Organisation and inspection of GM crops cultivation in the Czech Republic

MINISTRY OF AGRICULTURE
OF THE CZECH REPUBLIC

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List of abbreviations

Bt	Bacillus thuringiensis
CAFIA	Czech Agriculture and Food Inspection Authority
CC GMO	Czech Committee for GMO Handling
at MoE	the Ministry of the Environment
CEI	The Czech Environmental Inspectorate
CISTA	Central Institute for Supervising and Testing in Agriculture
CR	Czech Republic
CRI, p.r.i.	Crop Research Institute, Public Research Institution
DNA	Deoxyribonucleic acid
EC	European Commission
ECB	European Corn Borer (Ostrinia nubilalis)
EFSA	European Food Safety Authority
EU	European Union
FPLB	farmer‘s part of land block
GM	Genetically Modified
GMO	Genetically Modified Organism(s)
GMO Committee	Committee for the Use of Genetically Modified Organisms and Genetic Products
at Moa	at the Ministry of Agriculture
LPIS	Land Parcel Identification System
MoA	Ministry of Agriculture
MoE	Ministry of the Environment
NRL	National Reference Laboratory
LB	land block
SPA	State Phytosanitary Administration
RAARA	Regional Agencies for Agriculture and Rural Areas
SAIF	State Agricultural Intervention Fund

1. Introduction

Genetically modified (hereinafter referred to as GM) or transgenic plants are such plants, within which hereditary material has been changed through genetic technologies (genetic engineering). GM plants are characterised by specific characteristics, among which there are particularly resistance against pests or tolerance to non-selective herbicides. Newly gained characteristics are generally to bring direct advantages, especially for farmers.

Within the European Union (hereinafter referred to as EU), the Czech Republic´s (hereinafter referred to as CR) experience with GM crops can be described as very advanced. On the EU territory, thus also in the CR, there is only one GM crop (maize) which is cultivated for commercial purposes; it is sometimes marked as "Bt maize". This is a GM plant with inserted gene from soil bacteria *Bacillus thuringiensis* (hence BT maize), which makes the maize resistant against the harmful European Corn Borer - *Ostrinia nubilalis* (hereinafter referred to as ECB). Recently, there has not been a single other modified crop that would be submitted to the strict and demanding approval process and simultaneously approved. In 2010 it was for the first time that GM potatoes with determined use outside food industry were cultivated; this was in particular the potato variety Amflora, which is characterised by modified starch content (amylopectin at the expense of amylosis). These potatoes were cultivated in the Vysočina region on the area of 150 hectares. The potatoes Amflora were not cultivated in the following years. Multiplying areas for production of GM potatoes planting stock were located in Sweden and in Germany. The company BASF finished the whole project in 2012.

While on a worldwide scale the share of the area of genetically modified plants increases annually, the share in the European Union stagnates or even decreases. A similar trend is also in the CR, where based on the register of the Ministry of Agriculture (hereinafter referred to as MoA), the area of genetically modified maize reached 1 754 ha in 2014, which is by 806 ha less than in the previous year. The number of growers dropped to nearly a half. According to information directly from growers, the main reasons for such a sharp drop are administrative burden, abiding by coexistence rules in practice and more expensive price of planting stock.

Czech growers who continued with GM maize cultivation see the advantages especially in its usable simplicity and reliability concerning protection against the ECB (crops of genetically modified maize show nearly 100% effectiveness against pests), in decreased inputs into crops (fewer chemical agents and mechanization rolling on the field when the ECB occurs) and quality harvest (crops that are not broken or lodged). The result is higher yields compared to cultivating through traditional forms; harvested material is more quality due to lower moulding by fungi of the family *Fusarium*. Production of GM maize is in most cases used as feed for farm animals, from a lesser part as a raw material for production of bio-ethanol or biogas. The cultivated GM maize is not used for food processing purposes in the Czech Republic.

On the other side, cultivating GM crops brings certain drawbacks as well. Growers tend to be increasingly dissatisfied with legal-administrative burdening which is inextricably linked with cultivation and generally with any GMO use in the EU. From the economic point of view, growers point out to higher costs concerning production inputs (more expensive seed stock) and also issues with marketing of the production. Purchasers´ concerns and unwillingness to buy GM crops products, possibly also animals which were fed by such crops still prevail. These issues are related to prevailing, generally negative perception of GMO in the EU.

Development of the areas and a number of GM maize growers since the beginning of cultivation:

Year	area (ha)	Number of growers
2005	**150**	**51**
2006	**1 290**	**82**
2007	**5 000**	**126**
2008	**8 380**	**167**
2009	**6 480**	**121**
2010	**4 680**	**82**
2011	**5 090**	**64**
2012	**3 050**	**41**
2013	**2 560**	**31**
2014	**1 754**	**18**

In comparison with other parts of the world, the EU treats GM crops with a high level of caution, with the principle of preliminary caution and thus does not use GM crops in such an extent like e.g. USA where "new technologies" find more and more applications.

The process of introducing GM crops into the environment is long, financially and administratively demanding.

Applying GM crops on the EU market is very problematic due to higher costs and due to considerable mistrust of inhabitants. It can be expected, given the current situation in the GMO area in the EU, that Czech growers´interest in technology based on GM crops will develop proportionally with the extent of GMO tolerance by European consumers and related development of legislation in the EU. In the CR there will continue to be the possibility to choose between cultivating GM maize and conventional or organic cultivation. Therefore, GM crop cultivation is a free will choice of every grower.

This publication is aimed, above all, at all inspecting authorities in the CR and in the EU. (It is also published in the English version). Furthermore, it is aimed at agricultural entities, expert and lay public. It is a comprehensive and complex overview of involved State Administration Authorities, tasks and rules which are adopted in the CR and applied in order to successfully manage the inspection of GM crops cultivation. The publication contains all effective requirements, references and methods in individual parts

of GM crops cultivation inspection, especially in chapter 4. Obligations for growers, in chapter 5. State Agricultural Intervention Fund methodical guideline (hereinafter referred to as SAIF) and in chapter 9 Central Institute for Supervising and Testing in Agriculture (hereinafter referred to as CISTA) and Crop Research Institute, Public Research Institution (hereinafter referred to as CRI, p.r.i.) methodical guideline, which are an inextricable part of this publication.

2. Overview, relations and competences of the State Administration Authorities in the Czech Republic and the European Commission

2.1. Overview of Authorities and Bodies of State Administration

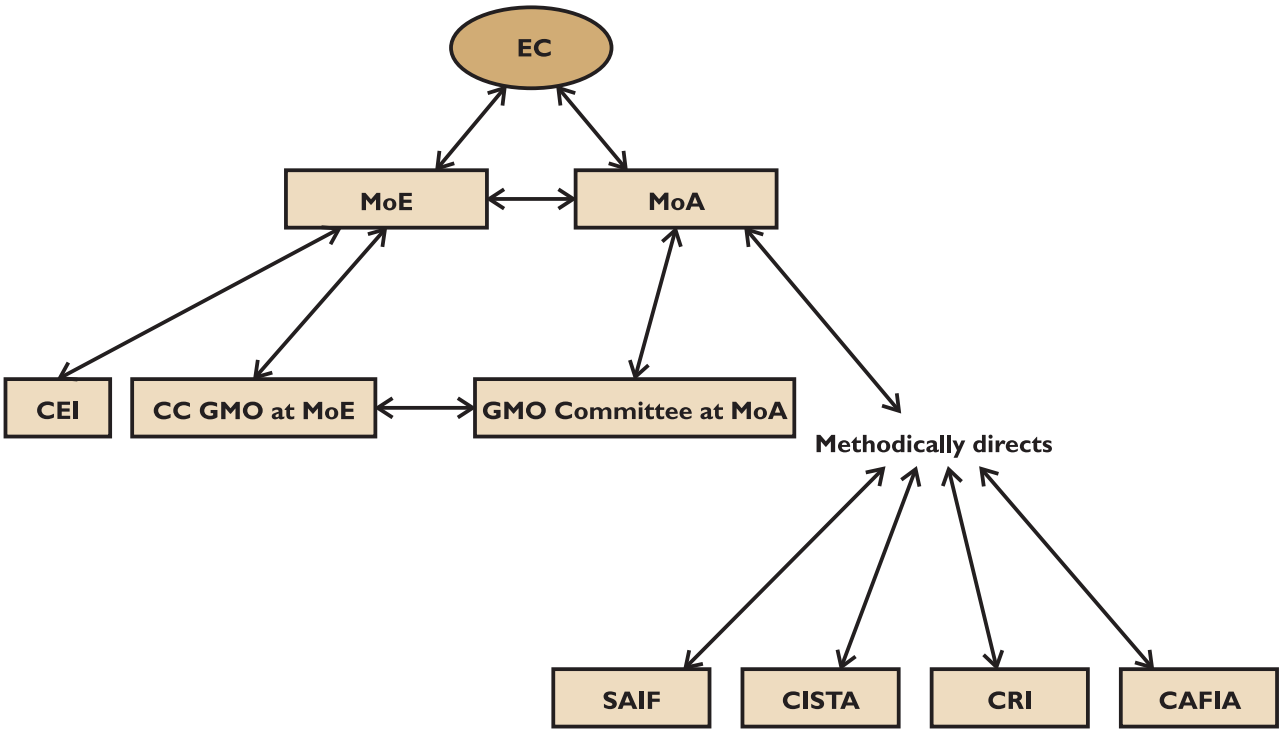
State Agricultural Intervention Fund
The Czech Environmental Inspectorate
Ministry of Agriculture
Ministry of the Environment (hereinafter referred to as MoE)
Czech Agriculture and Food Inspection Authority (hereinafter referred to as CAFIA)
Central Institute for Supervising and Testing in Agriculture (hereinafter referred to as CISTA)
Crop Research Institute, p.r.i- National Reference Laboratory for GMO identification and DNA fingerprinting

A part of the CISTA are certain departments, which until 31.12.2013 belonged with State Phytosanitary Administration (hereinafter referred to as SPA). These departments are, by their share, responsible for inspecting rules for cultivation - coexistence. They carry out inspections regarding resistance of the ECB population to GM maize and assess impacts of cultivation on the environment. Field inspectors of regional units of Agriculture Inspection Department within CISTA are responsible for inspections carried out on places where transgenic crops are cultivated. In this publication, the organisation SPA is already stated under the new name CISTA.

Furthermore, in relation with legislative change within Act Amendment No. 252/1997 Coll. on Agriculture, as amended, and other related acts, as of 1.1.2015 in the area of inspecting rules of GM crops cultivation in the CR, there has been a change of office carrying out preliminary check of reported data with the grower in field, i.e. a change from locally authorized Regional Agencies for Agriculture and Rural Areas of the Ministry of Agriculture (hereinafter referred to as RAARA) to the State Agricultural Intervention Fund.

In order to take advantage of professional capacities of Central Institute for Supervising and Testing in Agriculture and also due to the fact that inspection of cultivation rules is not connected with any subsidy and is only a technical matter, activities connected with operating LPIS database and physical preliminary check of reported data of a grower in field are delegated from the MoA to SAIF. However, other technical operations of inspections are carried out by CISTA. For this reason, the amendment of Act on CISTA has been carried out as well.

2.2. Relations of State Administration Authorities in the CR and the EC



2.3. Competences of State Administration Authorities

The Czech Republic closely cooperates with European Food Safety Authority (hereinafter referred to as EFSA). At the same time, the Czech Republic does not accept any information from abroad without having it inspected. The Czech Republic has at its disposal advisory and executive authorities and bodies, which consist of erudite experts in a given area and work of these authorities and bodies is subordinated only to scientific basis in combination with ethical standards.

The Ministry of the Environment is the fundamental, authorized GMO authority in the Czech Republic, technical assessment is carried out by Czech Committee for GMO Handling. The area of production GM crops cultivations belongs with the authority of the Moa and its GMO Committee at the MoA. Simultaneously, the following authorities work in an intertwined way: CAFIA, CEI, CISTA, SAIF, CRI. Altogether these form a network, which processes all the information on GMO. The GMO issue is evaluated thoroughly and in a length, from both points of view, i.e. the legislative one and also from the risk, trial and introducing into circulation and the like.

Framework Opinion of the CR on GMO was approved on the governmental level on 9th January 2008 in relation to decision making within the EU and it was updated on a governmental level on 22nd February 2010.

By approving the opinion, the CR supports modern biotechnologies applications for purposes of research and industrial and agricultural production supposing ensuring a high level of health and environmental protection based on the cooperation with a wide range of experts and relevant administrative departments.

The Czech Republic stems from three basic principles when assessing scientific evaluation and when formulating conclusions of GMO handling:
- Adequately applies preliminary caution principle,
- Proceeds according to the principle "from case to case",
- Decision on GMO handling does not represent violation of international trade connected with possible sanctions against EU member states.

3. Legal Framework

The issue of GMO and its products is a part of the EU policy, which has one of the most strict legal frameworks in the world concerning GMO. The Czech Republic unconditionally stems from the EU effective legal regulations.

The actual approval process of new GMO in the EU is very thorough: after the actual GMO development and processing the application for its introduction onto market, the application is administratively assessed by relevant authority of the member state in which the application was filed. Furthermore, the application is assessed by an EU technical body, which is EFSA. Subsequently, the application is announced and member states and public can raise comments on it. The EC prepares proposal of the decision, followed by voting on the proposal on the member states level. If the proposal does not get "in favour" or "against" by qualified majority, the application is forwarded to the so called Appeal Committee (The EC body). If the qualified majority is not reached in favour or against the proposal on introducing new GMO onto market, subsequently the EC shall decide on the proposal.

The European Parliament approved Directive No. 412/2015 on 13.1.2015, which enables the EU member states to limit or entirely prohibit cultivating GM crops on their territories. Permission concerning GM crops cultivation in the EU are to be decided jointly by all member states by the same procedure as up to now, based on the EFSA scientific risk assessment. Permission concerning introducing particular GM crop onto the market shall be, in case of positive decision, issued on the EU level. The approved amendment of original Directive No. 2001/18/ EC provides member states with the legal support and the possibility to subsequently limit or prohibit cultivation of these crops, approved for cultivation in the EU. Otherwise, no member state separately can permit GM crops cultivation. The new legal regulation brings legal support for bans on GM crops cultivation in individual states, and its result will definitely not be a spread of using these technologies.

3.1. Fundamental legislation in the CR

Act No. 78/2004 Coll., on handling GMO and genetic products, as amended:
- act amendment on handling GMO and genetic products with the effective date as from 1st January 2014: „§ 23 section 3: Everybody who cultivates genetically modified organisms approved for introduction to circulation pursuant to section 2, is obliged to provide the Ministry with written information on the location of their cultivation, 60 days at the latest from the beginning of cultivation, in case they have not done so according to a different legal regulation[20)]. The Ministry discloses locations of GMO cultivation pursuant to § 10. b)." *(Explanation.: [20)] Act No. 252/1997 Coll., on Agriculture, as amended),*
- MoE has the main competence,
- MoA: proposes to MoE procedures of risk evaluation connected with GMO and genetic products handling from the agricultural point of view; Moa makes statements from the point of view of its responsibilities concerning applications for introducing GMO into circulation (marketing) and into the environment (field experiments with GM crops). Furthermore, it makes statements concerning announcement of enclosed handling (laboratory and greenhouse experiments, closed industrial production etc).

Act No. 252/1997 Coll., on Agriculture, as amended (GMO dealt with in amendments No. 441/2005 Coll. and No. 291/2009 Coll.) - § 2i, §3 and section. 1, § 4a, § 5, § 5a.

Decree No. 209/2004 Coll., on closer conditions of GMO and genetic products handling, as amended – Implementing Decree to Act No. 78/2004 Coll.

Decree No. 89/2006 Coll., on closed conditions of cultivating genetically modified variety, in Decree amendment No. 58/2010 Coll.

- Implementing Decree to Act No. 252/1997 Coll., on Agriculture -up to § 2i.

"Agreement on delimitation of competences and the way of cooperation" (between MoA a SAIF).

3.2. Fundamental EU Legislation

Directive of the European Parliament and of the Council, by which Directive No. 2001/18/EC is amended, as for the possibility of member states to prohibit or limit GMO cultivation on their territories and the amendment No. 412/2015.

Regulation No. 1829/2003, on genetically modified food and feed products, as amended

- approving GM foodstuffs and feed, but also crops for cultivation purposes
- labelling of GM foodstuffs and feed, the Moa bears the main competence (Food Safety Department).

Regulation No. 1830/2003, on traceability and labelling of GMO and traceability of food and feed products produced from GMO and amending Directive No. 2001/18/EC, as amended

- system of labelling and traceability of GMO, the MoA bears the main competence (Food Safety Department).

Regulation No. 1946/2003, on trans-boundary movements of genetically modified organisms

- the GMO issue in connection with third countries (imports and exports outside the EU).

4. Obligations for GM maize growers in the Czech Republic

(effective of 1st January 2015)

GM maize growers are to comply with legislation in force. With the effective date as of 1st January 2014 there has been amendment in Act No. 78/2004 Coll., on GMO and genetic products handling, as amended. Hereby, duplicity of reporting GM maize cultivation to the MoA and simultaneously to the MoE is repealed. Thus, newly only reporting obligation of growers to authorised departments of SAIF remains.

In connection with legislative change within Act amendment No. 252/1997 Coll., on Agriculture, as amended and other related acts, as of 1st January 2015 there has been a change in the Czech Republic concerning the inspection rules of GM crops cultivation, i.e. the authority that carries out preliminary check of reported data of growers in field – it used to be Regional Agencies for Agriculture and Rural Areas of the Ministry of Agriculture, however, now it is SAIF.

For reasons of using the CISTA expert capacities and also due to the fact that the inspection of cultivation rules is not connected with any subsidy and is only a technical matter, all activities related to operating LPIS database and physical preliminary check of reported data of a grower in field have been delegated from MoA to SAIF. However, other expert operations shall be carried out by CISTA. For this reason, act amendment on CISTA has been made.

Reporting duty to neighbouring growers can be considered one of the key obligations. This duty is topical in the course of February. Everybody who is going to grow GM maize within the particular year has to report their intention to neighbouring growers (see further information below). Reporting can be carried out orally. However, if the grower decides to provide the information in written, they can use a ready made application form of MoA, which can be found in electronic version on internet websites: (http://eagri.cz/public/web/mze/, section "Agriculture", item "GMO - genetically modified organisms" and subsequently "Forms").

All-embracing summary of rules for GM maize growers are as follows – everybody who grows (or is going to grow) Bt maize has to:

1. Inform a neighbouring grower, at the latest by 1st March, about the intention to sow GM maize (this does not apply if up to the distance of 140 metres from the land parcel, where GM maize is going to be cultivated, there are their own land parcels and simultaneously up to the distance of 400 metres there shall be no land parcel farmed in the organic farming scheme.) Reporting does not have to be in written, however, if needed, application form *Reporting GM crops BEFORE starting the cultivation* can be used.
2. Keep a minimum distance of 70 metres between GM maize crops and another land parcel with non-modified maize (possibly to sow typical maize which when harvested is considered GMO, according to a scheme when one row of typical maize with a minimum width of 70 cm around GM maize makes up for 2 metres of a minimum separation distance – e.g. when there are closely adjoining land parcels with maize, it is necessary for GM hybrids to sow by a minimum of 35 rows of conventional maize variety.).
3. Keep a minimum distance of 200 metres between GM maize crops and another land parcel with maize, which is cultivated in the organic farming scheme.
4. Inform a neighbouring grower about GM maize sowing within 15 days from sowing (this does not apply if up to the distance of 140 metres from the land parcel, where GM maize is cultivated, there are only their own land parcels and simultaneously up to the distance of 400 metres there shall be no land parcel farmed in the organic farming scheme.) Reporting does not have to be in written, however, if needed application form *Reporting GM crops AFTER starting the cultivation* can be used.
5. Inform authorized department of SAIF about sowing GM maize in written, at the latest 30 days from sowing (for these purposes it is SAIF that issues the forms).

6. After harvesting, it is essential to label the GM maize product as "genetically modified organism" including relevant identification code – for hybrids of maize type MON810 the code is MON-ØØ81Ø-6 (this information shall be submitted in written to GM maize purchaser) The conventional maize, which formed buffer crops, shall be labelled in the same way. Animal products of animals fed by GM maize do not have to be labelled.
7. keep a record of GM maize handling and keep the data in company for at least 5 years. Particular required data are stated in Decree No. 89/2006 Coll., on more detailed conditions for genetically modified variety (respectively in its amendment No. 58/2010 Coll.). Further information on GM crops cultivation in the Czech Republic can be found on the above stated internet web sites of the portal e-AGRI.

5. GM Crops Cultivation Inspection System

5.1 Inspection of farmer's part of land block (hereinafter referred to as FPLB) neighbouring with FPLB with GM maize (SAIF)

Staff of SAIF departments carry out preliminary check of compliance with set conditions, that is the so called coexistence rules – see the following guideline for carrying out GM maize cultivating inspections intended for SAIF:

The guideline for carrying out GM maize cultivating inspections intended for SAIF
(updated as of 1st January 2015)

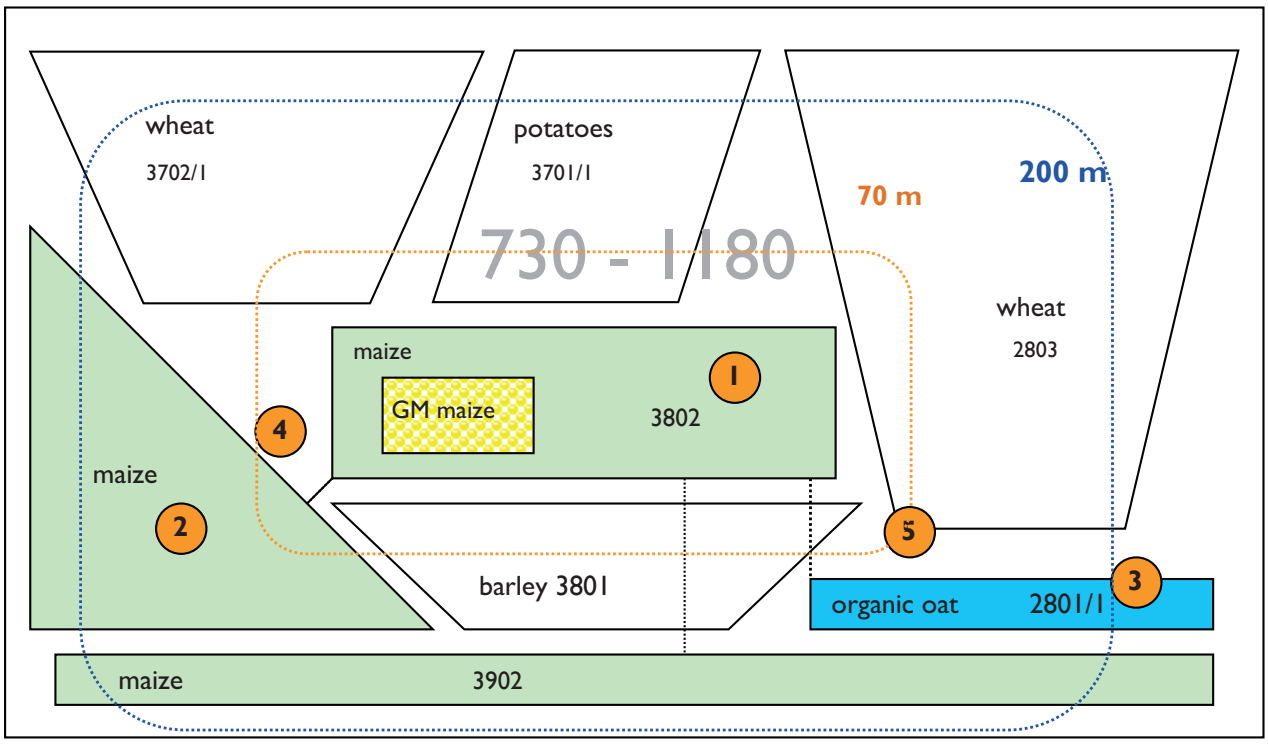
Starting points and objectives of the inspection
Inspections of GM maize cultivation are carried out based on § 4a section 10 Act No. 252/1997 Coll., on Agriculture, as amended (hereinafter referred to as "Act") and then based on Implementing Decree No. 89/2006 Coll., on Detailed Conditions for Cultivation of Genetically Modified Varieties, as amended by Decree No.58/2010 Coll. (hereinafter referred to as "Decree").

Based on the quoted legislation, the GM maize growers are obliged to:

a) keep the fixed minimum separation distance - 70 metres between GM maize cultivation and the place of maize variety cultivation which is located on a different FPLB and it is not genetically modified (§ 2i, section 2, a) of the Act),

b) keep the fixed minimum distance - 200 metres between GM maize crops cultivation and another land parcel with maize variety, which is cultivated in the organic farming scheme (§ 2i, section 2, b) of the Act).

Picture: GM maize cultivating inspection



Note 1: If on the inspected FPLB there is apart from maize (GM and non-GM) **a different crop** crucial for inspection (identification of neighbouring crops and measuring of distances), then **the maize is the boundary of crop**, not the whole FPLB.
Note 2: Staff of SAIF departments do not enter maize crop.

The obligation to keep the fixed minimum separation distance of GM maize cultivation can be fulfilled by the grower if they **sow** GM maize buffer crops by another maize which is not genetically modified, within the same FPLB and in the extent stipulated by the Decree; when harvesting, maize from buffer crops is considered genetically modified (§ 2i, section 3 of the Act).

The inspections objective is to verify whether the following is observed, i.e. the minimum separation distances of GM maize crop from a different maize crop which is not genetically modified and which is cultivated by a different user.

From this standpoint it is necessary to identify land parcels where FPLB with GM maize are neighbouring with other FPLB sown by non-modified maize – this is to be carried out on all registered GM maize crops by the SAIF departments.

In other stages of inspections, it is checked on selected FPLB whether maize which is declared as buffer crops is really made up by non-modified maize - this is to be carried out by CISTA with methodical help of CRI, p.r.i.

GM maize cultivation inspections are carried out in three steps:

1) **mapping of FPLB which are neighbouring with FPLB with GM maize (SAIF departments),**
2) *taking samples from selected localities (CISTA),*
3) *analysis of taken samples (CRI, p.r.i.).*

Inspection method for SAIF departments (point No. 1)
SAIF departments inspect land parcels which are situated in the vicinity of FPLB, on which GM maize cultivation is registered and which fall within their authority. The output of this is a report which is made for FPLB with GM maize registered in LPIS, which is neighbouring with FPLB of a **different user** and simultaneously on which **non-modified maize** is cultivated. The report contains the following information (a map printed from LPIS can be attached for illustration):

1) **Identification of inspected FPLB** (registration number according to LPIS including square), on which GM maize is cultivated *(according to the picture: 730-1180).*
2) **A list of neighbouring FPLB** (LPIS registration numbers), on which there is maize, which is not genetically modified (thus it is not registered in LPIS as GMO) and at the same time this FPLB is located within the distance of **70 metres from the external boundary of FPLB** with GM maize; possibly the information that there is no other maize crop up to 70 metres; in the list of neighbouring FPLB with maize it is necessary to state the **user of these FPLB.**
3) **A list of neighbouring FPLB** (LPIS registration numbers) **with organic farming scheme** which are located up to the distance of **200 metres from the external boundary of FPLB** with GM maize, including the specification of cultivated crop variety *(according to the picture: FPLB No. 2801/1, oat)*; possibly the information that there is no such land parcel up to the distance of 200 metres.
4) **The distance** of FPLB with GM maize from the neighbouring FPLB stated in point 2 (thus ranging from 0 to 70 metres).
   Note.: This distance is measured from the **external boundary** of FPLB with GM maize, not from the GM maize crops *(according to the picture: 45 metres)*.
5) **The distance** of FPLB with GM maize from the neighbouring FPLB stated in point 3 (thus ranging from 0 to 200 metres).
   Note.: This distance is measured from the external boundary of FPLB with GM maize, not from the GM maize crops *(according to the picture: 100 metres)*.
6) **Information** about the fact whether the grower states **buffer crops** and to what extent (based on the data obtained from a grower when reporting) around GM maize within touched FPLB.

5.2 Analysis of sent reports on inspection of SAIF departments through LPIS system (CISTA)

An authorized CISTA staff carries out analysis and inspection of reports check sent from the SAIF departments, among others, through LPIS system. It is inspected whether growers comply with all coexistence rules. Localities with growers who breach rules are selected from reports. Subsequently, CISTA field inspectors inspect these localities, possibly along with the Moa from the methodical supervision point of view.

5.3 Taking samples from selected localities (CISTA)

The CISTA Guideline determines for the field inspectors the procedure during the samples-taking of maize plant from field crops in order to determine the presence of GM maize in buffer crops as a part of inspection for compliance with coexistence rules, especially compulsory separation distances between GM crops and conventional (non-modified) maize hybrids.

CISTA carries out sampling which is to provide material (sample) for subsequent laboratory testing on presence of GM maize and then its submission to experimental laboratory. The taken samples for an inspected land parcel have to be representative to a maximum possible extent. CISTA is in charge of selecting inspected land parcels, commissioning experimental laboratory and writing the final report on growers inspection.

5.4 Analysis of taken samples (NRL, CRI, p.r.i.)

For GMo and DNA fingerprinting the NRL carries out sample analysis taken by CISTA field inspectors. The final report is submitted to CISTA and MoA.

In case of discrepancy between CISTA and inspected entity concerning solution of the event on spot, CISTA is entitled to impose administrative measures or special administrative measures. If the CISTA´s administrative measure or special administrative measure is not complied with, the CISTA waits for analysis results of taken samples from the CRI and then it submits them along with other materials in the form of act to SAIF which initiates proceedings with inspected entity.

6. Imposing sanctions system

For natural persons and legal entities SAIF carries out inspection of compliance with obligations and conditions for GM crop varieties, which are stipulated by provision § 2i of Act No. 252/1997, on Agriculture, as amended.

SPA carried out on spot land parcel inspections with identified problems from the cultivation rules point of view until 31st December 2013, which was united with CISTA as of the effective date 1st January 2014. Ever since this date the SPA competences have been delegated to CISTA. When carrying out inspections, CISTA proceeds towards inspected persons pursuant to effective provision § 4a of Act on Agriculture and pursuant to Act No. 255/2012 Coll., Auditing Guideline. CISTA, based on the carried out inspection, makes a report on inspection, to which an inspected person can raise objections (§ 13 and § 14 of Auditing Guideline). An inspected person can be given a fine up to 500 000 CZK (§ 15 to §17 of Auditing Guideline) when breaching obligations resulting from Auditing Guideline.

With procedures of both audit authorities there is always an assessment based on from case to case principle; the extent of divergence is taken into account based on which sanctions are imposed in respective legal framework. When considering individual violations, SAIF/CISTA proceeds based on the fact whether the grower who has made a mistake:

a) has made a mistake for the first time and cooperates during the remedy process,
b) has made a mistake for the first time and does not cooperates during the remedy process,
c) has made a mistake more times (has not cooperated during the remedy process).

Furthermore, the extent of damage caused by the grower´s mistake is considered, i.e. economic specification of the amount of damage and its impact on affected entities.

From the point of view of factual localities solution with identified problems concerning cultivation rules, the following procedure is applied:

1st degree
If CISTA, by means of on the spot checks, finds out breaching of coexistence conditions resulting from provision § 2i of Act on Agriculture and its Implementing Decree No.89/2006 Coll., firstly **out-of-court settlement** of arisen situation is preferred. This means that crops of the same crop on the neighbouring land parcel, which is not genetically modified but is located in smaller than the fixed minimum distance from the place of genetically modified crop cultivation shall be handled at the costs of a grower with genetically modified crop who has not complied with the fixed minimum distance in the following way: based on a written agreement, a grower of GM crop provides the damaged owner or leaseholder of the neighbouring land parcel with adequate financial compensation or provides them with adequate natural performance compensating for adequate amount of a crop considering differences in marketing possibilities and using harvest from given crop. At the same time, it unconditionally applies that crops of the same crop on a neighbouring land parcel which is not genetically modified have to be worked by a grower of GM crop as if it were a GM crop.

2nd degree.
If an agreement is not reached, CISTA orders the owner or leaseholder of land parcel to remove the affected crops, where it is proceeded pursuant to § 75 or § 76 of Act No. 326/2004 Coll., on Phytosanitary Care, as amended. The right of the owner or leaseholder of a neighbouring land parcel to claim damages of incurred damage from a grower of GM crop in a private law way is not affected by the stated administrative procedure.

3rd degree
If remedy is not reached even after CISTA measures, collected facts, including analysis results from CRI are submitted to SAIF which is by Act on Agriculture entrusted with the authority to consider violation or administrative violation pursuant to effective version of § 5 section 1 d) or § 5a section 1 e) of the same Act.

As soon as CISTA submits relevant materials to SAIF, then SAIF is entitled to initiate administrative proceedings with a grower of GM crop on imposing a fine for violation of administrative violation pursuant to § 5, § 5a and § 5b of Act on Agriculture, as amended. As mostly all growers of GM crop are businessmen, the initiated proceedings will be in a form of proceedings on administrative violation, which is governed by Act No. 500/2004 Coll., Administrative Guideline. For a committed administrative violation, SAIF can impose a fine up to 250 000 CZK. When determining the height of fine, the following are considered: gravity of administrative violation, especially the way of committing the violation, length of duration, its consequences and circumstances under which the administrative violation has been committed.

The damaged person can exempt themselves from liability for administrative violation if they prove that they have made all efforts possible to claim to prevent not compliance or violation of legal obligation.

7. Main tasks overview in the process of GM crops cultivating inspection

Month	JANUARY – FEBRUARY		
Task name	Annual sending of news/updates in coexistence rules to SAIF	Update of GM crops permitted for cultivation in the EU, information about current rules on GM crops cultivation	Update of the worldwide GM crops area for the previous year
A way of performing the task	Director of Plant Commodities of MoA Department writes a letter to 1st Deputy of SAIF, for the attention of Department Director of LPIS concerning news on coexistence rules and with a request concerning potential application into internal directive of SAIF and distribution of effective rules to authorized SAIF departments and concerning GM crops growers. (Cover letter sent to SAIF Departments, top-level guideline of MoA – Organisation and inspection of GM crops cultivation in the Czech Republic, Obligations for growers)	Publishing a brief article in a medium guaranteeing national access (information about current rules for GM crops cultivation), based on approval and coordination with Communication Department	Update of the worldwide GM crops area for the previous year based on international sources: e.g. www.transgen.cz www.isaaa.org and the like
The task will be carried out by organisation, department	MoA, Plant Commodities Department	MoA, Plant Commodities Department	MoA, Plant Commodities Department
Deadline	In the course of 2nd ten-day period of January	In the course of February	In the course of February

Month	MARCH
Task name	Data update on GM crops cultivation
A way of performing the task	Information update for media: Green Book, Agriculture, etc.
The task will be carried out by organisation, department	MoA, Plant Commodities Department
Deadline	In the course of March

Month	APRIL – MAY
Task name	Physical preliminary check of reported data of a grower in field
A way of performing the task	GM crop grower reports information on cultivation to authorized SAIF department, which, in turn, enters the data into LPIS database and carries out physical preliminary check of reported data of a grower in field. Subsequently, reports are made containing findings from the field and SAIF sends these to CISTA for information summarisation, final data inspection and for assessment. MoA carries out methodical supervision and provides consultations to CISTA as for selected localities and growers who have not met coexistence rules. SAIF provides data from the MoA preliminary check based on the current requirement
The task will be carried out by organisation, department	SAIF
Deadline	In the course of April-May, as a follow-up to sowing date and reporting, Time shift is possible with regard to climate conditions of the year

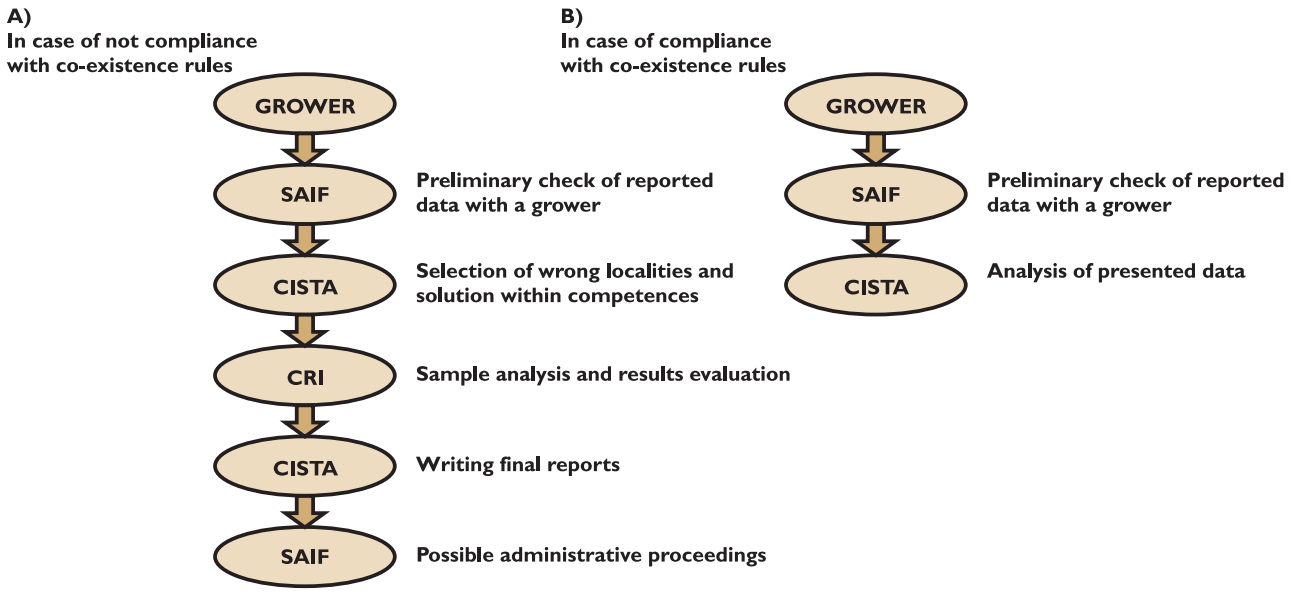
Month	JUNE	
Task name	Methodical supervision and consultations of CISTA with MoA on inspection of compliance with coexistence rules based on reports from preliminary check of SAIF departments, which CISTA received from SAIF	Statistics on GM crops cultivation
A way of performing the task	A CISTA staff in charge of GMO issue selects, based on the information from SAIF preliminary check of data, localities with detected violation of rules and asks for methodical consultation with MoA	MoA works out statistics of GM crops cultivation for the relevant year (total and average areas based on territorial self-governing units, the whole of the Czech Republic and based on growers, possibly further indicators) based on submitted data on GM crops cultivation for the relevant year from SAIF
The task will be carried out by organisation, department	CISTA and MoA (Plant Commodities Department)	SAIF and MoA (Plant Commodities Department)
Deadline	In the course of June	In the course of June

Month	JULY	
Task name	Inspection of selected localities and sample taking	Announcing of GM crops cultivation localities
A way of performing the task	CISTA carries out field check of rules with involvement of CISTA field inspectors and moreover possible involvement of a MoA staff (arrangement with chief inspector concerning date and further information on inspection)	Compiling a chart of GM crops cultivation localities based on the sent data from SAIF. Subsequently, MoA sends the data to MoE for subsequent announcement on the web sites of MoE
The task will be carried out by organisation, department	CISTA and possibly MoA, Plant Commodities Department	MoA and SAIF
Deadline	In the course of July	In the course of July

Month	AUGUST
Task name	MoA press release
A way of performing the task	MoA press release publishing on GM crops cultivation in the CR for relevant year. It is necessary to state the total GM crops area, which was cultivated during the relevant year, number of growers, reasons for decrease, possibly increase (this can be found out through SAIF departments or growers can be directly addressed)
The task will be carried out by organisation, department	MoA, Plant Commodities Department
Deadline	In the course of August

Month	NOVEMBER/DECEMBER	
Task name	Inspection termination on selected GM maize localities	CISTA solves initiatives with problematic GM crops growers
A way of performing the task	Termination of inspections with GM maize growers (time-wise based on submitting analysis from CRI, p.r.i. laboratories), writing up final reports and their signing by the grower. Forwarding the matter to SAIF, in case the matter is not solved, it is forwarded to CISTA within competences	Administrative proceedings
The task will be carried out by organisation, department	CISTA	SAIF
Deadlines	In the course of November – December	Following up the completed process at CISTA

Postup kontroly dodržování pravidel koexistence



A GM crop grower reports information about cultivation to an authorized SAIF department, which carries out physical preliminary check of reported data with a grower in field and enters the obtained data into LPIS database. Following completed reports with findings from the field, it is SAIF that sends this data to CISTA for information summarisation, for final data check and for assessment. MoA carries out methodical supervision and provides consultations to CISTA concerning selection of given localities, finds out and sorts out the state, possibly takes samples and submits them for subsequent analysis and assessment, which is carried out by CRI, p.r.i.. CISTA finishes inspections with GM maize growers (time-wise based on submitting analysis from CRI laboratories), makes final reports and submits them for signing to growers. SAIF solves initiatives from CISTA with problematic GM crops growers and it is in charge of administrative proceedings. Moreover, CISTA carries out analysis on effectiveness of cultivated GM crop (only maize is permitted) against the ECB (no chemical spraying is carried out against the ECB).

8. Contact points

Ministry of Agriculture
Těšnov 65/17, Praha 1, 110 00
Development and Project Managing IT Department
Central Registers Office Department (for SAIF)
Plant Commodities Department (for others)

State Agricultural Intervention Fund
Ve Smečkách 33, Praha 1, 110 00
LPIS and External Registers Department
Integrated Administrative and Controlling System

Central Institute for Supervising and Testing in Agriculture
Zemědělská 1a, Brno, 656 06
Methods of Integrated Plant Protection Department

Crop Research Institute, p.r.i
National Reference Laboratory for GMO identification
Drnovská 507/73, Praha 6 - Ruzyně, 161 06

9. Appendix

Application form on reporting GM crop BEFORE sowing/starting the cultivation.

Application form on reporting GM crop AFTER sowing/starting the cultivation.

CISTA Guideline: "Sample taking for purposes of determining GM plants in maize crops as a part of inspections on compliance with the coexistence rules for cultivating GM varieties of maize."

CRI Guideline: "GMO Detection in maize buffer crops. Assessing presence of MON810".

Form – GM crop reporting BEFORE sowing/starting the cultivation

1. Data concerning the grower

NP*

Surname and first name of the grower (NP)

NP´s address of seat/permanent residence

Address of NP´s running business place

LE*

Trade/company name including legal type

Address of company´s registered office (when LE)

2. Data on intention to cultivate GM crops

land parcel serial No.	crop name	UIC GMO**	square	land parcel code, land parcel unit				/			expected area	sowing date/sowing (day, month, year)			Will you carry out buffer crops ?*** NO	YES	number of rows
								/			,						
								/			,						
								/			,						
								/			,						
								/			,						
								/			,						
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								/			,						
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								/			,						

Date of making notification (day, month, year)

Signature, possibly also stamp of announcer

* mark with cross; NP = natural person, LE = legal entity

** UIC GMO = Unambiguous Identification Code of GMO (e.g. maize type MON810 … MONØØ81Ø-6; GM potatoes Amflora … BPS-25271-9)

*** mark with cross; NO – in case you are not planning to carry out buffer crops of GM crop with a non-modified crop, YES – in case you are planning to carry out buffer crops of GM crop with a non-modified crop

Form – GM crop reporting AFTER sowing/starting the cultivation

1. Data concerning the grower

Trade/company name including legal type

Registration Number (RN)

NP*

Surname and first name of the grower (when NP)

NP´s address of seat/permanent residence

Address of NP´s running business place

User code in the land register (LPIS)

LE*

Address of company´s registered office (when LE)

2. Data on GM crop cultivation

land parcel serial No.	crop name	UIC GMO**	square	land parcel code, land parcel unit	GM crop area in ha (to 2 decimal places)	crop sowing date (day, month, year)	Have you carried out buffer crops of GM crop?*** NO YES .. number of rows
				/	,		
				/	,		
				/	,		
				/	,		
				/	,		
				/	,		
				/	,		
				/	,		
				/	,		
				/	,		

Are maps with marked GM crop cultivation attached to this report? NO YES

Number of pieces:

Stamp of the reporting place ****

Receipt date (day, month, year)****

Received by (surname in capital letters, signature)****

Date of making notification (day, month, year)

Signature of announcer (when LE pursuant to Commercial Register)

* * mark with cross; NP = natural person, LE = legal entity

** UIC GMO = Unambiguous Identification Code of GMO (e.g. maize type MON810 … MONØØ81Ø-6; GM potatoes Amflora … BPS-25271-9)

*** mark with cross; NO – in case you have not carried out buffer crops of GM crop with a non-modified crop, YES – in case you have carried out buffer crops of GM crop with a non-modified crop (state the extent)

State Phytosanitary Administration

Ref. no.: SRS 036112/2010 Prague, on 20 September 2010

SPA Guideline

A/OMIOR/3/2010

Collection of Samples for the Purpose of Identification of Genetically Modified Plants in Maize Crops as a Part of Check of Compliance with Rules of Coexistence in Cultivation of Genetically Modified Maize Varieties

Date of entry into force: 1 October 2010 Prepared by: Ing. Rostislav Hrubý, CSc.,

For the attention of: SPA Department of Territorial Units
SPA Department of Protection against Harmful Organisms

For information: MoA Department of Plant Commodities
SPA Director Secretariat

Ing. Richard Ščerba
SPA Director

Ing. Dita Vrbová
Head of Department of Protection against Harmful Organisms

Annexes: 1 - 6

Guarantor of the accuracy: Ing. Rostislav Hrubý, CSc.

Subject Matter of the Guideline

The present Guideline defines the procedure of collecting samples of maize plants in field crops for the purpose of identifying the presence of GM maize in buffer crops as a part of check of compliance with coexistence rules, in particular, the compulsory separation distances between GM and non-GM (conventional) maize varieties. Other inspection activities connected to coexistence rules are carried out by the Ministry of Agriculture (MoA).

The State Phytosanitary Administration shall only collect samples in order to obtain material (the samples) for further laboratory testing for presence of GM maize and submit it to the contracted laboratory. Maximum representativeness of the samples taken from the inspected area must be ensured.

Selection of inspected areas, commissioning of the contracted laboratory including payment for the sample analysis, drawing up a final protocol on inspection with the grower and, if necessary, the administrative procedure continues to fall within the remit of the MoA Department of Plant Commodities.

The scope of inspection is defined for each calendar year on the basis of agreement between MoA and SPA (the State Phytosanitary Administration).

Regulatory Framework and Related Documents

Act no. 252/1997 Coll., On Agriculture, as amended (Section 2i(3))

Act no. 255/2012 Coll., On Inspection (Inspection Code)

Decree no. 58/2010 Coll., (Section 7) amending Decree no. 89/2006 Coll., Detailed Conditions for Cultivation of Genetically Modified Varieties.

MoA, Department of Plant Commodities requests for cooperation in check of compliance with coexistence rules in cultivation of GM varieties of maize (Ref. no. 27061/2009-17220).

Terms Used and Definitions

1. **Sampler:** an SPA employee authorized to collect samples,
2. **Official sample**: a sample collected and completed by sampler,
3. **Individual sample:** a small, approximately equal amount of plant material (approx. 5 g) which is taken from determined parts of one sampled plant; 2 samples for laboratory testing, 1 back-up sample and 1 sample for archiving are taken. (Thus 4 individual samples, marked “A”, “B”, “R”, “Arch.”, are taken from one plant)
4. **Contracted laboratory:** an accredited laboratory dealing in GMO detection which is commissioned by MoA to establish the presence of GMO in official samples,
5. **Completion of samples:** activities such as packing, closing and labelling of samples.

1. Activities Preceding the Visit of the Inspected Land Parcel

1. MoA shall submit to OMIOR (Department of Integrated Plant Protection Methods) relevant maps and numbers of the land parcels where the samples shall be collected. OMIOR shall send these supporting documents to the relevant territorial department (OBO). (**MoA, SPA-OMIOR, OBO**)
2. Setting a date of collection of official samples. In principal it is possible to collect samples from plants before the flowering stage of the crops and/or after the flowering stage until the foliage show significant losses of chlorophyll as a result of their ageing. (**SPA-OMIOR**)
3. OMIOR shall ask the relevant OBO for cooperation in sample collection. (**SPA-OMIOR, OBO**)
4. OBO shall contact the grower, agree on a date of inspection and collection of official samples and find out facts and conditions needed for relevant sample collection (when

were the agrochemicals applied in the crops and what is the development stage according to BBCH scale). (**SPA-OBO**)
   a) Phone call verification of whether the GM maize is separated from non-GM maize by buffer crops (in the event that the grower asserts that the required buffer crops have not been planted, OBO shall immediately contact OMIOR which shall inform MoA; in such case the samples shall not be taken),
   b) Application of agrochemicals in the crops (the date of sampling has to be agreed in a way that it does not follow immediately after crop-spraying),
   c) Stage of the crops development (if the crops are in the flowering stage, the collection of samples shall be postponed until the flowering stage is over),
   d) Variety of the buffer crops maize.
5. Contacting the contracted laboratory in order to agree on the procedure and exact date of submitting of the official sample. (**SPA-OMIOR**)
6. Preparation of supporting documents needed in order to carry out the inspection in a specific locality:
   a) Map showing the inspected land parcel – location and size of the land parcel, access roads, location of the declared buffer crops and place of inspection – part of the land parcel adjacent to the neighbouring land parcel covered with non-GM maize (Annex 1). (**SPA-OMIOR, the supporting documents shall be provided by MoA**),
   b) Sampling scheme – scheme of individual sample collection. Specification (adding the distances) shall be made on site during the inspection. (Annex 1). (**SPA-OMIOR**)
7. Preparation of tools needed for the sample collection (Annex 2). (**SPA-OBO**)
8. Preparation of sample containers and sample containers package (Annex 3). (**SRS-OBO**)
9. Preparation of the following official documents:
   a) Protocol on Sample Collection in 3 copies (the protocol form is defined in Annex 5) (**SPA-OBO**),
   b) Protocol on Delivery of Samples in 3 copies (the protocol form is defined in Annex 6). (**SPA-OMIOR),**
   c) Protocol on Inspection generated in IS – Monitoring / Surveillance and Inspection in 3 copies (see Annex 7). (**SPA-OBO**)

2. Activities Performed in the Place of Inspection and Sample Collection

1. The inspection is carried out simultaneously by **OMIOR and OBO** representatives.
2. Verification of the subject of inspection, identification of the land parcel by means of an aerial photograph or GPS device. (**SPA-OMIOR**)
3. Assessment of the conditions of the crops in terms of the inspection feasibility. In the event that the sampler finds that the crops have been exposed to conditions that would clearly influence the results of the laboratory test (e.g. **crops in flowering stage**, damaged crops, **recent application of agrochemicals**), he or she shall immediately inform OMIOR, which shall coordinate further steps with the MoA Department of Plant Commodities. (**SPA-OMIOR-OBO**)
   MoA contact person: ing. Daniel Froněk, tel.: +420 221 812 612
4. Determination of the vegetation stage according to the BBCH scale. (**SPA-OBO**)
5. Measuring the separation distance between the border of the inspected land parcel and the border of the adjacent land parcel with maize by a measuring tape and calculation of the inspected rows – for more detail see Annex 1. (**SPA-OBO**)

6. Adding distances between the points of sample collection and the reference points into the sampling scheme. Specification of the sampling scheme according to the real distances measured on site. (See Annex 1) (**SPA-OMIOR**)
7. Collection of individual samples: (**SPA-OBO**)
    a) The samples are taken according to the sampling scheme (see Annex 1) from 20 plants within the inspected area (the buffer crops), whereby plants which are significantly damaged or which have lost significant amount of chlorophyll are excluded.
        i. In case of plants before the flowering stage, the sample is taken from fully developed foliage of the upper part of the plant,
        ii. In case of plants after the flowering stage, the sample is taken from foliage of mid part of the plant.
    b) Four leaf laminas are cut from each sampled plant, the central vein and the base and the top part of the lamina are removed. All four cut leaf laminas are put one on top of the other and cut into 4 equal parts, i.e. individual samples (see Annex 4).
    c) Individual samples containing ¼ of each of the four collected leaf laminas are inserted into the sample container and labelled A, B, R and Arch. **All four individual samples must be taken from one plant!** (see Annex 3).
    d) The size of the individual samples is at least 5x5 cm (approx. 5g).
8. Between collection of individual samples from individual plants the sampler shall clean the sampling scissors by 60% alcohol (it is not necessary to use pure spirit) and distilled water.
9. Inserting the closed labelled sample containers into prearranged packages, completion and sealing of the samples (Annex 3). **(SPA-OBO)**
10. Indicating the time and duration of the sample collection (the beginning and the end of the collection).
    Completion of Protocol on Sample Collection, Protocol on Inspection and its confirmation/signing by the grower, the sampler (OBO) and the representative of OMIOR. The protocols shall be archived by OBO, OMIOR and the grower in case they are needed in future (the protocol form is defined in Annex 5). (**SPA-OBO – OMIOR – Grower**)

3. Activities Following the Inspection and Sample Collection

1. Delivery of the samples in shortest time possible to the contracted laboratory commissioned to carry out the analysis of the samples. (**SPA-OMIOR**). The samples must be stored and transported in dry place with a maximum temperature of 25 °C, protected from direct sunlight and submitted to the contracted laboratory within 12 hours after their collection. When justified (the sampling has been finished late in the day), the samples may be stored for a limited time (no more than 24 hours after their collection) at temperatures 0 – 10 °C before their delivery into the laboratory.
2. Filling all necessary data into the Protocol on Delivery of Samples (Annex 6) and its confirmation/signing by the SPA representative who submits the samples and by a representative of the contracted laboratory which is carrying out the sample analysis. (**SPA-OMIOR – Contracted Laboratory**)

4. Entry into the IS – Monitoring

The inspections are entered into the surveillance and inspection module in the same way as in case of other inspections.

The protocol is made in 3 copies.

Protocol status – submitted

Subject of inspection – to be selected from a prepared code list

The date of commencement and completion of inspection is indicated – the same day

Submitter – OBO representative

Advice of appeal – item PS01 to be selected from the code list – The subject of inspection may lodge an objection against the protocol in writing stating the reasons thereof within 15 days from the day when the subject of inspection became familiar with the protocol confirmed by his or her signature; the objections may be lodged to the controlling officer. The subject/-s of inspection have been made familiar with the content and the advice stated above and they confirm the acceptance of the protocol by their signature.

Name of the site – fill in the site name

Type of land – agricultural land

Number – number of the inspected land parcel (on the basis of materials provided by MoA)

Land register – to be selected from the code list

Type of inspection – to be selected from the code list – 7000 – Inspection in the area of GM varieties cultivation (for MoA) pursuant to Section 72 (12) Act no. 326/2004 Coll. in connection to Section 2 (2i) Act no. 252/1997 Coll., On Agriculture.

Subject matter of inspection – the following shall be filled in: "Collection of samples for the purpose of identification of genetically modified plants in maize crops as a part of check of compliance with rules of coexistence in cultivation of genetically modified maize varieties as laid down in Decree no. 58/2010 Coll., Detailed Conditions for Cultivation of Genetically Modified Varieties".

Findings of the inspection – the following shall be filled in: "The collected samples were submitted to the contracted laboratory, which is commissioned by MoA to establish the presence of GMO in the official sample. The results of the inspection shall be officially served on the subject of inspection directly by MoA."

Type of result – the following shall be filled in: "Comprehensive results of the inspection of compliance with coexistence rules as laid down in Decree no. 58/2010 Coll. shall be communicated to the subject of inspection directly by MoA."

Participants – the names of persons who participate in the sample collection and the representative of the subject of inspection shall be filled in.

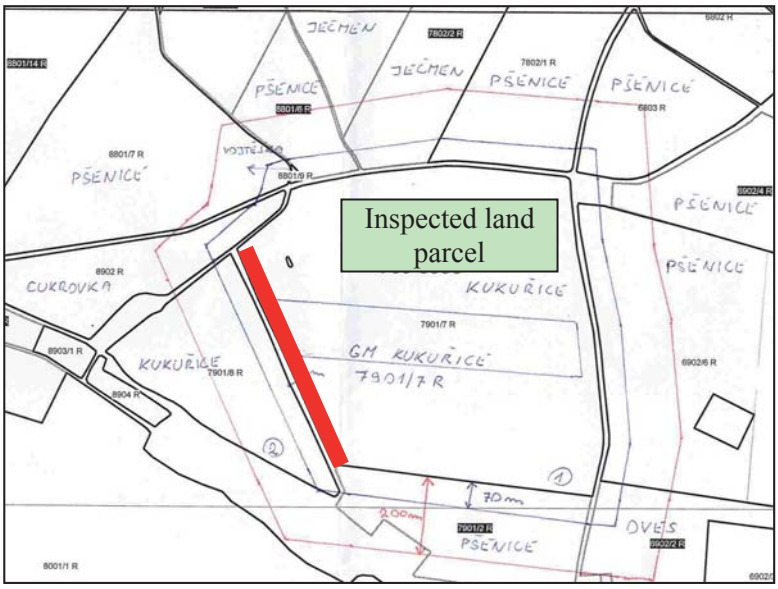
Distribution – OMIOR, the relevant OBO, the subject of inspection.

Annexes to the SPA Guideline

Annex 1 – Maps and the Sampling Scheme
Annex 2 – List of Equipment and Tools for the Sample Collection
Annex 3 – Sample Container
Annex 4 – Sampling Procedure
Annex 5 – Protocol on Sample Collection
Annex 6 – Protocol on Delivery of Samples

Annex 1
Map Showing the Inspected Land Parcel
- According to the supporting documentation provided by MoA (or, regional agencies for agriculture and rural areas)

Inspected part of the land parcel



Sampling Scheme
The sampling scheme is a scheme of sample collection/collection points proposed for the concrete inspected locality. The sampling scheme is derived from the maps provided by MoA and from LPIS maps. Selection of the collection points is based on assessment according the shape and size of the land parcel or the inspected part thereof (adjacent to the neighbouring land parcel with non-GM maize crops), and on the number of the buffer crops rows necessary for compliance with Act no. 252/1997 Coll., On Agriculture, as amended (Section 2i (3)) and the implementing decree no. 89/2006 Coll., Detailed Conditions for Cultivation of Genetically Modified Varieties, as amended (with allowed deviation of 2 rows).

The compulsory separation distance between GM maize and non-GM maize is, as per the Decree, 70 m in case of conventional maize and 200 m in case of organic farming, and these separation distances may be replaced by a corresponding number of buffer crops rows composed of non-GM maize.

The replacement of the separation distance by the buffer crops is determined according to the following formula: 1 row of buffer crops (which is at least 70 cm wide) replaces 2 m of separation distance. In case of land parcels closely adjacent to each other it is therefore necessary to plant buffer crops in no fewer than 35 rows. The inspection (sample collection) is carried out on the 1st to 33rd row. In the event that the inspected land parcel is 10 m distant from the neighbouring field with non-GM maize (the minimum number of buffer crops rows is 30), the inspection is carried out on the 1st to 28th row of the buffer crops etc.

Samples are taken from a total of 20 plants in the inspected area (the buffer crops) with regular distance intervals between them. The interval and the line of transfer between rows are assessed according to a V-shaped scheme. The collection points are, for instance, every 10 – 20 m each 3 rows on the diagonal line of collection.

Annex 2
List of Equipment and Tools for the Sample Collection

- Portable cooling boxes for transportation of the collected samples into the contracted laboratory and a sufficient number of ice packs
- Polyethylene bag (approx. 40x80 cm) for the used material

- Sample containers – PE or microtone bags (approx. 15x10 cm, ideally with a side fastener)
- Packages for sample containers – PE bags (of larger size, approx. for 20 sample containers)

- Scissors from stainless steel (2 pc)

- Clean cellulose napkins (approx. 20x20 cm) for cleaning the scissors – 40 pc,

- Wash bottle with distilled water and a reserve of distilled water (approx. 1 l)
- Wash bottle with 60% solution of alcohol (pure spirit is not necessary) and a reserve of the 60% alcohol solution (approx. 1 l)

- Sticky labels (or a hangtag) for labelling the package for sample containers
- Permanent markers for marking the samples
- SPA official stamp
- Transparent tape for protecting the labels
- Elastic band (or rope) for closing the bags
- Tape for securing the package for sample containers
- GPS for verifying the location of the land parcel
- Measuring tape

- Other (facultative) tools – e.g. camera

Annex 3
Sample Containers

The sample containers must be still closed, both before and after inserting the samples, and they must be stored and transported in clean environment in order to prevent their contamination. The sample containers must be secured in authorized (sealed) closed transportation containers which must be constantly under the supervision of the sampler until their delivery to the contracted laboratory.
Before the samples are inserted, it must be checked whether the sample containers are not damaged or whether they meet the above-mentioned criteria.

Sample Containers Labelling

Each individual sample must be marked in a way that clearly shows the relation between the inspected crops, the plant and the sample.
The mark shall be in the following format:
Order number of the sampled plant followed by "A", "B", "R" or "Arch" / year of sample collection / first three letters of the name of the locality. E.g.: 3B/10/Mik

It is advisable to prepare and mark the sample containers before the inspection.
Note: Four samples of approximately equal size are taken from each sampled plant and inserted into four separate sample containers marked as A, B, R and Arch, i.e. in total there will be 80 sample containers (if there are 20 collection points / sampled plants).

Package for Sample Containers

The sample containers containing the individual samples shall be inserted into a suitable package for sample containers in a way that all containers with individual samples collected from the inspected locality (from 20 plants) which are marked as "A" are packed in one package. Similarly, the "B", "R" and "Arch" individual samples are packed in separate packages. (Thus there are 4 packages of individual samples for one locality.) The packages with individual samples shall be sealed by the sampler and clearly labelled.

The label of the packages shall contain the following data:

a) Identification of the sample containers in the package, e.g. "A Mik", "B Mik",
b) The number of sample containers,
c) Identification of the sampler – stamp and signature
d) Date of the sample collection.

Identical label shall be inserted inside the package before closing it.

In case of paper labels used for labelling the package for sample containers, the quality of the paper must be adequate with respect to its purpose. Edges of the hole (loop) of the label shall be reinforced and the label shall be fixed to the package for sample containers by a seal (stamp and signature) of the sampler. Data given on the label must be written in a way that they cannot be deleted.

Annex 4
Sampling Procedure

At least 4 equivalent individual samples from a segment of four leaf laminas are taken from each tested plant at the collection point and inserted into 4 sample containers (see picture below):

- Laboratory sample A
- Laboratory sample B
- Back-up sample (R)
- Sample for archiving (Arch)

Each individual sample (A, B, R and Arch) contains ¼ of the 4 collected leaf laminas. A and B samples are designated for laboratory tests (establishment of genetic modification). R and Arch samples are used in case that a repetition of the laboratory test is requested.
After each sample collection it is necessary to disinfect the scissors by 60% alcohol solution.

Cut 4 leaf laminas from 1 plant

Put the leaf laminas on top of each other

Cut the leaves in 4 parts (individual samples) and remove the central vein

Insert each individual sample into a marked sample container

Annex 5

Protocol on Sample Collection

Identification of the grower:
(Name and Commercial Reg. No.)

Identification of the inspected land parcel:
(Land block/ land block part No. and square No.)

Identification code of the Bt maize:

Number of buffer crops rows necessary as per conditions of Section 7 of the Decree no. 58/2010 Coll.:

Description of samples: Segments of maize leaf lamina, sealed samples, variety…….

Data about the collected individual samples:
Amount: ……pc, approx. 5g each.

Date and time of collection: ……

Identification of samples:
(unique identification indicated on the sample containers)

Samples were collected according the attached sampling scheme and handled according to **A/OMIOR/3/2010** guidelines. The samples were taken for the purpose of establishing the presence of Bt maize plants. The collected samples have been sealed and they shall be immediately submitted to a representative of the contracted laboratory.

Samplers:
(name and signature of persons collecting the samples)

Grower:
(name, signature and, if possible, stamp)

The present protocol has been issued in:…… on (date):…….

Annex 1: Sampling Scheme

Annex 6

Protocol on Delivery of Samples

Subject of delivery: Segments of maize leaf lamina, sealed samples, variety …….

Data about the delivered material:
Amount: ……pc, approx 5g each
Date and time of collection: ……
Date and time of delivery:……

Sampler collector/-s:
(names of persons who participated in the sample collection)

Identification of samples:
(unique identification indicated on the sample containers)

The samples shall be hereinafter handled in accordance with MoA guidelines ref. no.: 31105/2007-17220.

Submitter:
(name, address and signature of the person submitting the samples)

Receiver:
(name, address and signature of the person receiving the samples)

The present protocol has been issued in:…… on (date):…….

GMO Detection in Maize Buffer Crops
Establishing the Presence of MON810 (MON-ØØ81Ø-6)

Table of Contents:

Receipt of Samples

In order to ensure protection of the test samples the following principles are observed:

1. The samples are stored in accordance with the sample storage requirements laid down in the binding procedure regulation for individual tests (Standard Operating Procedures)

2. The laboratory facility has adequate equipment preventing loss of value, damage or loss of the sample during storage

3. Each sample is clearly identified by a label during its stay in the laboratory

4. The tests are performed according to valid test procedure guidelines (SOP)

5. The tests are carried out by workers with the required qualification

6. Preparation of the sample for testing (obtaining the test portion, homogenisation) is carried out in accordance with relevant test procedure guidelines in a way that prevents influencing the characteristics of the sample

7. Each activity related to the test sample (receipt, labelling, drawing of protocols, analysis and evaluation) is documented. All documents related to the collection, acceptance and analysis of a test sample are archived.

Each sample which is submitted to analysis (via post, courier service or by the customer), is registered in the Registry of Accepted Samples upon which a registration number is allocated to the sample. The registration number is clearly marked on the sample package.
The receiving worker verifies the data on the order sheet, checks the integrity of the sample packaging, weighs the sample and fills in the Sample Accompanying Document.
A copy of the Sample Accompanying Document is provided to the customer upon request.
The sample is subsequently stored at a temperature of approx. -80°C.
In the event that upon receipt of a sample any deviations from the standard binding procedures are identified or if there is a doubt as to the adequacy of the sample for the intended testing, the receiving worker notes such circumstances in the Sample Accompanying Document.

Isolation of DNA from Fresh and Frozen Foliage Samples

DNA isolation is conducted following a method based on selective precipitation in CTAB environment. Extraction control is included for each series of samples (a sample without addition of the tested matrix processed identically as the test sample).

Preparation of a Sample

Preparation of the Working Area

1. The room is treated with UV light
2. The desktops are mopped with a 20% disinfectant (SAVO brand) solution (freshly prepared)
3. The desktops are mopped with 70% ethanol solution

Working Tools Check

1. Grinding mortars and pestles (sterile, kept at the room temperature)

2. Liquid nitrogen
3. Container for liquid nitrogen
4. Sterile spatula
5. Sterile scalpel
6. Gloves
7. Pipette 100 – 1000 µl
8. Pipette 0.5 – 10 µl
9. 65°C water bath
10. Centrifuge
11. Vortex mixer
12. Shaker
13. Sterile pipette tips 100 – 1000 µl
14. Sterile pipette tips 0.5 – 10 µl
15. Sterile tweezers
16. Loops
17. Sterile Falcon test tubes, 15 ml (orange caps)
18. Sterile micro-tubes, 1.5 ml
19. Parafilm strips approx. 1x4cm

Solutions Check:

1. Sterile H_2O
2. Extraction buffer – do not shake as this makes the solution froth – preheated to 65°C
3. RNAse A marked RA-8/1
4. Proteinase K marked PK-7/1
5. 1.2 M NaCl
6. Chloroform: IAA 24:1
7. Precipitation buffer CTAB – do not shake as this makes the solution froth – in case of cloudiness or the presence of crystals heat and mix by turning the bottle gently
8. 99.8% 2-propanol
9. 70% ethanol
10. TE buffer

THE ISOLATION PROCEDURE:

1. Put the 15 ml Falcon test tubes in a stand and put the stand in the hood
2. Add 5 ml of CTAB buffer into each Falcon 15 ml tube
3. Add 10 µl of 2-merkaptoethanol into each Falcon 15 ml tube
4. Pre-cool the grinding mortar by liquid nitrogen
5. Homogenize 1 g of fresh or liquid nitrogen frozen leaves (segments of leaves) in a pre-cooled grinding mortar

   Homogenization

   1. Insert the sample of plant tissue or a part thereof (e.g. after cutting it by sterile scissors or sterile scalpel) carefully into the liquid N_2 container
   2. Grind approx. 1 g of the plant material by a pestle in liquid N_2 environment in the pre-cooled sterile grinding mortar
   3. After the nitrogen evaporates, add new nitrogen and continue grinding (repeat as necessary), until a very fine powder is obtained
   4. Transfer quantitatively the content of the grinding mortar by a cooled sterile scalpel blade or by steel spatula into a 15 ml test tube containing the solution for DNA extraction

6. Mix the mixture properly by a loop and by a vortex mixer
7. Incubate in water bath for 1 hour at the temperature of 60°C, mix the mixture 2 or 3 times during the incubation by turning the test tubes and subsequently by vortex mixer
8. After the incubation, put the test tubes into an ice container and incubate for 5 minutes on ice

9. Add chloroform: isoamyl alcohol = 24:1 in the amount of 1 times the volume (approx. 5.5 ml) of the mixture using a pipette
10. Mix for 30 minutes at the laboratory temperature and then centrifuge for 30 minutes at 4500 revolution per minute until phase separation
11. Remove the top water phase immediately by a pipette and put it into a new sterile 15 ml test tube, add again chloroform: isoamyl alcohol = 24:1 in the amount of 1 times the volume of the mixture
12. Mix for 10 minutes and then centrifuge for 10 minutes at 4500 revolution per minute
13. Remove the top phase immediately by a micro-pipette and put it into a new 15 ml Falcon test tube which is marked identically to the original test tube, add cooled (-20°C) 2-propanol in the amount of 1 times the volume of the mixture and let it precipitate for 30 minutes at 4°C on ice.
14. Pour off the organic phase into the relevant waste receptacle in the hood
15. Coil the DNA around a sterile glass hook and put it into a new sterile test tube containing 2 ml of 70% ethanol
16. Leave for 1 hour at the temperature of 4°C on ice, then pour off the ethanol, replenish to 2 ml by new 70% ethanol and leave in a refrigerator overnight
17. Remove the hook with the DNA carefully, turn it and let the DNA get dry. Note: if the DNA falls from the hook, coil it again and "squeeze it dry" against the test tube walls
18. Leave the DNA on the hook approx. 5 minutes to let it dry
19. Prepare 1.5 ml micro-tubes marked according to the name of the sample
20. Transfer the DNA carefully into the sterile 1.5 ml micro-tube containing 500 µl of TE buffer
21. After dissolution add 15 µl of RNase A, mix by turning the tube and let it incubate in a block bath for 30 minutes at 37°C
22. Put the micro-tubes containing the DNA into a refrigerator, 4°C.

Detection of GMO and Maize Specific Sequences

The test is based on amplification of PCR of the product of the transgenic DNA target sequence corresponding to the sequence for nopaline synthase terminator *Agrobacterium tumefaciens* and its electroforetic separation on agarose gel, whereby the amplified part of the gene is identified on the basis of **the presence of a product of a previously defined size**, which is visible in UV light.

Preparation of the Working Area

1. UV light treatment
2. 20% disinfectant (SAVO brand) treatment
3. 70% ethanol solution treatment

Working Tools Check:

1. Pipette 100 – 1000 µl
2. Pipette 20 – 200 µl
3. Pipette 10 – 100 µl
4. Pipette 2 – 20 µl
5. Pipette 2 – 20 µl
6. Pipette 0.5 – 10 µl
7. Pipette 0.1 – 2 µl
8. Mini-centrifuge
9. Vortex mixer
10. Pipette tips with a sterile filter
11. 0.2 ml sterile micro-tubes

12. 0.5 ml sterile micro-tubes
13. 1.5 ml sterile micro-tubes
14. 2 ml sterile micro-tubes
15. Gloves
16. Ice container
17. Micro-tubes stands

Solution Check:

1. Ultra Pure H_2O for PCR,
2. AmpliTaqGold Polymerase – to be taken out of the freezing box, centrifuged gently and placed in ice
3. 10x PCR Gold buffer – to be taken out of the freezing box, defrosted, placed in ice, mixed before use in a vortex mixer and immediately centrifuged gently
4. $MgCl_2$ for ApliTaqGold– to be removed out of the freezing box, defrosted, placed in ice, mixed before use in a vortex mixer and immediately centrifuged gently
5. 10mM dNTP mixture – to be removed out of the freezing box, defrosted, placed in ice, mixed before use in a vortex mixer and immediately centrifuged gently on a mini-centrifuge
6. The analyzed DNA with a concentration of 20 ng/µl (if the concentration is lower, it must be noted in the Protocol on Establishing the Internal Gene by the "!C!" symbol)
7. Primers for the examined amplicon F (10pmol/µl), R (10pmol/µl) – to be removed out of the freezing box, defrosted, placed in ice, mixed before use in a vortex mixer and immediately centrifuged gently on a mini-centrifuge

A Table of the Primers Used

amplicon	Primer F	Primer R	Length of the product
invertase	5´- ggC Cgg ATC gTC ATg CTC TAC A- 3´	5´- TTg gCg TCC gAC TTg ACC CAC T - 3´	122 bp
CaMV _ 35S promotor	5´- CCg ACA gTg gTC CCA AAg ATg gAC -3´	5´- ATA TAg Agg AAg ggT CTT gCg AAg g - 3´,	162 bp
T-NOS	5´- gCA TgA CgT TAT TTA TgA gAT ggg-3´	5´-gAC ACC gCg CgC gAT AAT TTA TCC-3´	118 bp
MON810	5‘- TCg Aag gAC gAA ggA CTC TAA Cg-3´	5‘- TCC ATC TTT ggg ACC ACT gTC g-3´	170 bp

Preparation of Reaction Mixture

To be carried out on ice.

- A necessary number of micro-tubes are prepared
- The micro-tubes are closed and labelled in a planned order as per the protocol

- A necessary amount of mastermix is prepared. It is recommended to put no more than 500 µl of mastermix in a 1.5 ml micro-tube in order to be able to mix correctly.

The reaction amount for one sample is 25 µl (20 µl of the reaction mixture and 5 µl of the analyzed DNA).
The total prepared volume of the reaction mixture is $V = V_1 . (n+1)$, where V_1 is a volume of the reaction mixture which is necessary for 1 sample, n is the number of samples including controls and 1 volume for 1 sample is added for pipetting error. One additional sample is added in the reaction mixture preparation for each 10 analyzed samples.

1. The individual components are pipetted into the micro-tube in the following order: Ultra Pure PCR water , PCR buffer, solution $MgCl_2$, dNTP, primers and Gold polymerase
2. Mix the mastermix properly by turning the micro-tubes and by the vortex mixer or a micro-pipette tip
3. Put 20 µl into 0.2 ml PCR reaction micro-tubes by a pipette

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The following controls must be included in each series of samples for PCR reaction:

- **Positive control for the transgene** – contains mastermix and modified DNA (0.1% and 1% IRMM or referential DNA). It must be **positive** in a given position in order to confirm the adequacy of PCR and verify the functionality of all steps of the process.
- **Negative control for the transgene** – contains mastermix and 0% IRMM. It must be **negative**.
- **CTRL-ex extraction control** – contains only mastermix, extraction reagents and buffers used in DNA isolation. It does not contain template DNA and it must be **negative**, otherwise it means that contamination occurred during the extraction.
- **Mastermix control** (MM control and MM-o) – contains only mastermix and it does not contain template DNA which is replaced by water. MM control is included 2 times (one micro-tube with mastermix remains opened during the entire procedure of pipetting the samples and it monitors the laboratory environment). MM controls must be **negative**, otherwise it means that the mastermix or the laboratory environment has been contaminated during the preparation thereof and the pipetting.

Pipetting order of the samples:

- MM control – o — add 5µl H_2O
- MM control — add 5µl H_2O
- CTRL-ex — add 5µl of extraction control
- Samples — add 5µl of DNA sample
- 0% Control — add 5µl of DNA IRMM 0%
- 0,1% Control — add 5µl of DNA IRMM 0,1%
- 1% Control — add 5µl of DNA IRMM 1%

DNA with a higher concentration is first diluted by water for PCR to a concentration of 20 ng/µl.

1. Add template DNA by micro-pipette, close the test tube accurately, mix in a vortex mixer, centrifuge in a mini-centrifuge and insert into a thermal cycler
2. Launch a programme for amplification of DNA specific sequence (see Annex 1)
3. After the amplification is completed, centrifuge the micro-tubes again in a mini-centrifuge, preventing aerosol release

Programme for Amplification of PCR Product Invertase Sized 122bp

Activation of AmpliTaq Gold polymerase	95°C 12 min.
Amplification – 40 cycles Denaturation Annealing Extension	 95°C 30 s 70°C 1 min. 72°C 30 s
Final extension	72°C 10 min.

Programme for Amplification of PCR Product T- NOS sized 118bp

Activation of AmpliTaq Gold polymerase	95°C 10 min.
Amplification - 50 cycles Denaturation Annealing Extension	 95°C 15 s 60°C 15 s 72°C 15 s
Final extension	72°C 7 min.

Programme for Amplification of PCR Product CaMV _ 35S promotor sized 162 bp

Activation AmpliTaq Gold polymerase	95°C 12 min.
Amplification – 40 cycles Denaturation Annealing Extension	 95°C 30 s 66°C 30 s 72°C 30 s
Final extension	72°C 10 min.

Programme for Amplification of PCR Product MON810 sized 170 bp

Activation of AmpliTaq Gold polymerase	95°C 12 min
Denaturation Amplification – 40 cycles Annealing Extension	95°C 30 s 63,4°C 30 s 72°C 30 s
Final extension	72°C 10 min.

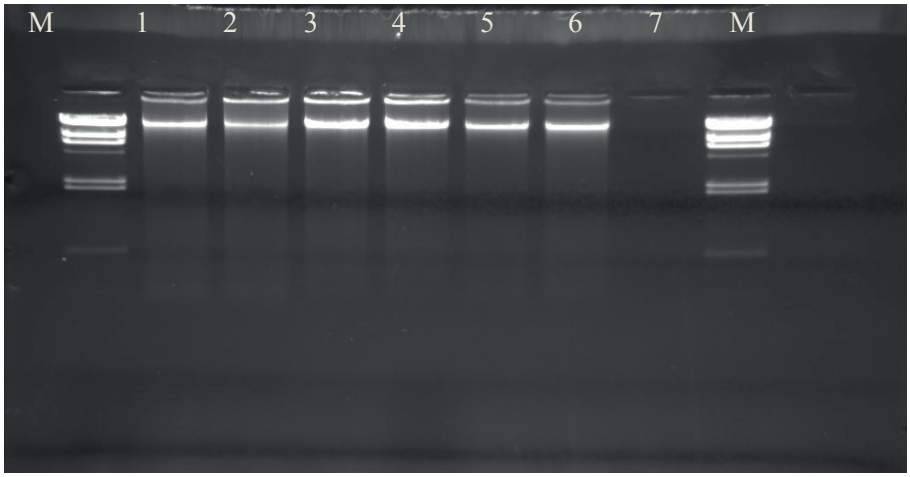

Fig. 1: DNA isolated from maize leaves.
M – size marker (Hind III), track 1 – 6: maize samples, track 7: extraction control.

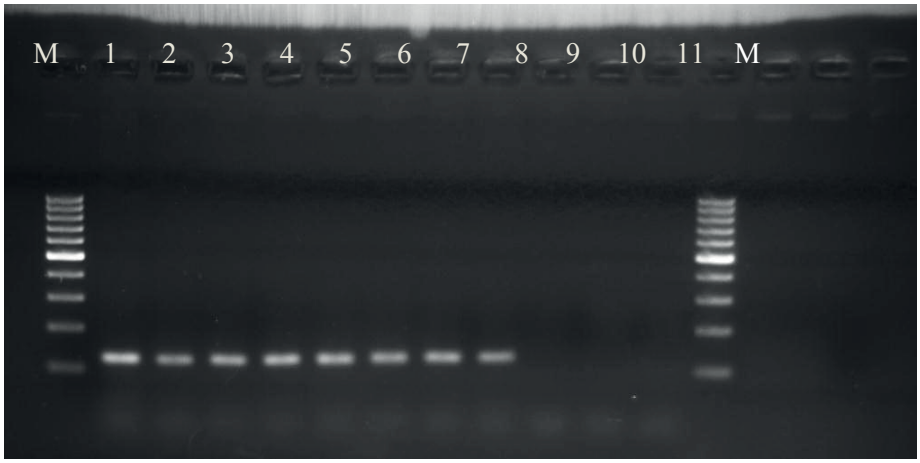

Fig. 2: Amplification of the internal maize gene – invertase
M – size marker (100 bp ladder), track. 1: 0.1% NK603, track 2: conventional maize, track 3 – 8: maize samples, track 9 – 11: controls.

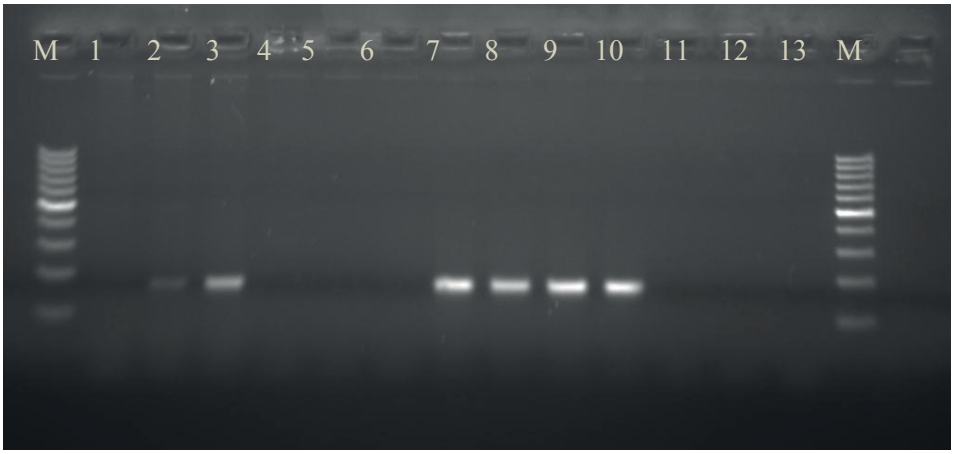

Fig. 3: Amplification of maize transgene – MON810
M – size marker (100 bp ladder), track 1: 0 % MON810, track 2: 0.1 % MON810, track 3: 1 % MON810, track 4: conventional maize, track 5 – 10: maize samples, track 11 – 13: controls.

Evaluation:

If a sequence originating from an internal gene of maize (e.g. invertase) has been amplified in the DNA isolate from the given sample, it is possible to consider the isolated DNA satisfactory and to continue with establishing GMO. If the presence of DNA sequence specific for 35S promotor CaMV (P35S CaMV) and nopaline synthase gene terminator (T-NOS) has been established, it is possible that the sample is taken from a GM maize plant. Conducting a test of presence of DNA sequence originating from MON810 confirms the presence of transgenic maize MON810 (unique identification code MON-ØØ81Ø-6). No other GM maize varieties are allowed to be grown in the EU.

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