

Review

Edible safety requirements and assessment standards for agricultural genetically modified organisms

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Abstract

This paper describes the background, principles, concepts and methods of framing the technical regulation for edible safety requirement and assessment of agricultural genetically modified organisms (agri-GMOs) for Shenzhen Special Economic Zone in the People's Republic of China. It provides a set of systematic criteria for edible safety requirements and the assessment process for agri-GMOs. First, focusing on the degree of risk and impact of different agri-GMOs, we developed hazard grades for toxicity, allergenicity, anti-nutrition effects, and unintended effects and standards for the impact type of genetic manipulation. Second, for assessing edible safety, we developed indexes and standards for different hazard grades of recipient organisms, for the influence of types of genetic manipulation and hazard grades of agri-GMOs. To evaluate the applicability of these criteria and their congruency with other safety assessment systems for GMOs applied by related organizations all over the world, we selected some agri-GMOs (soybean, maize, potato, capsicum and yeast) as cases to put through our new assessment system, and compared our results with the previous assessments. It turned out that the result of each of the cases was congruent with the original assessment.

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Keywords: Agricultural genetically modified organism; Edible safety assessment; Criteria; Hazard grade

Abbreviations: agri-GMOs, agricultural genetically modified organisms; ANZFA, Australia New Zealand Food Authority; ArAA, aromatic amino acid; *Bt*, *Bacillus thuringiensis* δ -endotoxin gene; CAC, Codex Alimentarius Commission; CaMV 35S, 35S promoter from Cauliflower Mosaic Virus; *CAD*, cecropin AD; *CDB*, cecropin DB; cDNA, DNA complementary to an RNA strand; *cp4-epsps*, 5-enolpyruvylshikimate-3-phosphate; *CryIA(b)*, a synthetic gene encodes the first 648 amino acids, insecticidal-active truncated product identical to that of *CryIA(b)* gene of *Bacillus thuringiensis* subsp.; DNA, deoxyribonucleic acid; EC, European Community; *E. coli*, *Escherichia coli*; EPA, Environmental Protection Agency; FAO, Food and Agriculture Organization of United Nations; FDA, Food and Drug Administration; GM, genetically modified; GMFs, genetically modified foods; GMOs, genetically modified organisms; HSP70, Heat-Shock Proteins 70; MHLW, Ministry of Health, Labour and Welfare; mRNA, messenger RNA; NOS, terminator of nopaline synthase gene; *npt II*, neomycin-3'-phosphotransferase gene; OECD, Organization for Economic Co-operation and Development; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; WHO; World Health Organization of the United Nations.

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1. Introduction

Agricultural genetically modified organisms (agri-GMOs) means “animals, plants, microorganisms and their products whose genomic structures have been modified by genetic engineering technologies for the use in agricultural production or processing”, which include: “genetically modified animals, plants (including plant seeds, breeding livestock and poultry, aquatic fry and seeds) and microorganisms”; “products of genetically modified animals, plants and microorganisms”; “products directly processed from genetically modified agricultural products”; “seeds, breeding livestock and poultry, aquatic fry and seeds, pesticides, veterinary drugs, fertilizers, additives and other products containing the ingredients derived from genetically modified animals, plants and microorganisms or their products” (State Council of P.R. China, 2001). Agri-GMOs are a wide category and with a wide applicability range and the connotation of the safety is very broad, including both ecological safety and edible safety. Ecological safety mainly refers to the impact of agri-GMOs on the environment, especially the impact on biological diversity. Edible safety mainly involves the influence of agri-GMOs on organismic health, especially the influence on the health of human. Edible safety becomes the main focus, when agri-GMOs are applied in producing and processing foods. Genetically modified foods (GMFs) are the foods or food additives derived from agri-GMOs. Generally, GMFs involve GM plants, animals or microorganism products and their direct processing products, i.e., the foods or food additives which are produced by using GMOs or their direct processing products as raw materials (Ministry of Health of P.R. China, 2002). As a result, it is considered that the crucial aspects involved in GMF safety are the edible safety of the agri-GMO itself and the procedures and conditions of the GMF’s production and processing (Ministry of Agriculture of P.R. China, 2002).

Although agri-GMOs may provide modern agriculture with the improvements in efficiency and other benefits, genetic engineering technology inevitably confers agri-GMOs with special problems of edible safety. Since the current safety criteria for agricultural organisms and foods are based on the traditional manufacturing techniques and history of long-term application, they lack specificity and sensitivity to the special problems of edible safety produced by genetic engineering technology. Accordingly, many international organizations and countries throughout the world began to draw up a set of administrative statutes, policies, assessment principles and testing methods for agri-GMOs and their products (König et al., 2004). The United Nations Food and Agriculture Organization/World Health Organization (FAO/WHO) first published a safety assessment strategy for modern biotechnology products in 1991 (WHO, 1991a,b), and promulgated guidelines in 1996 and 2000 for the safety assessment of organisms and foods which are produced by utilization of the current biotechnology or genetic engineering technology, including

evaluation principles, indexes and methods (FAO/WHO, 1996, 2000). With the support of the FAO/WHO, the Codex Alimentarius Commission (CAC) promulgated a set of principles and guidelines for edible safety assessment of foods derived from modern biotechnology and DNA recombination techniques in 2003 (CAC, 2003a). In 1993, the Organization for Economic Co-operation and Development (OECD) put forward the concept and principles of safety evaluation of foods derived from biotechnology (OECD, 1993), then followed this by the guideline for safety assessment, toxicity and nutrition test (OECD, 1996, 1998). The European Community (EC) has controlled agri-GMOs and their products through legislation since 1990, and has promulgated and modified a set of statutes, principles and methods about the administration and safety assessment of agri-GMOs (EC, 1990, 1997, 2003a; European Food Safety Authority, 2005). Similarly, many countries, such as the United States, Canada, Japan, Australia and New Zealand have successively promulgated administration regulations for agri-GMOs (US FDA, 1992; Health Canada, 1994; MHLW, 2000 ANZFA, 2000). China has also promulgated policies on agri-GMOs, for instance, the “Regulations on Administration of Agricultural Genetically Modified Organisms Safety” and the “Implementation Regulations on Safety Assessment of Agricultural Genetically Modified Organisms” as well as “Administration Measures for Genetically Modified Food Hygiene”, among which edible safety assessment of agri-GMOs is an important part (State Council of P.R. China, 2001; Ministry of Agriculture of P.R. China, 2002; Ministry of Health of P.R. China, 2002). In order to ensure the edible safety assessment system was properly executed, the Ministry of Agriculture of P.R. China promulgated the “Guideline for Safety Assessment of Foods from Genetically Modified Plants and derived products”. This guideline prescribes that when the edible safety assessment is undertaken, several aspects must be included, i.e., safety assessment of the gene recipient organisms, gene donor organisms and genetic manipulation, toxicity assessment of agri-GMOs and their products, key components analysis and the nutrition assessment of the GMFs, accumulation assessment of exogenous chemical substances, and assessment of pesticide resistance (Ministry of Agriculture of P.R. China, 2006).

Since different international organizations and countries held differing opinions on the safety issues of agri-GMOs beforehand, different principles have been used when legislation on agri-GMOs were drawn up. Typically, there were two patterns of the assessment and administration: one focused on the procedure, considering that the genetic engineering technique itself has a certain potential hazard, so the safety of the procedure should be the crucial part of assessment; the other one focused on the products, suggesting that the genetic engineering technique has no essential difference with traditional biotechnology, so the safety assessment should be aimed at the final products. However, currently, with far more widespread recognition of food

safety issues and genetic engineering techniques and more frequent research cooperation between countries, different countries have gradually reached agreement on the safety assessment issues and put forward some basic principles, including the risk analysis principles, the substantial equivalence principle and the necessity for case-by-case assessment (König et al., 2004).

In 1995, FAO/WHO established risk analysis principles to evaluate the impact on health of chemicals found in food, consisting of three parts – risk assessment, risk management and risk communication. These principles have played an important role in assessing potential hazard to human or animals, formulating international sanitary standards for food safety management and harmonizing the procedures and administration policies between countries. Risk assessment, which is a scientifically based process consisting of hazard identification, hazard characterization, exposure assessment and risk characterization, evaluating the existence, content, source and degree of risk for human in food, forms the core of risk analysis (FAO/WHO, 1995, 1997, 1998, 2006; CAC, 2003b; EC, 2003b). Recently, based on these risk assessment principles and long-term accumulation of data, we considered the toxicity, allergenicity and anti-nutrition effects of agri-GMOs as the three possible risk elements that could be harmful to human health. Those risk elements mentioned above can be present in agri-GMOs or emerge during the process of products manufacture or processing. As a result, the safety assessment targets of agri-GMOs should be the recipient organisms, genetic manipulation, unintended effects and the impact of the production process on the existence, contents and the degree of hazard of the three risk elements.

The potential risk elements, which include the toxicity, allergenicity and anti-nutrition effects, widely exist in both agri-GMOs and traditional agricultural organisms and foods. Nevertheless, some of the risks are peculiar to agri-GMOs, due to the specific manufacturing technique. In 1990, FAO and WHO pointed out that the comparison between GMFs and traditional foods contribute a key part to the safety assessment of GMFs. Following this, OECD developed the substantial equivalence principle and suggested it should be the basic rule and practical approach for the safety assessment of foods derived from modern biotechnology (OECD, 1993). So far, this principle has been widely used by many international organizations and countries to assess the safety of agri-GMOs, therefore, it has a critical impact as a starting point for the safety assessment of agri-GMOs. In accordance with the basic rule of the substantial equivalence principle, the starting point of safety assessment of agri-GMOs should be the comparisons made with the counterpart traditional products for sale in the current market. The aspects for comparisons should be the agronomical traits, the morphological traits, the macro-nutrients, the micro-nutrients, the key toxins, the key allergens, the key anti-nutrients and so forth. Through comparing the various aspects of chemical composition listed above with those of the traditional com-

parator food, the similarities and differences can be identified. If the compositions of the two are substantially equivalent, then they can be regarded as of equal safety; if they are not substantially equivalent, a further analysis of the differences is needed.

The case-by-case principle, which is applied by many international organizations and countries, is considered to be the basic rule for ascertaining the precise approaches and methods for the safety assessment of agri-GMOs (FAO/WHO, 2000). For various GMOs, due to the difference in many aspects including category and nature of the recipient organisms, the sources and functions of the exogenous genes, the introduced methods and sites of exogenous genes, the usage of GM products, the effects arising from various GMOs and their impacts on human and animal health are impossible the same. In particular, the current introduction technology cannot ensure exogenous genes are directionally integrated to a fixed-point in a recipient organism's genome. In addition, the alteration of the genomic constitution and the non-isoloci integration of exogenous genes may intensify the discrepancy of genetic structure in homologous chromosomes of recipient organisms. This means that the heterogeneity of the exogenous gene and the randomness of foreign gene integration could result in new mutation hot spots in the exogenous genes inserted into a recipient organism's genome and in the altered genome itself. It is predictable that the probability and frequency of mutation in the phenotypic characteristics and the hereditary features of GMOs could be much higher than that of their traditional counterparts. It is clear that the unintended effects of agri-GMOs may be concealed, progressive, diverse and uncertain, therefore, they may affect the edible safety of GMOs to different degrees. For instance, if the targeted phenotypic or hereditary features designed by the genetic engineering scheme disappear, or the non-targeted phenotypic or hereditary features produce unintended changes, it may lead to unintended changes in toxicity, allergenicity or anti-nutrition effects of GMOs. In addition, the horizontal transfer of antibiotic resistance genes integrated into a GMO genome may induce antibiotic resistance of bacteria living in the human or animal gastrointestinal tract, as well as the environment, which is an important safety issue for GMOs (Bennett et al., 2004; EC, 2003d; ILSI, 2000; Kuiper et al., 2004; Van den Eede et al., 2004). In other words, we cannot assess the safety issues, which include the potential, acute, short-term and long-term edible safety of GMOs, unless the unintended effects have been sensitively detected and a precise appraisal of these effects has been made. Clearly, unintended effects of GMOs should be considered as one of the most difficult aspects among all the items in edible safety assessment and they also are the aspects in which there is the most remarkable discrepancy in safety appraisals comparing agri-GMOs with their traditional counterparts (FAO/WHO, 2000; EC, 2003b; König et al., 2004; Cellini et al., 2004). According to the case-by-case principle, for each agri-GMO, a scientifically event-specific safety

assessment procedure should be established based on its recipient organism, genetic manipulation and the target of gene modification. In brief, the safety assessment scheme for a GMO should have enough specificity and sensitivity to find any hidden dangers in edible safety, especially any generated from unintended effects, but should avoid any unnecessary evaluation steps in order to simplify the assessment process.

In the frame of the three principles mentioned above, a universal assessment procedure has been built to evaluate the safety of agri-GMOs and their products, including the assessment of recipient organisms, the assessment of genetic manipulation (such as gene reconstruction methods, exogenous genes and donor organisms), and the assessment of novel proteins or metabolic products. By comparing the related traits of GMOs with those of their recipient organisms, the safety of gene reconstruction can be determined, and whether or not the gene reconstruction will have a harmful impact on the health of human beings can be demonstrated (König et al., 2004). In the meantime, the related techniques and methods, which are applied to test for toxicity, allergenicity, anti-nutrition effects and the unintended effects of agri-GMOs and their products, have been extensively researched. Some of these techniques and methods have gained widespread approval and extensive application in the safety assessment of agri-GMOs and products by many international organizations and countries. These include (i) toxicity testing methods, such as whole foods animal testing methods, in vitro and in vivo methods for assessing the recombinant protein, the novel compound or the novel metabolite, (ii) allergenicity assessment methods, such as the decision tree analytical method, amino acid sequence homology analysis method, methods for testing resistance of recombinant protein to proteolysis by simulated gastric enzymes or pepsin, heat resistance of recombinant protein, specific serum and the target serum screen methods, and animal models for testing the recombinant protein allergenicity, (iii) methods for detection of anti-nutrition effects, such as the quantitative analysis method for anti-nutrients, and animal testing models for nutrition efficiency and (iv) unintended effects assay methods, such as the overall comparison and analysis methods for characteristics or composition, the comparison and analysis methods for targeted and non-targeted characteristics or the composition (FAO/WHO, 2000, 2001; OECD, 1998, 2001, 2002; EC, 2003c; Ministry of Agriculture of P.R. China, 2006; König et al., 2004; Cellini et al., 2004; Jacobsen, 1996; Deng et al., 2003, 2004; Li et al., 2004; Jia et al., 2004; Prescott and Hogan, 2006).

However, there are also some difficulties when the risk analysis principle, substantial equivalence principle and case-by-case principle are applied in the safety assessment of agri-GMOs. For instance, when evaluating the risk of some new gene expression products, it can prove difficult to find any data on epidemiology, in vitro or in vivo animal tests. As a result, the hazard identification, hazard characterization, exposure assessment and risk characterization

cannot be done; for example, when approaching the comparison section of an agri-GMO constructed using a new recipient organism, there are no counterpart data from a traditional agricultural organism, so the substantial equivalence analysis cannot be done. Thus, when the safety assessment of agri-GMOs is carried out, the results may be very different due to different appraisal approaches.

Although, the current basic principles for assessing agri-GMOs tend to be congruous in different countries throughout the world, when it comes to the assessment indexes, the requirements, the precise procedures and the approaches, there are still some uncertainties, inconsistencies, non-standardization and opacity. In particular, in some proposals for assessing the edible safety of agri-GMOs, the emphasis only lies in the indexes to which attention should be paid and how to obtain information about such indexes, but the safety requirements for GMOs or their products, expressed as the hazard grades, has never been focused on. Such a demerit of the present safety assessment system weakens the authority of assessment results. It can lead to doubts among consumers and the public about the safety of agricultural GMFs, which inhibit the development of agri-GMOs. In order to improve the accuracy and the authority of safety assessment reports and enhance public confidence in the safety of agri-GMOs, and promote good practice in the GMF industry, international organizations and countries should focus on improving the evaluation approaches for agri-GMOs' safety. In particular, these efforts should be concentrated on strengthening the versatility of assessment standard, the consistency of appraisal procedure, the adaptability of assessment approaches, so that the comparability of assessment results can be enhanced, the trade barriers in GMFs between countries and districts can be reduced, whilst at the same time, the rights to health, information and choice of consumers can be protected.

Since August 2005, with the support of the People's Government of Shenzhen Municipality and natural science funds of Guang Dong Province, the Center for Quality Crop Seed of Shenzhen Municipality, Shenzhen Center for Disease Control and Prevention and Tsinghua Shenzhen Longgang Research Institute of China National Hybrid Rice Research and Development Center have built up a group to devise the technical regulation of "Requirement and Assessment of Edible Safety for Agriculture Genetically Modified Organisms" for Shenzhen Special Economic Zone. This regulation was authorized and published on August 15th, 2006, by the People's Government of Shenzhen Municipality and has been implemented since February 15th, 2007 (People's Government of Shenzhen Municipality P.R. China, 2006). Shenzhen Special Economic Zone has local legislative power in China, and it also is a research demonstration center for agri-GMOs and a main distributing center for import and export trade of GMFs. To ensure sound development of agri-GMOs, and with reference to the basic principles set up by FAO, WHO, CAC, OECD and EC, in accordance with the

relevant statutes and the guidances of our country, we have developed edible safety requirements and assessment processes for agri-GMOs. In this paper, we set out the assessment indexes, compulsory requirements, hazard grades, evaluation procedures and run several cases (soybean, maize, potato, capsicum and yeast) through the assessment requirements, comparing the results with the original assessments to validate the applicability and the congruency of our safety assessment requirements.

2. Edible safety requirements for agri-GMOs

2.1. Concepts

2.1.1. Edible safety

“Food security (food safety)” and “edible safety” are closely related to each other. But the two concepts have different meanings. Food security characterizes the safety attribute of food while edible safety defines the degree of food safety assurance. However, these two concepts are often interchanged in the evaluation of food hygiene and safety management. According to the “Food Hygiene Law of the People’s Republic of China”, “Food shall be nontoxic and harmless, conform to proper nutritive requirements and have appropriate sensory properties such as colour, fragrance and taste” (People’s Republic of China, 1995). In China, the “Administration Measures for Genetically Modified Food Hygiene” has regulated that GMFs should meet the “Food Hygiene Law of the People’s Republic of China” as well as other relevant laws, regulations, rules and standards, and should not result in any acute, chronic or other potential health hazards to human (Ministry of Health of P.R. China, 2002). The above regulation emphasizes the safety attributes of foods and also legally defines the safety of foods and GMFs. On the other hand, the definition of food safety from WHO is “an assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use” (WHO, 1996), whose focus lies in the promise and requirements for food security. Therefore, in essence, the “food safety” defined by WHO refers to “edible safety”. This indicates that “food security” is an absolute concept, with which the evaluation of a food will yield a conclusion of either “yes” or “no”. However, no food can be absolutely safe, and even in some traditional foods there exist some known or may be some unknown health risk factors. Therefore, the absolute safety requirement for GMFs lacks a scientific basis and is difficult to meet. On the other hand, “edible safety” is a relative concept whereby the “edible safety” evaluation can give a conclusion such as “superior”, “inferior”, or other different grades, an approach that is more readily applicable to the management and evaluation of food security. Currently, relative food security manifests increasing importance and the substantial equivalence principle, based on the concept of relative safety, is generally recognized in the global evaluation and management of GMF safety. According to the concept

of substantial equivalence, if a genetically modified food or GM food ingredient has the equivalence in substance with a known food or food ingredient, they should be considered to have the same food security (OECD, 1993). In China, the “Administration Measures for Genetically Modified Food Hygiene” has also proposed relative safety standards for GMFs, that is, the edible safety and nutritional quality of GMFs should not be inferior than the corresponding traditional food (Ministry of Health of P.R. China, 2002). We have adopted the concept of edible safety raised by WHO and the standards of relative safety of GMFs proposed by the Ministry of Health of the People’s Republic of China.

2.1.2. Risk factors

Presently, there are several risk factors for agri-GMOs which have been generally accepted by some relevant international organizations and countries. These risk factors are likely to cause harm to human and animal health and they include toxicity, allergenicity, anti-nutrition effects, and unintended effects. Accordingly, we have taken toxicity, allergenicity, anti-nutrition effects and the unintended effects as the indicators for edible safety evaluation. Given the current limitations at the technical level, there may be unknown factors related to agri-GMOs that are harmful to human health. Thus, the evaluation standard we have developed may need to be modified with the development of the research and understanding about the edible safety of agri-GMOs.

2.1.2.1. Toxicity. Toxicity in our standard is defined as any adverse effect in human or animals caused by the intake of biotoxins present in agri-GMOs. The toxic components contained in agri-GMOs can be classified into exogenous and endogenous toxic components. Exogenous toxic components are introduced into agri-GMOs during the process of production and processing of agri-GMOs and they can cause adverse effects in human and animals, including pathogenic microorganisms and parasites, pathophoric insects, pesticide residues, residues of veterinary drugs, metals, organic and inorganic compounds. On the other hand, the endogenous toxic components are natural biotoxins, including protein toxoid, polypeptide toxoid, glycoprotein toxoid, alkaloid toxoid and mutagens, which are produced by plants, animals and microorganisms themselves and which are capable of causing adverse effects in human or animals. The source of exogenous toxic components and their content in agri-GMOs have no apparent correlation with genetic modification. Moreover, the existing health and safety standards regarding agricultural organisms and products have developed a set of specific requirements for exogenous toxic components. Therefore, this standard for agri-GMOs does not include any specific considerations about possible adverse effects caused by exogenous toxic components. On the other hand, the source of endogenous toxic components and their content in agri-GMOs are related to the genetic modification.

Moreover, so far, there is not an assessment and requirement system about endogenous toxic components in the existing health and safety standards regarding agricultural organisms and products. Therefore, the assessment and requirement about the possible adverse effects caused by endogenous toxic components in agri-GMOs are involved in this standard.

2.1.2.2. Allergenicity. In this standard, allergenicity is defined as the allergic effect in human or animals caused by intake of bio-allergens contained in agri-GMOs. Biological allergens are the bioactive substances which are produced by plants, animals and microorganisms themselves and can result in allergic reaction in human or animals. The chemical essence of most biological allergens is protein. On account of the correlation between the allergic effect arising from allergenic components in agri-GMOs and genetic modification, as well as the absence of such considerations in the present health and safety standards of agricultural organisms and products, the assessment and requirement about the allergic effects caused by allergens in agri-GMOs are involved in this standard.

2.1.2.3. Anti-nutrition effects. In this standard, the anti-nutrition effect is defined as the inhibition in animals or human of the absorption and utilization of nutritional ingredients in foods, which is caused by the intake of anti-nutrients in agri-GMOs. Anti-nutrients are produced by plants, animals and microorganisms themselves, and they can result in inhibition of the absorption and utilization of nutritional ingredients of foods. Based on the similar consideration with toxicity and allergenicity mentioned above, the requirement and assessment for anti-nutrition effects of agri-GMOs are developed in this standard.

2.1.2.4. Unintended effect. In the standard, unintended effect is defined as the deviation of phenotypic and hereditary characteristics of agri-GMOs from the designed target of genetic engineering, or the horizontal transfer of antibiotic resistance genes. Deviation can occur in the periods of propagation, growth, development and metabolic processes of agri-GMOs, caused by the introduction of exogenous genes. Based on the similar consideration with other risk factors mentioned above, the requirement and assessment for unintended effects of agri-GMOs are involved in this standard.

Phenotypic characteristics of agri-GMOs include the main agronomy traits and nutrition traits. The agronomy traits refer to the features in such aspects as production, processing and economy, which are related to the production and processing of agri-GMOs. The nutrition traits refer to the types, contents and functions of the nutrients produced by agri-GMOs themselves, including the targeted nutrition traits designed by genetic engineering and other non-targeted nutrition traits. The deviation of agronomy and nutrition traits from the designed target of genetic

engineering of agri-GMOs is defined as phenotypic characteristics variation. Because of the influence of various factors, phenotypic characteristics variation may occur in both traditional agricultural organisms and agri-GMOs, which may result in adverse effects on edible safety.

Hereditary features of agri-GMOs include the integration, transcription and expression traits of exogenous genes, and genome transcription traits. Integration traits refer to such features as the type, copy number, integration site and the number of base pairs and nucleotide sequences of exogenous genes; transcription traits refer to the nucleotide sequences and the number of base pairs of messenger RNA (mRNA) produced by exogenous gene transcription; expression traits refer to the molecular weight, the amino acid sequences and the biological activity of the expressed products of exogenous genes; genome transcription traits refer to the types of the mRNA produced by transcription of agri-GMO genome. The deviation in the above-mentioned traits from the designed targets of agri-GMOs is called as hereditary feature variation. Such hereditary feature variation may occur in not only agri-GMOs but also traditional agricultural organisms, which may result in adverse effects on edible safety.

The antibiotic resistance genes are the exogenous genes that can express antibiotic resistance products. Antibiotic resistance genes integrated into the genome of agri-GMOs could transfer into other non-targeted biological cells (such as the bacteria living in human or animal gastrointestinal tract) in the process of the production, utilization and consumption of agri-GMOs. Antibiotic resistance genes of agri-GMOs can be transferred into non-targeted cells by horizontal transfer, which is also regarded as an adverse effect on the edible safety of agri-GMOs.

2.1.3. Hazard grade

In 2001, State Council of the People's Republic of China promulgated the "Regulations on Administration of Agricultural Genetically Modified Organisms Safety", in which the safety grades of agri-GMOs were classified into four levels according to the hazard degree of agri-GMOs for human, animals, plants, microorganisms and ecological environment ([State Council of P.R. China, 2001](#)). In 2002, the "Implementation regulations on Safety Assessment of Agricultural Genetically Modified Organisms" promulgated by Ministry of Agriculture of P.R. China, further regulated the safety grades of agri-GMOs. According to the regulations the safety grades of agri-GMOs from I to IV respectively signify no harmful effect or no risk, low risk, moderate risk and high risk ([Ministry of Agriculture of P.R. China, 2002](#)). This standard follows the above-mentioned classification principles.

2.1.3.1. Toxicity grade. According to the toxicity of recipient organisms or agri-GMOs and its hazard degree for human and animals, four toxicity grades can be defined: grade I signifies no toxicity; grade II and grade III, respectively signify the toxicity whose effects can be completely

and mostly avoided if certain safety control measures are adopted; grade IV signifies the toxicity whose effects cannot be prevented due to no appropriate control measure being currently available.

2.1.3.2. Allergenicity grade. According to the allergenicity of recipient organisms or agri-GMOs and its hazard degree for human and animals, four allergenicity grades can be defined: grade I signifies no allergenicity; grade II and grade III, respectively, signify the allergenicity whose effects can be completely and mostly avoided if certain safety control measures are adopted; grade IV signifies the allergenicity whose effects cannot be avoided due to no appropriate control measure currently available.

2.1.3.3. Anti-nutrition effect grade. According to the anti-nutrition effects of recipient organisms or agri-GMOs on the absorption and utilization of nutritional ingredients in foods, four anti-nutrition effect grades can be defined: grade I signifies no anti-nutrition effects; grade II and grade III, respectively, signifies the occurrence of anti-nutrition effects, but certain safety control measures can completely and mostly prevent the anti-nutrition effects; grade IV signifies the occurrence of anti-nutrition effects but there is no appropriate control measure currently available to prevent such effects.

2.1.3.4. Unintended effect grade. According to the unintended effects and the impact degree of recipient organisms or agri-GMOs on edible safety, four unintended effect grades can be defined: grade I signifies that neither phenotypic characteristics nor hereditary features variation occur, and that no horizontal transfer of antibiotic resistance genes occurs; grade II signifies that some phenotypic or hereditary characteristics variations occur, but the variations have no harmful impact on edible safety, and that no horizontal transfer of antibiotic resistance genes occurs; grade III signifies that some phenotypic and hereditary characteristics variations occur, but the variations have no harmful impact on edible safety, and that no horizontal transfer of antibiotic resistance genes occurs; grade IV signifies one of the following two statuses occur: one status is that some of phenotypic or/and hereditary characteristics variations occur, and the variations have a harmful impact on edible safety; the other status is that horizontal transfer of antibiotic resistance genes occurs.

2.1.4. Impact type of genetic manipulation

Genetic manipulation is the technology used to introduce recombinant DNA into recipient organisms by physical, chemical or biological means. Three impact types of genetic manipulation on the edible safety of recipient organisms were specified by the “Implementation regulations on Safety Assessment of Agricultural Genetically Modified Organisms”. Impact type 1 signifies an increase in the edible safety of recipient organisms; impact type 2 signifies no effect on the edible safety of recipient organ-

isms; impact type 3 signifies a reduction in the edible safety of recipient organisms (Ministry of Agriculture of P.R. China, 2002). According to the classification principles, this standard specifies the impact types of genetic manipulation on the toxicity, allergenicity and anti-nutritional effects of recipient organisms.

2.1.4.1. Impact type of genetic manipulation on toxicity.

Such aspects as the vectors, the expression products, controlling products or metabolic products of target genes, and the expression products of reporter genes and marker genes involved in genetic manipulation may affect the presence or content of the natural biotoxins originally contained in recipient organisms, and even novel toxins may emerge in the process of genetic manipulation. Based on the effects of genetic manipulation on the presence and content of the natural biotoxins of recipient organisms, this standard defines three types of genetic manipulation on the toxicity of recipient organisms: type 1 signifies elimination or reduction in the toxicity of recipient organisms; type 2 signifies no effect on the toxicity of recipient organisms; type 3 signifies enhancement of the toxicity of recipient organisms.

2.1.4.2. Impact type of genetic manipulation on allergenicity.

The expression products of target genes, reporter genes and marker genes involved in genetic manipulation may have effects on the presence or content of the natural biological allergens originally contained in recipient organisms, and new allergens may even be produced in the process of genetic manipulation. With respect to the effects of genetic manipulation on the presence and content of the natural biological allergens of recipient organisms, this standard classifies the impact type of genetic manipulation on the allergenicity of recipient organisms into three types: type 1 signifies elimination or reduction of the allergenicity of recipient organisms; type 2 signifies no effect on the allergenicity of recipient organisms; type 3 signifies enhancement of the allergenicity recipient organisms.

2.1.4.3. Impact type of genetic manipulation on anti-nutrition effects.

The expression products of target genes, reporter genes and marker genes involved in genetic manipulation may affect the presence or content of the natural biological anti-nutrients originally contained in recipient organisms, and new anti-nutrient may even emerge in the process of genetic manipulation. according to the effects of genetic manipulation on the presence and content of the natural biological anti-nutrients of recipient organisms, this standard defines the three impact types of the genetic manipulation on the anti-nutrition effects of recipient organisms: type 1 signifies elimination or reduction of the anti-nutrition effects of recipient organisms; type 2 signifies no effect on the anti-nutrition effects of recipient organisms; type 3 signifies enhancement of the anti-nutrition effects of recipient organisms.

2.2. Requirements for the edible safety of recipient organisms

Recipient organisms refer to the plants, animals, or microbes into which recombinant DNA is introduced by genetic manipulation in the production of agri-GMOs. Recipient organisms include traditional and non-traditional organisms in agriculture. The former refers to the agricultural organisms which have been widely used as foods by human for a long period in both domestic and overseas regions. The latter includes the following kinds of agricultural organisms: (i) those which have not been used as foods by human either in domestic or overseas regions until now; (ii) those which have been used as foods only in few overseas countries; (iii) those which have been used as foods only in few domestic regions. In addition, the organisms, in which the presence of natural biotoxins, biological allergens or anti-nutrients is definite or unknown, also act as recipient organisms. Therefore, recipient organisms are perhaps the main source of the natural biotoxins, biological allergens and anti-nutrients of agri-GMOs, especially when non-traditional agricultural organisms are used as recipient organisms. Therefore, this standard specially proposes safety requirements for recipient organisms, in accordance with the principles and theories of risk assessment. The standard identifies that organisms that may have harmful effects on human health and no measure is available to avoid such effects, should not be used as recipient organisms of agri-GMOs. The specified requirements are as follows: organisms for which the toxicity grade, the allergenicity grade or anti-nutrition effect grade is grade IV, should not be used as recipient organisms.

2.3. Requirements for the edible safety of agri-GMOs

Agri-GMOs are constructed by the genetic manipulation of recipient organisms. Therefore, the edible safety of agri-GMOs depends not only on their recipient organisms, but also on the genetic manipulation itself and any unintended effects of the genetic manipulation. According to the concept of edible safety, the criteria of relative safety of transgenic foods and substantial equivalence principle, this standard sets out the edible safety requirements for agri-GMOs, and requires that genetic modification should not increase the hazards to human health. Specifications are provided as follows: the toxicity grade, allergenicity grade and anti-nutrition effect grade of agri-GMOs should not be higher than the corresponding recipient organisms; the unintended effect grade of agri-GMOs should not be higher than grade III.

3. Edible safety assessment of agri-GMOs

3.1. Assessment procedures

The edible safety assessment of agri-GMOs includes such aspects as the hazard grade of recipient organisms, impact type of genetic manipulation and the unintended effect grade of the agri-GMOs. The assessment procedures are listed in Fig. 1.

The assessment standards for the toxicity, allergenicity and anti-nutrition effect grades with respect to recipient organisms and agri-GMOs, the impact types of the genetic

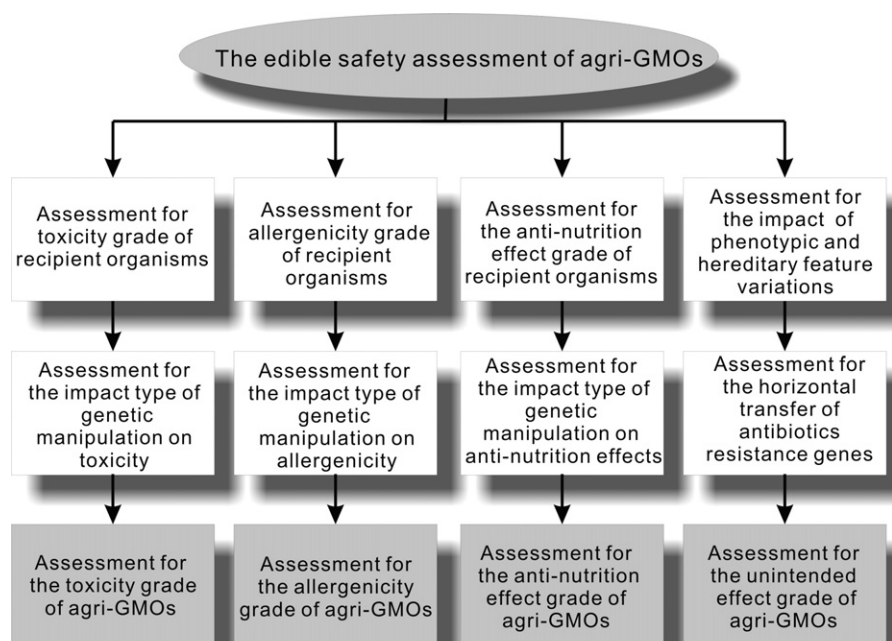


Fig. 1. Procedures for the edible safety assessment of agri-GMOs.

Table 1
Assessment standard for the toxicity grade of recipient organisms

Grade	Index
Grade I	(1) Recipient organisms which are traditional agricultural organisms without natural biotoxin Or (2) Recipient organisms which are non-traditional agricultural organisms proved reliable for foods by the 1st, 2nd stage assays provided in <i>Procedures for toxicological assessment of food (GB 15193.1-2003)</i>
Grade II	(1) Recipient organisms which are traditional agricultural organisms with natural biotoxin, but the adverse effects can be completely avoided by some proper safety control measures Or (2) Recipient organisms which are non-traditional agricultural organisms proved reliable for foods by the 1st, 2nd, 3rd stage assays provided in <i>Procedures for toxicological assessment of food (GB 15193.1-2003)</i>
Grade III	(1) Recipient organisms which are traditional agricultural organisms with natural biotoxin, and the adverse effects can be mostly avoided by some proper safety control measures Or (2) Recipient organisms which are non-traditional agricultural organisms proved reliable for food by the 1st, 2nd, 3rd, 4th stage assays provided in <i>Procedures for toxicological assessment of food (GB 15193.1-2003)</i>
Grade IV	(1) Recipient organisms which are traditional agricultural organisms with natural biotoxin, and the adverse effects cannot be avoided by any safety control measures yet Or (2) Recipient organisms which are non-traditional agricultural organisms with no traditional agricultural counterparts, and are shown to be unacceptable for use as foods by the assays provided in <i>Procedures for toxicological assessment of food (Ministry of Health of P.R. China/Standardization Administration of P.R. China, 2003. GB 15193.1-2003)</i>

Table 2
Assessment standard for the impact of exogenous genes on recipient organism toxicity

Type	Index
Type 1	(1) The natural biotoxin originally contained in recipient organisms can be eliminated due to the introduction of exogenous genes Or (2) The content of natural biotoxin in recipient organisms can be reduced due to the introduction of exogenous genes
Type 2	(1) Exogenous genes derive from traditional agricultural organisms without natural biotoxin Or (2) Exogenous genes derive from traditional agricultural organisms with natural biotoxin, but the amino acid sequences of their expression products have no homology with the natural biotoxin Or (3) Exogenous genes derive from non-traditional agricultural organisms, the amino acid sequences of their expression products have no homology with natural biotoxin, but they have homology with food proteins Or (4) Exogenous genes derive from non-traditional agricultural organisms, the amino acid sequences of their expression products have no homology with either natural biotoxin or food proteins, and the expression products are shown to be reliable for food by the assays provided in <i>Procedures for toxicological assessment of food (GB 15193.1-2003)</i> Or (5) The introduction of exogenous genes has no influence on the sort or content of natural biotoxin in recipient organisms
Type 3	(1) Expression products of exogenous genes have homology with natural biotoxin Or (2) Exogenous genes derive from non-traditional agricultural organisms, the amino acid sequences of their expression products have no homology with either natural biotoxin or food proteins, and the expression products are shown to be unacceptable for use in foods by the assays provided in the <i>Procedures for toxicological assessment of food (GB 15193.1-2003)</i> Or (3) The introduction of exogenous genes can increase the sort or content of natural biotoxin in recipient organisms

Table 3
Assessment standard for the impact of vectors on recipient organism toxicity

Type	Index
Type 1	(1) The vectors that have already been licensed for use by no less than 2 international organizations or countries Or (2) The vectors derived from traditional agricultural organisms without natural biotoxin
Type 2	(1) The vectors have already been licensed for use by one international organization or country Or (2) The vectors derived from traditional agricultural organisms with natural biotoxin, but the adverse effects can be completely or mostly avoided by some safety control measures
Type 3	(1) The vectors have not yet been licensed for use by any international organization or country Or (2) The vectors derived from traditional agricultural organisms with natural biotoxin, and the adverse effects cannot be avoided by any safety control measures Or (3) The vectors derived from non-traditional agricultural organisms for which it is not possible to identify whether natural biotoxin are contained in them

manipulation on recipient organisms and agri-GMOs, together with assessment standards for the impact type of exogenous genes, vectors and genetic manipulation on tox-

icity, allergenicity and anti-nutrition effect, are shown in Tables 1–11. The assessment standards for unintended effects grade are shown in Table 12.

Table 4

Assessment standard for the impact of genetic manipulation on recipient organism toxicity

Type	Index
Type 1	The impact of both exogenous genes (including target genes, reporter genes, marker genes) and the vectors, used in genetic manipulation, on recipient organism toxicity is type 1
Type 2	All or part of the impact of both exogenous genes (including target genes, reporter genes, marker genes) and the vectors, used in genetic manipulation, on recipient organism toxicity is type 2
Type 3	All or part of the impact of both exogenous genes (including target genes, reporter genes, marker genes) and the vectors, used in genetic manipulation, on recipient organism toxicity is type 3

Table 5

Assessment standard for the toxicity grade of agri-GMOs

Toxicity grade of recipient organisms	The impact of exogenous genes on recipient organism toxicity	Toxicity grade of agri-GMOs
Grade I	Type 1	Grade I
	Type 2	Grade I
	Type 3	Grade IV
Grade II	Type 1	Grade I
	Type 2	Grade II
	Type 3	Grade IV
Grade III	Type 1	Grade II
	Type 2	Grade III
	Type 3	Grade IV
Grade IV	Type 1	Grade IV
	Type 2	Grade IV
	Type 3	Grade IV

Table 6

Assessment standard for the allergenicity grade of recipient organisms

Grade	Index
Grade I	Recipient organisms which are traditional agricultural organisms without biological allergens
Grade II	Recipient organisms which are traditional agricultural organisms with biological allergens, but the adverse effects can be completely avoided by certain safety control measures
Grade III	(1) Recipient organisms which are traditional agricultural organisms with biological allergens, and the adverse effects can be mostly avoided by certain safety control measures Or (2) Recipient organisms which are non-traditional agricultural organisms and have been shown to be without biological allergens by animal allergenic testing
Grade IV	(1) Recipient organisms which are traditional agricultural organisms containing biological allergens, and the adverse effects cannot be avoided by any safety control measures yet Or (2) Recipient organisms which are non-traditional agricultural organisms and have been shown to contain biological allergens by animal allergenic testing

4. Congruency validation

In order to validate the congruency on assessment results of GMO edible safety between this assessment system and the appraisal proposals applied currently by other countries and districts, we selected several cases including GM soybean, maize, potato, capsicum and yeast, which have already undergone edible safety assessments by various organizations, to run through this standard. Based on the data and information about these GM cases, we have reappraised the cases mentioned above using the indexes, requirements and procedures of this new standard, and have reached unanimous conclusions by comparing the results of this new appraisal system with those of the assessments published previously by others.

4.1. Edible safety assessment of roundup ready soybean (RRS)

4.1.1. Materials and resources

Genetically modified soybean 40-3-2 (roundup ready soybean, RRS), with resistance to herbicide, made by Monsanto Company in America is the commercial genetically modified soybean strain that is now broadly applied in foods and feedstuffs. This GMO was authorized to use as foods for human and feedstuffs for animals by different countries as follows: United States of America, 1994; Canada, Argentina, Japan, Holland, Switzerland, 1996; Uruguay, 1997; Brazil, Mexico, 1998; Russia, 1999; Korea, Roumania, 2000; South Africa, 2001.

Table 7
Assessment standard for the impact of exogenous genes on recipient organism allergenicity

Type	Index
Type 1	(1) Biological allergen in recipient organisms can be eliminated due to the introduction of exogenous genes Or (2) The content of biological allergen in recipient organisms can be lowered due to the introduction of exogenous genes
Type 2	(1) Exogenous genes derive from traditional organisms without biological allergen Or (2) Exogenous genes derive from traditional agricultural organisms with biological allergens, and their expression products are shown to have no homology with the amino acid sequences of the biological allergens and no allergenicity identified by specific serum screening Or (3) Exogenous genes derive from non-traditional agricultural organisms, the amino acid sequences and the molecular characteristics of the exogenous genes' expression products have no homology or similarity with biological allergen Or (4) Exogenous genes derive from non-traditional agricultural organisms. The amino acid sequences of the exogenous genes' expression products have no homology with biological allergen, but the molecular characteristics of these expression products have similarity with biological allergen. The expression products of exogenous genes are proved no allergenicity by digestive enzyme test, heat resistance test and animal allergenic test Or (5) The sort or content of biological allergen in recipient organisms cannot be changed by the introduction of exogenous genes
Type 3	(1) The amino acid sequences of exogenous genes' expression products have homology with biological allergen Or (2) Exogenous genes derive from non-traditional agricultural organisms. The amino acid sequences of exogenous genes' expression products have no homology with biological allergen, but the molecular characteristics of these products have similarity with biological allergen. It is proved that the expression products of exogenous genes have allergenicity by resistance test of protein to proteolytic digestion, resistance test of protein to heating and animal allergenic test Or (3) Exogenous genes derive from traditional agricultural organisms containing biological allergen, and their expression products have homology with the amino acid sequences of biological allergen Or (4) The sort or content of biological allergen in recipient organisms can be increased by the introduction of exogenous genes

Table 8
Assessment standard of the allergenicity grade of agri-GMOs

Allergenicity grade of recipient organisms	The impact of exogenous genes on recipient organism allergenicity	Allergenicity grade of agri-GMOs
Grade I	Type 1	Grade I
	Type 2	Grade I
	Type 3	Grade IV
Grade II	Type 1	Grade I
	Type 2	Grade II
	Type 3	Grade IV
Grade III	Type 1	Grade II
	Type 2	Grade III
	Type 3	Grade IV
Grade IV	Type 1	Grade IV
	Type 2	Grade IV
	Type 3	Grade IV

Table 9
Assessment standard of the anti-nutrition effect grade of recipient organisms

Grade	Index
Grade I	Recipient organisms are traditional agricultural organisms without anti-nutrient factors
Grade II	(1) Recipient organisms are traditional agricultural organisms with anti-nutrient factors, but the adverse effects can be completely avoided by safety control measures Or (2) Recipient organisms are non-traditional agricultural organisms which can be shown to be without anti-nutrition effects by certain testing
Grade III	Recipient organisms are traditional agricultural organisms with anti-nutrient factors, but the adverse effects can be mostly avoided by safety control measures
Grade IV	(1) Recipient organisms are traditional agricultural organisms with anti-nutrient factors, and the adverse effects cannot be avoided by any safety control measures yet Or (2) Recipient organisms are non-traditional agricultural organisms which have anti-nutrition effects

The exogenous gene integrated in RRS is *cp4-epsps* gene, which is regulated by the 35S promoter from Cauliflower Mosaic Virus (CaMV35S) and the terminator of Nopaline Synthase gene (NOS). Information about the

safety assessment of RRS was published by OECD (Fuchs et al., 1996). The assessment conclusion was as follows: genetically modified soybean RRS is substantially equivalent to traditional soybean.

Table 10

Assessment standard of the impact of exogenous genes on the anti-nutrition effects of recipient organisms

Type	Index
Type 1	(1) The anti-nutrient factors in recipient organisms can be eliminated due to the introduction of exogenous genes Or (2) The content of anti-nutrient factors in recipient organisms can be reduced due to the introduction of exogenous genes
Type 2	(1) Exogenous genes derive from traditional organisms without anti-nutrient factors Or (2) Exogenous genes derive from traditional agricultural organisms with anti-nutrient factors, but the amino acid sequences of the exogenous genes' expression products have no homology with the anti-nutrient factors Or (3) Exogenous genes derive from non-traditional agricultural organisms. The amino acid sequences of the exogenous genes' expression products have no homology with anti-nutrient factors, and it is shown that the exogenous genes' expression products have no anti-nutrition effects by nutrition efficacy testing Or (4) The sort or content of the anti-nutrient factors in recipient organisms cannot be changed by the introduction of exogenous genes
Type 3	(1) The amino acid sequences of exogenous genes' expression products have homology with anti-nutrient factors Or (2) Exogenous genes derive from non-traditional agricultural organisms. The amino acid sequences of the exogenous genes' expression products have no homology with anti-nutrient factors, but it can be proved that exogenous genes' expression products have anti-nutrition effects by nutrition efficacy test Or (3) The sort or content of the anti-nutrient factors in recipient organisms are increased after the introduction of exogenous genes

Table 11

Assessment standard of the anti-nutrition effect grade of agri-GMOs

Anti-nutrition effect grade of recipient organisms	The impact of exogenous genes on recipient organism anti-nutrition effect	Anti-nutrition effect grade of agri-GMOs
Grade I	Type 1 Type 2 Type 3	Grade I Grade I Grade IV
Grade II	Type 1 Type 2 Type 3	Grade I Grade II Grade IV
Grade III	Type 1 Type 2 Type 3	Grade II Grade III Grade IV
Grade IV	Type 1 Type 2 Type 3	Grade IV Grade IV Grade IV

Table 12

Assessment standard of the unintended effect grade of agri-GMOs

Grade	Index
Grade I	It has been proved that neither phenotypic nor hereditary feature variation occurs and the horizontal transfer of antibiotic resistance gene does not occur
Grade II	It has been proved that phenotypic or hereditary characteristics variations occur, but the variations have no adverse effects on edible safety, and the horizontal transfer of antibiotic resistance gene does not occur
Grade III	It has been proved that both phenotypic and hereditary characteristics variations occur, but the variation has no adverse effects on edible safety, and the horizontal transfer of antibiotic resistance gene does not occur
Grade IV	It has been proved that one of the following two statuses occur: one is that some phenotypic or/and hereditary characteristics variations occur, and the variations have a harmful impact on edible safety; the other is that horizontal transfer of antibiotic resistance genes occurs

4.1.2. Hazard grade assessment of recipient organism

Soybean has been cultivated as an edible crop for thousands of years in China. In the last 100 years, it has been introduced to other countries around the world. Worldwide, about 71,000,000 km² of land has been used to plant soybean each year and the annual production reaches to 15.5 billion kilograms. Since soybean has been used as food for a long period in domestic and overseas regions, and no natural biotoxin has been found in it, it belongs to traditional agricultural organism without natural biotoxin. According to this assessment standard (Table 1), the toxicity grade of the recipient organism is grade I. It is known that some of adults and children are hypersensitive to soybean, which contains an allergenic protein, but the adverse effects can be completely avoided by certain safety control measures. According to the standard (Table 6), it can be concluded that the allergenicity grade of the recipient organism is grade II. Soybean contains multiple anti-nutrients, such as protease inhibitor and lipoxidase, but they can be completely avoided by certain safety control measures. According to the standard (Table 9), the anti-nutrition effect grade of the recipient organism is grade II.

It has been proved that one of the following two statuses occur: one is that some phenotypic or/and hereditary characteristics variations occur, and the variations have a harmful impact on edible safety; the other is that horizontal transfer of antibiotic resistance genes occurs

4.1.3. Assessment of the impact of genetic manipulation

(1) *Donor organism of target gene:* The donor organism of *cp4-epsps* gene, *Escherichia coli* (*E. coli*), is a non-traditional agricultural organism without natural biological allergen.

(2) *Expression products of target gene:* The amino acid sequences of the expression product, *cp4-epsps* protein, have no homology with the known natural biotoxin, and *cp4-epsps* protein shows no acute toxicity in rat gavage test. The molecular weight of *cp4-epsps* protein is similar to natural biological allergen, but this protein is not glycosylated and has no resistance to digestive enzymes or heat. The amino acid sequences of *cp4-epsps* protein have no homology with natural anti-nutrients, but *cp4-epsps* protein is a type of enzyme involved in the aromatic amino acid (ArAA) synthetic route, which probably has impact on the content of ArAA in soybean and thereby has influence on the nutrition efficacy of soybean. While testing results showed that there was no significant difference in the content of amino acids (including ArAAs) in RRS and that in traditional soybean. It has also been shown that there is no statistically significant difference in the transformation efficiency of feedstuff and nutrition effects on animal growth and development, when RRS and traditional soybean are used as feedstuff to feed rats, chickens, milk cows, cat fish and quails.

(3) *Marker gene, reporter gene, controlling gene and vector:* RRS contains neither marker gene nor reporter gene. CaMV35S promoter and NOS terminator, used as controlling genes, do not code for any expression products. Detailed data on the vector was not available.

If we did not take the vector of RRS into account, based on Tables 2, 4, 7 and 10 of the standard, the impact of the genetic manipulation on toxicity, allergenicity and anti-nutrition effects of the recipient organism were all type 2.

4.1.4. Hazard grade assessment of RRS

Given that the toxicity, allergenicity and anti-nutrition effect grades of the recipient organism utilized by RRS were grade I, grade II and grade II, respectively. The impact of the genetic manipulation on toxicity, allergenicity and anti-nutrition effect of the recipient organism were all type 2. According to Tables 5, 8 and 11 of the standard, the toxicity grade of this agri-GMO was grade I, the allergenicity grade and the anti-nutrition effect grade of RRS were both grade II.

4.1.5. Assessment of the unintended effect grade of RRS

(1) *The testing for target agronomy traits:* field experiments showed that the tolerated dose for herbicide (glyphosate) of RRS was much higher than that of the recipient.

(2) *The testing for non-target nutrition traits:* The testing results from 1000 samples showed that the contents of nutrients (including protein, fat, fiber, ash content, carbohydrate and water) and anti-nutrients (including digestive inhibitor, phytohematoagglutinin, genistein, soybean glycoside, phy-

tate, gossypose and stachyose) of RRS were not substantially different from that of the recipient organism.

(3) *The testing for the variation of inheritance characters:* The nucleotide sequences of *cp4-epsps*, CaMV35S and NOS sequences in RRS were the same as the theoretical values. The molecular weight and the amino acid sequences of *cp4-epsps* protein detected from RRS were the same as the theoretical values.

Based on the testing results of the variation of the phenotypic and hereditary characteristics, according to Table 12 of the standard, it can be concluded that the unintended effect grade of the GMO was grade I.

4.1.6. Assessment conclusions

(1) Given that the recipient organism of RRS was of toxicity grade I, allergenicity grade II and anti-nutrition effect grade II, this recipient organism met the requirement of this assessment standard for the edible safety of recipient organisms.

(2) RRS was of toxicity grade I, allergenicity grade II, anti-nutrition effect grade II and unintended effect grade I. Given that the grades of the toxicity, allergenicity, and anti-nutrition effects were not higher than the conventional agriculture organisms, and the unintended effects that showed influence on edible safety did not occur, we can confirm that RRS met the requirement of the standard for the edible safety of GMOs.

Based on the updated data and information on the genetically modified soybean with *cp4-epsps*, we can conclude that the assessment result of the new standard was the same as that of the original assessments.

4.2. Assessment for the allergenicity of the genetically modified soybean with 2S albumin gene

4.2.1. Materials and resources

The genetically modified soybean, with 2S albumin gene derived from Brazil nut, is known as the only agri-GMO that has not been permitted for commercial use after edible safety assessment. The target gene of this GM soybean is 2S albumin gene derived from Brazil nut, while the data about the marker gene and the controlling gene are not known. Information on the allergenicity assessment of this GM soybean has been published by OECD (Nordlee et al., 1996). The assessment conclusion was as follows: The novel protein in this GM soybean could cause a certain allergic reaction in human, therefore the GM soybean cannot be permitted for commercial use.

4.2.2. Assessment for the allergenicity grade of recipient organism

Although it is well known that soybean is a common allergenic food for adults and children because of the allergenic protein contained in soybeans, the hazard element can be eliminated by some safety control measures. According to the standard (Table 6), the allergenicity grade of the recipient organism is grade II.

4.2.3. Assessment for the impact of the exogenous gene on the allergenicity of recipient organism

The donor organism that provides 2S albumin gene is Brazil nut. Brazil nut is a non-traditional agricultural organism, which has a safe consumption history only in a few foreign countries. The nut is considered to be a common allergenic food and a proportion of persons produce allergic reaction when it is ate. As a result, the donor organism of 2S albumin gene is considered to be a non-traditional agricultural organism with natural biological allergen.

The result of the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis showed that an additional band of the novel protein can be detected in the GM soybean, and this additional band has the same mobility with the 2S albumin used as reference. When analyzing the protein extracted from the Brazil nut, the same band was shown again, while in the recipient organism this band was absent. The molecular weight of this novel protein in the genetically modified soybean was around 9 kDa, which shares similarity with natural biological allergens (which have molecular weights around 10–80 kDa). In the serum taken from nine persons who had the experience of allergic reactions to Brazil nut, the novel protein in the genetically modified soybean had an affinity reaction with seven of the serum samples, and both the reference 2S albumin and the protein extracted from Brazil nut had affinity reactions with eight of the serum samples, while the protein extracted from the recipient soybean had no affinity reaction with any of the serum samples. It indicated that the novel protein would cause allergic reactions in human.

From the above results, it can be concluded that the introduction of the target gene into the recipient organism can augment the category or the content of biological allergens, and the impact of the exogenous gene on the allergenicity of the recipient organism was type 3 based on this standard (Table 7).

4.2.4. Assessment for the allergenicity grade of the 2S albumin transgenic soybean

Given that the allergenicity grade of the recipient organism utilized by the genetically modified soybean was grade II, and the impact of the exogenous gene on the allergenicity of the recipient organism was type 3, we can conclude that the allergenicity grade of the 2S albumin transgenic soybean was grade III, according to the assessment standard (Table 8).

4.2.5. Assessment conclusions

(1) Since the allergenicity grade of the recipient organism of the 2S albumin transgenic soybean was grade II, it met the allergenicity requirements for recipient organisms, which is regulated by this standard.

(2) Since the allergenicity grade of the 2S albumin transgenic soybean was grade III, which was higher grade than the relevant recipient organism, it did not meet edible

safety requirements for GMOs, which is regulated by this standard.

(3) Based on the updated data and information on the genetically modified soybean, it can be concluded that the assessment result of the new standard was the same as that of the original assessment record.

4.3. Edible safety assessment of genetically modified maize MON810

4.3.1. Materials and resources

Genetically modified maize MON810 (*CryIA(b)* gene was integrated in), which has resistance to European maize borer and was developed by Monsanto Company in America, has been assented for commercial production. This GMO has been authorized by different countries for applying in food for human and feedstuffs for animals as follows: United States of America, 1996; Canada, South Africa, Japan, 1997; Argentina, European Commission, 1998; Switzerland, Australia, 2000.

The exogenous structure gene contained in MON810 is anti-insect protein gene *CryIA(b)*, which is regulated by CaMV35S promoter. There is no marker gene, and Heat-Shock Proteins 70 (HSP70) was used as regulatory element, which derived from the 0.8 kb intron of the heat shock protein gene. Information on the edible safety assessment for MON810 has been published (Fred et al., 2000), and information on the edible safety assessment for the *CryIA(b)* protein has been published by the OECD (Kuiper and Noteborn, 1996). The results of these assessments were that MON810 is substantially equivalent to the recipient maize strain.

4.3.2. Assessment of the hazard grade of recipient organism

Maize is one of the three major crops around the world, and also one of the most important forage crops, which plays an essential role in global food production. Maize is a traditional agricultural organism with a long history of safe consumption and it does not contain natural biological toxins, natural biological allergens or anti-nutrient factors. According to Tables 1, 6 and 9 of the standard, it can be concluded that the toxicity grade, allergenicity grade and anti-nutrition effect grade of the recipient organism were all grade I.

4.3.3. Assessment of the impact of genetic manipulation

(1) The donor organism of target gene: The donor organism of the *CryIA(b)* is *Bacillus thuringiensis*, which is one of the non-traditional agricultural organisms without natural biological allergens.

(2) Expression products of target gene: The amino acid sequences of *CryIA(b)* protein have no homology with known natural biological toxins, natural biological allergens or anti-nutrient factors. It was reported that there was no specific receptor for the *CryIA(b)* protein in mammalian digestive tract (including human), and this protein

had no histopathologic effects on alimentary tract mucosa. Acute oral gavage administration of the purified *CryIA(b)* protein expressed in *E. coli*, mouse as test object and at a dose of 100,000 times than the average intake in human, indicated that the *CryIA(b)* protein had no toxicity. The microbial pesticide containing the *CryIA(b)* protein has been safely utilized for more than 40 years, and no toxic or allergenic effect has been produced in human or animals.

(3) Marker gene, reporter gene, controlling gene and vector: This genetically modified maize contained no marker gene. CaMV35S promoter and HSP70 controlling gene do not code for any expression products. Since the exogenous gene was introduced into the recipient organism by particle gun transformation, there was no vector in the whole process.

Based on Tables 2, 4, 7 and 10 of the standard, the impact of the genetic manipulation on toxicity, allergenicity and anti-nutrition effect of the recipient organism were all type 2.

4.3.4. Assessment of the hazard grade of MON810 transgenic maize

The toxicity, allergenicity and anti-nutrition effect grade of the recipient organism utilized by this GMO were all grade I. The impact of the genetic manipulation on toxicity, allergenicity and anti-nutrition effect of the recipient organism were all type 2. According to Tables 5, 8, and 11 of the standard, it can be confirmed that the toxicity grade, allergenicity grade and anti-nutrition effect grade of the genetically modified maize MON810 were all grade I.

4.3.5. Assessment of the unintended effect grade of genetically modified maize MON810

(1) *Testing for the target agronomy trait*: Referring to the results of field experiments, which have been held in 1994 and 1995 in America, and in 1995 and 1996 in Europe, GM maize MON810 can tolerate the potential hazards of lepidoptera insects (including moth and butterfly) effectively and continuously during the whole growing seasons.

(2) *Testing for non-target nutritional trait*: In the field experiments in America, MON810 showed no statistically significant difference from the recipient organisms in the following aspects: the contents of the nutrients (including protein, fiber, fat, carbohydrate, ash content and moisture content), amino acid ingredients, fatty acid composition and the contents of calcium and phosphorus. In the field experiments in Europe, MON810 maize showed no statistically significant difference from the recipient in the following aspects: fat, ash content, neutral detergent fiber, acid detergent fiber, carbohydrate and dry weight. The protein level of MON810 was significantly higher than that of the recipient. However, this difference had no biological relevance.

(3) *Testing for the variation of hereditary features*: CaMV35S and *CryIA(b)* genes can be detected in

MON810 maize. The *CryIA(b)* gene can be inserted into the maize genome stably, and displays single gene isolation phenomenon. The gene heredity, which is transferred by pollen, follows Mendelian inheritance law. The nucleotide sequences of the *CryIA(b)* gene were the same as the theoretical sequences. The molecular weight and the amino acid sequences of the *CryIA(b)* protein detected from MON810 maize, were the same as the theoretical values. The anti-insect activity of *CryIA(b)* protein expressed in MON810 maize was similar to that in natural *B. thuringiensis*, but the expression level of the former was much higher than the latter.

Based on the testing results of the variation of phenotypic and hereditary characteristics, it can be concluded that the unintended effect grade of genetically modified maize MON810 was grade I, according to this standard (Table 12).

4.3.6. Assessment conclusions

(1) Given that the toxicity grade, allergenicity grade and the anti-nutrition effect grade of GM maize MON810 were all grade I, the results met the requirement of our standard for the edible safety of recipient organism.

(2) Given that the toxicity grade, allergenicity grade and the anti-nutrition effect grade of MON810 were all grade I, and the unintended effects grade was grade I, it can be concluded that the grades of the toxicity, allergenicity, and the anti-nutrition effects of genetically modified maize MON810 were not higher than those of the recipient organism, and no unintended effect that had impact on edible safety was found, so the *CryIA(b)* transgenic maize met the requirement of our standard for the edible safety of GMOs.

(3) Based on the updated data and information on GM maize MON810, we can conclude that the assessment result of the new standard was the same as that of the original assessment record.

4.4. Edible safety assessment of genetically modified potato containing *Cry3A* gene

4.4.1. Materials and resources

The genetically modified potato *Bt6*, *Bt10*, *Bt12*, *Bt16*, *Bt17*, *Bt18* and *Bt23*, with resistance to insects, containing *Cry3A* gene and Neomycin-3'-phosphotransferase gene (*nptII*), made by Monsanto Company in America, has been permitted for commercial production and use. This GMO has been authorized to be applied as foods for human and feedstuffs for animals by several countries, which are as follows: United States of America, 1994; Canada, 1995; Japan, 1996.

The exogenous gene integrated in the GM potato is *Cry3A*, which encodes for an anti-insect protein and is regulated by CaMV35S promoter and *rbcS* terminator. The marker gene is *nptII*, which is regulated by CaMV35S promoter and NOS terminator. Information on the edible safety assessment of the GM potato has been published

by WHO (Fuchs et al., 1995). The result of this assessment was that the GM potato is substantially equivalent to the recipient potato.

4.4.2. Assessment of the hazard grade of recipient organism

Potato is an annual tuberous plant, which is one of the four main crops in the world. The cultivated area of this crop is only less than wheat, rice and maize. In China the cultivated area of potato reaches 300,000 km²/year. China is the second largest producer of potato in the world, and the potato is a traditional agricultural organism with a long history of safe consumption. It contains a natural biotoxin called solanine, which is usually found in the *Solanum nigrum* species. When potato is stored inappropriately, the content of solanine may markedly increase and it cannot be eliminated efficiently by common cooking methods. In such cases, those who eat it can be poisoned. However, the toxic hazard can be avoided by some appropriate measures. Potato is a traditional agricultural organism without natural biological allergen but with some anti-nutrients, such as solanine. However, this hazard can be totally avoided by certain safety control measures. According to Tables 1, 6 and 9 of the standard, it can be concluded that the toxicity grade, allergenicity grade and anti-nutrition effect grades of the recipient organism were grade II, I and II, respectively.

4.4.3. Assessment of the impact of genetic manipulation

(1) *The donor organism of target gene*: The target gene integrated in the genetically modified potato is *Cry3A*, which is derived from *B. thuringiensis*. The donor is a non-traditional agricultural organism without natural biological allergen.

(2) *Expression products of target gene*: The expression product of *Cry3A* gene, *Cry3A* protein, belongs to Bt protein family. It is shown that the amino acid sequences of the *Cry3A* protein have no homology with known natural biological toxins, natural biological allergens or anti-nutrients. The purified Bt protein expressed by *E. coli* had no toxic effects in rats in acute toxicity testing. The content of the Bt protein was lower than 0.01% of the total protein in the GM potato, so this protein was not a major protein in the GM potato. When this protein was degraded by digestive enzymes in vitro, its half-decay period was shorter than 30 s, which indicated that it had no resistance to digestive enzymes and the protein is unlikely to cause allergic reactions in human. In a 28-day test, rats were fed on the feedstuff made by the GM potato or non-GM potato, and there were no significant differences in food intake, growth rate or organ gross weight.

(3) *Marker gene, controlling gene and vector*: The marker gene utilized by the GM potato is *nptII*. Although this marker gene is derived from the bacterial Tn5 transposon, the expression products of the marker gene were not bacterial toxin. By comparing the amino acid sequences of the *nptII* protein with the known toxic protein, natural biological allergens and anti-nutrients, there was no significant

sequence homology between them. Acute toxicity testing in animals has shown that the *nptII* protein has no adverse effects following a dose of this protein larger than 5,000,000 times of the average consumption of human. In 1994, FDA and EPA authorized that *nptII* marker gene could be applied in genetically modified plants. In 1996, the Nordic Council of Ministers permitted that *nptII* marker gene could be used as the first safe marker gene, and considered that as a marker gene, the *nptII* gene itself would be safe, as well as its expression products. The content of the *nptII* protein was lower than 0.005% of the total protein in the GM potato, so this protein was not the major protein in the GM potato. The half-decay period of *nptII* protein in simulated digestive system was shorter than 20 s which indicated it had no resistance to digestive enzymes; the protein did not cause any remarkable allergic reaction in human. The controlling genes utilized by the GM potato are CaMV35S promoter, *rbcS* and NOS terminators, and they do not code for expression products. The information on the vector is uncertain.

Regardless of the vector, based on Tables 2, 4, 7 and 10 of the standard, the impact of the genetic manipulation on toxicity, allergenicity and anti-nutrition effect of the recipient organism were all type 2.

4.4.4. Assessment of the hazard grade of the GM potato containing *Cry3A* gene

The toxicity, allergenicity and anti-nutrition effect grades of the recipient organism utilized by this GMO were grade II, I and II, respectively. The impact of the genetic manipulation on toxicity, allergenicity and anti-nutrition effect of the recipient organism were all type 2. According to Tables 5, 8 and 11 of the standard, it can be concluded that the toxicity grade of the GM potato containing *Cry3A* gene was grade II, the allergenicity grade was grade I and the anti-nutrition effect grade was grade II.

4.4.5. Assessment of the unintended effect grade of the genetically modified potato containing *Cry3A* gene

(1) *Testing for target agronomy traits*: The content of the Bt protein in the GM potato leaves can reach 5.4–28.3 µg/g, and the content of the *nptII* protein can reach 5.4–28.3 µg/g. The contents of the Bt protein and the *nptII* protein were both remarkably higher than that of the recipient organism.

(2) *Testing for non-target agronomy traits*: There are no differences between seven strains of this GM potato and the recipient organism in the following aspects: yield, growth tendency, potato characteristics and the sensitivity to diseases and insects.

(3) *Testing for non-target nutrition traits*: The contents of nutrients (including protein, fat, carbohydrate, dietary fiber, ash content, vitamin C, vitamin B₆, vitamin B₁, vitamin B₂, niacin and folic acid), mineral matters (including calcium, copper, iron, iodine, phosphorus, potassium, sodium and zinc) and anti-nutrients (including solanine) of the GM potato were the same as the recipient organism.

(4) *Testing for the variation of hereditary features:* CaMV35S, NOS, *Cry3A* genes and the *nptII* gene were detected in the GM potato. The nucleotide sequences of the *Cry3A* gene and the *nptII* gene were the same as the theoretical sequences. The molecular weight and amino acid sequences of the *Cry3A* protein detected from the genetically modified potato were the same as the theoretical values. The *Cry3A* protein expressed in the GM potato had the same specific anthelmintic activity, similar molecular weight and immune reaction with the Bt protein contained in *B. thuringiensis* pesticide available in market.

Based on the testing results of the variation of phenotypic and hereditary characteristics, it can be confirmed that the unintended effect grade of the transgenic potato was grade I according to this new standard (Table 12).

4.4.6. Assessment conclusions

(1) Given that the toxicity, allergenicity and the anti-nutrition effect grades of the GM potato with *Cry3A* gene were grade II, I and II, respectively, the results met the requirement of our standard for the edible safety of recipient organisms.

(2) The toxicity, allergenicity and the anti-nutrition effect grades of the GM potato with *Cry3A* gene were grade II, I and II, respectively, and the unintended effect grade was grade I. It can be concluded that the toxicity grade, the allergenicity grade, and the anti-nutrition effect grade of GM potato were not higher than those of the relevant recipient organism, and no unintended effect that had effects on edible safety occurred. These testing results met the requirement of our standard for the edible safety of agri-GMOs.

(3) Based on the updated data and information on the GM potato with *Cry3A* gene, it can be concluded that the assessment result of our new standard was the same as that of the original assessment record.

4.5. Edible safety assessment of the genetically modified capsicum with *Cecropin DB* gene

4.5.1. Materials and resources

The genetically modified capsicum with *Cecropin DB* (CDB) gene, which has resistance to Bacterial Wilt (*Ralstonia solanacearum*), was made by the Vegetables Research Institute of Guangdong Agricultural Sciences Academy in P.R. China. This project was funded by Guangdong Natural Science Foundation (970677). In 2001, the safety grade of the GM capsicum was confirmed as grade I and approved to enter intermediate test by Ministry of Agriculture of P.R. China.

The target genes utilized by the GM capsicum are the antibacterial peptide D (*Cecropin D*) gene and the antibacterial peptide B (*Cecropin B*) gene, which are regulated by CaMV35S promoter and NOS terminator; the marker gene is *nptII* gene. Information on the edible safety assessment of the GM capsicum has been published (Deng et al., 2003). The assessment result was that the GM capsicum

has substantial equivalence with the recipient capsicum, and the nutritional quality is not lower than the recipient.

4.5.2. Assessment of the hazard grade of recipient organism

The recipient organism of the GM capsicum is capsicum “Zaofeng No. 1”, which has a long history of safe consumption in many countries. This capsicum is one of the major vegetables and has no harmful impact on human or animals. Capsicum is a traditional agricultural organism without natural biotoxin, biological allergen or anti-nutrient. According to Tables 1, 6 and 9 of the standard, it can be concluded that the toxicity, allergenicity and anti-nutrition effect grades of the recipient organism were all grade I.

4.5.3. Assessment of the impact of genetic manipulation

(1) *The donor organism of target gene:* The antibacterial peptide D gene is derived from China oak silkworm chrysalis and the antibacterial peptide B gene is derived from wild silkworm chrysalis. The silkworm chrysalis is traditional food in China, and has not been found any toxic effect on human or animals. Antibacterial peptide is an immune haemolymph component in silkworm chrysalis induced by external bacteria and is one of the natural components of silkworm chrysalis. Therefore the donor organisms of the target genes are traditional agricultural organisms without natural biotoxin. It is known that some persons are allergic to silkworm chrysalis.

(2) *Expression products of target gene:* The theoretical molecular weight of the antibacterial peptide D is 3806 Da, and the theoretical molecular weight of the antibacterial peptide B is 1400 Da. They have no similarity with biological allergens (with molecular weights of 10,000–80,000 kDa). The amino acid sequences of the antibacterial peptide have no homology with biotoxin, natural biological allergens or anti-nutrients. The CDB have resistance to digestive enzymes and heat. Allergenic testing in animals did not shown allergic reaction.

(3) *Marker gene, controlling gene and vector:* The marker gene utilized by the GM capsicum is *nptII* gene, and its coding products are considered safe. Controlling genes in the GM capsicum are CaMV35S promoter and NOS terminator, which do not code for any expression products. The vector utilized by the GM capsicum is pCDB-II, and the introduction of recombinant gene was mediated by *Agrobacterium tumefaciens*. The vector and the introduction method have been broadly applied in genetically modified plant products, authorized by many international organizations such as FDA and EPA.

Based on Tables 2–4, 7 and 10 of the standard, the impact of the genetic manipulation on toxicity, allergenicity and anti-nutrition effect of the recipient organism were all type 2.

4.5.4. Assessment of the hazard grade of the GM capsicum

The toxicity, allergenicity and anti-nutrition effect grades of the recipient organism utilized by this GMO were all grade I. The impact of the genetic manipulation on

toxicity, allergenicity and anti-nutrition effect of the recipient organism were all type 2. According to Tables 5, 8 and 11 of the standard, it can be confirmed that the toxicity, the allergenicity and the anti-nutrition effect grades of the GM capsicum were all grade I.

4.5.5. Assessment of the unintended effect grade of the CDB transgenic capsicum

(1) *Testing for target agronomy traits:* The results of laboratory and field experiments had shown that the resistance of Bacterial Wilt (*Ralstonia solanacearum*) of the GM capsicum was generally superior to that of the recipient capsicum. Ten to thirty days after inoculating *Ralstonia solanacearum*, the survival rates of the GM capsicum were, respectively, 98–49%, while the rates of the recipient capsicum were, respectively, 60–20%.

(2) *Testing for the activity of target components:* Target expression products were extracted from fruits and leaves of the T₀–T₃ generation GM capsicum and used for bacteriostatic activity testing, the expression products showed bacteriostatic activity to *E. coli* K₁₂D₃₁, *E. coli* persister, *E. coli* type strain, *Staphylococcus aureus* and *Candida albicans*.

(3) *Testing for non-target agronomy traits:* The agronomy traits of the GM capsicum, including the adaptability, breeding, dormancy stage, strain type, growth potential, fruit shapes, showed no significant difference from the recipient capsicum.

(4) *Testing for non-target nutrition traits:* By testing and comparing the major nutrients (protein, fat, vitamin C, β -carotene, vitamin B₁ and vitamin B₂) and the amino acid contents of the GM capsicum with that of the recipient capsicum, there were no significant difference between the two.

(5) *Testing for the variation of hereditary features:* CaMV35S, NOS, *Cecropin D* gene fragments and *nptII* gene were detected in the GM capsicum. The nucleotide sequences and the molecular weight of the detected *Cecropin D* gene were congruous with the theoretical nucleotide sequences and molecular weight. When target expression products were extracted from the fruits and leaves of the GM capsicum for bacteriostatic activity testing, the target expression products showed the same bacteriostatic activity with the natural antibacterial peptide D extracted from the immune haemolymph of China oak silkworm chrysalis. The antibacterial peptide B cannot be detected in the transgenic capsicum, but it did not affect the edible safety of the GM capsicum.

Based on the testing results of the variation of phenotypic and hereditary features and this standard (Table 12), it can be concluded that the unintended effect grade of the CDB transgenic capsicum was grade II.

4.5.6. Assessment conclusions

(1) The toxicity, allergenicity and anti-nutrition effect grades of the recipient organism of the GM capsicum were

all grade I, which met the requirement of our standard for the edible safety of recipient organisms.

(2) The toxicity grade, the allergenicity grade and the anti-nutrition effect grade of the GM capsicum were all grade I, and the unintended effect grade of the GM capsicum was grade II. The toxicity grade, the allergenicity grade and the anti-nutrition effect grade of the GM capsicum were not higher than those of the recipient organism, and the unintended effect of the GM capsicum had no impact on edible safety, which met the requirements for the edible safety in the standard.

(3) Based on the updated data and information on the GM capsicum, it can be concluded that the assessment result, according to our new standard, was the same as that of the previous assessment record.

4.6. Edible safety assessment of the genetically modified yeast with *Cecropin AD (CAD)* gene

4.6.1. Materials and resources

The GM yeast with *CAD* gene was produced by South China Agricultural University and Shenzhen Yi Peng Biotechnology Limited Company of China and was used as a feedstuff additive for chickens, pigs and prawns to prevent and treat inflammation in alimentary canal, such as diarrhea caused by *E. coli* or *Salmonella*. In 2004, the GM strain, with safety grade I, has been approved to be applied in production by Ministry of Agriculture of P.R. China. The foreign target gene of the GM yeast is *CAD* gene, which is regulated by α -factor promoter and 2 μ m terminator; the reporter gene is a Leucine deficiency gene. Information on the safety assessment of the GM yeast had been published (Deng et al., 2004). The assessment results showed that the safety grade of the GM yeast is grade I, which suggests that this genetically modified feedstuff additive will not be harmful to human health.

4.6.2. Assessment of the hazard grade of recipient organism

The recipient organism of the GM yeast is bakers' yeast, which is one of the *Saccharomyces cerevisiae* used since ancient times in China and has a long history of safe consumption. Up to now, no records show that it transforms to some harmful organisms, or exhibits biological toxicity or pathogenicity in animals or human. Bakers' yeast is considered to be a traditional agricultural organism without natural biotoxins, biological allergens or anti-nutrients. According to Tables 1, 6 and 9 of the standard, it can be concluded that the toxicity, allergenicity and anti-nutrition effect grades of the recipient organism were all grade I.

4.6.3. Assessment of the impact of genetic manipulation

(1) *The donor organism of target gene:* The donor organisms of the *CAD* gene are wild silkworm and China oak silkworm, which are regarded as traditional food organisms and have no toxic effects on human or animals, but some persons have allergic reactions to them.

(2) *Expression products of target gene*: The *CAD* gene is a hybrid gene, which is reconstructed according to the natural antibacterial peptide genes derived from wild silkworm and China oak silkworm. Its expression products are heterotic antibacterial peptide *A* and antibacterial peptide *D*. This heterotic antibacterial peptide *AD* is a new artificial material rather than the natural ingredient of silkworm chrysalis, and the amino acid sequences of the heterotic antibacterial peptide *AD* are quite different from that of the natural antibacterial peptide. The heterotic antibacterial peptide *AD* has been detected in acute toxicity test, Ames test, mouse bone marrow micronucleus test, mouse teratospemia test, dominant lethal test, 40 weeks feeding test in rat and the safety test of animal filial generation. The results of these tests have shown that the heterotic antibacterial peptide *AD* has no toxicity to animals. The molecular weight of the antibacterial peptide *AD* is about 3800 Da, which has no similarity with natural biological allergens (with molecular weight of 10,000 to ~80,000 kDa). The amino acid sequences of antibacterial peptide *AD* had no homology with natural allergens. The antibacterial peptide *AD* is difficult to degrade by digestive enzymes, in other words, it retains its bacteriostatic activity after being exposed to digestive enzymes. The antibacterial peptide *AD* retains its bacteriostatic activity even in an aqueous bath at 100 °C for 30 min. The animal allergenicity test showed that the antibacterial peptide *AD* has no allergenicity towards animals. In addition, the amino acid sequences of the antibacterial peptide *AD* had no homology with those of natural anti-nutrients.

(3) *Marker gene, reporter gene, controlling gene and vector*: For the marker gene, *nptII*, it has been shown that all traits of its coding products are safe. Leucine deficiency reporter gene is derived from yeast, and it does not contain any natural biological toxins. The Leucine deficiency reporter gene belongs to inducible expression gene. When no Leucine is contained in nutritive medium, this gene can express and synthesise Leucine. Therefore, the strain containing this special gene can grow in Leucine defective nutritive medium, which is benefit for screening the transformed strain. The expression product of this special gene is amidocaproic acid, which is a normal nutrient in food with no toxicity, allergenicity or anti-nutrition effect. Two controlling genes utilized in the GM yeast were α -factor promoter and 2 μ m terminator, which are both derived from yeast. The vector applied in the genetic manipulation is pCLAW2 plasmid, which is derived from yeast, and the recombinant plasmid was transformed into bakers' yeast by lithium salt method. The vector and the transformation method have both been permitted to be applied in genetically modified microorganism products by many international organizations such as FDA and EPA.

According to Tables 2–4, 7 and 10 of the standard, the impact of the genetic manipulation on toxicity, allergenicity and anti-nutrition effect of the recipient organism were all type 2.

4.6.4. Assessment of the hazard grade of the GM yeast with *CAD* gene

The toxicity, allergenicity and anti-nutrition effect grade of the recipient organism utilized in this GMOs were all grade I. The impact of the genetic manipulation on toxicity, allergenicity and anti-nutrition effect of the recipient organism were all type 2. According to Tables 5, 8, and 11 of the standard, it can be confirmed that the toxicity, allergenicity and the anti-nutrition effect grades of the GM yeast containing *CAD* gene were all grade I.

4.6.5. Assessment of the unintended effect grade of the genetically modified yeast with *CAD* gene

(1) *Testing for target agronomy traits*: The GM yeast was subjected to target agronomy traits testing. After chickens were fed with this feedstuff for 40 days, and pigs were fed for 30 days, the survival rate of chickens was 88–89% (in the antibiotics control group, the survival rate was 75–78%); the survival rate of pigs was 97%, and the net increase in weight was 9.56 kg/pig (in the antibiotics control group, the survival rate was 97%, and the net increase in weight was 8.45 kg/pig). The feeding test indicated that the GM yeast can totally replace antibiotics, and there is a much higher survival rate and a net increase in weight of animals.

(2) *Testing for non-target agronomy traits*: The growth and development traits of the GM yeast were similar to the recipient yeast. The major specific indexes are as follows: the optimal temperature was 30–32 °C; the thallus is easily killed at 45 °C; the lethal temperature of the scospore is 55 °C, it will stop growing below 4 °C, and it can be stored for long time at –80 °C; 90% relative humidity will ensure good growing state, and it can be stored in arid conditions for half a year; the optimal pH was pH 6.5, and it cannot grow normally below pH 4 or above pH 10; its fermentation needs qualified airfeed; its growth has no special requirement for illumination, but sunlight or ultraviolet light may kill the thallus; by monitoring successive generations (over 3 years) of the GM yeast, we found that all traits of the GM yeast were the same as the recipient yeast, which means it can remain stable. After fermentation for several times, both the density (cfu/ml) of the bakers' yeast in broth and the output of the moist thallus remain stable. These testing results have shown that the biological traits of the GM yeast remain stable.

(3) *Testing for the variation of hereditary features*: the *CAD* gene sequences detected from the GM yeast after fermentation showed the same nucleotide sequences as the theoretical sequences. The molecular weight of the antibacterial peptide *AD* in the GM yeast was about 3800 Da, which showed the same molecular weight as the theoretic value. When the GM yeast was successively fermented for five times in the same nutrient medium and the same fermentation conditions in a 50-l fermentation tank, the sterilization potency remains at the level of

5000–500 U/ml for each fermentation. The fermentation products derived from the GM yeast can work effectively both on gram-negative bacteria and gram-positive bacteria, especially *B. aeruginosus*, *S. aureus*, *E. coli* and *Salmonella* bacteria. The indexes of the fermentation products, including the bactericidal ability, the sterilizing potency, the viable count of residual yeast and the stability of sterilizing potency, have met the quality standard.

(4) *Testing for the transfer of the GM yeast and its exogenous gene*: Yeast fungus was isolated from environmental samples (collected in pigsty, prawn pool and hen coop) and the intestinal contents of animals, which ingested the GM yeast. The GM yeast fungus was screened using selective culture medium, but the viable GM yeast fungus could not be detected ultimately. The testing result showed that the GM yeast applied in the feedstuff had not been transferred into the environment or the animal's alimentary tract.

When *E. coli* was isolated from the feeding environment and the intestinal contents of animals, the DNA extracted from the viable *E. coli* was amplified to test *CAD* gene and *nptII* gene fragments by PCR. While *CAD* gene and *nptII* gene fragments could not be detected in the *E. coli*. These results indicated that the exogenous genes of the GM yeast had not been transferred into alimentary tract bacteria. The *CAD* gene fragment could not be detected in meat, blood or liver of pigs and chickens, which were fed with the feedstuff containing the GM yeast by PCR. The result indicated that there were no *CAD* gene residues in the fed animals.

Based on the testing results, we can conclude that the phenotypic and hereditary features of the GM yeast have not been altered, and the GM yeast and the exogenous genes have not been transferred horizontally. According to this standard (Table 12), it can be confirmed that the unintended effect grade of the agri-GMO was grade I.

4.6.6. Assessment conclusions

(1) The toxicity, allergenicity and anti-nutrition effect grades of the antibacterial peptide GM yeast were all grade I, which met the requirement of our new standard for the edible safety of recipient organisms.

(2) The toxicity, allergenicity and anti-nutrition effect grades of the GM yeast were all grade I and the unintended effect grade was grade I. The toxicity grade, the allergenicity grade and the anti-nutrition effect grade of the GM yeast were not higher than the relevant recipient organism, and the unintended effect that has harmful impact on edible safety did not occur. All of these results met the requirement of our new standard for the edible safety of GMOs.

(3) Based on the updated data and information on the GM yeast, it can be concluded that the assessment result of our new standard is the same as that of the original assessment record.

5. Conclusions

The technical regulation of “edible safety requirement and assessment of agricultural genetically modified organisms” for the Shenzhen Special Economic Zone in the People's Republic of China has been implemented by the People's Government of Shenzhen municipality since Feb 15th, 2007. It provides a set of systematic criteria for edible safety requirement and assessment process for agri-GMOs. According to the criteria, the hazard of recipient organisms and agri-GMOs on human and animal health is classified into four grades, and the impact of genetic manipulation on recipient organisms is defined as three types. In the criteria, the assessment standards involved in many aspects are developed, including the assessment standards for the hazard grades of toxicity, allergenicity, anti-nutrition effects of recipient organisms; the assessment standards for the impact of vectors, exogenous genes and their expression products on toxicity, allergenicity, anti-nutrition effects of recipient organisms; the assessment standards for the hazard grades of toxicity, allergenicity, anti-nutrition effects of agri-GMOs. In addition, according to the impact degree of phenotypic and hereditary feature variation and horizontal transfer of antibiotic resistance genes, the assessment standards for the hazard grades of unintended effects of agri-GMOs are provided in the criteria.

Based on the assessment standards for different hazard grades of recipient organisms and agri-GMOs, the edible safety requirements for recipient organisms and agri-GMOs are proposed: the organisms, when their hazard grades of toxicity, allergenicity, anti-nutrition effects is determined as grade IV, cannot be used as recipient organisms; the hazard grades of toxicity, allergenicity, anti-nutrition effects of agri-GMOs should not be higher than those of counterpart traditional organisms; hazard grades of unintended effects of agri-GMOs should not be higher than grade III.

The assessment procedure for the edible safety of recipient organisms and agri-GMOs is also provided in the criteria. The assessment for edible safety of agri-GMOs is based on the evaluation of two aspects: the hazard grade of recipient organisms and the impact type of genetic manipulation on recipient organisms. The hazard grade of recipient organisms refers to the hazard grades for toxicity, allergenicity, anti-nutrition effects, which are evaluated by the analysis of related data and toxicological tests, animal allergy tests and anti-nutrition factor test; the impact type of genetic manipulation is determined by the impact degree of exogenous genes and vectors on recipient organisms, which is evaluated by the analysis of the donor organisms, expression products and designed functions of exogenous genes and a serial of safety tests for expression products of exogenous genes. Another important aspect of assessment for edible safety of agri-GMOs is the hazard grades of unintended effects of agri-GMOs. The evaluation considers the impact of phenotypic and

hereditary feature variation and horizontal transfer of antibiotic resistance genes.

According to this new assessment system, several agri-GMOs, such as GM soybean, maize, potato, capsicum and yeast were reassessed. The comparison of the reassessment results with the previous assessment results suggested that the new evaluation conclusion of each case was congruent with the original assessment. It can be concluded that the novel criteria are suitable for the edible safety assessment of agri-GMOs, on account of their clear indexes, normative procedure and consistent methods.

Conflict of interest statement

None.

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