PROJECT OF IMPLEMENTATION OF
NATIONAL BIOSAFETY FRAMEWORK FOR TURKEY

TECHNICAL GUIDELINES FOR THE RISK ASSESMENT OF GENETICALLY ENGINEERING CROPS AND DERIVED FOOD AND FEED

REPUBLIC OF TURKEY
MINISTRY OF FOOD AGRICULTURE
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FOREWORD

In an effort to protect the environment and biodiversity against the potential risks of genetically modified organisms, the Cartagena Protocol on Biosafety -the first international document that is of a binding nature in this area- took effect around the world on 11 September 2003 and in Turkey on 24 January 2004. The protocol seeks to ensure an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, also considering its risks to human health, and specifically focusing on transboundary movements.

The Biosafety Law, which was prepared by taking the Cartagena Protocol, the EU Acquis, the situation and needs of the country into consideration, was approved by the Turkish Grand National Assembly on 18 March 2010, published in the Official Gazette no. 27533 of 26 March 2010 and entered into force on 26 September 2010. The Biosafety Law aims to establish and implement a biosafety system in order to prevent the potential risks of the genetically modified organisms and products thereof obtained through modern biotechnological means within the context of scientific and technological advancements and protect human, animal and plant health; safeguard and ensure the sustainable use of the environment and biological diversity and to determine the procedures and principles governing the control, regulation and monitoring of these activities.

Within the scope of the Biosafety Law the “Regulation on the Genetically Modified Organisms and Their Products” and the “Working Principles and Procedures of the Biosafety Board and Committees” were published on the Official Gazette No. 27671 of 13 August 2010.

In order to develop the capacity needed for ensuring biosafety within the scope of the national and international legislations, the project titled “Support for

the Implementation of the National Biosafety Framework of the Republic of Turkey" was prepared and accepted by the Global Environment Facility (GEF). The project was implemented between 2013 and 2017 under the coordination of the Directorate General of Agricultural Research and Polices (DGARP). Within the scope of the project, five guidelines were prepared by considering the works of national consultants and the contributions of the relevant partners obtained during the workshops, which were conducted at the preparation stages of some of the guidelines. The following guidelines have been developed: "Application Guideline", "Technical Guideline for the Risk Assessment of Genetically Engineering Crops and Derived Food And Feed", "Socio-economic Evaluation Criteria for the Decision-Making Process Regarding GMOs and Products", "Guidelines on Control and Traceability of Genetically Modified Organisms and Products" and "Legal Guideline".

Our General Directorate considers the works conducted for raising public awareness during the project, the documents prepared as outputs of the project and overall project experience significant gains. I hope that these guidelines, which were prepared within the scope of the project, will be useful. I also congratulate and thank everyone who contributed to the project, especially the UNEP-GEF Portfolio Manager (Biosafety) Alex Owusu-BINEY, Project Assistant Birgül GÜNER, Project team consisting of Hilal YÜCE ARSLAN, Ayfer ŞAHİN and Serdar AYDEMİR, national consultants Professor Emine OLHAN, Professor Mustafa Fadıl YILDIRIM, Associate Professor Remziye YILMAZ, Dr. Seval ÜNALAN and Fatih KAYA.

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Project Coordinator

PROJECT OF IMPLEMENTATION OF NATIONAL BIOSAFETY FRAMEWORK FOR TURKEY

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Project Coordinator	:	Dr. Yusuf ARSLAN
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CHAPTER 1

INTRODUCTION

Technical guideline for the risk assessment of genetically modified plants and derived food and feed have prepared under the auspices of the UNEP/GEF National Biosafety Implementation Project for Turkey. Genetically modifications are likely to be detected through the comprehensive comparison of agronomic, phenotypic, molecular, and compositional characteristics of the GM crop and derived food or feed with those of near-isogenic and other conventional non-GM varieties conducted as part of the assessment. Risk assessment should be carried out in a scientifically sound and transparent manner, and can take into account expert advice of, and guidelines developed by relevant international organizations. Before preparation of this guideline, two workshops organized at the international level (15-17th December, 2015) and national level (16-17th March, 2017) in Antalya, Turkey. International workshop focused on key critical thematic issues in risk assessment, risk management and socio-economic considerations in support of biosafety decision making and the guideline. In summary, Ministry of Food, Agriculture and Livestock, industry and NGOs and academia were very active partners in this workshop, there were undoubtedly significant discussion on the need for networking and knowledge sharing is critical especially as the biosafety

obligations at the plenary sessions with two experts from European Union (EU), three experts from U.S. Department of Agriculture (USDA) and Michigan State University (MSU) and five experts from Turkey (TR).

The last workshop with national stakeholders was on the theme of risk assessment methodology in TR. The workshop was divided into a series of presentations on the

- "Brief Information about the Project of Support for the Implementation of the National Biosafety Framework"
- "Introduction to Risk Assessment Methodology" and
- "A Case Study: Safety Assessment of NK 603 Maize Event"

followed by two deliberative sessions with participants. Participants were drawn from a range of governmental, civic and private organizations representing scientists, traders and non-governmental organizations. The following organizations were represented: Ministry of Food, Agriculture and Livestock; Biosafety Board; Scientific Risk Assessment Committee; Social Economic Risk Assessment Committee; The Scientific and Technological Research Council of Turkey (TUBITAK), Turkish Feed Producers Association (TÜRKİYEM-BİR), Turkish Poultry Meat Producers and Breeders Association (BESD-BİR).

In this workshop recognized overall needs are:

- to review of the regulatory system after new developments of the modern biotechnology such as genome editing and omics technologies.
- to review the international obligations in the legal text and the implementation.

The technical guideline for the risk assessment of genetically modified plants and derived food and feed is for the use of risk assessors and notifiers who intend to apply for the commercial release of genetically modified plants and derived food and feed under national legislation in Turkey and/or for the commercial

authorization of genetically modified (GM) food or feed, i.e. food or feed containing, consisting of or produced from genetically modified plants. This document does not cover genetically modified animals, or micro-organisms or medicinal products for human or animal use. At the same time, issues such as containment or risk management are not within the scope of this document and thus the post-market monitoring of GM crops and derived food and feed is not addressed specifically.

The guideline prepared under the auspices of the "Support for Implementation of the National Biosafety Framework" project and does not have any regulatory status, but elaborates on the information needed for the risk assessment of genetically modified plants and derived food and feed. It seeks to provide guidance to both notifiers and risk assessors and also aims to assist notifiers in the preparation of dossiers. The risk assessor or the regulator may require additional information on a case-by-case basis. Notifiers must adhere to the requirements laid down in the appropriate Biosafety Law and related Regulations in TR.

People have been growing crops for thousands of years to feed themselves and their animals. Over time crops became domesticated from their wild predecessors and later were improved by conventional breeding. Conventional breeding depends on the ability to cross two closely related individuals capable of producing viable offspring. However, in some cases an important trait such as disease resistance is not available within the crop and its close relatives. Therefore, people have searched for new techniques to develop agriculture. The ability to work with genes and to transfer them from one organism to other, has allowed for the development of modern agricultural biotechnology. Genetic engineering provides a direct method to introduce one or more useful genes within a short period of time. A desirable trait or property from one species can be transferred into same or different species to make a crop or plant better in terms of its defenses against insects, diseases or

weeds, resistance to drought or other environmental stresses, nutritional content, and other desired characteristics.

Genetic engineering, by definition, involves genes. All living organisms (plants, animals, microbes) have genes. Genes which are located chromosomes, encode hereditary information that is passed from one generation to the next, and in encoded form, provide all the instructions that are needed to produce a functional organism. The information provided by genes in the form of instructions for producing proteins, not the least of which are all of the enzymes necessary to perform all of the biochemical reactions in a cell. The coding capacity of genes is derived from their molecular structure. Genes are made of DNA, a helical molecule composed of strings of four type nucleotides, A (adenine), T (thymine), G (guanine), C (cytosine). The order of the nucleotides specifies the sequence of amino acids that is specific to each kind of protein. The language of DNA is critical factor underlying genetic engineering techniques, which makes possible to move genes from one organism to another. Genetic engineering depends on two types of technologies primarily developed 1970s and 1980s; recombinant DNA technology and transformation technologies. The process of producing a GE crop can be broadly divided into five general steps:

- Obtain and engineer the desired gene (recombinant DNA technology)
- Introduce the gene into individual cells/chromosomes (transformation)
- Regenerate the transformed cell into a whole plant (tissue culture technology)
- Verify the presence and expression of the introduced gene and desired new trait (laboratory, greenhouse and field studies),
- Incorporate the new trait into a high-performing variety (conventional breeding).

The first genetically engineering (GE) crop entering the market for human consumption was tomato in 1994. Since then several crops have become available for both human and animal consumption in developed and developing counties. Today, the most widely produced GE crops around the world are corn, soybean and cotton with 185.1 million hectares planted worldwide in 2016. A list of GM crops approved for commercialization in the US has been complied by the International Service for the Acquisition of Agri-biotech Applications (ISAAA) as shown in Table 1.

Human have continuously improved the yield and quality of agricultural and food crops and the scales of human numbers are ecologically unprecedented. Food and Agriculture Organization of the United Nations (FAO) estimates that global agricultural production needs to grow by 70 percent if the estimated 9 billion people that will inhabit the planet in 2050 are to be fed. On the other hand, possible risks of genetic engineering technology have been and continue to be the subject of extensive evaluation. Especially, with respect to food safety, potential risks such as allergens, toxins, and possible unintended effects must be evaluated scientifically for each crop or GE food on a case by case basis before going to market. The producer of a crop/seed must provide information and data that characterize the inserted gene, the protein it makes; how it was introduced into the crop plant; tests to determine whether there is possible toxicity and allergenicity of the new protein; and any effects on nutritional composition and safety of the engineered plant.

The key question is the final product substantially different from the non-engineered crop? Safety assessment of GE crop/food depends on the comparative approach. The concept of substantial equivalence is used to evaluate the differences in toxicity, allergenicity and nutritional value of a new GM crop in comparison to the traditional crop.

Table 1 | Current status of GM plant/crop categorized based on trait approved for commercialization in U.S. or currently commercially produced in U.S. or other countries 2016(*) (data from ISAAA, 2016)

Trait	Aim	Crop
Insect resistance	Making plant resistance to certain insects	Maize*, Soybean*, Rice*, Cotton*, Poplar, Eggplant*, Potato*, Tomato
Disease resistance	Provide resistance to plant to defend against virus specific for crop	Bean, Papaya*, Plum, Potato*, Squash, Sweet pepper, Tomato
Herbicide tolerance	Increase tolerance of crop to certain herbicides (e.g. glyphosate and glufosinate) to improve weed control	Maize*, Alfalfa*, Canola*, Cotton*, Potato*, Rice*, Soybean*, Sugar Beet*, Chicory*, Carnation*, Creeping Bent grass, Flax*, Tobacco, Wheat
Modified product quality	Modified fatty acid and starch content, change in flower color and mannose metabolisms, increase in amino acid production, reduction in acrylamide, non-browning appearance	Alfalfa, Soybean*, Maize*, Rice, Canola*, Apple, Carnation*, Melon, Petunia, Potato*, Rose, Tomato*, Tobacco
Environmental stress tolerance	maintaining normal functions of cell under drought stress conditions	Maize*, Soybean*, Sugarcane
Altered growth/ yield	providing faster growth, increase in biomass enhanced photosynthesis	Maize, Soybean, Eucalyptus*
Pollination control system	Mail sterility	Maize*, Canola*, Chicory*

Depending on the requirements and guidelines provided by responsible agencies of each country, all questions must be clarified based on scientific results before going into approval stage for commercialization.GE crops are also evaluated for environmental safety before entering the market. In response to concerns expressed by farmers and the public about replacement of traditional crops with GE crops, scientists and regulators investigate the possibility of risks for other species and environments in which they will be grown. The effects of GE crops on non-target organisms, the possibility of new GE plant to become weeds, the level of invasiveness into wild types, and possible development of resistance to pests need to be addressed for environmental risk assessment of each crop or each trait.

The regulators must then evaluate the submitted data to perform risk assessment. After assessing the risks, risk management strategies are used to develop plans and actions to manage any risks described for the GE crop. The major aim of a management plan is to eliminate the hazard itself, or decrease the chance that a hazard can cause harm to human, plant, animal and environment. This would be analogous to management plans to minimize risks associated with any technology, such as appropriate dosages in medicine, vaccination for flu, pesticide usage in agriculture, seatbelts in automobile, and hard hats for cycling.

Notably, the promise of genetically engineering will likely expand the number of traits and species for which new approaches on risk assessments will be needed. Last decade there are numbers of recognized genes editing methods to obtain new traits or crop species. Gene editing (or genome editing) is the insertion, deletion or replacement of DNA at a specific site in the genome of an organism or cell. It is usually achieved in the laboratory using engineered nucleases also known as molecular scissors. Genome editing allows scientists to perform the same types of loss and gain of function experiments, but manipulate genes of interest at the endogenous level. In future, it needs to lay a robust and comprehensive epistemic foundation of genome editing suitable for risk assessment and legal audiences.

CHAPTER 2

REGULATORY SYSTEMS FOR GENETICALLY ENGINEERED PRODUCTS

Genetically engineered (GE) products are regulated under the laws of different agencies throughout the world. While some countries already have legislations, laws and regulatory systems for biotechnological products, others have been recently developing their regulatory processes. Turkey established the "Regulation of The Principles and Procedures of Biosafety Board and Committee" (No 27671) in 2010 after the acceptance of the Biosafety Law in 2010. This regulation gives the Biosafety Board responsibility for evaluating applications for use of GE crops and their products as food/feed and release into environment for research. The Biosafety Board consists of nine members representing the Ministry of Food, Agriculture and Livestock; the Ministry of Health; the Ministry of Environment and Forest; the Ministry of Industry and Trade; and Ministry of Economy. The Biosafety Board appoints the scientific committee members (totally 11 members) for each application for GE crops or products.

The establishment of the Turkish regulatory system largely drew upon the systems of the European Union (EU) and the Cartagena Biosafety Protocol in accordance with Global Environment Facility (GEF) Project implemented by United Nations Environment Program (UNEP). In here, it is summarizing that the regulatory system in EU and Turkey by describing guidelines and agencies responsible for the regulation of plants, food and feed derived from genetic engineering.

2.1. Regulation in Turkey

The biosafety process in Turkey began by the acceptance of 'Instructions of Field Trials for Transgenic Crops' in 1998. According to this instruction, the field trials at certain locations could be done by research institutes before importation of GE plants. In order to minimize the risks related with GE crops, this instruction limited the field trials to GE crops that were approved in the producer's country for a minimum of three years before the application to Turkey for importation. Also by the guidance of this instruction, only transgenic plants that did not have any related varieties in Turkey were allowed for field trials. The first field trials of GE potato, corn and cotton varieties were done in 1999 by the acceptance of this instruction. In 2000, risk assessment processes were implemented to evaluate safety of these GE varieties.

After 10 years, the "Regulation of Importation, Processing, Exportation, Control and Regulation of Genetically Modified Organisms and Their Products for Food and Feed" was instituted by the Ministry of Agriculture. This regulation gave the responsibility to the Ministry of Agriculture and Rural Affairs for the selection of committees to perform risk assessment of GE crops and their product for human and animal consumption. From 2002 to 2005, the " Project on the Development of National Biosafety Frameworks" was conducted as a part of "The Global Environment Facility (GEF)" of "United Nations Environment Program (UNEP)".

The National Biosafety Framework which consists of legal systems, organization of the committee, risk assessment and management systems, and a control system for monitoring and identification of GE organisms was specified within the scope of the project.

In 2010, Biosafety Law No 5977 was prepared by a commission composed of 27 experts from different ministries, universities and non-profit organizations. This law was based on the Cartagena Protocol, EU legislation and national requirements. It includes provisions for research, development, marketing, monitoring, usage, importation, exportation, transportation, storage, packing and labeling of GE organisms and their products. This law states that the decision for importation, exportation, release into environment for experiments and marketing of GE organisms is made according to the risk assessments performed in accordance with scientific principles and with opportunity for public consultation. The approval process for GE organisms in Turkey is shown in Figure 1B. In 2016, Turkey has approved 7 soybean and 25 corn genetically modified events currently for feed use only, and the biosafety legislation has banned the cultivation of GM crops and it requires all genetically modified organisms, including imports, to be approved for use and establishes a strict policy of testing for food, feed and seed potentially containing GMOs. For the GMO analysis, each laboratory should be established the verification on method performance criteria and calculation of measurement uncertainty. As in the EU, food and feeds containing greater than 0.9% GE organisms or their products are labeled in Turkey.

2.2. Regulation in European Union

The EU has established a legal framework to ensure that the development of modern biotechnology, and more specifically of GE organisms, takes place in safe conditions.

The legal framework aims to:

- Protect human and animal health and the environment by introducing a safety assessment of the highest possible standards at EU level before any GE organism is placed on the market.
- Put in place harmonized procedures for risk assessment and authorization of GE organisms that are efficient, time-limited and transparent.
- Ensure clear labeling of GE organisms placed on the market in order to enable consumers as well as professionals (e.g. farmers, and food feed chain operators) to make an informed choice.
- Ensure the traceability of GE organisms placed on the market

The building blocks of the GE organism legislation are:

- Directive 2001/18/EC on the deliberate release of GE organisms into the environment
- Regulation (EC) 1829/2003 on genetically modified food and feed
- Directive (EU) 2015/412 amending Directive 2001/18/EC as regards the possibility for the Member States to restrict or prohibit the cultivation of GE organisms in their territory
- Regulation (EC) 1830/2003 concerning the traceability and labeling of genetically engineered organisms and the traceability of food and feed products produced from genetically modified organisms
- Directive 2009/41/EC on contained use of genetically engineered micro-organisms. Regulation (EC) 1946/2003 on transboundary movements of GMOs

These main pieces of legislation are supplemented by a number of implementing rules or by recommendations and guidelines on more specific aspects.

The legal framework for GE products in the EU was established through EU Directive 2001/18/EC which covers the regulations for environmental release of GE organisms and post marketing monitoring. Then, the Regulation EC No 1829/2003 on genetically modified food and feed was accepted for food or feed derived from GE organisms and those containing GE organisms or their products. This regulation states that risk assessment for GE organisms, foods and feeds is done by the European Food and Safety Authority (EFSA). The European Commission (EC) then gives a recommendation to the Standing Committee on the Food Chain and Animal Health for acceptance or rejection based on the risk assessment report of EFSA. If it is accepted by the committee, EC accepts its approval. If it is not accepted, the Council evaluates the decision of the committee. A final decision is given by the Commission based on the Council's decision. The regulatory process in the EU is shown in Figure 1A. Today, only one GM crop (MON810 maize) is allowed for cultivation, and 57 GM crops are approved for importation and processing for food and feed in Europe. These GM crops include maize, soybean, rapeseed, sugar beet and cotton. The food and feeds containing GE organisms or their products are labeled if they exceed the threshold level of 0.9 %.

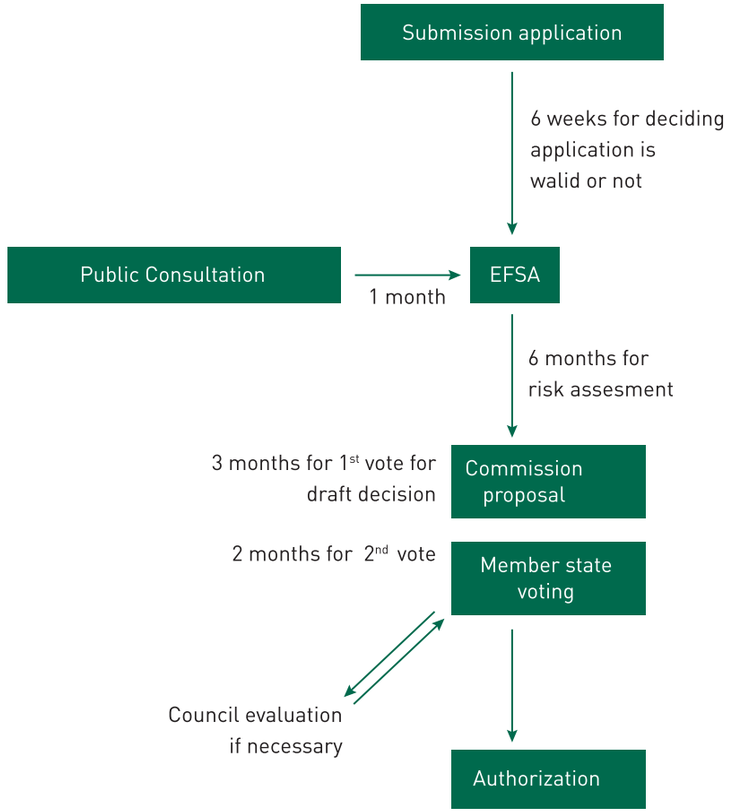

Figure 1 | a. Summary of evaluation processes for approval of GE organisms; in EU.

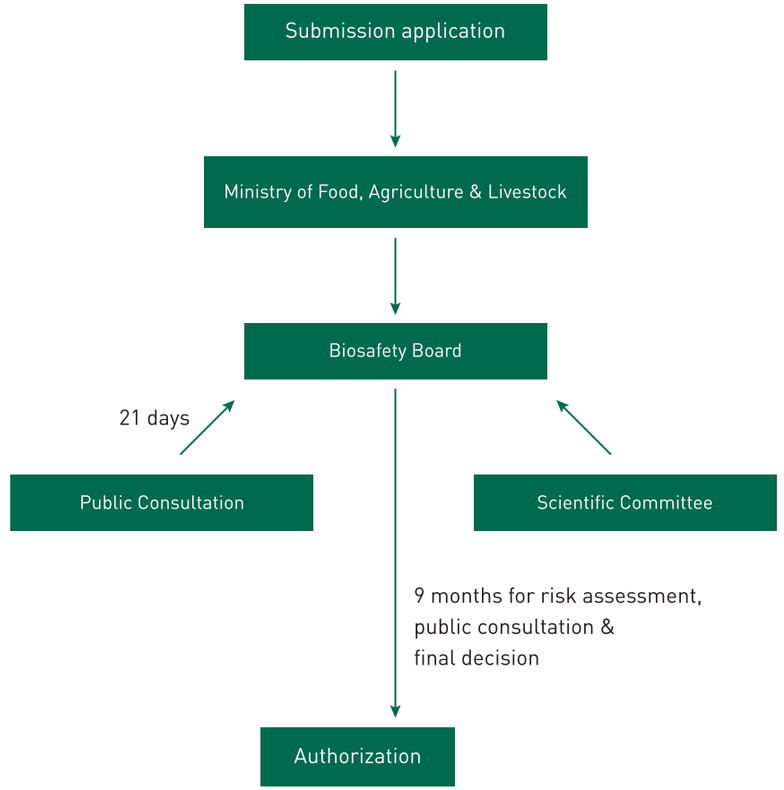

Figure 1 | b. Summary of evaluation processes for approval of GE organisms; in Turkey.

CHAPTER 3

TECHNICAL GUIDELINE FOR RISK ASSESSMENT

The principles refer to risk analysis as including three components: risk assessment, risk management, and risk communication. Risk assessment, an evidence-based process for characterizing the risks posed by a product, is a critical component of the risk analysis. In order to eliminate the concerns about the safety of GE crops, foods and feeds derived from GE crops general comparative approach has been taken as the basis for deciding the safety assessment process. Primarily, **risk assessment can be defined** as "a process of evaluation including the identification of the attendant uncertainties, of the likelihood and severity of an adverse effect(s) /event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s)". **A risk assessment includes four main phases** to identify characteristics which may cause adverse effects, evaluate their potential consequence, assess the likelihood of occurrence and estimate the risk posed by each identified characteristic of the GEs:

- Hazard identification: A hazard is the potential of a risk source to cause an adverse effect. The identification of the type and the adverse effects that an agent has inherit capacity to cause in an organism, system or (sub) population.
- Hazard characterization: The qualitative and, wherever possible, quantitative description of the inherent property of an agent or situation having potential to cause adverse effects.
- Exposure assessment: Evaluation of the exposure of an organism, system, or (sub) population to an agent and its derivatives.
- Risk characterization: The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system or (sub) population, under defined exposure conditions.

In the spirit of the text in the Cartagena Biosafety Protocol, biosafety can be defined as the regulatory systems and risk assessment procedures designed to ensure the safe use genetically modified organisms, a product of modern biotechnology. In this sense, **the general principles of risk assessment** are following:

- Risk assessment should be carried out scientifically sound and transparent manner and can take into account expert advices and international level guidelines.
- Lack of the scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk.
- Risks associated with genetically modified organisms or products thereof should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment.

- Risk assessment should be carried out on a case-by-case basis. The required information may vary in nature and level of detail from case to case, depending on the GMO concerned, its intended use and likely potential receiving environment.

There are important **issues to be considered for the risk assessment** of GE crops and food and feed derived from GE crops. Indeed, the risk assessment should take account of the following aspects:

- Description of the GE plant, the crop involved and the nature of the genetic modification event or events.
- Description of the host plant and its use as a food, including the host plant's cultivation and breeding development and any known toxicity or allergenicity issues.
- Description of donor organisms, including any toxicity or allergenicity issues associated with them.
- Description of the genetic modifications, including details of the method of transformation, the DNA used, the vectors used, and any intermediate hosts that might have been used in the process.
- Characterization of the genetic modifications, including the number and nature of DNA insertions and border regions, the expression of the inserted DNA sequences, and a determination as to whether the expression of any other genes in the host plant has been affected.
- Expressed substances (non–nucleic-acid substances); an examination of the toxicity of any expressed products resulting from the genetic event and an evaluation to ensure that toxic components from a donor organism have not been inadvertently transferred. In the case of proteins, it is expected that amino acid sequences will be characterized and the potential for allergenicity determined.

- Compositional analysis of key components, an examination of key components of the host plant in comparison with the transformed plant. Plants are generally field-trialed under conditions that closely resemble commercial production, and natural variations
- in key components are considered in any evaluation.
- Evaluation of metabolites; An evaluation of metabolites that might be produced in the GE plant but not in the original host. The metabolites, if present, need to be assessed for their potential
- effect on human health.
- Food processing; studies that explore the effects of food-processing treatments on components or metabolites of GE foods. The focus is to determine whether an altered protein or metabolite might become toxic after processing in contrast with components of the non-GE counterpart.
- Nutritional analysis; same as the compositional analysis except when the genetic insertion is intended to change a key nutritional component, in which case additional testing may be needed to determine the level of the nutrient in question and its effects on human health, taking into account normal consumption patterns and the stability of the trait in multiple production environments.

The risk assessment should take into account any potential impact of horizontal DNA transfers between plant or plant components and micro-organisms in relevant environments. Genes integrated in the GE crop should also be subjected to risk assessment with respect to the possible effects of ingestion of the protein expressed in plant parts. Different outcomes of a genetic transformation event can be envisaged: Intended effects are those that are targeted to occur from the introduction of the gene(s) in question and which fulfill the original objectives of the genetic transformation process. Unintended effects are considered to be consistent

differences between the GE crops and its appropriate control lines, which go beyond the primary expected effect(s) of introducing the target gene(s). They may be evident in the phenotype or composition of the GE crop when grown under the same conditions as the controls. Additionally, molecular and biochemical analyses can be used to determine changes at the level of transcription and translation that could lead to unintended effects.

The comparison of the GE crop or product with its non-GE counterparts is the starting point of the safety assessment which then focuses on any intended or unintended differences identified. Established and validated protocols should be used throughout and the data analyzed using appropriate statistical techniques. The possible outcome of the comparative approach will further structure the safety assessment procedure, which may include additional toxicological and nutritional testing. It is obvious that the insertion of genes and other associated DNA from a donor organism into the host will result in a plant that is not completely identical to the parent. The risk assessment process therefore concentrates on the outcomes of the transformation process using appropriate comparators. To this end the concepts of familiarity and substantial equivalence were developed by the OECD and further elaborated by WHO/FAO for the assessment of the environmental safety of GEs and the safety of genetically modified foods/feeds, respectively.

Mostly, GE crops are developed from organisms such as crop plants the biology of which is well understood. It is not a risk/safety assessment in itself but familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of similar crops including GE crops into the environment. Familiarity is related to the knowledge and experience available for conducting a risk/safety analysis prior to scale-up of any new plant line or crop cultivar in a particular environment.

Substantial equivalence provides assurance that the GM food/feed may be as safe as the traditional counterpart, or that no comparison can be made because of the lack of an appropriate comparator. Analysis of substantial equivalence involves not only a comparison of the chemical composition between the new and the traditional food or feed, but also of the molecular, agronomical and morphological characteristics of the organism in question. Such comparisons should be made with GM and non-GM counterparts grown under the same regimes and environments. When the degree of equivalence is established as substantial, a greater emphasis is placed on the newly introduced trait(s). Where substantial equivalence does not occur, this does not necessarily identify a hazard. Where a trait or traits are introduced with the intention of modifying composition significantly and where the degree of equivalence cannot be considered substantial, then the safety assessment of characteristics other than those derived from the introduced trait(s) becomes of greater importance.

3.1. Risk Assessment in Cartagena Biosafety Protocol

The Cartagena Biosafety Protocol defines a living modified organism as any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. Risk assessment (Box 1-1) is a basic requirement under the Cartagena Biosafety Protocol for all GE organisms that are to be intentionally introduced into the environment. Risk assessment is a tool to assist in decision making on release of GE organisms into the environment. The objective of risk assessment is to identify and estimate the possible adverse effects of the GE organisms on the conversation and sustainable use of biological diversity, taking into account risks to human health. Risk assessment should be carried out in a scientifically sound and stepwise manner, as described in Annex III. These steps normally include identifying the possible adverse effects, estimating the likelihood that each of these effects will occur, and evaluating the extent of damage should the adverse effects be realized. Parties may request that a risk assessment be carried out by the notifier and the cost be borne by the notifier.

BOX 1-1 Cartagena Biosafety Protocol Article 15, Annex III

Article 15 - 1. Risk assessments undertaken pursuant to this Protocol shall be carried out in a scientifically sound manner, in accordance with Annex III and taking into account recognized risk assessment techniques. Such risk assessments shall be based, at a minimum, on information provided in accordance with Article 8 and other available scientific evidence in order to identify and evaluate the possible adverse effects of living modified organisms on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

2. The Party of import shall ensure that risk assessments are carried out for decisions taken under Article 10. It may require the exporter to carry out the risk assessment.

3. The cost of risk assessment shall be borne by the notifier if the Party of import so requires.

Annex III: Risk Assessment

Objective

1. The objective of risk assessment, under this Protocol, is to identify and evaluate the potential adverse effects of living modified organisms on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health.

Use of risk assessment

2. Risk assessment is, inter alia, used by competent authorities to make informed decisions regarding living modified organisms.

General principles

3. Risk assessment should be carried out in a scientifically sound and transparent manner, and can take into account expert advice of, and guidelines developed by, relevant international organizations.

4. Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk.

5. Risks associated with living modified organisms or products thereof, namely, processed materials that are of living modified organism origin, containing detectable novel combinations of replicable genetic material obtained through the use of modern biotechnology, should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment.

6. Risk assessment should be carried out on a case-by-case basis. The required information may vary in nature and level of detail from case to case, depending on the living modified organism concerned, its intended use and the likely potential receiving environment.

Methodology

7. The process of risk assessment may on the one hand give rise to a need for further information about specific subjects, which may be identified and requested during the assessment process, while on the other hand information on other subjects may not be relevant in some instances.

8. To fulfil its objective, risk assessment entails, as appropriate, the following steps:

(a) An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;

(b) An evaluation of the likelihood of these adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;

(c) An evaluation of the consequences should these adverse effects be realized;

(d) An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;

(e) A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks; and

(f) Where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the living modified organism in the receiving environment.

Points to consider

9. Depending on the case, risk assessment takes into account the relevant technical and scientific details regarding the characteristics of the following subjects:

a) Recipient organism or parental organisms. The biological characteristics of the recipient organism or parental organisms, including information on taxonomic status, common name, origin, centres of origin and centres of genetic diversity, if known, and a description of the habitat where the organisms may persist or proliferate;

b) Donor organism or organisms. Taxonomic status and common name, source, and the relevant biological characteristics of the donor organisms;

c) Vector. Characteristics of the vector, including its identity, if any, and its source or origin, and its host range;

d) Insert or inserts and/or characteristics of modification. Genetic characteristics of the inserted nucleic acid and the function it specifies, and/or characteristics of the modification introduced;

e) Living modified organism. Identity of the living modified organism, and the differences between the biological characteristics of the living modified organism and those of the recipient organism or parental organisms;

f) Detection and identification of the living modified organism. Suggested detection and identification methods and their specificity, sensitivity and reliability;

g) Information relating to the intended use. Information relating to the intended use of the living modified organism, including new or changed use compared to the recipient organism or parental organisms; and

h) Receiving environment. Information on the location, geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin of the likely potential receiving environment.

3.2. Risk Assessment in Biosafety Law

In Turkey, genetic engineering products have been regulated on the basis of the risk posed by a product's intended use or characteristics. The national regulatory system address both biosafety concerns (food safety and environmental protection) and biosafety considerations to address socioeconomic concerns, such as consumer right-to-know and protection of farmers of non-GE crops from unintended gene flow from GE crops. The Biosafety Law (5977, 2010) puts main elements of the risk assessment for all applications for placing on the market of GE organisms, whether they concern the GE organisms themselves or food and feed products derived there from (Box 1-2).

Box 1-2 Biosafety Law (Article 2, 4, 5, 6)

Article (2)

(ü) Risk Assessment: 4 phase process including identification by means of scientific methods such as test, analysis and trial; specification; evaluation and determining of risk factors, risk resources and risks that may be resulting from GMO and GMOPs on human, animal and plant health with environment and biological diversity owing to genetic modifications,

Article (4)

(1) Risk assessment and socio economic assessment in compliance to scientific principles are made separately for each application in accordance with this Law. In the case that submitted information in application is found inadequate; the new test, experiment, analysis and research can be requested from applier. Expenses, connected with transactions of risk assessment and socio-economic assessment, are met by applier.

(2) In applications; different risk assessments are made separately for each application. Submitting the results of field trials including laboratory, greenhouse, cropland tests with food analyses, toxicity and allergy tests in company with other required tests, by applier is obligatory in risk assessment.

(3) In order to constitute a basis on rendering decision about each application; Socio economic assessment is made for conservation and enabling sustainability of biodiversity, and determination impacts on consumers and users.

(4) Risk management principles are designated based on risk assessment and socio-economic assessment results for applications of GMO and GMOPs. A detailed plan connected with risk management is prepared. Applier is responsible for preparing and implementing risk management plan.

(5) Procedures and principles connected with enforcing of this Article are arranged by regulations.

Prohibitions

Article 5

(1) Doing following actions connected with GMO and GMOPs is prohibited:

a) Placing GMO and GMOPs on the market without approval.

b) Using or making GMO and GMOPs available to use in violation of Commission decisions.

c) Making production of genetically modified plants and animals

d) Using GMO and GMOPs out of the purpose and area designated by Commission in scope of place on the market

e) Using GMO and GMOPs in baby food and baby formulae, follow-on baby food and follow- on formulae, baby and kid's nutritional supplements.

Simplified procedure

Based on the Biosafety Law there is the simplified procedure for the risk assessment. The decision process based on previous risk assessments and existing information on that there is no any risk that may be due to GMO and GMOPs, and that there is no any harmful effect on human, animal and plant health with environment and biological diversity.

Article (6)

(1) Simplified procedure can be implemented for applications having no any risk and based on existing information on that there is no any harmful effect on human, animal and plant health with environment and biological diversity, and based on previous risk assessments, also taking into account socio-economic assessment results.

(2) During the application to simplified procedure; fulfilling conditions stated below excluding other matters to be set by Ministry is obligatory:

a) Being known the gene resource with the taxonomy and biology of transferred living organism.

b) Existing adequate information about GMO's effects that may be on human, animal and environment health with biological diversity.

c) Existing information regarding absence of adverse effect obtained from previous risk assessments that are available for GMO's relationship with other living organisms.

ç) Existing detail method and information for identification of transferred genetic material and detection it in recipient living organism.

(3) Procedures and principles connected with enforcing of this Article are arranged by regulations.

3.3. Main elements of the risk assessment procedures for regulated products in the concept of Biosafety Law

Analysis of risks and benefits associated with a technology is often considered to involve the difficult but straightforward scientific task of reviewing the most relevant and highest-quality scientific papers on the technology and drawing up a set of statistically supported conclusions and recommendations. Turkey make use of the OECD, the Food and Agriculture Organization, the World Health Organization, and the Codex Alimentarius Commission standards in developing scientific risk assessment of biosafety and in shaping its national regulatory system. It has a technical expert body to conduct a risk assessment of a product seeking regulatory approval. On the other hand, the biosafety approach obviously differs from that of the other countries because it is based on existing national laws. It has taken a more precautionary approach to approving the commercialization of GE crops such as case-by-case screening also for scientific uncertainties owing to novelty of genetic engineering process. Notably, the biosafety approach to the decision of what kinds of new GE products require regulatory review has been comprised stringent regulations and labeling requirements.

The elements of scientific risk assessment are broadly similar among regulatory systems, but policy decisions, which inherently reflect different political and cultural perspectives on risks and benefits, vary considerably. For food, feed and environmental safety, the risk-assessment process used in Turkey starts with the fundamental idea of comparison of a GE variety with a known, conventionally bred counterpart. Risk assessment focuses on the intended and unintended differences and considers the effects of the differences on relevant endpoints. For food and feed, the primary issues to be considered include the potential effects of compositional changes on nutritional elements, toxicity, and allergenicity. Environmental issues include effects on nontarget organisms, changes in invasiveness or weediness, and potential for unwanted gene transfer to related species. In every case, developers are required to submit a package of data from field trials and other sources to show that the GE variety poses risks no greater than its non-GE counterpart. The one of the main problem is dossiers of the risk assessment is not drafted by the developers in Turkey, that's why submit package of data is not coming from directly developers. Figure 2 shown that main elements of the risk assessment procedures for regulated products in the concept of Biosafety Law.

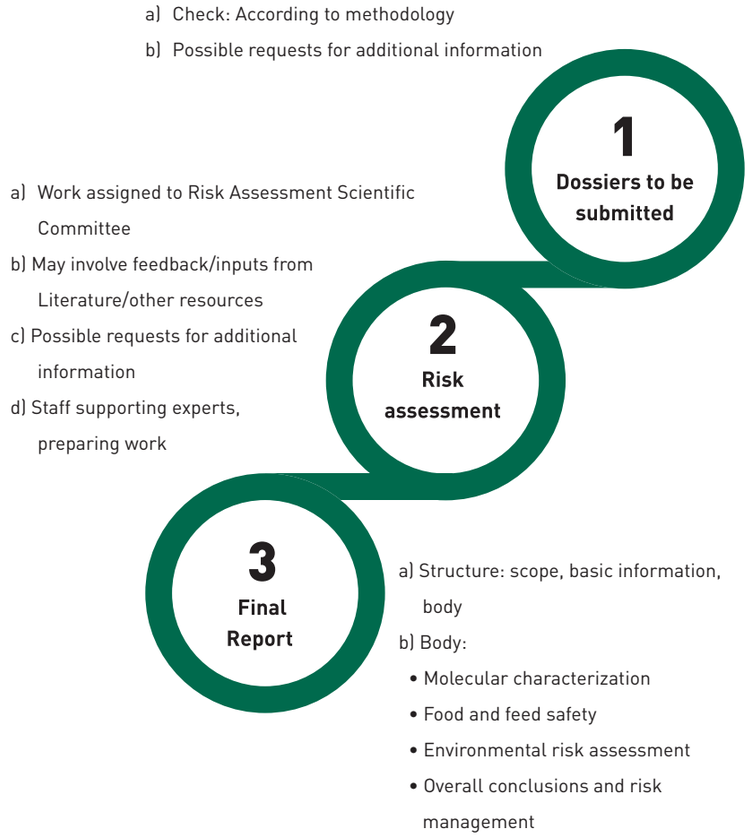

Figure 2 | Main elements of the risk assessment procedures in the concept of Biosafety Law

3.4. A case study of safety assessment; NK603 corn

In order to clarify the requirements for safety assessment documents of GE crops submitted by companies and regulatory dossiers reported by agencies, a case study is explained in the following section. This section demonstrates the general information about a GE corn event NK603, a summary of its safety assessment supplied by importing institutions and what type of information/data or analyses are present in the regulatory documents reported by Scientific Risk Committee (Table 2-5) and it contains brief timeline about its approval process (Figure 1) in Turkey. There have been several GE crops developed for different purposes such as resistance to insect and disease, nutritional enhancement, and tolerance for drought since 1996. However, as compared with other GE crops, herbicide tolerant GE crops have the largest area for planting over the world. Among other herbicides, glyphosate is the most common herbicide that is targeted for the development of herbicide tolerant GE crops. The basic principles underlie the action mechanism of glyphosate is the blocking of key enzyme in the pathway for aromatic amino acid production. Therefore, the strategy for glyphosate tolerant GE crop's development is the introduction of a gene responsible for the expression of modified enzyme which can have lower affinity for glyphosate or degrade glyphosate.

In this case, GE corn event NK603 was produced by a foreign private company to develop herbicide tolerance crop for weed control in farm lands. This biotech corn has a modified form of an enzyme that has lower binding affinity toward glyphosate as compared with conventional corns, thus its function of aromatic amino acids production is not affected upon an application of glyphosate for weeds. Glyphosate with a trade name of Roundup® herbicide normally acts on plant's unmodified form of 5-enolpyruvyl shikimate-3-phosphate synthase (ESPSP) and blocks the shikimate pathway responsible for the production of aromatic amino acids in plants. By changing binding affinity of the enzyme toward glyphosate, genetic

engineering has provided herbicide tolerant crops with agricultural application for weed control.

To address the concerns about the safety of GE crops and foods derived from GE crops, a general comparative approach, 'substantial equivalence' has been taken as the basis for the safety assessment process. The principle for 'substantial equivalence' as defined by the Organization of Economic Cooperation and Development (OECD) (OEDC, 1993) and widely accepted by many agencies throughout the world, is that "existing organisms used as food or a source of food can be used as the basis for comparison when assessing the safety of human consumption of a food or food component that has been modified or is new". In accordance with this concept, Turkey has accepted Biosafety Law No 5977 in 2010 and established the organization for regulation and safety assessment for GE crops and foods/feeds derived from them. This white paper presents a case study of the regulatory process for GE corn NK603 for use in Turkey. The following information is provided.

- the general information about GE corn NK603,
- a summary of its safety assessment
- a summary of the type of information/data or analyses that were presented in the regulatory documents reported by Scientific Risk Committee in Turkey (Table 1-4)

3.4.1. General information about GE corn NK603

GE corn event NK603 was produced to develop tolerance to the herbicide, glyphosate, to facilitate weed control in farm lands. Glyphosate, with a trade name of Roundup, normally kills weeds by binding to a key enzyme [5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS)], needed for production of aromatic amino acids. This biotech corn has a modified form of the enzyme that has lower

binding affinity toward glyphosate as compared with conventional corn varieties. By changing binding affinity of the enzyme toward glyphosate, it is now possible to spray the field with glyphosate and kill the weeds but not the crop.

3.4.2. Summary of its safety assessment

Molecular characterization of NK 603 corn event

NK603 corn varieties were developed by the insertion of two adjacent gene cassettes containing two copies of the EPSPS genes at a single location. The introduced genes did not undergo genetic rearrangements in the recipient corn and were stably inherited when studied for nine generations. The EPSPS proteins were similar to other EPSPS proteins that are ubiquitous in nature. Expression of the genes was sufficient to confer tolerance to glyphosate. The methods used for molecular characterization of NK603 and the results of these methods are given in Table 1, summarizing the Company's report on NK603.

Molecular characterization showed that the EPSPS gene introduced into NK603 corn, and the CP4 EPSPS and CP4 EPSPS L214P proteins are similar to other EPSPS proteins that are ubiquitous in nature.

Table 2 | The summary of methods and results from a molecular characterization study of NK603 corn

Methods	Results
Sequencing inserted DNA confirmed	• single insert containing; a single complete copy of the linear DNA of PVZMGT32 used for transformation, • two intact CP4 EPSPS gene cassettes, within the single insert, • expected cp4 epsps coding region regulated by Pract1 • altered cp4 epsps coding region regulated by e35S (two nucleotide changes, one of which encoding proline instead of leucine at 214th position in protein called CP4 EPSPS L214P)
PCR and DNA sequencing confirmed	• expected 5′ and 3′ ends of insert • native flanking sequences
Western Blot analysis verified	• expression of only two full-length CP4 EPSPS proteins in NK603 plants, undistinguishable as expected • No longer than full-length CP4 EPSPS protein
RT-PCR analyses showed	• initiation of mRNA transcription in either one of the two promoters of the NK603 insert • proceeding through the NOS 3′ polyadenylation sequence continuing into the corn genomic DNA flanking the 3′ end of the insert as expected.
Inheritance study confirmed	• Mendelian segregation pattern as expected for single genetic loci
Stability test done	• through more than nine generations of crossing and one generation of self-pollination • multiple site-progeny test for NK603 in US and EU • no instability of insert detected during extensive field testing and commercial production
Enzyme linked immunosorbent assays (ELISA)	• CP4 EPSPS protein levels measured in forage and grain of NK603 corn; mean 25.6 µg/g fw, 10.96 µg/g fw, respectively (for the combination of the CP4 EPSPS and CP4 EPSPS L214P proteins) • CP4 EPSPS proteins detected in event NK603 samples and not detected in the non-modified control line, as expected • low levels of CP4 EPSPS protein expression in line NK603 are sufficient to confer tolerance to glyphosate.

Safety assessment of the introduced protein (the EPSPS enzyme) NK603 corn

CP4 EPSPS and CP4 EPSPS L214P proteins were shown to be structurally and functionally equivalent to their counterparts in nature which have a long history of safe use. They were characterized for their potential toxicity and allergenicity. Based on lack of sequence similarity between newly expressed proteins and known toxins and acute oral toxicity tests on mammals, it was concluded that these proteins were non-toxic to humans. Also, they were shown not to be allergens according to lack of similarity to known allergens and digestibility test in gastric juices. Table 2 shows the summary of safety assessment methods and results for newly expressed proteins in NK603 based on the report.

By the safety assessment of newly expressed enzyme for glyphosate tolerance, the report of Company report summarized that; CP4 EPSPS and CP4 EPSPS L214P proteins are not toxic to non-target organisms, including humans, animals and beneficial insects.

Compositional analysis of key components of NK603 corn

Compositional analysis is the method by which substantial equivalence is tested. The levels of 44 components including proteins, fats, carbohydrates, minerals and vitamins were compared between GE and conventional crops that were grown under similar environmental conditions, and to 19 non-transgenic commercial corn varieties. In addition, the possible effects of different growing seasons and different growing temperatures on composition of key nutrients were determined to provide guidelines for ranges of levels occurring in conventional crops. Feeding trials with NK603 corn also were performed with chickens and rats. The summary of the compositional analysis and nutritional assessment of NK603 corn is shown in Table 3.

By the compositional analyses and nutritional assessment of NK603 Corn, the report summarized that Roundup Ready corn plants containing corn event NK603 were shown to be as safe and nutritious as conventional corn varieties.

3.4.3 Final Report for NK603 corn

After submission of documents to regulatory agency, they were evaluated and final reports were published by the Scientific Risk Committee of Turkey. The final report of Scientific Risk Committee of Turkey was further evaluated by the Biosafety Board in Turkey. Because of the withdrawal of application by the applicant, there is no final decision of Biosafety Board on this application for food. The reports of NK603 were summarized in Table 4.

In this case the final report concluded:

With the comparative analysis, it is determined that GE NK603 corn varieties are as safe as conventional corn varieties, it does not change in terms of allergenicity, and there is no difference in terms of nutritional content or agriculture properties. It is concluded that in the case of unintended flow of GE NK603 corn varieties into the environment, the probability of causing different environmental effects as compared with conventional varieties is very low.

Based on the information provided, the Scientific Risk Assessment Committee decided that the use of GE NK603 corn grain containing cp4 epsps genes for glyphosate tolerance as food is not different from non-GM equivalent varieties in terms of causing unintended effects to human and environment. The scope of application contained the import and processing, food, and feed. Application of NK603 corn for food use was withdrawn by applicants. Therefore, there is no final decision of Biosafety board on this event. On the other hand, GM NK 603 corn and its products are used as feed.

Table 3 | A summary of safety assessment for NK603 corn event

Summary	Methods and Results
protein characterization based on - lack of similarity to known toxins - determination of homology by comparison between amino acid sequence of introduced protein to known toxins - if homology identified- experiments conducted - if no homology identified- general oral toxicity screening done - lack of similarity to known allergens shown by physicochemical and human exposure profile (factors contributing to exposure, such as stability to digestion, prevalence in the food, and consumption (amount) of the specific food **Long history of safe consumption of similar proteins digestibility in vitro** simulated mammalian gastric and intestinal digestive mixture used for proteolytic digestion of proteins **Lack of acute oral toxicity in mice of the CP4 EPSPS protein** - Mice administered CP4 EPSPS protein by oral gavages at dosages up to 572 mg/kg	**Analytical and three-dimensional modeling analyses showed;** • CP4 EPSPS and CP4 EPSPS L214P proteins structurally and functionally equivalent comparison of aa sequences of active site residues showed; • structural relationship between CP4 EPSPS and CP4 EPSPS L214P EPSPS and CP4 EPSPS L214P and other EPSPS proteins found in food **X-ray crystal structure analyses demonstrated;** • proline residues naturally occur near position 214 in extant EPSPS proteins • L214P substitution not alter the predicted secondary and tertiary structure of CP4 EPSPS and enzymatic activity for both proteins **Bioinformatics assessments of CP4 EPSPS and CP4 EPSPS L214P proteins showed;** • similarity only to proteins of the EPSPS gene family, and are not similar to toxins or other pharmacologically active proteins contained in the PIR, EMBL, SwissProt and GenBank protein sequence databases **Western blot analyses showed;** • rapidly degradation of CP4 EPSPS protein by the components of the in vitro digestive system • half-life for CP4 EPSPS protein of less than 15s in the simulated gastric system and less than 10 min in the simulated intestinal system. • half-life for the CP4 EPSPS L214P protein less than 15 s in simulated gastric fluid • a minimal risk of conferring novel toxicity or allergy comparable to other safe dietary proteins **Acute oral toxicity test showed;** • CP4 EPSPS protein not acutely toxic to mammals as expected **Concentration analyses for allergenicity showed;** • CP4 EPSPS proteins present at extremely low levels approximately 0.01% of the total protein found in the grain of Roundup Ready corn. **comparison of aa sequences of introduced protein with allergen protein showed;** • CP4 EPSPS and CP4 EPSPS L214P proteins not share any meaningful amino acid sequence similarity with known allergens assembled from publicly available genetic databases (GenBank, EMBL, PIR and SwissProt) **History of safe results showed;** • these proteins from a family of proteins with a long history of safe consumption of CP4 EPSPS protein expressed in corn event NK603 due to the use of Roundup Ready soybean expressing the same protein for glyphosate tolerance

Table 4 | A summary of Compositional Analysis and Nutritional Assessment of NK603 Corn event

Summary	Methods and Results
Compositional analysis; • Based on comparison between Roundup Ready corn and conventional corn present in marketplace • Grain and forage composition measured • Samples collected from Kansas, Iowa, Illinois, Indiana, and Ohio in 1998, also from Italy and France in 1999 • 19 conventional, commercial hybrids used a reference samples • Roundup Ultra® herbicide used to treat NK603 plants • 51 different compositional components evaluated **Nutritional Assessment;** • Measure of growth performance of animals fed withNK603 grain or grain fraction to measure adverse effects • Two key animal feeding studies completed using diets incorporating raw corn grain or ground grain containing corn event NK603; **√ a 42-day chicken study** - From days 1-20, chickens fed a starter diet containing approximately 55% w/w corn - From days 20-42, chickens fed a grower diet containing approximately 60% w/w corn **√ a 90-day rat study** diets containing 11 or 33% (w/w) corn event NK603 or control corn grain or diets containing 33% (w/w) reference control grain	**Statistical analyses;** • 44 components in grain resulted from difference between initial 59 components minus 16 components excluded because of low level of quantification. • commercial reference lines data collected from1999 study not included in analyses • Population tolerance intervals determined for each component (with 95% confidence, 99% of values expressed in population of commercial lines) **Compositional analysis showed;** • grain and forage of corn event NK603 comparable in their composition to those of the control corn and to conventional corn • few statistically significant differences observed are most likely due to random chance and unlikely to be of biological relevance • composition of NK603 corn fall within the 99%tolerance interval for components in 19 non-transgenic commercial corn varieties grown in 1999 field trials in Europe • composition of NK603 corn fall also within range of values reported for non-transgenic corn in the literature as well as in historical data **Nutritional analyses showed;** • corn event NK603 did not produce significant changes in 51 biologically and nutritionally important components • chicken study showed; **√** live weight at day 0, live weight at day 42,total feed intake and feed efficiency similar across all treatments **√** broilers fed diets containing NK603 corn, non-modified control and one of the five commercial reference lines had similar adjusted feed efficiency **√** diets containing the other four reference lines had 2.3% poorer adjusted feed efficiencies than corn event NK603 **√** live weight, chill weight, breast meat, thighs, drums and wings were not affected by diets **√** fat pad and breast meat weights of the corn event NK603 birds were significantly lower than the non-modified line within the range of literature values rat study showed; **√** clinical parameters (hematology, clinical chemistry, urinalyis) and gross and microscopic pathology findings confirmed comparability of corn event NK603 to the non-modified control and the commercial grain diets • **summary;** **√** compositional and nutritional equivalence of corn containing the event NK603, **√** the absence of any significant pleiotropic or unintended effects **√** the absence of toxicity of the CP4 EPSPS andCP4 EPSPS L214P proteins **√** human and animal health safety of NK603corn

Table 5 | A summary of final reports written by Scientific Risk Committee for NK603 corn

Content in Scientific Risk Committee's report	Summary	Final decision
1. Vector construct and transformation process; • Analysis of molecular structure, expression and stability of inserted gene 2. Risk analysis of chemical composition and agricultural properties; • Chemical compositional analysis • Agricultural properties analyses 3. Toxicity assessment 4. Allergenicity assessment 5. Environmental risk assessment; • Potential invasiveness resulted from genetic modification • Gene flow from plant to other plant • Gene flow from plant to bacteria 6. Food processing technologies 7. Risk management	With the comparative analysis, it is determined that GM NK603 corn varieties are as safe as conventional corn varieties, it does not change in terms of allergenicity, and there is no difference in terms of nutritional content agricultural properties. It is concluded that in the case of unintended flow of GM NK603 corn varieties into environment, the probability of causing different environmental effects as compared with conventional varieties is very low. Based on information, Scientific Risk Assessment Committee decides that the use of GM NK603 corn grain containing cp4 epsps genes for glyphosate tolerance as food is not different from non-GM equivalence varieties in terms of causing unintended effects to human and environment. The scope of application contained the import and processing, food, and feed.	**Scientific Committee decision:** It is concluded that the use of corn for the production of fully refined oil, sugar syrup, dextrins and starches may not pose risk when GM NK603 corn and its products are used as food. **Biyogüvenlik Kurulu kararı:** Application of NK603 corn for food use was withdrawn by applicants. Therefore, there is no final decision of Biosafety board on this event. On the other hand, GM NK 603 corn and its products are used as feed.

CHAPTER 4

GLOSSARY

Adenine - A compound that is one of the four constituent bases of nucleic acids. A purine derivative, it is paired with thymine in double-stranded DNA.

Agriculture - The science and business of producing crops and/or livestock that provides food, fabric, and fuel.

Agrobacterium tumefaciens - A soil bacterium used in biotechnology to transfer genes to plant cells as a result of its ability to naturally transfer DNA into a plant host.

Bacillus thuringiensis (Bt) - A soil microorganism that is used as a biological insecticide by farmers—including organic farmers—to control pests. Additionally, the cry gene from this microorganism has been engineered into some crops to confer insect resistance.

Biotechnology - The practice of using tools from cellular biology, molecular biology, genetics, and biochemistry to improve genetic attributes of plants, animals, and other organisms.

Breeding - The human-facilitated mating of plants or animals with the objective of genetic improvement through selection.

Chromosome - Determines the inheritance of traits; made up of proteins and a molecule of DNA combined in a long, threadlike structure.

Cisgenics- Transfer of gene(s) from one plant line to another using recombinant DNA technology—but only using sequences from sexually-compatible species.

Classical breeding - Classical plant breeding uses deliberate interbreeding (crossing) of closely or distantly related individuals to produce new crop varieties or lines with desirable properties. Plants are crossbred to introduce traits/genes from one variety or line into a new genetic background.

Cloning - Creating a genetic replica of DNA (be it a fragment or an entire organism) without sexual reproduction.

Cytosine - a compound found in living tissue as a constituent base of nucleic acids. It is paired with guanine in double-stranded DNA.

Deoxyribonucleic acid (DNA) - Carries genetic information in living systems. The molecule's characteristic double-helix structure is made up of four base proteins and a sugar-phosphate backbone.

Gene - The functional unit of heredity, found on a chromosome. The "blueprint" in DNA that encodes information leading to cellular structure and function.

Gene silencing - The use of recombinant DNA technology to precisely decrease or eliminate the expression of a specific gene.

Genome - The complete genetic material found in the chromosomes of a particular organism.

Genome selection - A form of marker-assisted selection in which genetic markers covering the whole genome are used so that all quantitative trait loci (QTL) are in linkage disequilibrium with at least one marker.

Guanine - A compound that occurs in guano and fish scales, and is one of the four constituent bases of nucleic acids. A purine derivative, it is paired with cytosine in double-stranded DNA.

Hazards - Any potential cause of harm irrespective of how likely or unlikely that potential harm.

Herbicide - Specialty crop chemicals used for the control of weeds. This is a class of pesticide.

Herbicide tolerance - Genetic adjustment of plant structures or metabolism that interferes with action of compounds toxic to plants. One can therefore apply the specific herbicide directly to the field without damaging crop.

Hybrid - The offspring resulting from the cross of two parental lines chosen by desired traits or a potentially likely benefit from mixing of genetics.

Hybrid seed - Most commonly, the seed resulting from mating two elite plant lines with the intention of moving all positive traits into a common background.

Inheritance - The process by which genetic information is passed on from parent to offspring.

Insecticide - Specialty crop protection chemicals used for the control of insects. This is a class of pesticide.

Marker-assisted breeding (MAS) - An indirect selection process where a trait of interest is selected based on a marker (morphological, biochemical, or DNA/RNA variation) linked to a trait of interest (e.g. productivity, disease resistance, abiotic stress tolerance, and quality), rather than on the trait itself. This process is used in plant and animal breeding.

Molecular breeding - The application of molecular biology tools, often in plant breeding. The areas of molecular breeding include QTL mapping or gene discovery, marker assisted selection and genomic selection, and genetic engineering.

Molecular markers - In genetics, a molecular marker (identified as genetic marker) is a fragment of DNA that is associated with a certain location within the genome. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA.

Molecular scissors (restriction enzymes) - An enzyme produced chiefly by certain bacteria, having the property of cleaving DNA molecules at or near a specific sequence of bases.

Mutations - The changing of the structure of a gene, resulting in a variant form that may be transmitted to subsequent generations, caused by the alteration of single base units in DNA, or the deletion, insertion, or rearrangement of larger sections of genes or chromosomes.

Natural selection - The process where a given trait increases in prevalence in a population due to its positive effect on an organism, conferring an advantage to reproduce.

Nucleases - An enzyme that cleaves the chains of nucleotides in nucleic acids into smaller units.

Pest resistance - Plants with an inherent structural or chemical deterrent to insect, arthropod, or fungal pests as a result of specific breeding or genetic engineering techniques.

Pesticide - Including insecticides, herbicides, fungicides, and rodentcides, pesticides are used to rid of specific pest organisms.

Precautionary principle - The philosophy requiring the elimination of potential hazards when there is little information about potential bad outcomes.

Protein - Any of a class of nitrogenous organic compounds that consist of large molecules composed of one or more long chains of amino acids and are an essential part of all living organisms, especially as structural components of body tissues such as muscle, hair, collagen, etc., and as enzymes and antibodies.

Recombinant DNA - DNA that has been formed artificially by combining constituents from different organisms.

Ribonucleic acid (RNA) - A nucleic acid present in all living cells. Its principal role is to act as a messenger carrying instructions from DNA for controlling the synthesis of proteins, although in some viruses RNA rather than DNA carries the genetic information.

Substantial equivalence - The concept that two genetically-different plant lines are deemed the same based on composition and safety.

Thymine - A compound that is one of the four constituent bases of nucleic acids. A pyrimidine derivative, it is paired with adenine in double-stranded DNA.

Trait - a genetically determined characteristic.

Transgene - Of, relating to, or denoting an organism that contains genetic material into which DNA from an unrelated organism has been artificially introduced.

Uncertainty - A circumstance in which an individual decision maker is not aware of all possible outcomes of their decision and/or the probabilities with which those outcomes may occur given their decision.

Utility - The level of satisfaction one obtains from a particular experience. Economists assume that individuals make choices in order to achieve the greatest level of utility.

CHAPTER 5

REFERENCES

Biosafety Law (No 5977) http://www.tbbdm.gov.tr/Libraries/Regulations/BiyoguvenlikKanunu.sflb.ashx (Accessed 25 May 2017)

CAC (Codex Alimentarius Commission). 2003a. Guideline for the Conduct of Food Safety Assessment of Foods Using Recombinant DNA Plants. Doc CAC/GL 45-2003. Rome: World Health Organization and Food and Agriculture Organization.

CAC (Codex Alimentarius Commission). 2003b. Principles for the Risk Analysis of Foods Derived from Modern Biotechnology. Doc CAC/GL 44-2003. Rome: World Health Organization and Food and Agriculture Organization.

Commission Decision (2002/623/EC) of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the

Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official

Journal L 200: 22-33. http://europa.eu.int/eur-lex/pri/en/oj/dat/2002/l_200/l_20020020730en00220033.pdf (Accessed 25 May 2017)

Commission recommendation 97/618/EC of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under

Regulation (EC) No 258/97 of the European Parliament and of the Council. http: //europa.eu.int/smartapi/cgi/sga_ doc? smartapi! celexapi!prod! CELEX numdoc&lg=EN&numdoc=3 1997H0618&model=guichett (Accessed 25 May 2017)

Council for Biotechnology Information. 2001. "Substantial Equivalence in Food Safety Assessment"http://thebeuselaer.weebly.com/uploads/6/3/8/4/6384873/substantial_equivalence.pdf (Accessed 25 May 2017)

Draft National Biosafety Framework For Republic Of Turkey Prepared In The Scope Of The UNEP-GEF Project On The Development Of The National Biosafety Framework By Ministry Of Agriculture And Rural Affairs General Directorate Of Agricultural Research http://www.unep.org/biosafety/files/TRNBFrep.pdf (Accessed 25 May 2017)

EC (European Commission). 2010a. A Decade of EU-funded GMO Research (2001–2010). Brussels: European Commission.

EC (European Commission). 2010b. Directive 2010/63/EU of the European Parliament andof the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union 276:33–79.

EC (European Commission). 2013. Commission implementing regulation (EU) No 503/2013of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1 981/2006. Official Journal of the European Union 157:1–48.

EFSA (European Food Safety Authority). 2007. Statement of the Scientific Panel on Genetically Modified Organisms on the Analysis of Data from a 90-day Rat Feeding Study with MON 863 Maize. Available at http://www.efsa.europa.eu/sites/default/files/scientific_ output/files/main_documents/GMO_statement_MON863%2C0.pdf. Accessed December 13, 2015.

EFSA (European Food Safety Authority). 2010. Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 8:1700.

EFSA (European Food Safety Authority). 2011a. Guidance on risk assessment of food and feed from genetically modified plants. EFSA Journal 9:2150.

EFSA (European Food Safety Authority). 2011b. Scientific opinion on guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA Journal 9:2438.

EFSA (European Food Safety Authority). 2011c. Statistical significance and biological relevance. EFSA Journal 9:2372.

EFSA (European Food Safety Authority). 2012. Review of the Séralini et al. (2012) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology. EFSA Journal 10:2910.

EFSA (European Food Safety Authority). 2013. Scientific Opinion on application EFSAGMO-NL-2007-45 for the placing on the market of herbicide-tolerant, high-oleic acid, genetically modified soybean 305423 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer. EFSA Journal 11:3499.

EFSA (European Food Safety Authority). 2015. Conclusion on the peer review of the pesticide risk assessment for the active substance glyphosate. EFSA Journal 13:4302.

Environmental safety of genetically crops. 2011. Edited by Rebecca Grumet, James F. Hancock, Karim M. Maredia, and Cholani Weebadde. Michigan State University, 234 pages.

EPA (U.S. Environmental Protection Agency). 2000. Risk Characterization Handbook. Washington, DC: EPA.

Gıda Amacıyla İthali İstenen Genetiği Değiştirilmiş Nk603 Mısır Çeşidi ve Ürünleri İçin Bilimsel Risk Değerlendirme Raporu http://www.tbbdm.gov.tr/Files/arsiv/gida/misir/risk/NK603.pdf (Accessed 25 May 2017)

GM Crop Database, Center for Environmental Risk assessment http://cera-gmc.org/GMCropDatabase (Accessed 25 May 2017)

GRACE, 2012–2015, available at http://cordis.europa.eu/project/rcn/104334_en.html, (accessed May 9, 2016)

International Service for the Acquisition of Agri-biotech Applications (ISAAA) 2016. Commercial GM Traits List. http://www.isaaa.org/gmapprovaldatabase/commercialtraitlist/default.asp (Accessed 25 May 2017)

Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32003R1829 (Accessed 25 May 2017)

Report of the Scientific Steering Committee's Working Group on Harmonisation of Risk Assessment Procedures in the Scientific Committees advising the European Commission in the area of human and environmental health - 26-27 October 2000. http://europa.eu.int/comm/food/fs/sc/ssc/out83_en.pdf (Accessed 25 May 2017)

Safety Assessment of Roundup Ready Corn Event NK603 http://www.monsanto.com/products/documents/safety-summaries/corn_pss_nk603.pdf (Accessed 25 May 2017)

Schauzu, Marianna (Apr 2000). "The concept of substantial equivalence in safety assessment of foods derived from genetically modified organisms" (PDF). AgBiotechNet. 2.

Safety Considerations for Biotechnology: Scale-up of Crop Plants, OECD, 1993. http://www.oecd.org/pdf/M00034000/M00034525.pdf 16. (Accessed 25 May 2017)

Safety evaluation of foods derived by modern biotechnology: concept and principles. OECD, 1993. http://www.oecd.org/pdf/M00033000/M00033002.pdf 17. (Accessed 25 May 2017)

Safety aspects of genetically modified foods of plant origin. Report of a joint FAO/WHO expert consultation on foods derived from biotechnology, 29 WHO/FAO, 2000. http://www.fao.org/es/ESN/food/pdf/gmreport.pdf (Accessed 25 May 2017)

Safety evaluation of foods derived by modern biotechnology: concept and principles. OECD, 1993. http://www.oecd.org/pdf/M00033000/M00033002.pdf 1 (Accessed 25 May 2017)

National Academies of Sciences, Engineering, and Medicine. 2016. Genetically Engineered Crops: Experiences and Prospects. Washington, DC: The National Academies Press. doi: 10.17226/23395.

OECD (Organisation for Economic Co-operation and Development). 1986. Recombinant DNA Safety Considerations. Paris: OECD.

OECD (Organisation for Economic Co-Operation and Development). 2006. An Introduction to the Food/Feed Safety Consensus Documents of the Task Force. Series on the Safety of Novel Foods and Feeds, No 14. Paris: OECD.

OECD (Organisation for Economic Co-Operation and Development). 2015. Safety Assessment of Foods and Feeds Derived from Transgenic Crops, Volume 2, Novel Food and Feed Safety. Paris: OECD.

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TECHNICAL GUIDELINES FOR THE RISK ASSESMENT OF GENETICALLY ENGINEERING CROPS AND DERIVED FOOD AND FEED

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