

JRC SCIENTIFIC AND POLICY REPORTS

European Coexistence Bureau

Best Practice Documents for coexistence of genetically modified crops with conventional and organic farming

2. Monitoring efficiency of coexistence measures in maize crop production

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2014







European Commission

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Abstract

The present technical report deals with monitoring the efficiency of measures/strategies for coexistence between genetically modified (GM) and non-GM maize crop production. The report is a follow up of the best practices for coexistence in maize crop production proposed by the Technical Working Group (TWG) for Maize of the European Coexistence Bureau (ECoB).

The ECOB TWG maize held three meetings in October 2010, June 2012 and November 2012 and examined state-of-art-knowledge from scientific literature, research projects and empirical evidence provided by numerous finished and ongoing studies looking at the appropriate level of monitoring, monitoring strategy, sampling and testing issues, detection methods, analysis of results and possible follow up.

The review of this information (coming from a total of 55 references) is presented in a structured manner in Section 3 and 4 of the document.

The overview of the activities carried out by EU Member States for monitoring effectiveness/efficiency of coexistence measures in maize crop production (Section 3), shows a still limited experience in practical terms, due to the limited experience in commercial cultivation of GM maize in most EU Member States. However, the present report provides technical guidance to those responsible for monitoring the efficiency of coexistence strategies.



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This best practice document is the result of work carried out by the European Coexistence Bureau — Technical Working Group for Maize, consisting of the following European Commission staff and experts nominated by EU Member States:

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Executive Summary

The present technical report deals with monitoring the efficiency of measures/strategies for coexistence between genetically modified (GM) and non-GM maize crop production. The report is a follow up of the best practices for coexistence in maize crop production proposed by the Technical Working Group (TWG) for Maize of the European Coexistence Bureau (ECOB) (¹).

The ECoB TWG Maize held three meetings in October 2010, June 2012 and November 2012 and examined state-of-art-knowledge from scientific literature, research projects and empirical evidence provided by numerous finished and ongoing studies looking at the appropriate level of monitoring, monitoring strategy, sampling and testing issues, detection methods, analysis of results and possible follow up.

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¹ Czarnak-Kłos, M, Rodriguez-Cerezo, E (2010) Best Practice Documents for coexistence of genetically modified crops with conventional and organic farming, Maize crop production, EUR 24509 EN.

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1. Introduction

The European coexistence framework promotes the ability of farmers to choose between the cultivation of genetically modified (GM) and non-GM crops. The coexistence rules support market forces to operate freely in compliance with the Community legislation (European Commission, 2006).

Cultivation of GM crops should be regulated in the way that different agricultural systems can exist side by side in a sustainable manner. For the management of the economic risk to non-GM farmers, the European Union (EU) coexistence framework adopts a quantitative purity model. Within this framework, preventive segregation measures ensure that any 'adventitious presence' of GM material complies with the established tolerance threshold. The margins for admixing of genetically modified organisms (GMOs) in non-GMO products are in line with market demands.

1.1. Legislative context for coexistence and its monitoring

The legislative basis in the EU for the coexistence of GM and non-GM crops is established by the relevant legislation for the release of GMOs into the environment and food and feed legislation for the labelling requirements of tolerance admixture containing GMOs. Both pieces of legislation provide a harmonised approach to the assessment of all potential environmental and health risks which might potentially be connected to placing GMOs on the market.

Directive 2001/18/EC (¹) on the deliberate release of GMOs into the environment and Regulation No 1829/2003 (²) on genetically modified food and feed ensure strict control of placing on the market GMOs in the EU. All GMOs and food and feedstuffs derived from them have to be clearly labelled to ensure freedom of customer choice. In addition to that, as an exception to the labelling requirements, the European legislation takes into consideration the presence of

technically unavoidable or adventitious traces of GM material. Directive 2008/27/EC (³) which amended Directive 2001/18/EC established the threshold of 0.9 % for commodities intended for direct processing, below which traces of market approved GM products do not require labelling. The Regulation (EC) No 1829/2003 establishes the same threshold for food and feed. These labelling rules are also valid for organic products, including food and feed, according to Regulation (EC) No 834/2007 (⁴).

Adequate technical and organisational measures during cultivation, on-farm storage and transportation may be needed to ensure the ability of the agricultural sector to efficiently maintain different production systems and thus ensure freedom of choice throughout the food chain. As local environmental conditions and farm structures may have a significant impact on the effectiveness and efficiency of coexistence measures their development is under the remit of individual Member States.

Recommendation 2010/C 200/O1 (5) of the EC provides guidelines for the development of national coexistence measures to avoid the unintended presence of GMOs in conventional and organic crops, replacing Commission Recommendation 556/2003 (6). Recommendation 2010/C 200/O1 recognizes that the market demand for particular food crops may result in economic damage to operators who would wish to market them as not containing GMOs, even if GMO traces are present at a level below 0.9 %. Therefore, Member States may establish different thresholds for adventitious and technically unavoidable presence of GMOs in non-GM harvests, taking into account the demands of the consumers and their market. The Recommendation also takes into consideration the extreme diversity of European

¹ Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106. 17.4.2001. p. 1–39.

² Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

³ Directive 2008/27/EC of the European Parliament and of the Council of 11 March 2008 amending Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, as regards the implementing powers conferred on the Commission. OJ L 81, 20.3,2008, p. 45-47.

⁴ Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. OJ L 189, 20.7.2007, p. 1-23.

⁵ OJ C 200, 22.7.2010, p. 1-5.

 $^{6\,}$ Commission Recommendation 556/2003 of 23 July 2003 on guidelines for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming. OJ L 189, 29.7.2003, p. 36.

farming systems, natural and economic conditions and the experience gained over recent years regarding coexistence and clarifies that under certain climatic and/or agronomic conditions Member States may exclude GMO cultivation from large areas, if other measures are not sufficient to ensure coexistence.

Current Community legislation does not require the establishment of any methods to monitor the effectiveness and efficiency of the coexistence measures in place. However, the EU does have legal requirements in place concerning the monitoring of possible environmental impacts of GM products placed on the EU market. This post-market monitoring is not the subject of this paper and is not related to the monitoring of effectiveness and efficiency of coexistence.

1.2. The role of the European Coexistence Bureau

Although the development of coexistence measures is under the remit of individual EU Member States, the European Commission retains several roles in this process. One important role is the technical advice offered to Member States through the European Coexistence Bureau (ECOB).

The mission of the ECoB, created in 2008, is to organise the exchange of technical and scientific information on the best agricultural management practices for coexistence and, on the basis of this process, to develop consensually agreed crop-specific guidelines for technical coexistence measures. The ECoB is managed by and located on the premises of the Joint Research Centre (JRC) of the European Commission.

The ECoB is organised into crop-specific Technical Working Groups consisting of experts nominated by EU Member States. Their main task is to develop Best Practice Documents (BPDs).

The first Technical Working Group (maize crop production) started work in 2008. In 2010 it published a BPD for coexistence in maize crop production (Czarnak-Klos and Rodriguez-Cerezo, 2010). The BPD covers the cultivation of GM maize up to the first point of sale. It deals with three types of production: grain, whole plant and sweet maize. The ECoB analysed the potential sources of admixture and reached a set of consensually agreed, best agricultural management practices that will ensure coexistence while maintaining the economic and agronomic efficiency of the farm. For example, among other practices, the ECoB proposes isolation distances of 15-50 m to reduce cross-pollination between GM maize and non-GM maize to limit GMO content in non-GMO harvests to levels below 0.9 % (the legal labelling threshold). Larger distances are proposed for lower targets of admixture levels.

One question arising during the development of the ECoB's document on maize coexistence was the issue of evaluation of the efficiency of coexistence practices. Presently GM maize

is grown in a few EU Member States only, and experiences with monitoring the efficiency of coexistence strategies are quite limited. Member States' current monitoring activities differed significantly in frequency and scope (see section 3). Also, the lack of commonly agreed methodologies and indicators to define a coexistence strategy as 'efficient' does not allow comparisons or general conclusions to be drawn.

This situation is reflected in the 'Report on the coexistence of genetically modified crops with conventional and organic farming' prepared by the European Commission in 2009 (DG AGRI, 2009), which stated that further experience is necessary in this field. The report commits the ECoB to develop guidelines in the area of monitoring coexistence efficiency.

1.3. Scope of the BP document

This document focuses on the best practices for monitoring the efficiency of coexistence measures for maize crop production — it is a BPD for monitoring. It does not address the issues of: legal compliance with the regulated binding labelling thresholds, or compensation for damage caused by an adventitious presence of GM material as a result of the correct application of coexistence measures or as violation of the coexistence rules.

The monitoring of the efficiency of coexistence strategies addressed in this BPD should not be confused with the 'post-marketing environmental monitoring' (PMEM) of GM crops which is conducted according to the requirements laid down in Directive 2001/18 and has different objectives.

The BPD for monitoring is elaborated on the basis of coexistence measures previously recommended by Czarnak-Klos and Rodriguez-Cerezo (2010). Consequently its scope is limited to GM maize containing single transformation events and is applicable to both insect-resistant and to herbicide-tolerant GM maize. The BPD for monitoring does not cover maize seed production.

Efficiency in general can be defined as, 'accomplishment of or ability to accomplish a task with a minimum expenditure of resources and effort'. Therefore, for the purpose of this document, efficiency of coexistence measures is defined as the ability to reduce the GM content in non-GM crops to a requested level at minimum cost and with minimum burden for the farmer.

The BPD for monitoring is intended to assist Member States in assessing the efficiency of existing or proposed national or regional coexistence approaches and to offer a basis for objective comparison of the different national strategies. In consequence, by definition of the best practices for monitoring efficiency of coexistence measures this paper can help to develop a reliable database for the assessment of individual coexistence measures and combinations of measures.

2. Definition of efficiency of coexistence measures

In the BPD for monitoring, the efficiency of coexistence measures is defined as the ability to reduce the GM content in non-GM crops to a required level at minimum cost and with minimum burden for the farmer. In that respect the relevance of the following variables is considered:

- Ability to limit the GM content in non-GM harvests to the required level;
- Cost of applied measures;
- Feasibility of the measures from the farmer's point of view

2.1. The ability to limit the GM content in non-GM harvests to the required level

Assessment of the ability of different coexistence measures to limit GM content would require appropriate monitoring plans. Important elements of such a plan are the sampling strategy and analytical protocol utilized for the determination of GM presence at field level and/or in harvested bulk content of non-GM crops located at, or beyond, the isolation distance required by national coexistence measures. The result of this analysis will prove if the level of GM admixture in a non-GM harvest is above or below the required threshold, and thus if the coexistence measures in place are effective.

Providing that the number of sampling points and their location ensures representativeness of the results, the compliance rate for the particular measurement could be determined as number of cases that met the coexistence target per total number of analysed samples.

2.2. Cost of applied measures

The costs associated with coexistence measures depend on their nature and the interaction between them. The main costs of coexistence are assumed to be borne by the farmer cultivating the GM variety. However, the cost structure of non-GM farms is also altered by transaction costs and risk exposure. Therefore, the European Commission formulated that 'coexistence measures should not go beyond what is necessary in order to ensure that adventitious traces of GMOs stay below the labelling threshold in order to avoid any unnecessary burden for the operators concerned' (European Commission, 2006). Moreover, low cost coexistence measures induce regulatory compliance and defend the freedom of choice between production systems. Consequently, the costs of coexistence measures directly influence their efficiency.

The cost of coexistence can be approximated roughly to the difference in the gross margins of GM and alternative crops, or — as the utilization of coexistence measures on part of the farm. The gross margins obtained by farmers can be defined as the difference between a farmer's income and variable costs, i.e. costs that depend on production such as costs of seeds, fertilizers, pesticides, costs of fuel used for machinery, labour costs etc. Thus the cost-benefit analysis of GM cultivation is included in the assessment of the efficiency of coexistence measures. Additionally, on top of the costs relating to the restriction of GM cultivation due to the coexistence measures in place, so-called opportunity costs should be considered. These stem from the management of two different production systems within a field or farm, which obviously comes at an extra cost.

The main coexistence measures: isolation distances, buffer zones, temporal segregation and administrative and notification obligations (Czarnak-Klos and Rodriguez-Cerezo, 2010) are varied costs.

2.3. Feasibility of use of coexistence measures

Quantitative assessment of the feasibility of certain coexistence measures depends on their perception from farmers. The most suited instrument is therefore a farm survey to elicit the feasibility as perceived by farmers given their institutional environment. This could take the form of questionnaires sent to farmers, in which they classify the measures into predefined categories, e.g. easy, moderately difficult and very difficult.

This indicator may be used to distinguish between coexistence regimes of similar efficiency, implying that a measure is assessed as more efficient if it is easier to apply.

3. Overview of monitoring activities of coexistence carried out by EU Member States

Nowadays only a few European countries (i.e. Spain, Portugal, Czech Republic, Slovakia and Romania) cultivate GM maize on a limited area and therefore the experience in terms of monitoring effectiveness/efficiency of coexistence measures in maize crop production is still limited.

In this section we review the activities on monitoring of coexistence in maize in EU Member States. This section of the document was developed, based on the information provided by the Members of the Technical Working Group for Maize and the representatives of Competent Authorities responsible for coexistence issues in EU Member States.

Portugal

Portugal started GM maize cultivation in 2005 and by 2012 the cultivation area was 9278 ha.

The cultivation of GM plants in Portugal is regulated with national Decree-Law 160/2005 of 21 September 2005, based on the principles established by Commission Recommendation 2003/556/EC (7).

The regular monitoring of the effectiveness of coexistence measures conducted in Portugal includes sampling of conventional maize fields near the GM fields, as well as farmers' questionnaires.

Between 2006 and 2011, samples were collected by official inspectors from 106 conventional fields, the ones in which the highest GM content was expected. Sampling points were evenly distributed in the field and were collected manually (between 80 and 100 maize cobs per hectare) or directly collected during the harvesting. The analysis is performed in a sample of 3000 kernels and the results are given as a percentage of the transgenic copies per haploid genome.

Additionally, the farmer growing GM maize was asked to answer about 20 questions (organized in a questionnaire), among others, regarding his assessment of feasibility of coexistence measures.

Both, results of monitoring and results of control of compliance with coexistence measures are published in annual reports issued by the Portuguese Ministry of Agriculture (De Carvalho, 2012).

Slovakia

Slovakia started cultivation of GM maize in 2006 with 33 ha. The maximum of maize MON810 cultivation was in 2008 with 1931 ha and it declined in 2011 to 761 ha (Horvath et al., 2012).

The cultivation of GM plants in Slovakia is regulated by national Act No 184/2006 of 16 March 2006 and the technical rules for GM plants' maintenance are established with the Decree of Ministry of Agriculture of Slovakia No 69/2007 from 14 August 2007. The minimum isolation distance between GM and conventional maize fields is 200 m, or the equivalent number of buffer rows (1 row is equal to 2 m distance). The testing of GM admixture in neighbouring conventional maize is performed after harvesting.

In 2006, 3 samples from neighbouring conventional maize fields were collected; in 2007 and 2008 sample number increased to 40; 30 samples were analysed in 2009, whereas 23 samples were taken in 2010 and 2011. The detected GM admixture for the period 2006-2011 was between 0.01 % and 0.83 % (w/w) with a mean of 0.07 %. The flowering time of GM and non-GM maize was synchronous (10 days overlap).

Consumer and producer risks (α -risk and β -risk) were analysed by Horvath et al., 2012 for legally established 200 m isolation distances and a threshold of GMO admixture of 0.9 %. The value obtained for consumer β -risk was 4.8 % (or better) and of producer α -risk was 0 %. These values

confirmed the sufficiency of the minimum isolation distances of 200 m established in Slovakia.

France

France had performed a comprehensive monitoring of effectiveness of coexistence measures before discontinuing GM maize cultivation in 2008. The monitoring took place in two main maize growing regions in 2007: Midi-Pyrenees and Aquitaine. During that time no binding legislative framework for coexistence existed in France. GM maize was cultivated according to the recommendations of the Ministry of Agriculture which required 50 m of isolation distance between GM and non-GM fields and could be replaced by 24 rows of non-GM maize of the same maturity class sown as a buffer around the GM maize field. The recommendation required also the information of neighbors; however this issue was not regulated specifically. There were also no regulatory provisions concerning publication of a GM field register including number and size of GM maize fields per district.

Based on the identification of the fields with maximum risk of pollen flow, 10 % of in total 150 fields in Midi-Pyrenees and 50 fields in Aquitaine, i.e. 20 fields, were sampled and the level of GM admixture was determined by laboratory analysis. The risk was considered higher in the case when:

- the distance between the non-GM maize field and the GM maize field was less than 50 m or less than 20 m if the GM maize field was surrounded by a non-GM maize strip of at least 20 m width,
- the non-GM maize field was downwind from the GM maize field, and
- no landscape elements served as a barrier between the GM and the non GM maize fields.

Two different sampling protocols were used:

- In Midi-Pyrenees: a field sampling protocol developed by the Technical Institute Arvalis based on the experiences of the UK farm-scale evaluations published by Henry et al. (2003) was applied. Samples were collected from 3 evenly distributed transects in the non-GM maize field. Along each transect, ear samples were collected at distances of 2, 5, 10, 25, 50 and 150 m from the GM maize facing field edge. Each sample consisted of 3 ears, each from a separate plant at the sampling location. The ears were shelled and grains were homogenized. A sub-sample of 1 kg was analysed by the laboratory.
- In Aquitaine, sampling in the bin was applied following a protocol developed by Arvalis. Sampling with a probe that can reach 1 m depth and retrieves a sample of approximately 200 g was conducted. Twelve samples were taken per bin. Again grains were mixed and homogenised and a sample of 1 kg was analysed.

Quantitative PCR analysis was carried out by the National Laboratory of Plant Protection, accredited for GMO testing. Screening for 35S promoter was conducted followed by identification of MON810 with the method validated by JRC. Quantitative analysis was based on the presence of the promoter P35S. Results were expressed in % GMO (mass).

The major problem at that time was the identification of non-GM fields which should be sampled in order to assess the effectiveness of coexistence measures. Therefore a refinement of the protocols for data collection was considered necessary to improve the reliability of monitoring. The results of monitoring were published as a report (Dissémination d'OGM, 2006 and Culture du Maïs génétiquement modifié en, 2007).

Germany

In Germany the preliminary monitoring strategy was developed in January 2008 based on the German coexistence field trials (Langhof, M. et al., 2008 a, b, c) but due to discontinuation of GM maize production the monitoring did not take place. The strategy has not been further specified.

The monitoring was planned to focus on grain maize. Grain samples were planned to be taken from trailers or trucks in the official manner routinely used in the case of certified seed production. These individual samples should be combined for each trailer or truck to one single sample. Since in the case of bigger fields samples from several individual trailers or trucks have to be taken, the total number of samples for a given field should be limited to a maximum of 5 samples by mixing samples from different trailers or trucks. Nevertheless, remaining amount of these individual samples were planned to be kept.

It was planned to use the data from the German public GMO location register to get the address of GM maize farmers and to collect essential data like distance to conventional maize fields and owner of the neighbouring conventional maize field. In Germany special agreements between GM and non-GM farmers are possible. This may result in shorter distances than the legislation-based 150 m. Therefore, the investigation of varying distances in the range of 0 to 300 m, with special focus on shorter distances, was intended. In total, not more than 70 fields would have been monitored to keep the costs in an acceptable range.

The Netherlands

The approach towards monitoring of the effectiveness of coexistence measures is also being discussed in the Netherlands. According to the strategy being developed the non-GM fields to be sampled would be identified based on the analysis of the risk of pollen-mediated gene flow from nearby GM fields. The sampling of harvest according to the European recommendation 2004/787/EC is likely to be recommended and samples would be analysed by RIKILT, the Institute of Food Safety, according to EU standards.

Spain, UK and Belgium

Some Member States decided to launch scientific studies in order to assess the GM content in non-GM crops either in real coexistence situations like in Spain or in conditions simulating crop production of GM maize, for example in the UK and Belgium.

The results of the Spanish investigations concentrating on gene flow (Messeguer et al., 2006), flowering delays (Paladeumas et al., 2008), influence of GM volunteers (Paladeumas et al., 2009) and the results of farm-scale evaluations (Weeks et al., 2003) were published and taken into account during the elaboration of the best practice document on coexistence in maize crop production and their results will not be described here.

The Institute for Research in Agriculture and Fisheries of Belgium conducted a field trial in 2010 in cooperation with the EC's JRC in Ispra. Pollen was collected following the JRC method, vegetative and generative material was sampled during the growing season until the harvest of the maize both as silage maize as well as grain maize. During winter time samples were planned to be analysed in the lab to detect transgenic DNA in different plant parts and tissues. Special attention was paid to sampling methods and sample preparation in order to find a scientifically sound sampling procedure at acceptable labour and financial costs.

Others

No information regarding the monitoring of effectiveness of coexistence measures was obtained from the Czech Republic. No information on monitoring the effectiveness/efficiency of coexistence measures in EU Member States not represented in the TWG Maize is included in this document. The remaining Member States, currently not cultivating GM maize, have not developed strategies for such monitoring.

Some of the existing approaches, especially in the case of Austria and Lithuania, could easily be adapted for monitoring purposes however.

As an example, the Genetic Engineering Precautionary Act of the Austrian province of Salzburg foresees GMO field monitoring including sampling and GMO detection/ quantification by the laboratory. The monitoring is delegated by the provincial government to an official expert organisation (e.g. the Austrian Agency for Health and Food Safety). Presently the generally applied method suggests that in total 30 cobs should be randomly taken per each half hectare of monitored field. All the cobs are shelled and divided into 2–3 samples (one for lab testing, one as a reference for the provincial government and one for the farmer). Currently the follow up of such checks is described only in the case of illegal planting. Similar procedures exist in the event of violation of the coexistence rules in the legislation introduced by all nine

provinces in Austria. And the majority of the maize growing provinces already delegate rights to the Austrian Agency for Health and Food Safety to perform official monitoring on an annual basis

In the Order of the Minister of Environment of Lithuania (Official Gazette, 2006, No 111-4243) concerning the sampling of the surrounding environment general requirements, which may be used for ad hoc inspections, the following are specified:

- plant parts (not more than 0.3 kg) are collected from the investigated field (where GMOs are growing or admixture is expected) at random;
- the size of the whole sample should not be less than 0.5 kg;
- the content of the sample young plants or upper leaves, meristems, seeds or grains;
- the sample must be packed in plastic bags, labelled for identification, put in the portable refrigerator and delivered to the laboratory within 24 hours.

Monitoring is also foreseen in the event of the violation of coexistence rules — the samples must be taken from traditional and organic crops of the same family, genus or species as the investigated GMO which is grown nearer than is stated in the coexistence rules (due to a violation of the rules by the GMO farmer). In this case monitoring data can provide information for assessment of the level of crosspollination when the isolation distance applied is smaller than that required by coexistence rules. Such monitoring is carried out by a control body (State Plant Service under Ministry of Agriculture) when the need arises.

This overview of monitoring activities of coexistence carried out by EU Member States shows that presently only two Member States, Portugal and Slovakia, routinely monitor the effectiveness of coexistence measures. France also has some practical experience in this field.

Most of the countries which perform or envisage the monitoring of effectiveness of coexistence measures choose sampling in fields with the highest probability of GM admixture, identified by risk analysis. Some other countries such as Austria also stipulate the sampling of the nearest non-GM fields for detection of incorrectly applied coexistence rules.

In contrast, the sampling strategies which are used or foreseen by Member States differ significantly — samples are collected both at pre- and post-harvest stages and the sampling protocols vary. This makes comparison of the effectiveness of coexistence measures among countries difficult.

4. Assessment of effectiveness/ efficiency of coexistence measures

Assessment of the efficiency of coexistence measures should be carried out in relation to their ability to limit the GM content in non-GM harvests to the required level and should take into account related costs.

An important element for assessment the effectiveness of coexistence measures are analytical tests for determination of the level of GM admixture in non-GM material. For a proper comparison, the results of quantitative analyses should include information concerning percentage of maize in the landscape, percentage of GM maize fields and information on whether GM fields are dispersed or grouped (Lecroart et al., 2007 and Le Bail et al., 2010).

The efficiency and feasibility of an effective coexistence measure depends significantly on its cost. Therefore the applied coexistence measure should place the lowest possible burden on farmers.

4.1. Identification and selection of non-GM fields to be sampled

The identification of the non-GM fields from which the samples should be taken is one of the crucial issues for the monitoring of effectiveness of coexistence measures. Member States should establish the registers for recording the location of GMOs grown (according to article 31 of Directive 2001/18/EC). This same article gives the National Competent Authorities the ability to decide the level of definition of the register according to national provisions. If the register does not go down to field level it will be difficult to identify non-GM fields for sampling.

Member States focus their monitoring activities on those fields which are potentially the most exposed for the out-crossing with GM maize (see previous section of this document). This approach is economically justified, although if coexistence problems were to appear in the areas considered not problematic they may potentially not be identified.

4.2. Sampling at various steps of maize crop production

Samples for quantitative analysis may be taken during maize crop production either in the field at silage maize or kernel maize harvest stage or after harvest.

Sampling should be carried out using sound scientific and statistical protocols to achieve an appropriate level of confidence for detection (and quantification) of GMOs. The Commission issued a dedicated Recommendation (2004/787/EC10 (a)) 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, which addresses the sampling at seed and commodity level.

Sampling is further addressed in Commission Regulation (EU) No 619/2011 of 24 June 2011, laying down the methods of sampling and analysis for the official control of feed as regards the presence of genetically modified material for which an authorisation procedure is pending or the authorization of which has expired.

4.2.1. Sampling in the field

In Recommendation 2004/787/EC there is no description of any sampling procedures on fields. Moreover, there are hardly any approaches available for sampling of plants, volunteers or grains in the field, therefore the need for 'fit for purpose' sampling methods was recognized and addressed, among others, by the SIGMEA (9) and Co-Extra (10) projects funded by the European Community.

8 OJ L 348, 24/11/2004 p. 18 — 26.

9 http://sigmea.group.shef.ac.uk

10 http://www.coextra.eu

Within the SIGMEA project an experiment aiming at the comparison of different field sampling schemes was conducted in a 1.2 km² landscape. 10 GM maize fields and 4 non-GM maize fields of varying sizes and orientation, adjacent to GM fields were studied.

Sampling in the field was conducted at kernel maturity stage. Five sample plans were examined:

- random sampling;
- sampling along two transects;
- sampling along four transects;
- sampling along cross-transects;
- sampling according to a method developed in Spain (Messeguer et al., 2009).

For comparison sampling was performed at harvest. 1 kg grains were taken at 30 evenly distributed time intervals from harvest machinery flow at each field.

Random sampling was found to be most accurate. The method developed in Spain performed at least as well as random sampling if the sample number exceeded 30. The least accurate approach was sampling on two orthogonal transects.

However, the results showed that regular and random sampling plans are more reliable than the Spanish method in case of GM content mostly originating from seeds and volunteers due to low number of sampling points in the field centre.

It was recognised that random sampling is not trivial as it is very sensitive to variation at the field edges. The position of randomly selected sampling points has to be determined very accurately e.g. by GPS (≤ 1 m), especially at field edges.

The highest suitability of the random sampling plan was also confirmed by the results of Šuštar-Vozlič et al. (2010). However, this plan was considered as not feasible to be applied routinely. Therefore, a simplified approach for estimating the outcrossing level in the whole field was developed. Two primary distances from the donor field (10 and 25 m) were used. In each case, 20 samples were collected along the field parallel to the donor field in one, two or three lines. All sampling schemes had a comparable precision; therefore sampling in two lines was recommended as compromise between walking distance and minimum sampling point number.

Messeguer et al. (2009) also proposed simplifications in field sampling methodology. The standard sampling method allows estimating total fields GM maize admixture and the specific contribution of particular Bt pollen donor fields but, according to the authors, the method is too expensive to be

used in agricultural practice since at least 28 real-time PCR analyses per field are needed. Analysing at harvest time is cheaper and could give a sufficiently reliable estimation of total GM content. It is not possible however to determine the origin of the pollen, what may be important in case of liability issues.

Three simplified sampling methods were tested: contour (8 samples of 3 cobs: 1 PCR analysis), transect (8 samples, 1 analysis) and stratified (12 samples, 1 analysis).

The results showed that the GM content quite often varied greatly between repetitions and a higher number of cobs may be needed to decrease variability. Also all three simplified methods underestimated the GM content of the total field, especially the transect system. Since the contour method showed the best fit to the standard values it was adjusted as follows: 10 instead of 3 cobs per sample were taken and the distance to the field border was fixed to 6m. With those improvements the contour method may in most cases allow to determine if the GM admixture in the field is below or higher than the labelling threshold of 0.9 %. Nevertheless, the still high variability introduces an uncertainty which could be too high for practical purposes, especially when close to 0.9 %.

The fact that it is difficult to obtain a reasonable certainty of measurement when adventitious GM presence is close to the labelling threshold of 0.9 % was also raised by Allnutt et al. (2008). An impractical number of samples is required in such cases. If GM content of the total field is e.g. 18 % higher than 0.9 % (i.e. 1.06 %) 70 to 190 random samples are needed to obtain at least a certainty of 95 %.

Other sampling schemes were tested to assess the GM content in non-GM receptor fields in experimental trials conducted in the Netherlands (Van De Wiel et al., 2009). In the case of 0.25 ha fields, at 250 m distance it was divided into 16 sections, from each one sample consisting of five cobs was taken. In the case of 1 ha fields at 25 m, samples were collected at pre-determined positions along three transects, starting at border row closest to GM source (total 21 samples per field). As mentioned with the field sampling methodology by Messeguer et al. (2009) above, this approach would be too costly for practical monitoring purposes.

4.2.2. Sampling at post-harvest stage

Two methods for sampling of bulk commodities are described in Commission Recommendation 2004/787/EC — the method for static sampling and the method to be used in the case of flowing commodities.

In the case of static sampling the increments should be collected at sampling points evenly distributed throughout the lot volume, while in the case of flowing commodities the sampling period should be defined as the total offloading time/total number of increments.

The number of increments or sampling points and the size of a bulk sample are defined according to the lot size (see table below):

Lot size in tonnes	Size of the bulk sample in kg	Number of incremental samples
≤ 50	5	10
100	10	20
250	25	50
≥500	50	100

This method of sampling was compared to the sampling method described in Regulation

401/2006/EC (11). The GMO sampling method turned out to be most fit for purpose in the case of a very low level of GMO presence (Miraglia, 2009).

Seed sampling methods could also be used in the case of sampling harvested maize grains. The EU recommendation requires that the general principles and methods for sampling seeds and other plant propagating material be in accordance with the International Seed Testing Association (ISTA) rules (ISTA, 2007) and associated ISTA Handbook on Seed Sampling (Kruse, 2004). Those rules are frequently used not only in seed testing laboratories for a wide range of tests but also by authorities and companies for management decisions (CO-Extra Deliverable 4.3).

The lot of grains to be sampled, in the case of maize, may not exceed a maximum size of 40 t (ISTA, 2007).

In order to obtain a sample which is representative of all seed lots it is necessary to take an appropriate number of primary samples of approximately the same size (25 000 seeds, at maximum 1 kg). For some specific tests a greater sample size may be required (Pietilä, 2008). The ISTA rules contain recommendations on the number of primary samples to be taken in the three cases, of which only one — for sampling of seed lots in containers of more than 100kg or in the seed stream — may be of interest in the case of onfarm monitoring of efficiency of coexistence measures. The minimum number of primary samples in this case is shown in the table below:

Sampling seed lots in containers greater than 100 kg and from the seed stream:

Lot size, kg	Minimum number of primary samples to be taken
Up to 500	At least 5 primary samples
501 — 3 000	One primary sample per 300 kg, but not less than 5
3 001 20 000	One primary sample for each 500 kg, but not less than 10
20 001 and above	One primary sample for each 700 kg, but not less than 40

The other recommendations address seed lots in containers smaller than 15 kg and in containers between 15 and 100 kg and will not be useful for monitoring purposes.

The ISTA Rules were not specifically developed for sampling for GMOs. To address this important issue a specific software tool, Seedcalc, was developed. It can be downloaded free of charge from the ISTA webpage (http://seedtest.org/en/statistical-tools-for-seed-testing-_content---1--1143--279. html) and can be used to design sampling/testing plans for estimation of adventitious GMO presence in non-GM seed lots. The software can also be applied for grains testing (Co-Extra — Deliverable 4.3). In the PRICE project is dedicated task for additional advancements of this topic.

Commission Regulation (EU) 619/2011 lays down the methods for sampling and analysis for the official control of feed as regards the presence of genetically modified material for which an authorisation procedure is pending or the authorization of which has expired. It recognizes that GM material shall be considered as a substance likely to be distributed non-uniformly throughout the feed. Therefore the rules regarding the size of the aggregate samples (shall not be less than the weight corresponding to 35 000 grains/seeds; in the case of maize 10 500 g) and the final sample (shall not be less than the weight corresponding to 10 000 grains/seeds; in the case of maize 3000 g) were established regarding the sampling procedures described in Commission Regulation (EC) No 152/2009 (12), which should be applied in the case of official controls of feed.

4.2.3. Sampling for specific case of whole plant use

The BPD for coexistence in maize crop production (Czarnak-Kłos and Rodriguez-Cerezo, 2010) recommended different isolation distances for grain maize and whole plant use based on the fact that in the case of whole plant use the grain

 $^{11\,}$ Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. OJ L 70, 9.3.2006, p. 12–34.

¹² Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, OJ L 054, 26/02/2009 P. 1-130.

(the product that may actually be susceptible to transgenic pollination) makes up no more than half the dry weight.

In the case of whole plant use the following observation should be taken into account for analysis of the GM content:

- The results of the trials conducted in Germany by Weber et al. (2005) showed virtually no difference between the GM content measured in whole plants and that found in the grains alone. The DNA content of the vegetative parts of the plant will be not sufficient at the end of the season, and due to the dying-off process it is quite feasible that the quality of the DNA extracted from vegetative parts will have deteriorated. The Dutch trials carried out in 2007 also investigated the effect of addition of vegetative parts to the quantification of GM content in fodder maize. The contribution of the stover (vegetative parts) appears to be relatively small (related to low amounts and quality of DNA from these parts), in line with Weber et al (2005) mentioned above. It was estimated that almost ten times less copies of the maize HMG gene that is used as representative for the number of genome copies (HGE), were detectable than in corresponding amounts of grain DNA (Dolstra et al. 2009). The results of another experiment, conducted in Germany, only showed a very high variation in the ratio of GM content of whole plants and kernels. An average ratio of approximately 1:2 was calculated (unpublished data by G. Rühl). In those experiments samples from 200 individual harvest points within the coexistence field trials were compared (directly adjacent sample points for whole plants and kernels only in a given field).
- In the case of complete harvests appropriate sampling is very complicated. Since the crop is relatively non-homogenous, it is difficult to obtain a homogenous mixture of all components and then to draw a representative sample from it. This opinion was also shared by the German expert (G. Rühl, personal communication). This was also the experience in the experiments of Dolstra et al (2009).
- In the case of ensiled maize the correct quantification of the GM content may be impossible. The pH of the silage is normally 3.5 - 4, which may cause a rapid breakdown of the DNA. Duggan et al. (2000) showed that maize DNA degrades within one minute in silage effluent, but it was proved possible to amplify a specific Bt gene sequence after at least 30 minutes using PCR. In silage trials carried out some years ago in Germany in the majority of cases (16/20) DNA was completely degraded after the silage making period of 49 days under a controlled temperature regime of constantly 20 °C. However, two Bt-maize samples as well as two samples from a conventional recipient plot still contained extractable DNA and the specific Bt-gene sequence (G. Rühl, personal communication). From the experiments by Dolstra et al (2009), it was concluded also that with silage, it was possible to obtain GM copy ratios according to the standard JRC protocol for MON810, but their accuracy was rather low due to the poor recovery

of DNA in the extraction from silage. With digestation (remnants of biogas production from silage maize that can be used as fertilizer), quantification proved impossible, even though some transgene could still be detected.

4.3. Quantification of GMO content

4.3.1. Requirements regarding GMO laboratories

Regulation (EC) No 882/2004 on official controls performed 'to ensure the verification of compliance with feed and food law, animal health and welfare rules' requires that National Reference Laboratories (NRLs) must be accredited according to EN ISO/IEC 17025 on 'General requirements for the competence of testing and calibration laboratories'. NRLs are assisted by the European Union Reference Laboratory for GMOs, operated by the JRC (http://gmo-crl.jrc.ec.europa.eu/).

4.3.2. Method for GMO analysis

Detection methods used by NRLs and by any accredited control laboratory should be described, published and must be validated by each control laboratory prior to use. Reference methods for GMO detection, identification and quantification can be found in the GMOMETHODS database (Bonfini et al., 2012) maintained and updated by the EU-RL GMO (http://gmo-crl.jrc.ec.europa.eu/gmomethods/). The selection of methods for inclusion in the reference database is carried out by the EU-RL GMO in collaboration with the European network of GMO Laboratories (ENGL, http://gmo-crl.jrc.ec.europa.eu/ENGL/ENGL.html) based on the validation status of each method.

The database includes qualitative and quantitative validated methods, targeting common genetic elements, GMO constructs or specific GM events. All methods included in the database were validated according to international standards, many by the European Reference Laboratory for Food and Feed (EURL-GMFF) within the framework of Regulation (EC) No 1829/2003. Each method is described in detail by general information, references (e.g. to the SOP), validation data, primers and probes sequences as well as the reaction setup and amplification conditions.

4.3.3. Measurement of uncertainty

According to Commission Regulation (EU) No 619/2011 in order to ensure a level of confidence of approximately 95 % the result of the quantitative analysis shall be reported as: $x \pm U$ (whereby x is the analytical result and U is the appropriate expanded measurement uncertainty). U shall be specified by the official laboratory for the whole analytical method using internal control data as described in the guidance document on Measurement Uncertainty for GMO testing laboratories developed by the JRC (Trapman et al.,

2009). The document describes two approaches for the estimation of measurement uncertainty which can be used by laboratories: one based on data from collaborative trials in combination with in-house quality control data; and one using data obtained from within laboratory samples.

According to ISO 17025, an accredited laboratory should establish measurement uncertainty linked to the analysis of the samples for which an analytical report is issued.

4.3.4. Units of measurement

The measurement unit of the certified reference material (CRM) determines in which unit the measurement result can be reported (Trapman et al., 2010). Using CRM with stated property values in mass fractions leads to the expression of results in mass fractions, whereas the results need to be expressed in DNA copy number ratios when the CRM used are certified for their copy number ratio.

Although the exact estimation of a mathematical relationship between the two measurement units for materials of known biological origin remains challenging, the application of new technologies like digital PCR offers the possibility to obtain reliable analytical data for which a realistic conversion factor can be established (13).

In order to facilitate the free choice of the measurement unit for GMO quantification results, the IRMM (Geel, Belgium) strives to make for some CRMs available calibration for both measurement units and matrix CRMs certified for their GMO content expressed in both measurement units.

4.4. Cost associated to different coexistence measures

The cost of coexistence in general depends on the nature of applied measures and their interplay as is already highlighted in section 2.2.

The cost increased proportionally to isolation distance as coexistence measure is closely linked to its magnitude. Demont et al. (2008) showed that large isolation distances might create a domino effect in which more farmers refrain from adoption despite the initial intention to do so, drastically increasing the opportunity costs.

Another spatial solution towards isolation distances is the use of buffer zones (Rühl and Langhof, 2011). The opportunity costs in this case are significantly reduced by the reduction in land use and despite that farmers growing GM maize are still required to manage two production systems. Moreover, buffer zones have the potential to be applied in a more flexible way, reducing the extent of the domino effect and making them more proportional to the aim of achieving coexistence (Demont, et al. 2009).

In the case of conventional maize being planted as an alternative crop (buffer or discard zone) a study of Gómez Barbero et al. (2008) can be taken into consideration. This study is based on a survey of commercial farms in three provinces of Spain. For the period 2002-2004 they found that the impact of Bt maize adoption on gross margins ranged, depending on the particular province, from being neutral to an increase of $\[\le \]$ 122 per hectare per year. The reasons for this were, first of all, increased yields and reduced pesticide use in Bt maize. The differences in seed prices and market prices of the harvest were taken into account.

The cost of temporal segregation, arising from the deviation from the optimal sowing date reducing the average yield is highly dependent on the regional climatic conditions. Messean et al., 2006 assessed the costs of temporal isolation when using varieties of different earliness (method more reliable than sowing delay). In the case of France 201 ϵ /ha as additional costs related to the changing of flowering time by using a late variety instead of the very late one (30 degree-days difference) were estimated, whereas in the case of a change from a late to a mid-early variety the cost was lower, around 46 ϵ /ha. While this is a feasible strategy in southern parts of Europe the yield loss in northern parts leads to higher opportunity costs and increased exposure to adverse weather conditions.

Apart of field cultivation, different administrative measures may evolve further costs. The costs of implementing bookkeeping rules, obtaining a certificate to grow GM crops or requiring administrative permits can be easily measured but are harder to generalize as they depend on the specific specifications of the measure in the different country.

A particular important part of the coexistence regulations from an economic point of view is the need for notification of other stakeholders. At first sight information provision increases the cost for the farmer. Besides the procedure to do so, depending on the target of the information provision, the effect might range from a deteriorated relation with neighbouring farmers, landowners and other societal actors to retaliation by opponents increasing production risks for the farmer. However, notification might be the gateway to communication and cooperation. If permitted by regulatory frameworks, communication might even lower the cost of coexistence by allowing for coordinated and flexible coexistence solutions (Devos et al., 2009) such as internalization in cooperatives (Skevas et al., 2009) or arrangements between farmers (Consmüller et al., 2012).

The relationship between *ex ante* measures and *ex post* measures significantly interferes with the final costs of coexistence (Beckmann et al., 2010). When *ex post* regulations (the legal framework for adventitious presence) are strong, farmers face less uncertainty and risk, potentially reducing the need for stringent *ex ante* measures (Demont et al., 2010). However, when uncertainty exists about maintenance of possible adventitious presence in non-GM production, both categories of farmers growing GM and non-GM varieties will overinvest in coexistence measures to minimize the exposure to this uncertainty. Therefore the lack of optimization in mixed coexistence measures will increase the cost and hence decrease the efficiency of coexistence measures at the farm level, eventually hampering the freedom of choice.

Although most of the coexistence measures are well understood theoretically, the practical implementation and the interplay is less well understood as European experience with GM cultivation and coexistence is limited. The most suited instrument is therefore a farm survey to elicit the costs as perceived by farmers given their specific environment. Examples of farm survey approaches can be found in literature (Consmüller et al., 2012;Gomez-Barbero et al., 2008; Skevas et al., 2010).

The European funded research project PRICE (http://price-coexistence.com/) is following the survey approach in order to assess the total cost of coexistence in 6 European countries with special focus on the interplay of different coexistence measures. One of the major novelties besides the extended geographical scope is the focus on the cost of coexistence for farmers growing non-GM crops in a GM producing area.

4.5. Feasibility of applying coexistence measures

The feasibility of the coexistence measures is highly variable and depends on local characteristics such as: scale of maize production, agricultural and crop management practices in place, environmental conditions (climate and agricultural landscape) as well as farmer preferences (Angevin et al., 2002; Messéan et al., 2006; Beckie & Hall, 2008; Messéan et al., 2009; Le Bail et al., 2010).

For the assessment of the feasibility of different coexistence measures, Gómez-Barbero et al. (2008) and Areal et al. (2012) Used a pre-coded structured questionnaire for the face-to-face farmer interviews.

4.6. Decision aid tools

Several decision aid tools have been developed to support the determination of sample size both in experimental and real life control conditions of the commodity market (CO-EXTRA Deliverable D 4.3):

- KeSTE (Kernel Sampling Technique Evaluation) to evaluate sampling techniques for the detection of impurities in large kernel lots (either virtual or actually sampled) (Paoletti et al., 2003);
- SISSI (Shortcut In Sample Size Identification) to estimate the optimal sample size in experimental data collection (Confalonieri et al., 2007);
- STAGED (Statistical Model for GMO Enforcement Detection) (14) mathematical model for combining information on the performance of all stages of GM event detection;
- OPACSA (Optimal Acceptance Sampling by Attributes) (15) to find the cheapest mode of control by attributes of the purity of grain lots.

Additionally, SeedCalc was developed by ISTA as a specific tool for the determination of seed sample size and may be downloaded free of charge from the ISTA website.

In contrast to the number of decision support tools aimed at the determination of sample size, the tools for identifying potentially problematic field arrangements to be sampled from a coexistence perspective are limited.

The predictive models can help decision-makers to assess the feasibility of coexistence measures (Angevin, 2012). However, its routine application is still challenging for farmers and the consultants because of complicated handling, and lack of data. This has led to the development of several decision aid tools during the European project SIGMEA (http://www7.inra. fr/sigmea). These can be used before sowing to estimate the risk of cross-pollination from field to field, to determine the need of segregation measures and help to select the most appropriate.

The 'GMcalculator' (Allnutt et al., 2008) or the global index, implemented in the software GIMI (Messeguer et al., 2006) are, in turn, based on a statistical analysis of large data sets and are being evaluated with results from trials and commercial fields followed by task for other crop cultivation (Alnutt, Messeguer, pers. comm.). This is a prerequisite before its application for other environmental and agricultural conditions because the modelling approach depends on the

¹⁴ http://www.gm-inspectorate.gov.uk/reportsPublications/documents/FinalreportfromDefrawebsite.pdf

¹⁵ http://www.coextra.eu/researchlive/reportage851.html

quality of datasets used for its development (Lavigne et al., 2004; Beckie & Hall, 2008).

The SMAC Advisor is a decision-support tool on maize coexistence (Bohanec et al., 2007), that evaluates feasibility of coexistence based on a multi-criteria model developed from two data sources:

- An extensive simulation of gene flow due to crosspollination (more than 8000), obtained by the MAPOD[®] simulator (Angevin et al., 2008).
- Decision rules obtained by expert opinions for other potential sources of mixtures.

The ongoing European-funded research project PRICE (Dec 2011 — Dec 2014; http://price-coexistence.com/) develops further models from the previous FP6 projects Co-Extra and SIGMEA. The Decision Support Tool (DST) of the PRICE project is designed to be user friendly and combines two types of modelling: in the first case by inputting of the required isolation distances is possible to predict expected GM admixture for assessing effectiveness of coexistence measures; in the second case by entering of the field sizes (GM and non-GM) in a set of gene flow models can be probabilistically predicted the adventitious admixture of GM grains by distance, in a respect to estimate the scale of coexistence measures, at which they will be efficient. Therefore the application of DST is flexible, more reliable,

and can be adapted to the different stages of crop production and adjusted according to the availability of data.

For example the DST can be applied at different times of the growing season:

- before sowing ('ex ante'), e.g. to allow farmers to manage the sowing plans;
- after sowing and before flowering ('ex post 1'), e.g. for policy-makers to plan monitoring;
- after flowering and before harvesting ('ex post 2'), e.g. for cooperatives to manage their harvesting plans.

The spatial data on field size and boundaries, plot attributes such as land use or type of crop, and date of sowing are editable by the DST interface and new parcels can be included. Climatic data for across Europe will be extracted from an external database. Further required data has to be entered by the users and is possible data exchange between users.

The DST can be easily accessed via a web platform by the different stakeholders such as policy-makers, cooperative managers, consultants, as well as farmers.

5. Best practices for monitoring of efficiency of coexistence measures

5.1. Appropriate level of monitoring

The monitoring of effectiveness and efficiency of coexistence measures should take into account all possible admixtures such as cross-pollination, contamination by sowing, harvesting, transportation, storage, etc. Therefore, monitoring the potential for adventitious presence of GM maize at the field level is appropriate. For optimization of monitoring cost efficiency, it could be combined and/or substituted with monitoring during the harvesting of the particular field on site, or afterwards from the trailers.

5.2. Monitoring scale

The number of monitored fields/trailers should be selected in a cost-efficient manner for achieving representative data. Initially, the focus should be on fields with an expected high out-crossing risk, and then based on these preliminary data the number of fields may be adjusted.

5.3. Monitoring strategy

The selection of sampled fields should be done in a rational manner and only the most exposed fields should be tested. If the coexistence measures are efficient in this case there is no need to assess adventitious presence in less risky situations.

For the evaluation of the potential for adventitious presence of GM maize in a particular field, the following should be considered:

- Location of the monitored fields in relation to the GM maize area and their size in relation to their out-crossing potential;
- Envisaged efficiency of applied coexistence measures; and
- Regional specificities, relevant for pollen mediated gene flow.

The location of the monitored non-GM fields could be obtained by using the registers for recording the location of GMOs grown. Depending on the particular data about the GM fields' location in these registers, may needs additional information to be obtained. Further refinement of monitored areas could be achieved with the utilization of the monitoring-aid tools.

5.4. Application of monitoringaid tools

Predictive modelling is quite a powerful tool for processing and concluding multivariable tasks. However, the monitoringaid tools currently available need to be validated for a large scale of variation of regional and environmental conditions for the assessment of coexistence between GM and non-GM maize. They should meet multi-user friendly requirements, for farmers, regulators and other stakeholders, in guiding coexistence measures. In this respect, the developments of the European-funded research project PRICE are guite promising as they are a continuation of the achievements of previous projects. The new monitoring-aid tool should combine the structure of cost of coexistence with the compliance rate to assess the effectiveness of coexistence measures. Those measures which effectively reduce the GM content in non-GM crops to the requested level should be ranked by their cost.

5.5. Sampling and testing issues

5.5.1. Sampling schemes

The sampling should be performed at the harvest stage for maize crop production in the field or the trailer. The stratified sampling strategy in the field or the random sampling on site during the harvesting or afterwards from trailers is recommended.

For monitoring of silage maize production the most reliable data will be obtained by analyzing kernels at harvested time, since taking a representative sample from silage maize field is still a matter of scientific investigation. It is already possible to determine the GM content of whole maize plants and maize silages but it is more laborious than in the case of kernels since the variation within these samples is high due to the different stage of DNA deterioration in the plants' green matter or in the silage.

5.5.2. Methods of sampling

Standardized methods of sampling are recommended. The ISTA sampling methodology, JRC Recommendation 2004/787/EU and the sampling approach for KeLDA protein analysis could be utilized.

5.5.3. Detection methods and expression of the results

Utilization of the standard Real-Time PCR method validated by the JRC is recommended. Results could be presented as the number of copies (recommendation of the EC) or the percentage of GM-DNA in terms of the haploid genome.

5.6. Analysis of results and possible follow up.

The monitoring of coexistence efficiency and analysis of its results should be done in a stepwise approach. If the effectiveness of coexistence measures for the fields most exposed to potential admixture is confirmed there is no need to assess adventitious presence in less risky situations.

During the monitoring process, further investigations should take place when GM admixtures above the labelling threshold were detected. In this case it has to be determined how the GMO admixture occurred and whether the adopted coexistence measures are effective.

5.7. Formatting and communication of monitoring results

Commission Regulation (EU) No 619/2011 (16) provides sufficient possibilities for uniform presentation of monitoring results.

16 The Commission Regulation (EU) NO 619/2011 of 24 June 2011 laying down the methods of sampling and analysis for the official control of feed as regards the presence of genetically modified material for which an authorization procedure is pending or the authorization of which has expired, OJ L 166, 25.6.2011, p 9-15.

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