

**Sampling and Detection**  
**of Living Genetically Modified Higher Plants**  
**in the Czech Republic**  
(pursuant to Article 18.2(a) of the Cartagena Protocol)

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## 1. Introduction

At present the Czech Republic belongs to countries which are using capabilities of modern biotechnologies even in the agricultural sector. Moreover, the Czech Republic is ranked among the EU Member States being more likely disposed to introduce GM crops.

In 2008, based on information of the Ministry of Agriculture, in the territory of the Czech Republic the GM maize resistant to corn borer (so-called Bt-maize; MON810, code: MON-ØØN81Ø) again increased in sown area. As stated in records of the Ministry of Agriculture, and in compliance with statistics of the Ministry of the Environment the total area of 8,380 ha of GM maize sown by 171 growers has been registered.

year	land area in ha	number of growers
2005	270	52
2006	1290	85
2007	5000	131
2008	8380	171

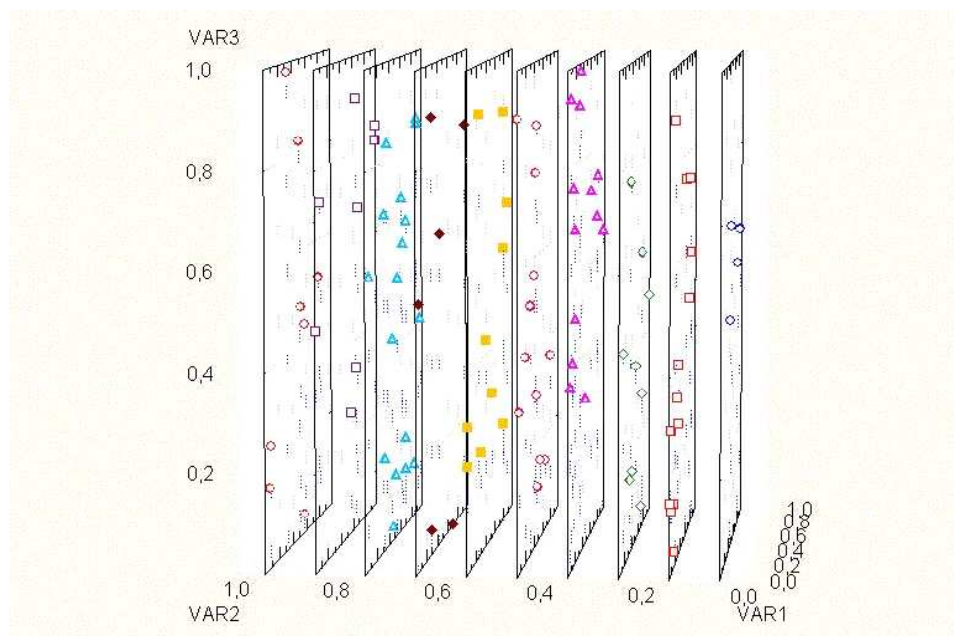
The obligations of growers of GM crops are stipulated particularly by provisions of §2i of the Act on agriculture (the Act No. 252/1997 Coll., on agriculture as amended by the Act No. 441/2005 Coll.) and by the implementing decree No. 89/2006 Coll. The notifying requirements and measures have been stipulated to prevent spreading of the pollen from GM crops onto neighbouring plots.

To ensure food and feed safety the Food Safety Co-ordination Unit and five advisory scientific committees have been established in the Czech Republic. Two out of them the Phytosanitary and Environment Committee ([www.phytosanitary.org](http://www.phytosanitary.org)) and the Scientific Committee for GM Food and Feed ([www.scgmff.cz](http://www.scgmff.cz)), are concerned according to the field of competence with the evaluation and the elaboration of standpoints relating to the effect of genetically modified organisms on food safety.

The analysis of information about risk sources based on the activity of members of the Phytosanitary and Environment Committee, and external co-workers includes among other things the risk assessment relating the presence of genetically modified organisms in the agro-eco-system.

## 2. Sampling requirements

The measures taken to ensure the availability of procedures for detection of living modified organisms should necessary include also the appropriate sampling methods. At present the need of harmonisation and new GMO sampling methods in the whole chain are discussed in relation to necessary traceability. The fact that in many cases the GMOs occurring in deliveries and products are unequally divided is the main reason why the sampling is a critical point.

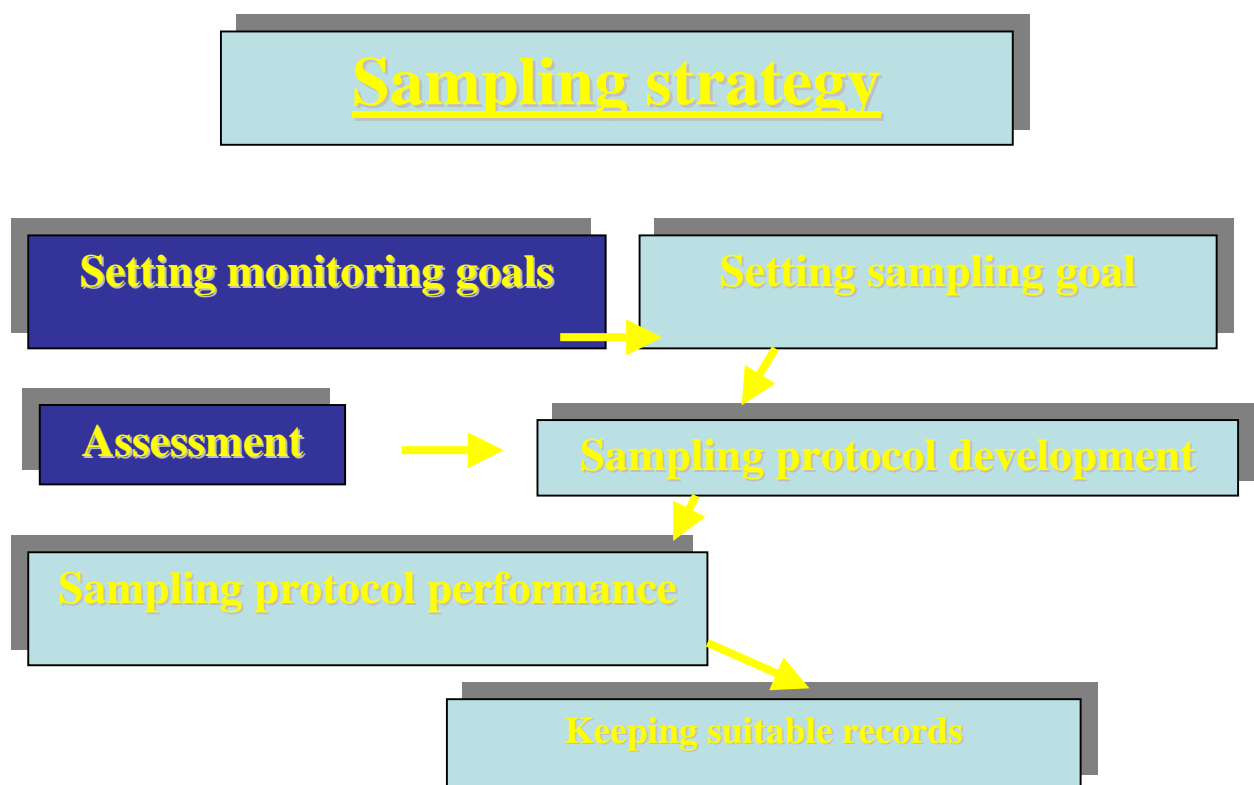


If the sampling is carried out inadequately the result of detection may be significantly influenced so that it may not be in accordance with reality, which means the content of genetically modified organisms in the delivery. Falsely negative or positive results if appropriate can therefore cause economic losses in relations between suppliers and customers or endanger final users.

To prepare the appropriate sampling plans it is necessary to unite the requirements for reducing the related uncertainty with the requirements for reducing the economic costs of the whole procedure, including costs of the subsequent laboratory tests.

Generally:

- The aim of sampling is to receive a representative sample which helps to decide whether a sampled entity (for example a set of maize plants on the plot) contains genetically modified plants.
- The sampling shall provide with a material (a sample) which may be subsequently used when applying the above mentioned analytical methods for proving the presence of genetically modified higher plants.
- The sampling must be performed so as to meet goals appointed by the supervisory body.
- The method of sampling depends on the purpose of the supervision (inspection), and that purpose must be predefined.



### 3. Sampling for the purpose of determining the presence of modified plants

Currently the procedures derived from procedures being used to take samples of food, feed and some agricultural commodities have been used for GMO sampling. Although, the results

of projects concerning the issue of GMO sampling have shown that these sampling plans often do not cover the specificity of GMO distribution. Particularly, the financial costs and technical complexity related to sampling and analyses can act as the reason for the small number of tests performed. The problems with limited available sources resulted in reducing inspections and supervisions in some EU Member States.

For the sampling of genetically modified organisms in food and feed the Commission Recommendation 2004/787/EC of 4 October 2004 has been issued on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No. 1830/2003. The main provisions in the Recommendation are concerning sampling of seed, agricultural commodity, food and feed lots. The official controls shall be carried out in every phase of manufacturing, processing and distributing of the product, which contains or may contain GMOs, as well as food and feed produced from GMOs, including the place of export.

This Recommendation is not concerned with methods for sampling plants in production vegetation, which means in the primary phase of the production of food and feed.

There is stated only generally that the other methods can be also applied aside from the sampling methods recommended by this regulation. Moreover, the further documents should be particularly taken into account, as follows: ISO standard 6644 (2002); ISO standard 13690 (1999); ISO standard 5725 (1994); ISO standard 2859 (1985) and ISO standard 542 (1990). It is recommended to take samples of bulk commodities (grain crops, oil seeds) in accordance with common principles and methods for sampling as described by the standards ISO 6644 and 13690. The sampling of material exceeded the size of grains (for example fruits, roots, potatoes) should be carried out in compliance with the standard ISO 2859. The sampling of oil seeds should be carried out in compliance with the standard ISO 542. The sampling of packed food and feed should be carried out under procedures described in the standard ISO 2859.

The Commission report on the implementation of Regulation (EC) No. 1830/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/ES stated that the Member States have problems when implementing the Recommendation 2004/787/EC on sampling and detection. Most of the Member States consider its implementation as costly and time consuming, particularly with regard to great shipments. They say that the controlling and sampling of imported bulk

consignments under technical guidelines laid down by the Recommendation places a great burden upon control authorities, and the results are not consonant with the time consumed and financial costs.

The recommendations for sampling within the planned official inspections are also included in the Regulation 882/2004. (REGULATION NO. 882/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL (EC) of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

In case of a need to manage some emergency situations (for example an occurrence of non-authorised genetically modified organisms/products), the regulations have been issued in the EU which deal besides analytical procedures also with the methods for taking official samples (see Commission Decision 2006/754/EC of 6 November 2006 amending Decision 2006/601/EC on emergency measures regarding the non-authorised genetically modified organism LL RICE 601 in rice products, the Annex Methods of sampling and analysis for official control regarding the non-authorised genetically modified organism LL RICE 601 in rice products).

#### **4. The other available standards and sampling methods for determination of the presence of modified plants in the Czech Republic**

ČSN EN 12305 (831022) Biotechnology – Modified organisms for the use in the environment – Guidelines for the sampling strategies for deliberate releases of genetically modified plants. This standard is a Czech version of the European standard EN 12305:1997. The European standard EN 12305:1997 has the status of the Czech technical standard.

This European standard provides with guidelines for the implementation of the sampling strategy that meets the objectives of the strategy for monitoring of genetically modified plants. The sampling shall provide with a material on which the analytical methods for genetically modified plants in question can be applied. This European standard is concerned only with the obtaining of the statistically significant samples from the experimental field according to the field trial plan. Thus the standard provides the experimentalists with a list of basic parameters having to be considered for the validity assessment of the proposed sampling strategy.

The standard ČSN EN 12468 (831022) Biotechnology – Modified organisms for the use in the environment – Guidelines for monitoring strategies for deliberate releases of

genetically modified plants. This standard is a Czech version of the European standard EN 12468:1997. The European standard EN 12468:1997 has a status of the Czech technical standard.

This European standard specifies guidelines concerning the factors and criteria considered when assessing the availability and validity of the plan, and the development and performance of the monitoring strategy for genetically modified plants. In testing the behaviour of genetically modified plants on the experimental field the valid monitoring strategy must be used, and the proposed monitoring strategy must be in conformity with specific plant species and experimental requirements. In this European standard the monitoring relates to the monitoring of the occurrence, persistence (stability) and/or the spread of genetically modified plant and/or the appropriate gene (genes). The monitoring includes the detection of genes and signs as well as the identification of genetically modified plants during deliberate releases thereof. Therefore, this European standard provides personnel staff carrying out the monitoring with factors and criteria that must be considered when assessing the validity of the proposed monitoring strategy.

The standard ČSN P CEN/TS 15568 (569906) - Food – Analytical methods for detection of genetically modified organisms and derived products – the Sampling Strategy has been elaborated on the basis of the Commission Recommendation 2004/787/EC, and it has the same scope of applications as well. Thus it does not cover a topic of the sampling of plants in vegetation for the purpose of detection the presence of genetically modified plants.

## **5. Areas of possible applications of plant sampling for the purpose of determination the presence of genetic modifications**

The sampling for purposes of checking the presence of genetically modified higher plants in the vegetation of field crops during the vegetation season is important for:

- verifying the use of GMOs when releasing into the environment (experimental vegetation)
- verifying the compliance with legal provisions in the framework of the co-existence

It is obvious that the official sampling can be carried out only for the purpose of verifying the characteristics of plants occurring on the plot, and it is not possible to carry out for the

purpose of checking the thresholds for accidental impurities of genetically modified plants because of labour intensity.

In terms of the official controls performed by the State Administration the Methodological guideline for sampling the maize vegetation for the purpose of detection the presence of genetically modified plants under § 2i of the Act No. 252/1997 Coll., on agriculture has been worked out in 2007 in the competence of the Ministry of Agriculture. The controls are focused on the detection of the occurrence of GM maize plants in buffer strips that means a genetically non-modified vegetation (hereinafter „non-GM“) of maize varieties.

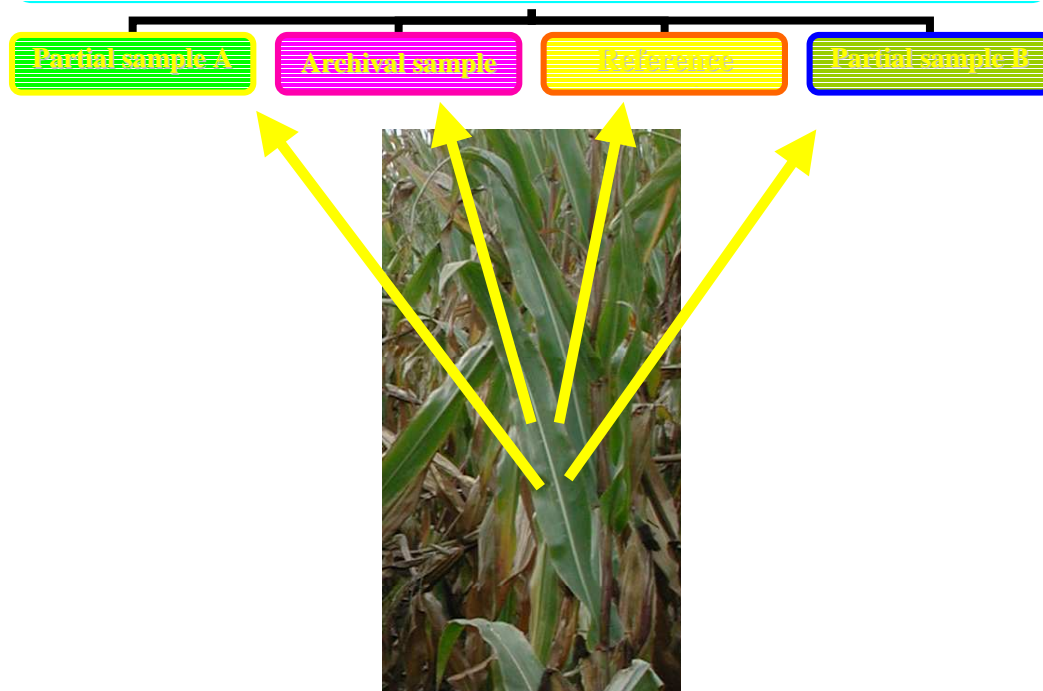
The Methodological guideline specifies a sampling method – the taking samples from maize plants during vegetation on land blocks and parts of land blocks if appropriate in order to detect the presence of genetically modified (hereinafter „GM“) maize plants. The aim of sampling is to provide with the representative material (a laboratory sample) for which the analytical methods (tests) can be subsequently applied as the evidence of the absence of GM plants in question. The samples shall be taken from the vegetation in such way that the 20 individual plant samples are taken in marked lines (the systematic sampling) on the block with non-GM maize variety or a part of the block if appropriate in regular intervals under the W scheme so that they are located on the whole lengths and width of the buffer strip surrounding the field.

In 2007 and subsequently in 2008 the official controls of buffer strips of selected growers of genetically modified Bt maize varieties (4+r growers) were carried out.

Besides the official controls above mentioned the 10 plots with the vegetation of MON810 maize of the total area of 310.3 ha (it means approximately 4 % of the area where the maize was cultivated in 2008) in 8 regions of the Czech Republic, and 10 plots with conventional maize of the total area of 266.7 ha in 7 regions of the Czech Republic, were assessed during the monitoring in 2008.

The results of the analyses of plant samples taken during the monitoring of maize growers in the territory of the Czech Republic (with a view to the utilisation of genetically modified varieties) were in conformity with the declared characteristics of producing vegetation.

## Scheme of the obtaining of representative samples when taking the parts of plants (leaves)



### 6. Detection of genetically modified plants

#### a. Testing laboratories

At the present time the National network of GMO laboratories under co-ordination of VÚRV, v.v.i. exists in the Czech Republic. 4 laboratories are included in the competence of the Ministry of Agriculture, and 1 laboratory belongs to the Ministry of Health. 1 laboratory is an academic workplace (VŠCHT). The Ministry of the Environment has not got the own laboratory network whilst using selected laboratories of the National network. The laboratories jointly meet obligations of the Czech Republic in accordance with the European legislation (Directive 2001/18/EC and Regulations of the European Parliament and Council 1829/2003, 1946/2003, 1830/2003, and 65/2003). The laboratories are also members of the network of European laboratories for the identification of GMOs (European Network of GMO Laboratories – ENGL), which is contributory to activities of the reference laboratory of the European Union (CRL – Community Reference Laboratory).

On October 23, 2006, the revised Annex EC 1829/2004 was issued, according to which the existence of five Czech GMO laboratories is verified by the European law.

List of laboratories authorised under ISO 17025 concerned with the detection of GMOs:

	Institution	Laboratory	Contact person	E-mail	Website
1.	Crop Research Institute, Prague, Czech Republic	Reference laboratory for GMO identification and DNA fingerprinting	RNDr.Jaroslava Ovesná, CSc.	<a href="mailto:ovesna@vurv.cz">ovesna@vurv.cz</a>	<a href="http://www.vurv.cz">www.vurv.cz</a>
2.	Institute of Chemical Technology Prague, Czech Republic	Testing laboratory of the Institute of Biochemistry and Microbiology	Prof.ing.Kateřina Demnerová, CSc.	<a href="mailto:demnerok@vsccht.cz">demnerok@vsccht.cz</a>	<a href="http://www.vsccht.cz">www.vsccht.cz</a>
3.	Czech Agriculture and Food Authority , Brno, Czech Republic	Laboratory of the Department for molecular genetics	Ing.Dagmar Vošmerová	<a href="mailto:dagmar.vosmerova@szpi.gov.cz">dagmar.vosmerova@szpi.gov.cz</a>	<a href="http://www.szpi.gov.cz">www.szpi.gov.cz</a>
4.	State Veterinary Institute Jihlava, Czech Republic	Laboratory for molecular biology	Ing.Martina Sedmíková	<a href="mailto:red@svujihlava.cz">red@svujihlava.cz</a>	<a href="http://www.svujihlava.cz">www.svujihlava.cz</a>
5.	Centre for the Hygiene of Food Chains in Brno - the National Institute of Public Health in Prague, Czech Republic	Centre for the Hygiene of Food Chains in Brno	RNDr.Irena Řehůrková, PhD.	<a href="mailto:sekretariat@chpr.szu.cz">sekretariat@chpr.szu.cz</a>	<a href="http://www.chpr.szu.cz/sluzby.htm">http://www.chpr.szu.cz/sluzby.htm</a>
6.	Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic	Laboratory of molecular genetic diagnostics	MVDr.Stanislav Malý, Ing.Jana Včeláková	<a href="mailto:stanislav.maly@ukzuz.cz">stanislav.maly@ukzuz.cz</a>	<a href="http://www.zeus.cz/index_lo.php">http://www.zeus.cz/index_lo.php</a>

b. Detection methods

The laboratory tests carried out in testing laboratories shall be realised under validated procedures authorised by CRL or under protocols stated in the appropriate technical standards.

Tab. 1 Basic standards for the detection of GMOs

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In principle, the sampling shall be carried out under EN/TS 15568

Then the presence of GMOs or parts thereof shall be determined according to the presence of DNA or proteins.

The determination of proteins shall be carried out under EN ISO 21572 (extraction, immunoassay, interpretation)

The determination of the transgenic DNA shall be carried out under

Extraction of nucleic acids	▶ EN ISO 21571
Screening	▶ EN ISO 21571
Identification	▶ EN ISO 21569
Quantification	▶ EN ISO 21570
Interpretation of results	

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## **7. Recommendation concerning the sampling of plants in field trial vegetation for the purpose of determining genetic modifications**

Addressed to the bodies of the state government and state supervisory organisations

To pay attention to the co-ordination of activities in the field of development and application of sampling methods for the detection of GMOs in departments of the Ministry of Agriculture of the Czech Republic and Ministry of the Environment of the Czech Republic.

For the area of research

To improve the methods for sampling and consequential tests in order to increase the reliability of the determination of GMO presence, and to ensure an acceptable rate of labour and costs.

Addressed to growers

To pay more attention to the duties laid down by legal regulations, particularly in the cultivation of genetically modified varieties – currently, that means mainly the maize.