Coll. Antropol. **35** (2011) 4: 1231–1236
Original scientific paper

Monitoring the Presence of Genetically Modified Food on the Market of the Republic of Croatia

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A B S T R A C T

From the beginning of the human race people have been applying different methods to change the genetic material of either plants or animals in order to increase their yield as well as to improve the quality and quantity of food. Genetically modified organism (GMO) means an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. Analysing the presence of GMO in food is done by detecting the presence of either specific DNA sequences inserted in the genome of transgenic organism, or detecting proteins as a result of the expression of the inserted DNA. In this work food testing for the presence of genetically modified organisms was conducted during the period from 2004 to 2007 in the GMO laboratory of the Croatian National Institute of Public Health. According to the regulations, among the samples in which the presence of GMO was detected, all those which had more than 0.9% of GMO content were either rejected from the border or removed from the market, because such GM food has to be appropriately labelled. Among the food samples which were analysed in 2004: 127 (2.37%) of a total of 1226 samples contained more than 0.9% of GMOs; in 2005 there was only one in 512 (0.20%) samples in total; in 2006 there were 4 out of 404 samples (0.99%), and in 2007: 7 of a total of 655 samples (1.07%) had GMO content above the allowed threshold of 0.9%.

Key words: *genetically modified food, health, market, monitoring, Croatia*

Introduction

From the beginning of the human race people have been applying different methods to change the genetic material of either plants or animals in order to increase their yield as well as to improve the quality and quantity of food. This traditional form of genetic manipulation was in fact a selective breeding. People willingly choose seeds of bigger and more resistant plants, cows that give more milk or pigs with more meat. As the global population has increased the problem of suffering from hunger seems to have been the main factors to find new ways of more effective food production in yield and costs. Using biotechnology to create new organisms, for example crops with herbicide tolerance and pest resistance, was the first solution. More recently, it has been tried to develop food with enhanced flavour, nutritive value, longer storage time and reduced toxicity as the aim for consumer benefit.

Genetically modified organism (GMO) means an organism in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination[1–3]. Genetic engineering (or recombinant DNA technology) creates a new combination of inherited material by inserting new DNA molecules constructed in vitro and then transported in host cells via viruses, plasmids or another form of carrier. These new DNA sequences provide new phenotypic characteristics which are capable of multiplication[4] and who will be inherited through next generations[5], where such horizontal gene transfer differs from the usual vertical transfer.

Received for publication June 09, 2010

However, many questions in connection with the use of genetic technology in food production remain open. Some scientists believe there are many reasons for using GM plants in the production of animal feed[6]. Some food and feed are cheaper and safer to produce. Potential benefits are bigger yields due to permanent protection from pests or disease resistance, easier and cheaper protection from weeds, better herbicide and low temperature tolerance, better nutritive value (higher content of amino acids, vitamins and others) and less consumption of pesticides and fertilizers, today's main source of pollution)[7]. In 1996, there were 1.7 million hectares (ha) cultivated surfaces with genetically modified (GM) plants in the world. In 1997 there were 11 million hectares and in 1998 were 28 millions. In 2005 more than 90 million hectares were cultivated with GM plants in 21 countries around the world, and we can say that cultivation of GM crops increases continuously by approximately 10% *per* year[8].

On a national level, there is a legal framework for GMO use. Legislative provisions are regulated by two acts: Act on Genetically Modified Organisms (Official Gazette 70/05) and Food Act (Official Gazette 46/07)[1,9]. Also important is the Regulation on the minimum threshold for genetically modified organisms in products below which the products placed on the market do not have to be labelled as products containing genetically modified organisms (Official Gazette 92/08)[10]. Therein it stipulates that products containing adventitious or technically unavoidable traces of the authorised GMOs in the level of 0.9% or less shall not be labelled as products containing genetically modified organisms.

Monitoring for the presence of GMOs in food on the market was conducted according to the above Regulation.

Different countries have different GMO labelling regulations. The threshold goes from 0.9% in the European Union to 5% in Japan[11].

Materials and Methods

Materials

The data on the presence of genetically modified food in Croatia are collected by the Laboratory for GMO detection, Croatian National Institute of Public Health for the period from 2004 to 2007. During the entire test period 2797 food samples were taken.

Sampling procedure was according to Ordinance on sampling procedure and methods for the analysis and super-analysis of food and objects of common use (Official gazette 58/98)[12] and according to the Commission Recommendation 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[13].

Among the analysed food samples the most common were soybean and corn as well as their products such as: soybean flour, soybean milk, soy oil, soy proteins, soy lecithin, soy sauce, corn, corn flour, corn grits, muesli, spice mixtures and other raw materials for further processing in the food industry and different foods like salads, dressings that contain or are produced from soybean and/or corn.

The request for testing food samples on the presence of genetically modified organisms came from the sanitary inspection and private parties. On the behalf of sanitary inspection there were 1524 requests (54.49%), and 1273 (45.51%) of private parties.

Methods

Monitoring the presence of GMO in food and/or its derivates is done by the presence of DNA or proteins, which occurs as a result of genetic modification. Performed tests were based on polymerase chain reaction (PCR technique) through which we determine the presence of DNA that has been inserted in plant genome or by determining the presence of proteins as a result of inserted gene expression by immunological reaction (ELISA)[14–18].

For monitoring the presence of GMO in food through DNA method we used the following kits: GMO Screen 35 S/NOS, GMO Quant IPC *35S* Screen Soy, and GMO Quant IPC 35S Screen Corn (Eurofins GeneScan GmbH, Germany)[18]. GMO Screen 35 S/NOS kit is used for detecting the most common regulatory DNA sequences (CaMV 35S promoter and *nos* terminator) in GMOs. The first step is DNA isolation from the sample. There are several methods for isolation, depending on the type of matrix, so that we can get a DNA of satisfactory purity and quantity. Standard procedure included DNA extraction using GENE*Spin* kit for isolation of high-quality DNA from food, feed and bacteria (Eurofins GeneScan GmbH, Germany) according to the manufacturer recommendation. The second step includes testing of particular DNA sequence through amplification by using polymerase chain reaction (PCR technique). PCR screening tests are designed to amplify CaMV 35 S-promoter and *nos*-terminator sequences. For this step GMO*Screen* 35 S/NOS kit (Eurofins GeneScan GmbH, Germany) for qualitative detection of GMO varieties in food, feed and seed was used according to the manufacturer recommendation. The products of amplification were separated by agarose gel electrophoresis and the size of amplicons was determined by comparison with the size of DNA length standard and/or with the amplification product of positive control. Instruments which were used in this phase were: Eppendorf Mastercycler gradient, BIO-RAD horizontal electrophoresis, and BIO-RAD GelDoc 2000.

GMO*Quant* IPC *35S* Screen Soy kit for quantitation of the CaMV 35S promoter in soy samples (Eurofins GeneScan GmbH, Germany) was used to determine the quantity of 35S promoter (regulatory nucleic sequence) in GMOs. There are two master mixes in the kit: the first one is 35S soybean master mix, which contains all components for specific detection of 35S promoter sequence from CaMV virus present in transgenic lines of soybean, and the second is soybean referent master mix used for

detection of soybean DNA, which is derived from both soybean lines, conventional and transgenic. The kit contains an exogenous system of internal control (IPC), which is used to distinguish the real targeted negative reaction from system inhibition. The kit is based on TaqMan chemistry and the quantification is done by using standard curve method applying the iCycler iQ™ Real-Time PCR Detection System software Version 3.0A according to the manual instructions (the iCycler iQ™ Real-Time Detection System Resource Guide) (20). Standard curve must be generated within each system using calibrated DNA standards. GMO*Quant* IPC 35S Screen Corn kit has the same procedure but adapted for corn detection. Quantification is done on real-time PCR instrument from BIORAD (iCycler).

For monitoring the presence of GMO in food through protein method we used the following kits: TraitChek Dipsticks (SDI, Strategic Diagnostics, Inc., USA) and GMOChek ELISA Tests (SDI, Strategic Diagnostics, Inc., USA). First there are TraitChek Dipsticks (SDI, Strategic Diagnostics, Inc., U.S.A.) which use immobilized double antibodies specific for the expressed protein attached to the reactive medium for colour and built in the nitrocellulose track. When this medium is putt in a plastic Eppendorf tube containing plant tissue extract where transgenic protein is hiding, a sandwich of antibodies is created. Positive reaction results in colouring of the reagent attached to the final antibody. Presence of only one (control) line on the membrane indicates a negative sample, and the presence of two lines indicates a positive result. Fast and economical, results are obtainable in five to ten minutes. Then there is GMOChek ELISA (Enzyme-Linked Immune Sorbent Assay) Test (SDI, Strategic Diagnostics, Inc., U.S.A.), which detects the presence of specific proteins (by specific bonding of antibody and antigen). We detected or measured the amount of target protein in sample that can contain many different proteins. We used one antibody to attach to the specific antibody (bounding antibody), another antibody to boost detection (detecting antibody) and the antibody to which the enzyme is attached from the substrate to create colour that can be visualized and quantified with an optic reader comparing with the standard positive control. Target protein is found in the sandwich between the bounding antibody and detecting antibody. Method is semi-quantitative, very sensitive and economic. Instrument that was used for ELISA tests is Columbus Washer, Tecan (Tecan Austria GmbH, Salzburg, Austria).

Results

During the period from 2004 to 2007, 2797 food samples were analysed. Most of the samples (1226) were analysed in 2004. That was the beginning of routine official control due to GMO laboratory establishment. During 2005, 512 food samples were analysed, while the smallest number was tested in 2006, namely 404 samples. In 2007, the number of samples increased to 655. The food samples that were analysed were basically consisted or produced from soybean and/or corn. Requests for testing food samples for the presence of GMOs came from the sanitary inspection and private parties. On the behalf of sanitary inspection there were 1524 requests (54.49%), while 1273 (45.51%) on behalf of private parties. During 2004 there were 733 demands (59.79%) for analysis by the sanitary inspection, as opposed to 493 (40.21%) requests by private parties. In 2005 private parties demanded testing in 264 (51.56%) cases, while the inspection in 248 cases (48.43%), which is considerably less in comparison with 2004. In 2006 there was an increase in the rate of private party requests (232 requests or 57.43%), as opposed to the 172 requests from the sanitary inspection (42.57%). In 2007, there was an increased demand for testing by the sanitary inspection again, namely 371 requests (56.64%), in comparison with the private parties' rate of 284 (43.36%).

TABLE 1
NUMBER OF FOOD SAMPLES ANALYSED FOR THE PRESENCE OF GMOS ACCORDING TO SUBMITTER AND YEAR

	2004 N %	2005 N %	2006 N %	2007 N %
Sanitary inspection	733 (59.79%)	248 (48.44%)	172 (42.57%)	371 (56.64%)
Private parties	493 (40.21%)	264 (51.56%)	232 (57.43%)	284 (43.36%)
Total	1226	512	404	655

TABLE 2
PRESENCE (+) OR ABSENCE (–) OF GMO IN FOOD SAMPLES ANALYSED *PER* YEAR FOR THE PERIOD FROM 2004 TO 2007

	2004 N %	2005 N %	2006 N %	2007 N %
Negative (–) samples	1131 92.25%	502 98.05%	394 97.52%	644 98.32%
Positive (+) samples	95 7.75%	10 1.95%	10 2.48%	11 1.68%
Total	1226	512	404	655

TABLE 3
FOOD SAMPLES WITH GMO CONTENT ABOVE THE ACCEPTED THRESHOLD (>0.9%) *PER* YEAR AND IN RELATION TO THE TOTAL NUMBER OF FOOD SAMPLES ANALYSED

	2004 N %	2005 N %	2006 N %	2007 N %
Samples with GMO content >0.9%	29 2.37%	1 0.20%	4 0.99%	7 1.07%
Total number of analysed food samples	1226	512	404	655

TABLE 4
FOOD SAMPLES WITH GMO CONTENT ABOVE THE ACCEPTED THRESHOLD (>0.9%) *PER* YEAR AND IN RELATION TO THE POSITIVE SAMPLES

	2004 N %	2005 N %	2006 N %	2007 N %
Samples with GMO content >0.9%	29 30.52%	1 10%	4 4%	7 63.64%
Positive samples	95	10	10	11

Discussion

Analytical results show that there is very little presence of genetically modified food on the market in Croatia. Foods containing GMOs in a quantity above the allowed threshold (0.9%) came mostly from the border inspection and were therefore not allowed to enter the Croatian market. In 2006, most positive results were found in samples like spice mixtures and other raw materials (based on soybean) for further processing in the food industry (meat, bakery industry) originating from import. The submitters of requests for testing food samples for the presence of genetically modified organisms were the sanitary inspection and private parties. In the whole period significantly more requests came from the sanitary inspection, except in 2005, when private parties submitted more requests.

To compare the results of our analyses with the available data from other European countries, (data provided by the Croatian Food Agency), we present data from Austria, Sweden, Ireland and Hungary.

Austrian data showed results from 2001 to 2007 (21). In 2001 there were 153 food samples, which was significantly less then in our case, while from 2002 to 2007 the number of food samples varied from 212 to 251, which more or less coincides with our sample size. When we look at the results *per* year, the percentage of positive results varied from 19.20% in 2003 to 0% in 2006. If we exclude year 2003, the percentage of negative results varied from 88.45% to 100%. Regarding samples with GMO content above 0.9%, in 2001 that percentage was 2.6%, while from 2002 to 2004 it varied from 0.4% to 0.8%. Between 2005 and 2007 the same rate was 0%. In comparison with our results from the Croatian market, we can say that the percentage of negative food samples varied much more significantly in Austria, while the percentage of samples with GMO content above 0.9% varied from 2.6% to 0%, as it did in Croatia (between 2.37% and 0.20%).

The data from Sweden[22] is available for years 2000, 2002 and 2006. The number of food samples analysed in 2000 was 101; in 2002, 44; and in 2006, 96, which is significantly less than in our country. The percentage of positive results varied from 27.08% to 36.36%. There were 9.9% of samples with GMO content above the threshold (0.9%) in 2000, in 2002 there were none, and in 2006 the percentage was 3.13%.

Food Safety Authority of Ireland (FSAI) is the competent body for GMO analysis in food. The available data of testing GM food cover the period from 2000 to 2006[23]. In total, there were 333 samples of food analysed, 75 of which (23.52%) tested positive for the presence of some traces of GMOs. However, none of the samples contained GMOs above the threshold, except for some GM rice which was not authorized to be placed on the market and was therefore later removed from it. That GM rice was originally from China and was later found in some other countries of the European Union, the information spread between the countries through European Rapid Alert System for Food and Feed (RASFF). During 2007 97 food samples containing rice, soybean and corn were analysed. None tested positive for the presence of GMOs above the established threshold.

In Hungary, during 2005 there were 169 food samples analysed, 58 of which (34.32%) tested positive for the presence of GMOs; only 5 samples thereof (2.96%) contained GMOs in the amount above the allowed threshold[24].

Conclusion

This study showed the results from the analyses of food samples tested for the presence of genetically modified organisms during the period from 2004 to 2007 performed at the GMO laboratory, Croatian National Institute of Public Health. Throughout this period 2797 food samples were analysed. The laboratory analysed an average of some 700 food samples *per* year, mostly food consisting of or produced from soybean and/or corn. The number of requests for analysis from both the sanitary inspection and private parties was equally distributed,

namely 1524 (54.49%) and 1273 (45.51%) requests, respectively. The overall rate of food samples analysed for the presence of GMOs during 2006 was 2.37% of the samples with the GMO content above the threshold (0.9%). In 2005 there was only one such sample, while in 2006, 4 samples with the GMO content above the allowed threshold were discovered. In 2007 there were 7 samples (1.07%) that contained more than 0.9% of genetically modified organisms. In comparison with the positive samples, the rates of samples with GMO content above the threshold were: 30.52% (29 samples) in 2004, 10% (1 sample) in 2005, 4% (4 samples) in 2006 and 63.6% (7 samples) in 2007.

The complete monitoring and screening of the Croatian market, as well as the border sanitary inspection of the suspected food samples was quite comprehensive and efficient. Comparable with other countries of EU, in Croatia can also be noticed the decreasing of the positive samples during the time as the laboratory and the whole system of official control, including relevant regulations has been established.

Acknowledgements

In this article we have shown the results of food sample analysis of the presence of genetically modified organisms in the GMO laboratory at the Croatian National Institute of Public Health. The laboratory was named GMO Quantification and Risk Assessment Unit which was authorized by the Ministry of Health in 2004 and accredited in 2009 in accordance with HRN EN ISO/IEC 17025:2007 by the Croatian Accreditation Agency.

REFERENCES

1. Act on Genetically Modified Organisms (Official Gazette 70/2005) accessed 5.02.2009. Available from: URL: http://narodne-novine.nn.hr/clanci/sluzbeni/ 2005_06_70_1372.html — 2. BOSNIĆ M, ANTUNOVIĆ B, FULGOSI H, CAPAK K, ZUPAN I, ŽAFRAN NOVAK J, LJUBENKO MIHELJ S: »Framework for the development of national biosafety strategy« (Zagreb, 2005). — 3. CELEC P, KUKUCKOVÁ M, RENCZÉSOVÁ V, NATARAJAN S, PÁLFFY R, GARDLÍK R, HODOSY J, BEHULIAK M, VLKOVÁ B, MINÁRIK G, SZEMES T, STUCHLÍK S, TURNA J, Biomed Pharmacother, 59 (2005) 531. — 4. CAPAK K, Medix, 53 (2004) 23. — 5. DELIĆ V, Molecular basis for horizontal gene transfer. In: RICHTER B, DELIĆ V, VUJAKLIJA D, TAMBIĆ-ANDRIJAŠEVIČ A, PAVELIĆ K, KOVAČ Z et al. (Eds) Horizontal spread of genes and human health (Croatian Academy of Medical Sciences, Zagreb, 2000). — 6. BORLAUG NE, Plant Physiol, 124 (2000) 487. — 7. PEJIĆ I, KEREŠA S, GRBEŠA D, Krmiva, 1(2000) 3. — 8. JAMES C, Global Status of Commercialized Transgenic Crops: 2003. Preview. ISAAA Briefs No. 30. (ISAAA, Ithaca, New York, 2003). — 9. Food Act (Official Gazette 46/2007) accessed 5.02. 2009. Available from: URL: http://narodne-novine.nn.hr/clanci/sluzbeni/ 2007_05_46_ 1554. html — 10. Regulation of the Government of the Republic of Croatia on the minimum threshold for GMOs in products below which the products placed on the market shall not have to be labelled as products containing GMOs (Official Gazette 92/2008). accessed 5.02.2009. Available from: URL: http://narodne-novine.nn.hr/clanci/sluzbeni/2008_ 08_92_2913.html — 11. DONG W, YANG L, SHEN K, KIM B, KLETER GA, MARVIN HJ, GUO R, LIANG W, ZHANG D, BMC Bioinformatics, 9 (2008) 260. — 12. Ordinance on sampling procedure and methods for the analysis and super-analysis of food and objects of common use (Official gazette 58/98). accessed 5.02.2009. Available from: URL: http://narodne-novine.nn.hr/clanci/ sluzbeni/1998_04_58_709.html — 13. Commission Recommendation 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. — 14. MICHELINI E, SIMONI P, CEVENINI L, MEZZANOTTE L, RODA A, Anal Bioanal Chem, 392 (2008) 355. — 15. URBANEK-KARŁOWSKA B, SAWILSKA-RAUTENSTRAUCH D, JEDRA M, BADOWSKI P, Rocz Panstw Zakl Hig, 54 (2003) 345. — 16. MIRAGLIA M, BERDAL KG, BRERA C, CORBISIER P, HOLST-JENSEN A, KOK EJ, MARVIN HJ, SCHIMMEL H, RENTSCH J, VAN RIE JP, ZAGON J, Food Chem Toxicol, 42 (2004) 1157. — 17. AHMED FE, Trends Biotechnol, 20 (2002) 215. — 18. MARMIROLI N, MAESTRI E, GULLÌ M, MALCEVSCHI A, PEANO C, BORDONI R, DE BELLIS G, Anal Bioanal Chem, 392 (2008) 369. — 19. AKIYAMA H, NAKAMURA F, YAMADA C, NAKAMURA K, NAKAJIMA O, KAWAKAMI H, HARIKAI N, FURUI S, KITTA K, TESHIMA R, Biol Pharm Bull, 32 (2009) 1824. — 20. The iCycler iQ™ Real-Time PCR Detection System Resource Guide, Bio-Rad Laboratories, Inc., USA. Available from: URL: http://www.bio-rad.com/ — 21. GMO Results Austria, Österreichishe Agentur für Gesundheit und Ernährungssicherheit GmbH, Wien, Austria; 2008. Available from: URL: http://www.ages.at/ — 22. KUROWSKA Z. Undersökning av tillämpning av lagstiftningen rörande genetiskt modifierade livsmedel (GMO). GMO-PROJEKT, LIVSMEDELSVERKET, Sverige, 2007. — 23. GM Food Survey 2007. Food Safety Information Centre, Food Safety Authority of Ireland, Dublin 1, Ireland; 2008 Feb. Available from: URL: http://www.fsai.ie/ — 24. Final Report of a Mission Carried out in Hungary (22 — 26 may 2006) in order to evaluate official control systems for food, feed and seed consisting of or produced from Genetically Modified Organisms (GMOs). European Commission, Health and Consumer Protection Directorate – General. 2006.

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PRAĆENJE PRISUTNOSTI GENETIČKI PREINAČENE HRANE NA TRŽIŠTU REPUBLIKE HRVATSKE

S A Ž E T A K

Od početka ljudske vrste ljudi su primjenjivali različite metode za promjenu genetskog materijala bilo biljaka ili životinja kako bi se povećala njihov prinos, kao i za poboljšanje kvalitete i količine hrane. Genetski modificirani organizam (GMO) je organizam u kojem je genetski materijal izmijenjen na način koji se ne pojavljuje prirodnim putem parenjem i/ili prirodnom rekombinacijom. Analiziranje prisutnosti GMO u hrani se radi otkrivanjem prisutnosti ili specifičnih DNA sekvenci umetnutih u genom transgeničnih organizama, ili otkrivanje proteina kao rezultat ekspresije umetnutih DNA. U ovom radu testiranje hrane na prisutnost genetski modificiranih organizama je provedeno u razdoblju 2004–2007 u GMO laboratoriju Hrvatskog zavoda za javno zdravstvo. Prema propisima, među uzorcima u kojima je prisutnost GMO-a otkrivena, svi oni s više s od 0,9% GMO-sadržaja ili su odbijeni na granici ili uklonjeni iz tržišta, jer takva GM hrana mora biti na odgovarajući način označene. Među uzorcima hrane koji su analizirani u 2004: 127 (2,37%) od ukupno 1226 uzoraka sadržavalo je više od 0,9% GMO-a. U 2005 bio je samo jedan od 512 (0,20%) ukupnih uzoraka, u 2006 su 4 od 404 uzoraka (0,99%), te u 2007: 7 od ukupno 655 uzoraka (1,07%) je imalo GMO sadržaj iznad dopuštenog praga od 0,9%.