conditions.....	15
6.4 Installing the equipment .....	16
6.5 Exposure time.....	17
6.6 Sampling at site.....	17
6.6.1 Sigma-2 passive sampler .....	17
6.6.2 PMF.....	17
6.7 Sample preparation.....	18
6.7.1 Preparation of slides for microscopy.....	18
6.7.2 Preparation of PMF samples .....	19
7 Microscopic pollen analysis .....	20
7.1 General.....	20
7.2 Sigma-2 passive sampler .....	20
7.2.1 Microscopic imaging methods.....	20
7.2.2 Qualitative analysis of the pollen diversity .....	21
7.2.3 Quantitative analysis of the pollen .....	21
7.3 PMF.....	21
7.3.1 Microscopic analysis .....	21
7.3.2 Qualitative analysis of the pollen (diversity).....	21
7.3.3 Quantitative microscopic analysis of pollen .....	21
8 Molecular-biological analyses of GMO.....	23

<b>9</b>	<b>Determination of the target parameters for GMO monitoring and representation of the results</b> .....	<b>24</b>
9.1	General .....	24
9.2	Sigma-2 passive sampler.....	24
9.2.1	Determination of pollen deposition per sampling period.....	24
9.2.2	Determination of the daily mean pollen deposition rate per sampling period.....	25
9.2.3	Determination of yearly pollen deposition.....	25
9.2.4	References to pollen dispersal models.....	25
9.3	PMF .....	25
9.3.1	Pollen count per sample $N_{i,PMF}$ .....	25
9.3.2	Relative frequency of pollen species $i$ .....	26
9.3.3	Determination of pollen flux per sampling period.....	26
9.3.4	Determination of the daily mean pollen flux rate per sampling period.....	26
9.3.5	Determination of the yearly pollen flux .....	27
9.3.6	Assessment of results from molecular-biological analyses .....	27
9.3.7	References to pollen dispersal models.....	28
<b>10</b>	<b>Performance characteristics of the methods</b> .....	<b>28</b>
10.1	General .....	28
10.2	Validation .....	28
10.3	Distribution of measured values.....	29
10.4	Methodical approach and determination of basic parameters.....	29
10.5	Sigma-2 passive sampler.....	31
10.5.1	Sensitivity, detection limit and reproducibility .....	31
10.5.2	Detection confidence level and required numbers of cases .....	33
10.6	PMF .....	35
10.6.1	Sensitivity, detection limit and reproducibility .....	35
10.6.2	Detection confidence level and required numbers of cases .....	38
10.7	Parallel measurements.....	40
10.8	Comparative measurements using a standard volumetric pollen trap (Hirst type).....	42
10.9	Pollen diversity .....	43
<b>11</b>	<b>Quality assurance and quality control</b> .....	<b>44</b>
11.1	General monitoring strategy and terms of reference of pollen monitoring with technical samplers .....	44
11.2	Site protocol.....	44
11.3	Accompanying documentation for samples.....	45
11.4	Parallel measurements.....	45
11.5	Comparative measurements using active samplers as calibration bases.....	45
11.6	Quality assurance and reference materials .....	45
11.7	Qualification .....	46
<b>Annex A (normative) Maize-specific requirements</b> .....		<b>47</b>
A.1	Scope .....	47
A.2	Basic principles .....	47
A.3	Sampling .....	48
A.4	Sample preparation .....	49
A.5	Quantitative microscopic pollen analysis.....	50
A.6	Molecular-biological analysis of maize DNA using PCR.....	51
A.6.1	General .....	51
A.6.2	DNA extraction.....	51

<b>A.6.3</b>	<b>Real-time PCR analysis.....</b>	<b>51</b>
<b>A.7</b>	<b>Determination of the target parameters for GMO monitoring and assessment of the results.....</b>	<b>52</b>
	<b>Bibliography.....</b>	<b>53</b>

## 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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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 and 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<sup>1)</sup>, *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*

**koniec náhľadu – text ďalej pokračuje v platenej verzii STN**

---

1) For application of the Sigma-2.