

English Version

**Ambient air - Monitoring the effects of genetically
modified organisms (GMO) - Pollen monitoring - Part 2:
Biological pollen sampling using bee colonies**

Air ambiant - Surveillance des effets d'organismes
génétiquement modifiés (OGM) - Surveillance du
pollen - Partie 2 : Échantillonnage biologique du pollen
à l'aide de colonies d'abeilles

Außenluft - Monitoring der Wirkungen von
gentechnisch veränderten Organismen (GVO) -
Pollenmonitoring - Teil 2: Biologische Pollensammlung
mit Bienenvölkern

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

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Contents	Page
European foreword.....	4
Introduction	5
1 Scope.....	6
2 Normative references.....	6
3 Terms and definitions	6
4 Basic principle of the procedure	8
5 Sample matrices	8
5.1 Honey.....	8
5.2 Pollen load	9
5.3 Bee-bread.....	9
6 Sampling procedure.....	9
6.1 General.....	9
6.2 Bee colony and hive.....	9
6.3 Sample site.....	9
6.4 Preparation and assembly.....	10
6.5 Exposure time.....	10
6.6 Sampling dates	10
6.7 Extraction, transport and storage.....	10
7 Palynology	11
7.1 General.....	11
7.2 From sample preparation to embedded slide preparation.....	11
7.2.1 General.....	11
7.2.2 Honey.....	11
7.2.3 Pollen load	11
7.2.4 Bee bread	11
7.3 Microscopic analysis	12
7.4 Pollen diversity	12
8 Molecular-biological analysis	13
8.1 General.....	13
8.2 Sample preparation.....	13
8.2.1 Honey.....	13
8.2.2 Pollen loads	13
8.2.3 Bee bread	14
9 Determination of the target parameters for GMO monitoring and representation of the results	14
9.1 Microscopic pollen analysis	14
9.1.1 General.....	14
9.1.2 Concentration in counts per gram of sample mass.....	14
9.1.3 Total number of pollen collected per exposure time and season.....	15
9.1.4 Relative frequency	15
9.2 Molecular-biological analysis	16
10 Performance characteristics of the methods.....	16
10.1 General.....	16

10.2	Bee colony	16
10.3	Foraging distance	17
10.4	Honey samples, bee-bread samples and pollen load samples	17
10.5	Microscopic pollen analysis	17
10.6	Molecular-biological analysis.....	17
11	Quality assurance and quality control	18
11.1	General measurement strategy and task of pollen monitoring with biological samplers.....	18
11.2	Site protocol.....	18
11.3	Accompanying documentation for samples.....	19
11.4	Parallel measurements.....	19
11.5	Quality assurance and reference materials	19
Annex A (normative) Maize-specific requirements		20
A.1	Scope	20
A.2	Basic principles	20
A.3	Sampling	21
A.4	Sample preparation	22
A.5	Molecular-biological analysis of maize DNA using PCR.....	23
A.5.1	General	23
A.5.2	DNA extraction.....	23
A.5.3	Real-time PCR analysis	24
A.6	Determination of the target parameters for GMO monitoring and assessment of the results	24
Annex B (normative) Rapeseed specific requirements		25
B.1	Scope	25
B.2	Basic principles	25
B.3	Sampling	26
B.4	Sample preparation	27
B.5	Molecular-biological analysis of rapeseed DNA using PCR for GMO detection	28
B.5.1	General	28
B.5.2	DNA extraction.....	28
B.5.3	Real-time PCR analysis	29
B.6	Determination of the target parameters for GMO monitoring and assessment of the results	29
Annex C (informative) Good beekeeping practice		30
Bibliography		31

European foreword

This document (CEN/TS 16817-2:2015) 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;*
- *Part 2: Biological pollen sampling using bee colonies* [the present document].

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 [6; 7]. 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). Hence, a monitoring procedure that involves recording and documentation of input and distribution of GMO via pollen in a monitoring network mirroring the natural environment is required. For this, technical (CEN/TS 16817-1) and biological sampling of pollen as well as PCR-screening (polymerase chain reaction) procedures are employed to provide evidence of GMO-exposure. The biological sampling system using honey bee colonies is described in the present Technical Specification.

VDI/Guideline 4330 Part 1 [3] presents the necessary fundamentals for the understanding of this Technical Specification. The sampling of pollen in the sample matrices honey, pollen load and bee-bread [5] needs to be viewed in conjunction with the technical sampling for the GMO-monitoring [4].

The use of the biological, actively foraging honeybee and the technical passive samplers complement each other in a manifold and positive way for pollen monitoring of GMO. Therefore it is reasonable to use both. The technical sampling (CEN/TS 16817-1) is based on stationary point-samplers [1]. They give a record of pollen exposure in the air at the sample site that correlates with the prevailing wind direction and relative position to the surrounding pollen sources. The biological sampling using honey bee colonies serves as indicator for GMO exposure in an area and for exposure to roaming insects. Bees display a spatially averaging sampling activity, which represents a cross section of the established, blossoming plants in the area according to the bees collection activities. A wide spectrum of pollen species is recorded using both sampling methods with the procedures complementing each other across the vegetation period [21].

1 Scope

This Technical Specification describes a procedure through which pollen – in particular pollen of genetically modified organisms (GMO) – can be sampled by means of bee colonies.

Bee colonies, especially the foraging bees, actively roam an area and are therefore area related samplers. Pollen sampling depends on the collection activity of the bees and the availability of pollen sources within the spatial zone according to the bees' preferences (supply of melliferous plants). A colony of bees normally forages over an area of up to 5 km radius (median 1,6 km, mean 2,2 km), in rare cases some bees may also forage in greater distances up to 10 km and more [26].

Foragers fix the gathered pollen on the outside of their hind legs (pollen loads, also known as pollen pellets). Inside the hive they place these pollen loads into comb cells close to the brood nest (bee bread). Furthermore, foragers gather nectar and honeydew. Nectar contains pollen which fell from the anthers of the blossom into the nectar drop, or pollen which was dispersed by the wind and sticks in the nectar of other blossoms or adheres to the sticky honeydew of plants. Nectar and honeydew are converted to honey and stored by the bees in the beehive.

Honey, pollen load and bee-bread may be used as sample matrices for the subsequent analysis of pollen as it is possible to concentrate sufficient amounts of pollen for microscopic and molecular biological diagnostics.

Microscopic analysis is used to identify the various pollen types and to quantify the exposure to the target pollen types in question. GMO exposure is analysed by molecular-biological methods: For analysis of pollen DNA quantitative PCR methods are used and described here in this Technical Specification. The analysis of GMO specific proteins and toxins in pollen is possible, too, using ELISA, but to this date the method has not been evaluated enough in pollen matrices for standardization in this Technical Specification.

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.

DIN 10760, *Analysis of honey — Determination of the relative frequency of pollen*

3 Terms and definitions

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

3.1

bee bread

pollen load stored in comb cells close to the brood nest

3.2

bee colony

colony of the honeybee species *Apis mellifera*

3.3

beehive

hive

container in which honeybees are kept by beekeepers