Cultivation of Biotech-Crops, Control of Co-existence and Environmental Monitoring of GM Plants in Slovakia

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Abstract

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The information on and selected results of biotech-crops cultivation, control of coexistence, and the environmental monitoring of genetically modified (GM) plants in Slovakia are presented. The cultivation of GM crops for commercial use in Slovakia started in 2006, the cultivated GM maize hybrids being based on the event MON 810. The testings of neighbouring conventional maize fields contamination were performed using a real-time PCR procedure for MON 810 maize quantification according to EN ISO 21570 and EU-RL GMFF methods. Minimum isolation distances according to the Slovak national legislation are 200 m for conventional maize and 300 m for ecological farming. The determined GM contamination of neighbouring fields varied between 0.01% and 0.83% (mean level 0.07%) in mass % of MON 810. The relationship between the GM contamination and isolation distance was documented. GM admixtures in harvested crops are caused due to combined factors as crosspollination, contamination by sowing, harvesting, transport, storage, etc. Consumer and producer risks (α-risk and β-risk) were analysed for minimum isolation distances in conditions of actual GMO limits, determined GM admixtures, and the testing procedures used. The calculated values gave good results for the conventional maize production, i.e. for 0.9% GMO limit, isolation distances of 200 m, and approximately 0.2% GMO level of impurities. The obtained value of consumer β-risk was 4.8% (or better), that of producer α-risk was 0%, and they both are sufficient for conventional maize production, confirming the optimum and sufficient value of minimal isolation distance (200 m) in Slovakia. No illegal cultivation of GM crops was found within the frame of environmental monitoring.

Keywords: biotech-crop; GMO; MON810 cultivation; isolation distances; impurities; environmental monitoring

Biotech-crop situation in the world, in the EU and in Slovakia: The cultivation of biotech-crops started in 1996. 2011 was the 16th year of biotech crops commercialisation, when the growth continued after a remarkable 15 consecutive years of increases, at a growth rate of 8%, reaching a record 160 mil. ha in 2011. It is noteworthy that of the 29 countries worldwide planting biotech-crops in 2011, 19 were developing and 10 were industrial countries. The top 10 countries each grew more than 1 mil. ha biotech-crops each, and the top nine each grew more than 2 mil. ha each, as published by James (2011).

USA grew 69 mil. ha biotech-crops (maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, and squash), Brazil grew 30.3 mil. ha biotech-crops (soybean, maize, cotton), Argentina grew 23.7 mil. ha biotech-crops (soybean, maize, cotton), India grew 10.6 mil. ha biotech-crops (cotton), Canada grew 10.4 mil. ha biotech-crops (canola, maize, soybean, sugar beet), and China grew 3.9 mil. ha biotech-crops (cotton, papaya, poplar, tomato, sweet pepper) (James 2011).

Biotech soybean continued to be the principal biotech-crop in 2011, occupying 75.4 mil. ha or 47% of global biotech area, followed by biotech maize (51 mil. ha at 32%), biotech cotton (24.7 mil. ha at 15%), and biotech canola (8.2 mil. ha at 5%) of the global biotech-crop area (James 2011).

Herbicide tolerance remains the dominant trait. In 2011, herbicide tolerance deployed in soybean, maize, canola, cotton, sugar beet and alfalfa, occupied 59% or 93.9 mil. ha of the global biotech area. In 2011, the stacked double and triple traits occupied a larger area (42.2 mil. ha, or 26% of global biotech-crop area) than insect resistant varieties (23.9 mil. ha) at 15%. The stacked genes were the fastest growing trait group between 2010 and 2011 at 31% growth (James 2011).

Six EU countries (Spain, Portugal, the Czech Republic, Poland, the Slovak Republic, and Romania) planted 114,490 ha of biotech Bt maize, by substantial 26% higher than in 2010, with Spain growing 85% of the total in the EU with a record adoption rate of 28%. Two countries (Sweden and Germany) planted a token 17 ha of the new biotech quality starch potato named Amflora for "seed" productiony (James 2011).

The planned release in 2014, subject to approval, of a new biotech potato named Fortuna resistant to late blight, is potentially an important product that can meet EU policy and environmental needs to make the potato production more sustainable by reducing heavy fungicide applications and decreasing production losses.

For the near, mid, and long terms there are numerous new products at different stages of development possessing:

– insect resistance – high priority is now being assigned to sucking pests (lygus and mirids) as they understandably have become the next top priority in the absence of the former top priority, bollworm family of pests, now effectively controlled by current biotech insect resistant cotton;
– disease resistance to the pathogens *Fusarium*, *Verticillium*, *Rhizoctonia*, *Pythium,* and *Cotton leaf curl virus* (CLCV) – the latter is critically important in Pakistan and some areas of the Punjab in India; nematode resistance is being explored;
– greater tolerance to abiotic stresses, particularly drought. Unlike maize where the critical stage for drought avoidance is the relatively short period of silking, in cotton it is required over a much longer period of flowering (James 2011).

The cultivation of GM crops for commercial use in the Slovak Republic started in 2006. In line with the lists of genetically modified organisms and products authorised in EU, the following genetically modified crops are registered for placing on the market in the Slovak Republic: cotton, maize, oilseed rape, soybean, potatoes, sugar beet, and carnation genetically modified for flower colour. No official statistics exists as to the modifications and amount of GM food and feed imported, processed and consumed in the Slovak Republic. Regarding GM crops cultivation, Slovak growers can use such GM varieties whose genetic modification has been approved at the EU level and that have been registered in the National Plant Variety Register of the Slovak Republic or in the Common Catalogue of Varieties of Agricultural Plant Species of the EU. So far only Bt maize line MON 810 (resistant to the European corn borer) and GM potatoes cv. Amflora (with modified starch content) have been authorised for cultivation in EU.

In Slovakia, growing is within the frame of coexistence for only one biotech-crop – Bt maize MON 810, and the share of this GM crop in total crop area remains very limited.

MON 810 (MON-ØØ81Ø-6) Yieldgard® Insect is resistant maize produced by inserting a truncated form of the *cry1Ab* gene from *Bacillus thuringiensis* subsp. *kurstaki* HD-1. The genetic modification affords resistance to attack by the European corn borer (ECB), *Ostrinia nubilalis*.

Maize event MON 810 was grown in 2006 on an area of 33 ha, in 2007 on 949 ha, in 2008 on 1931 ha, in 2009 on 875 ha, in 2010 on 1249 ha, and in 2011 on an area of 761 ha. In addition, a small scale field trials with GM maize events are performed in Slovakia for the research purposes as the deliberate release into the environment of GM organisms for any other purposes than placing on the market according to "Part B" of the Directive 2001/18/EC. Before undertaking a deliberate release of a GMO, a notification shall be submitted to the competent authority which is the Ministry of Environment of the Slovak Republic. The commission shall make available to the public the trial information contained in the "summary notification information format" (SNIF). According to the Directive 2001/18/EC the intentional introduction of a genetically modified organism or a combination of genetically modified organisms into the environment (deliberate release) is strongly regulated and a stepwise introduction into the environment is carried out. First, the test trial is performed with a significant limitation of reproduction and spreading of genetically modified organisms, and later on, after thoroughful evaluation of test trial, in compliance with the modern state of science and technology when no effects adverse to human beings and to the environment are foreseen according to the evaluation of

the risk level, the introduction can be performed in full scale, under controlled reproduction and spreading of genetically modified organisms in the environment. In the framework of the analysis of cumulative long-term effects the user is obliged to investigate the effects of genetically modified organisms on human health, animals and plants, soil fertility, food chain, ecosystems, biological diversity of plants and animals, and to resistance on the antibiotics used as human pharmaceuticals and veterinary pharmaceuticals. After completing the research and development phases, passing through confined field trials and receiving the approval from the competent authority, the respective a GM crop can be placed on the market and thus released into the environment. This is a different process compared with confined field trials. During the commercial release the risks are identified and judged to be negligible or manageable, hence no measures are in place to limit the exposure of the environment to the GM plant. Only the coexistence of GM cropsis in question.

Coexistence of GM crops and environmental monitoring of GMOs in the Slovak Republic: The coexistence control and legal and precautionary measures concerning GM and non-GM agricultural crops in Slovakia are based on Commission Recommendations No. 2003/556/EC of 23 July 2003 on guidelines for the development of national strategies and best practices to ensure the coexistence of genetically modified crops with conventional and organic farming (the new Commission Recommendation No. 2010/C 200/01 of 13 July 2010 on guidelines for the development of national coexistence measures to avoid the unintended presence of GMOs in conventional and organic crops).

On this base, the national Act No. 184/2006 of 16 March 2006 was established on the regulation of the GM plants cultivation in agriculture as well as the Decree of Ministry of Agriculture No. 69/2007 of 14 August 2007 with technical rules for the cultivation of GM plants in agriculture.

This Decree governs the details of technical measures associated with the cultivation and handling of genetically modified crops, specialised plans for the cultivation of modified crops, minimum isolation distances and training courses in the handling of modified crops.

The minimum isolation distances for the cultivation of modified crops in places where crops of the same botanical species, which are not genetically modified, are cultivated.

Minimum isolation distances for GM crops cultivated using conventional farming methods are 200 m for maize, 400 m for rapeseed, 50 m for sugar beet, and 20 m for potatoes.

Minimum isolation distances for GM crops cultivated using organic farming methods are 300 m for maize, 600 m for rapeseed, 50 m for sugar beet, and 20 m for potatoes.

Crop barrier shall means an area sown with one botanical species which is not genetically modified and has a minimum width of six rows for maize and six meters for rape.

For maize, one row of the crop barrier shall substitute for two meters of the isolation distance given in the annex and, for rape, one meter of crop barrier shall substitute for two meters of the isolation distance. Growers shall harvest crop barriers at the same time as the produce from the modified crops.

National competences

The Central Control and Testing Institute of Agriculture in Bratislava (CCTIA) executed activities and competences regarding the Act No. 184/2006 and the Decree of Ministry of Agriculture No. 69/2007.

The Department of Molecular Biology of the CCTIA is a reference laboratory for the control of coexistence and executes the detection, identification, quantification, and evaluation of GM admixtures in harvested crops in non-GM fields. The inspection of GM fields and neighbouring conventional maize fields (including field characteristics as distances, areas, flowering synchronicity, prevailing wind, etc.) and the sampling of harvested crops are ensured, by the seed inspectors of the CCTIA. Department of Molecular Biology NRL is a member of the European Network of GMO Laboratories, member of Biosafety Clearing House Network of LMO detection and identification laboratories, and it also cooperates with the European Coexistence Bureau (ECoB) within the Technical Working Groups for Maize. The Commission has set up the European Coexistence Bureau (ECoB), located at the Institute for Prospective Technological Studies (IPTS) of the Commission' Joint Research Centre in Seville (Spain), whose purpose is to develop technical reference documents for the best practices to achieve coexistence. The reference documents will be elaborated in Technical Working Groups (TWG) composed of national experts, and will

provide Member States with non-binding guidelines for technical coexistence measures.

The activity of the TWG for maize started in October 2008. TWG consists of technical experts nominated by interested Member States. In collaboration with TWG, the ECoB published The Best Practice Documents for coexistence of genetically modified crops with conventional and organic farming, 1. Maize crop production (BPD) in 2010.

The BPD is the first outcome of the collaboration. A reference document for the best practices for the coexistence of GM maize with conventional and organic maize contains a set of consensually agreed, best agricultural management practices that will ensure coexistence, while maintaining economic and agronomic conditions on the farm.

The BPD contains nine chapters e.g. Maize Cultivation in European Union; Review of the available information on management of adventitious GM presence in maize crop production; Best practices for coexistence measures in maize crop production, etc.

The chapter Best practices for coexistence measures in maize crop production describes the rules of the best agricultural management practices e.g. Best practices for seed purity, for seed driller management, for reduction of cross-pollination from GM fields, for harvester management, for dryer management, for transport and for storage (Czarnak-Klos & Rodriguez-Cerezo 2010). The observance of these rules guarantees the coexistence of GM maize with conventional and organic maize.

The activity of ECoB and TWG concerning coexistence continues. The ECoB together with TWG collect the data for the preparation of the second document: Monitoring efficiency of coexistence measures in maize crop production.

CCTIA is also a member of the Network Group for the Exchange and Coordination of Information (COEX-NET). The aim of COEX-NET, which involves representatives from Member States administrations in charge of co-existence, is to foster the exchange of information on the results of scientific studies as well as on the best practices developed within national strategies for coexistence among the Member States and the Commission.

Environmental monitoring. Since after its commercial release a GM plant is free to be grown on very large areas, scale-related unanticipated effects on the environment as possible. And since GMOs are living organisms, they interact with their environment and are subject to ecological laws and processes, possibly resulting in unpredictable effects and behaviour of the GMO following its release. In order to assess the impact of the identified risks of a GMO on the environment, identify unanticipated effects and evaluate the agronomic performance of the GMO, post-release monitoring is performed. Monitoring can be defined as "a procedure that involves the systematic measurement of selected variables and processes that may be affected by a given practice" (FAO 2005). The results of such monitoring programmes has to be used to formulate the additional precautions, influence the maintenance, renewal or withdrawal of the approval for a GMO, and they can be fed back into the risk assessment procedure. The release of a GMO could have impacts on the environment at a variety of levels, from single cells to organisms, populations, communities, and ecosystems. Due to the variance inherent to all life and ecosystems, the effects of GMOs may be difficult to predict in a spatial and temporal manner; they may appear immediately or only after long time spans, and may impact only on the initial site of the release or over wide distances and different ecological compartments. Variations will be observed between farming systems, crop types, and the environmental contexts. It is therefore recommended to design the monitoring plans for GMOs on the case-by-case basis, taking into account all the relevant information regarding the individual GMO and the receiving local environment. The choice and establishment of reliable monitoring indicators, which will allow the detection and quantification of adverse effects caused by the release of the GMO and that are based on specific protection targets, are crucial in this respect.

MATERIAL AND METHODS

The sampling procedures for the coexistence control and testing were done according to ISTA rules and in line with the EC Recommendation No. 2004/787/EC, on which basis was developed the CCTIA sampling procedure No. 7/2006 for the sampling of plant material from the area of GMOs cultivation for the estimation of GMOs in the neighbouring fields with non-GMO crops. The procedure is based on the sampling of harvested products from the individual neighbouring fields using standardised protocols (Tatarova 2009).

Analytical and control samples with minimum 3000 maize grains were taken out from an appropriate number of composite samples and used for the testing of the mean level of GMOs contamination.

Event-specific real-time PCR validated method according to EU-RL GMFF and/or construct-specific real-time PCR method according to EN ISO 21570, EN ISO 21569 and CTAB method for DNA extraction according to EN ISO 21571 were used for maize MON 810 detection and quantification. The maize samples were ground using LM 3303 laboratory mill, at least 100 g of the individual samples were homogenised, and 2 g of each sample were incubated at 60°C for 1 h in 10 ml of CTAB lysis buffer with proteinase K, followed by chloroform/isopropanol treatment and ethanol precipitation of DNA. The DNA was purified using Promega Wizard or JetQuick spin columns and dissolved in TE buffer. DNA samples were quantified using UV-VIS spectrophotometer and agarose electrophoresis, 200 ng of DNA sample per 25 µl PCR reaction volume was used. For construct-specific real-time PCR method MON 810 target sequence detection primers MON 810 2-5'sequence 5'-gAT gCC TTC TCC CTA gTg TTg A-3' and MON 810 2-3' sequence 5'-ggA TgC ACT CgT TgA TgT TTg-3' and TaqMan labelled probe MON 810-Taq sequence 5'-FAM- AgA TAC CAA gCg gCC ATg gAC AAC AA-TAMRA-3' were used. For maize reference sequence detection primers SSIIb 1-5 sequence 5'-CTC CCA ATC CTT TgA CAT CTg C-3' and SSIIb 1-3' sequence 5'-TCg ATT TCT CTC TTg gTg ACA gg-3' and TaqMan labelled probe SSIIb-Taq sequence 5'-FAM-AgC AAA gTC AgA gCg CTg CAA TgC A-TAMRA-3' were used. Real-time PCR testing was performed using ABI7900 HT System. IRMM maize powder certified reference material ERM-BF413 for MON 810 (5, 2, 1, 0.5, and 0.1% in mass fraction %) and ERM-AD413 Plasmid DNA fragments of MON 810 maize and ΔCt procedure and/or absolute quantification in three repetitions for each analysed sample were used.

For event-specific real-time PCR of MON 810 target sequence detection were used primers ZM1-F sequence 5'-TTg gAC TAg AAA TCT CgT gCT gA-3' and ZM1-R sequence 5'-gCT ACA TAg ggA gCC TTg TCC T-3' and TaqMan labelled probe ZM1 sequence 5'-FAM -CAA TCC ACA CAA ACg CAC gCg TA-TAMRA-3'. For maize reference sequence detection were used primers Mail-F1 sequence 5'-TCg AAg gAC gAA ggA CTC TAA CgT-3' and Mail-R1 sequence 5'-gCC ACC TTC CTT TTC CAC TAT CTT-3' and TaqMan labelled probe Mail-S2 sequence 5'-FAM-AAC ATC CTT TgC CAT TgC CCA gC-TAMRA P-3'.

All the liquid handling operations were performed using robotic epMotion Liquid Handling System (Eppendorf). Quantitative results were expressed in mass fractions, in DNA copy number, and in the relative number of GM maize grains. Consumer and producer risks (α-risk and β-risk) were analysed according to (Remund *et al.* 2001).

For the detection of other GM events and unauthorised GMOs for environmental monitoring of GM plants, similar DNA extraction and event or construct specific real-time PCR methods were used according to EU-RL GMFF with specific primers and probes and real-time PCR screening methods for the detection of genetic elements which are characteristic for appropriate GM events and/or unauthorised GMOs, as published by EURL-GMFF and ENGL (2010).

The technical guidance on the sampling of higher plants which are grown in the field trials were done by Slovak Inspectorate of Environment according to the rules of the analytical laboratory of Central Controlling and Testing Institute in Agriculture, Bratislava, Slovak Republic..

The sampling sites are plots of farmland and the test site is usually divided into small plots for cultivation with buffer zones between them. Samples are taken from the individual plots which are designed to satisfy the statistical criteria of data analysis. The number of samples taken should be sufficient to characterise all different parts of the site. This will vary depending on the site size. The plant sample is a segment of actively growing plant leaf near the top of the plant (earleaf). The precision of a sample-based estimate increases directly with its size. Prior to a sample drawing, the authorised person should calculate the size required to achieve the given precision level:

– from 10 to 100 plants on the selected area, the incriminating sample should be taken from each plant,
– from 100 to 800 plants on the selected area (the estimation), the incriminating sample should be taken from 80 plants,
– from 800 to 1000 plants on the selected area (the estimation), the incriminating sample should be taken from 80 to 100 plants,
– where the size of the selected area is over 0.1 ha, the incriminating sample should be taken from 100 plants according the "W" scheme.

The random rows for the needed number increment samples should be chosen, the number of rows is given as follows:

Number of rows on the site	Number randomly chosen row
1–3	all
4–10	3
11–50	5
51–100	7
> 100	1 from every next 10 rows

The number of samples taken to check the contamination will depend on the level of contamination suspected or the level of statistical assurance that is required. Examples of the required sizes of samples are:

100 plants give a 95% confidence of detecting a 3% contamination level;

200 plants give a 95% confidence of detecting a 1.5% contamination level;

300 plants give a 95% confidence of detecting a 1% contamination level;

3000 plants give a 95% confidence of detecting a 0.1% contamination level.

The samples intended for laboratory testing have to be submitted to the official laboratory without delay.

RESULTS AND DISCUSSION

For the analysis of the required level of GM and non-GM coexistence in Slovakia, lots of samples were harvested together from 3 neighbouring conventional maize fields in 2006, from 40 neighbouring conventional maize fields in 2007, from 40 neighbouring conventional maize fields in 2008, from 30 neighbouring conventional maize fields in 2009, from 23 neighbouring conventional maize fields in 2010, and from 23 neighbouring conventional maize fields in 2011. The individually taken samples and adequate numbers of bulk, laboratory, and control samples were prepared and tested for the presence of MON 810 maize (Horvath & Feketova 2008).

The distances between GM maize fields and conventional maize fields altered between 200 and 3000 m. Maize buffer zones of 35, 80 or 100 rows wide were applied around the individual GM maize fields and 200 m wide minimum isolation distances from the nearest conventional maize fields were respected. Minimum isolation distance between GM and conventional maize fields according to Decree No 69/2007 is 200 m, or corresponding buffer rows number (1 row is equal to 2 m distance). Testing of GM contamination in neighbouring conventional maize products was realised after the harvest of neighbouring maize fields (i.e. the measured GM admixtures in harvested crops were caused by all contamination factors as crosspollination, contamination by sowing, harvesting, transport, storage, etc.).

No detectable GM contamination of conventional maize fields was observed in 2006. GM contamination of neighbouring non-GM maize fields was detected together in 7 fields (~ 17% of tested fields) in 2007, in 19 fields (~ 47%) in 2008, in 17 fields (~ 57%) in 2009, in 4 fields (~ 17%) in 2010 and in 19 fields (~ 26%) in 2011. The GM contamination of neighbouring non-GM maize fields in the period 2006–2011 was found to be between 0.01% and 0.83% (w/w) and the mean contamination level was 0.07%. Flowering of both types of fields (GM and non-GM) was assumed as synchronous (< 10 days).

The quality of the sampling procedure was tested according to quantitative testing of independently prepared control samples. Both pairs of results were in good conformity and inside the expanded uncertainty interval (k = 2) (Horvath *et al.* 2007).

The most distant GM contamination of the neighbouring non-GM maize field was at 750 m (0.03%), therefore the distance extrapolated to 0% of GM contamination is about 1000 m.

Within 2006–2011, a relationship was found between the increased ratio and level and decreased isolation distances, which corresponds with common expectations. For the isolation distances between 200 and 400 m, very high GM contamination was observed and this data are not statistically consistent with the data for other distances.

Our explanation is that up to these distances (200–400 m), the agrotechnical activities are probably realised concurrently and use the same machinery which causes a higher probability of contamination (GM admixtures in harvested crops from the neighbouring non-GM maize fields are caused by combined factors such as crosspollination, contamination by sowing machines, harvesting machines, by transport, storage, etc.). This assumption is confirmed by our further finding, i.e. that the contamination of the neighbouring non-GM maize fields differs in the dependence on the owners of both field types – in the case that the owners of GM fields and non-GM neighbouring fields are identical, the contamination level of non-GM fields is usually higher.

Consumer and producer risks (α-risk and β-risk) were analysed for minimum isolation distances

(200 and 300 m) in conditions of actual GMO limits (0.9%), determined GM admixtures, and the testing procedures used. The calculated values give good results for conventional maize production, i.e. for 0.9% GMO limit, isolation distances 200 m, and approximately 0.2% GMO level of impurities, which supports the current production practice. The obtained value of consumer β-risk is 4.8% (or better) and that of producer α-risk 0%, and they are sufficient for conventional maize production. These values confirmed the optimum and sufficient value of minimum isolation distances (200 m) in Slovakia according to the Decree of Ministry of Agriculture No. 69/2007.

The ecological aspects of the biotech agricultural activities are yet unknown. There is limited experience in Slovakia as there is the need to develop technical capacity for the development and implementation of monitoring frameworks. It is necessary to develop the Slovak national strategy for monitoring the adverse effects that were identified but not addressed in the risk assessment, for monitoring the unanticipated adverse effects that were not identified in the risk assessment, and for the detection of GMOs whose release was not authorised, e.g. GMOs that were unintentionally released or that entered the country through illegal transboundary movements. The only data are available due to the environmental inspections of the Slovak Inspectorate of Environment (SIE). SIE is the state authority for the supervision over the use of genetic technologies and genetically modified organisms (state surveillance), and it performs the state surveillance and imposes fines for administrative infractions and also deals with delicts. The monitoring is not the standard inspection procedure in general, but achieving of the monitoring data has been enlarged by several pieces of legislation as there is a goal around the biosafety area to make the enforcement proactive with the use of information and intelligence instead of working reactive. One of the pieces of the legislation is the monitoring plan obligation. The monitoring plan is a part of the risk management strategy, the document is obligatory for the user of GMOs and the standard inspection involved in its keeping. The objective of the monitoring plan is to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMOs or its use in the risk assessment is correct, and to identify the occurrence of adverse effects of the GMOs or its use which were not identified in the risk assessment. The design of the monitoring plan should be detailed on the case by case basis, take into account the characteristics of the GMOs, its use and scale of use and the range of relevant environmental conditions, incorporate general surveillance for unanticipated adverse effects, provide for case-specific monitoring over a sufficient time period to detect immediate and direct as well as, where appropriate, delayed and indirect effects which have been identified in the risk assessment, and provide for the use of already established routine surveillance practices where appropriate. Standard inspection procedures include the examination and verification of relevant documentation, random sampling testing by accredited laboratory, and taking appropriate measures. The number of samples analysed per year is about 150. All the penalties regarding the illegal use of the GMOs were in the form of fines and administrative sanctions to date. The Act No.151/2002 Coll. on the use of genetic technologies and genetically modified organisms also provides for the confiscation of GMOs or any product thereof used in contravention of the Act and states that such ones shall be destroyed at the expense of the user. The Act No. 300/2005 Coll. Criminal Code also includes penalties that would involve prison sentences. However, since the adoption of the biosafety legislation, no case of serious and wilful misconduct in the illegal use of GMOs has occurred. In the period of 2007–2011, SEI made 1017 controls and only 32 breaking of the law were solved. There were very few events of the illegal use of GM crops (flax, soybean, and maize) which happened because the importers did not recognise that the organisms had been genetically modified. The sources of the illegal GMOs were determined and the cases were dealt with in conformity with EU regulations. These measures were intended to stop the use the GMOs because they were illegal at the time, not because of the identification of some adverse effects. It is because the biotech-crop is the product of modern biotechnologies, being as genetically modified organism under extensive biosafety legal regulation. Legal provisions to regulate biosafety issues exist at every level of the legal frameworks, that means at transnational, regional, national, and subnational levels as well, and the legal frameworks include binding and non-binding international and regional agreements and national laws, regulations and guidelines. The current Slovak *lex*

generalis regulatory framework is set up by the Act No. 151/2002 Coll. on the use of genetic technologies and genetically modified organisms as amended by the Acts No. 587/2004 Coll., No. 77/2005 Coll., No. 100/2008 Coll., No. 515/2008 Coll. and Act No. 117/2010 Coll., and by the implementing Decree No. 399/2005 Coll. amended by the Decree No. 312/2008 Coll. The Act transposes international agreements, EU Directives, and EC Regulations covering the GMO handling, packaging, and transport as well. Each user of GMO must take the necessary measures to ensure that GMOs are handled, packaged and transported under safety conditions in order to avoid the adverse effects on biodiversity conservation and sustainable use.

CONCLUSION

The objective of the above listed analyses was to verify the effectiveness of isolation distances stated by national legislation, technical rules, and production practice to ensure the coexistence of GM maize with conventional and ecological farming and summarise the basic environmental monitoring experience and outcomes in Slovakia.

The obtained values of producer α-risk (0%) and consumer β-risk (4.8%) confirmed he optimal and sufficient values of minimum isolation distance in Slovakia according to the Decree of Ministry of Agriculture No. 69/2007 for conventional maize production within the frame of coexistence with GM maize. These values are sufficient to ensure the contamination level of neighbouring non-GM fields under 0.9%, which is required for the conventional plant products in the EU. The production practices used in Slovakia according to the Act No. 184/2006 and the Decree of Ministry of Agriculture No. 69/2007 are sufficient to ensure the coexistence of GM maize with conventional farming. The data achieved due to the environmental inspections of the Slovak Inspectorate of Environment support the conclusion that the state authority is not aware of any serious illegal cultivation of genetically modified crops in the Slovak Republic.

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