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EDITOR'S COMMENT

The seminar of "Food Allergy and Safety Assessment", which was held in Beijing by Chinese Division of International Life Sciences Institute, ILSI; China National Center for Food Safety Risk Assessment; Health and Environmental Sciences Institute, HESI; International Food Biotechnology Committee, IFBiC ; and Key Laboratory of Food Safety Risk Assessment, Ministry of Health, April, 2013. The specialists discussed deeply three subjects on the seminar: 1. proteins allergy and toxicity assessment of genetically modified crops, and research progress in components analysis; 2. the diagnosis standards and new methods development of food allergy; 3. safety assessment procedure, surveillance and approval procedure of genetically modified cops, and how to apply them globally. Based on the suggestions and instructions from our chief editor- academician Junshi Chen, MS. Nancy G. Doerrer who is associate director of Health and Environmental Sciences Institute of International Life Sciences Institute and Dr. Xudong Jia who is from China National Center for Food Safety Risk Assessment were invited by Clinical Journal of Prevention Medicine to organize the subjects of meeting and opinions of specialists to write two review articles ("Agriculture biotechnology safety assessment" and "Food allergy: definitions, prevalence, diagnosis and therapy "), which were also translated into Chinese and published at the same time, it provided helpful reference for Chinese researchers. Presentations of seminar are available on HESI website (http://www.hesiglobal.org/i4a/pages/index.cfm? pageID=3618).

Agricultural biotechnology safety assessment

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ABSTRACT

Genetically modified (GM) crops were first introduced to farmers in 1995 with the intent to provide better crop yield and meet the increasing demand for food and feed. Soybean and maize are the primary GM crops along with cotton and canola, but other crops with combinations of herbicide tolerance, insect resistance, and nutritional improvements are being developed. GM crops have evolved toinclude a thorough safety evaluation for their use in human food and animal feed. Safety considerations begin at the level of DNA whereby the inserted GM DNA is evaluated for its content, position and stability once placed into the crop genome. The safety of the proteins coded by the inserted DNA and potential effects on the crop are considered, and the purpose is to ensure that the transgenic novel proteins are safe from a toxicity, allergy, and environmental perspective. In addition, the grain that provides the processed food or animal feed is also tested to evaluate its nutritional content and identify unintended effects to the plant composition when warranted. To provide a platform for the safety assessment, the GM crop is compared to non-GM comparators in what is typically referred to as composition equivalence testing. New technologies, such as mass spectrometry and well-designed antibody-based methods, allow better analytical measurements of crop composition, including endogenous allergens.Many of the analytical methods and their intended uses are based on regulatory guidance documents, some of which are outlined in globally recognized documents such as Codex Alimentarius. In certain cases, animal models are recommended by some regulatory agencies in specific countries, but there is typically no hypothesis or justification of their use in testing the safety of GM crops. The quality and standardization of testing methods can be supported, in some cases, by employing good laboratory practices (GLP) and is recognized in China as important to ensure quality data. Although the number of recommendedor, in some cases, required methods for safety testing are increasing in some regulatory agencies, it should be noted that GM crops registered to date have been shown to be comparable to their nontransgenic counterparts (Herman et al., 2009; Herman and Price, 2013) and safe (Weber et al., 2012). The crops upon which GM development are based are generally considered safe.

[Key words]Transgeres; Food ; Risk assessment; Toxic actions; Environment

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An important aspect of evaluating the safety of genetically modified (GM)crops for use as food and animal feed is a risk assessment of potential effects on human and animal health as well as the environment. This risk assessment is based on evaluations of potential allergenicity, toxicity, and unintended adverse effects.

The current state of the science for addressing the safety of protein allergens utilizes a globally recognized weight-of-evidence approach, as outlined by the Codex Alimentarius Commission (CAC, 2003)[1], recognizing that no single endpoint is sufficiently predictive of the allergenic potential of a novel protein. Toxicity assessments are necessary to complete the safety assessment of a novel protein by determining if a protein has characteristics similar to known toxic proteins and evaluating the purified protein for toxicity in a model organism. The GM whole product, typically harvested grain from maizeor soybean, may also be evaluated for toxicity in animal feeding studies. However, the predictive value of such toxicology data and appropriate study designs are debated (EFSA, 2008)[2], particularly longer-term feeding studies, and will be discussed. The GM crop product (grain) is also evaluated for its similarity to the grain from the same or similar genetic background, as well as reference comparators utilizing a comprehensive compositional analysis. Novel GM trait proteins and the crops into which they are transformed are assessed for potential adverse environmental effects by evaluating environmental exposure and potential effects on non-target organisms (NTOs) utilizing hypothesis-based testing on a case-by-case basis. Thorough molecular and protein characterizations of the inserted DNA and expressed novel proteins complete the evaluation and are utilized in the assessment of allergenicity, toxicity, and risk.

AGRICULTURAL BIOTECHNOLOGY BACKGROUND

One in seven people in the world will go to bed hungry on any given day. Making sure that every person has access to healthy, nutritious food is a critical global challenge. The United Nations (2009) predicts that by the year 2050, the world's population will be more than 9 billion people. To feed that many people, food production must increase through enhanced productivity of each acre farmed. According to the UN, food output must grow to 70% beyond today's level, and that increase in productivity mandates that farmers adopt

new technologies best fitted for their purposes(FAO, 2009)[3]. The majority of world population growth, i.e., 49%, is expected to occur in Sub-Saharan Africa, constituting an increase of one billion people by 2050. Asia will account for 41% of the growth, a projected increase of over 900 million people by 2050 (United Nations, 2010) [4].The Food and Agriculture Organization (FAO) also projects that protein-based diets will increase by one-third, meaning that the total supply of food measured in kilocalories will need to increase by more than 170% (FAO, 2009)[5].

Technology adoption and advancement is not new to agriculture. Farmers adopt technology due to technology's ability to increase yield. For example, the adoption of double-cross hybrids in maize increased the yield from ~30 bushels per acre to over 60 bushels per acre in the US from 1935-1960. The adoption of single-cross hybrids and biotech traits has continued to increase the yield in the US to over 120 bushels per acre from 1960 to 2000 (Sutch, 2008) [6].

GM plant products were first introduced in 1995 and represent another application of technology in agriculture. GM crops are developed using the precision tools of modern biotechnology to introduce desirable traits into a plant. Since introduction of the technology, the number and complexity of GM products in the agricultural sector has increased. This includes the rapid adoption by farmers of 'breeding stack' GM crops that allow several beneficial traits to be expressed in a single plant.

Breeding stacks are generated by the commercial breeding of two or more single trait GM crop varieties. Common examples of breeding stacks include breeding together a GM crop with herbicide tolerance with a GM crop with insect resistance to provide a single variety containing both traits. Through the use of stacked products, farmers are able to benefit from multiple technologies. Stacks allow farmers to capture more value for their efforts because yield can be increased. Based on the work from the International Service for the Acquisition of Agri-biotech Applications (ISAAA, http://www.isaaa.org), it is reported that stacked traits in stacked productsare a growing component of GM crops, indicating that the technology continues to be

adopted. In 2012, 13 countries planted stacked event products with two or more traits, with approximately 43.7 of 170 million hectares (26%) planted with stacked GM crops overall.

The lack of globally harmonized regulatory approaches for breeding stacks may adversely impact the availability of breeding stacks to farmers. To meet farmer needs, science-based regulations across countries are akey factor to delivering approved products to the marketplace. The safety assessment and the existing approvals of single trait crops (a single event)should be readily applied to the stacked crop product as there are no hypothesis-based risks associated with breeding multiple trait stack varieties. It is reasonable to expect that stacked products containing approved insect resistantand herbicide tolerant traits would not show any adverse safety effects and, therefore, the single event safety assessments can be used to assess the safety of the stacked product with limited repetitive studies on the stacked event. Data have shown that the composition of a stacked product is comparable to conventional comparators – the same conclusion found from having analyzed single trait products (Steiner et al., 2013; Weber et al., 2012)[7-8].

SAFETY ASSESSMENT PROCESS TO REGISTER GM PRODUCTS

Delivering GM products to market requires time and investment to ensure safety. In a survey by Phillips McDougall sponsored by CropLife International (www.croplife.org), companies provided information on the time and cost of discovering, developing, and registering a new GM trait that was introduced or scheduled to be introduced between 2008 and 2012. The results of the study reveal that themean cost associated with a GM crop is USD $136 million. Based on the results of the complete survey, the mean duration value for all crops to move from research and development to commercialization was 13.1 years (Phillips McDougall, 2011)[9].

Globally, GM crops undergo regulatory safety evaluation focused on feed, food, and environmental safety unlike crops produced through conventional breeding. The studies supporting the evaluation of both food and feed safety are typically identical for GM crops. A key component of the independent safety evaluations performed by regulators is a peer-reviewed evaluation of the science provided in the dossiers.The standard used to support food safety for GM crops is an approach called substantial equivalence. This approach asks the

question, “Is the plant or article of commerce from the GM plant substantially equivalent to the same materials derived from a non-GM comparator?” Absolute safety is unattainable for any food. However, substantial equivalence can be used because it is centered on the premise that existing crop products used as foods can serve as the basis for comparison. The safety assessment is therefore supported by a comparison of the GM food to its traditional (non-GM) counterpart with a focus on composition, toxicology, allergenicity, and nutritional content. The substantial equivalence principle was originally proposed in 1991 and has been endorsed byCodex Alimentarius and the Organisation for Economic Cooperation and Development (OECD) and adopted by many countries and regions as the main principle for biosafety assessment of GM products.

Demonstrating substantial equivalence takes a multidisciplinary scientific effort. Before any GM food can enter the market, it is exhaustively tested and independently evaluated for safety by expert scientists in industry that run the studies and by auditors in the government. These food/feed safety assessments are based on guidelines and include a description of the food product and its history of safe use; detailed information about its proposed use; and molecular, protein, biochemical, toxicological, nutritional, and allergenicity data.

The safety assessment of GM products includes an evaluation of intended and unintended changes in the crop. Evaluation of intended changes is focused on new proteins expressed in the GM crop, i.e., evaluationof toxicity potential and allergenic potential. *In silico* and *in vitro* evaluation of the protein is also conductedto test fortoxicity and allergenic potential. Examples include updatedbioinformatic comparisons between novel biotechnology proteins and known allergens and mammalian toxins conducted according to Codex recommendations, and an evaluation of the digestibility of the protein using simulated gastric fluid and simulated intestinal fluid in an *in vitro* assay.

Unintended effects of GM products are also evaluated. One common approach used to affirm that the nutritional and safety profile of the crop has not changed (between GM and non-GM) is compositional analysis. Many analytes are measured in order to provide a thorough analytical survey of important nutrients, toxins, and anti-nutrients(anti-nutrients are natural or synthetic compounds that interfere with the absorption of

nutrients).To date, insect control and herbicide-tolerant GM crops have shown no biologically relevantchanges in compositional analytes when they are compared to the natural range of variation in the crop of interest (Herman and Price, 2013)[10].

Safety assessment of GM products may include additional studies to confirm protein safety when they are indicated for specific characteristics of the novel protein trait. One study commonly used is a mouse acute oral toxicology study with purified novel protein. In some regulatory regimes, the safety of the article of commerce (i.e., grain) is further assessed using a 90-day rat feeding study or a broiler chicken feeding study.

Environmental safety assessments are also conducted on a case by case basis, and are dependent upon the crop, the biotech trait, and the environment. Generally, environmental safety assessments (otherwise known as environmental risk assessments [ERAs]) are only performed by governments when the commercial developer is seeking cultivation approval. ERAs are often not part of the overall safety assessment when the developer is seeking only to import the grain for food, feed, or processing.

In conclusion, GM crops have undergone significant testing. Proteins expressed by GM crops undergo extensive analyses to demonstrate that they are not allergenic or toxic. Analyses of GM crops, particularly grain/seed, undergo many scientific studies to demonstrate that they are substantially equivalent to and as safe as those from non-GM crops, and their use as food and feed is evaluated to support distribution in the commodity crop trade.

COMPOSITIONAL ASSESSMENT OF GM CROPS

The composition of GM crops is compared to conventional crops to establish similarity. Similarity is based on the principle of substantial equivalence,which supports the conclusion that the GM crop is not substantially different from and is “as safe as” its nontransgenic comparator. The comparison of the GM to the non-GM crop includes the measurement of key nutrients, anti-nutrients, and natural toxins. The key nutrients include proximates, amino acids, fatty acids, minerals and vitamins, etc. The endogenous antinutrients and natural

toxins should be tested and are specific to the type of crop being developed for a GM product. The statistical significance of any observed differences should be assessed in the context of the range of natural variations by examining OECD consensus documents and the ILSI crop composition database (www.cropcomposition.org). Compositional assessment of nutritionally improved GM crops proposes new challenges such as choosingthe propercomparator and reference range from the non-GM comparator in order to identify unintended effects. The detection methodsused to identifyunintended effects include targeted approaches and non-targeted approaches. However, before using profiling methods, baseline data need to be collected.

MOLECULAR AND PROTEIN CHARACTERIZATION OF GM PRODUCTS

The safety assessment of GM products begins with a molecular characterization of the DNA that is inserted into the host plant and drives the expression of the trait and selectable marker proteins. The characterization relies on asking key questions:

(1) What DNA was put into the crop?

(2) How many copies of this DNA were put into the crop?

(3) Where in the host genome is the inserted DNA located?

(4) Is expression of the gene(s) stable?

Similarly, characterization of the trait proteins relies on answering fundamental questions to support the safety assessment from a food and feed safety perspective:

(1) Is the transgenic protein expressed in the plant in a stable manner?

(2) Are the biophysical properties of the transgenic protein in the plant consistent with a safe protein?

(3) Is heterologous protein equivalent to the GM plant protein?

(4) Is the protein suitable for human and environmental toxicity studies?

The inserted DNA of a transgenic crop consists of gene(s) that express protein(s) with specific trait(s) as well as supporting DNA, such as promoters and terminators. A vector DNA construct containing the DNA of interest (e.g., circular plasmid DNA delivered to the target plant cells to be transformed through the agrobacterium vector delivery process) is verified as accurate prior to insertions into the host plant genome.

This construct is then used to insert the target DNA into the host plant genome. Extensive sequence analysis of the host plant and the inserted DNA are used to verify that the inserted DNA has remained intact. A key technical component of this assessment is the Southern blot method and answers the following questions:

(1) How many copies of the insert DNA are present in the host plant genome?

(2) Is there any backbone DNA from the vector present in the host plant?

Further sequencing analysis addresses whether an endogenous host plant gene has been interrupted. The host plant genome insertion site is identified and a scan of the genomic sequence on either side is performed, typically out to 1 kilobase. Once this host genomic sequence is known, bioinformatic searches are performed to determine nearby genes, if any.Part of this genome location analysis also identifies the chromosome upon which the insert DNA is located and the potential for novel open reading frames at the junction of the insert and genomic DNA. Once the insert sequence and its location are known, the stability of the insert DNA to maintain its expression (stability) is evaluated. Again, Southern DNA blotting is used to visualize a discrete DNA sequence from the insert and to identify this in successive breeding generations of the host plant. Finally, Mendelian inheritance is used to check the expected proportion of plants that maintain the insert DNA in its genome. To support GM crop registrations, molecular detection techniques are developed for specific recognition of registered products according to published standards[12].

Biochemical assessments are performed on purified trait and selectable marker proteins expressed in GM crops. Techniques involve using Western blot (molecular weight), activity assays (enzymatic or insecticidal assays), total protein concentration, % purity, mass spectrometry (intact mass analysis), and N-terminal amino acid sequence analysis. Most importantly, these assessments are used to verify that the new GM protein(s) are expressed *in planta*as intended. However, because trait proteins are typically expressed at very low concentration *in planta*, these same techniques are used to verify that purified trait proteins expressed in a surrogate organism (usually *Escherichia coli*) are the same as *in planta*(Raybould et al., 2013)[13]. Once verified as the same, the surrogate trait protein(s) from *E. coli* are produced in quantities necessary for further testing of allergy and toxicity potential.

Protein characterization to support allergy safety includes extensive bioinformatics (Ladics et al., 2011) [14]. First, it can be verified that the GM protein is not from an allergenic source. The protein can also be compared directly to known allergen sequences (CAC, 2003, 2009)[15-16] to determine risk of allergen cross-reactivity. Additionally, protein is tested in simulated gastric and simulated intestinal fluids (Goodman et al., 2008) [17]. Rapidly digested proteins are expected to pose a low likelihood of allergic sensitization. Further testing verifies that the protein is not glycosylated when expressed *in planta*.

Toxicity testing is also assessed to support both human and NTO safety; the focus of environmental assessments for NTOs is on insecticidal traits. Bioinformatics is used again to show that trait proteins are not similar to toxins. Proteins are also evaluated in a mouse model of acute exposure for 14 days using toxicological endpoints (OECD, 2001) [18]. Together, this data is used to support human and environmental exposure calculations and an overall risk assessment profile. Biotechnology cropproductsthat contain novel proteins that are shown to have negligible risk for toxicity and allergy are then registered as safe for animal and human food consumption.

PROTEIN ALLERGY ASSESSMENT OF GM CROPS

Regulatory agencies, companies, and the public want to know that GM crops are safe.To that end, there is a comprehensive safety assessment program in place. This program includes evaluation of both the introduced protein and the GM crop with the purpose of demonstrating the GM crop is ‘‘as-safe-as” non-GM comparators. The GM crop safety assessment includes allergenicity, toxicology, and ecotoxicology evaluations. One of the concerns for GM crops is the assessment of the expressed GM trait protein for potential food allergy. The primary risk is that there would be a Type I allergy response triggered. Protein-specific IgE is the key mediator in food allergy and can elicit symptoms such as hives, angioedema, asthma, diarrhea/vomiting, atopic dermatitis, and anaphylaxis in sensitized individuals upon ingestion of the offending food (Ladics, 2008) [19].

There are three protein allergenicity concerns with agricultural biotechnology products (listed in order of potential risk):

(1) transfer of an existing allergen or cross-reactive protein into another crop;

(2) creation of a food allergen *de novo* (i.e., the potential to become a new allergen); and

(3) alteration or quantitative increase of endogenous (existing) allergens.

Because no single endpoint is currently available to identify a protein as a potential allergen, a weight-of-the-evidence approach is utilized as recommended by Codex (CAC, 2003) [1]. This assessment is based on what is currently known about allergens and includes the following:

(1) history of exposure and safety of the gene source;

(2) *in silico* bioinformatics analysis of the novel protein's amino acid sequence to a database containing the amino acid sequences of food, dermal, and respiratory allergens;

(3) stability to pepsin and trypsin digestion *in vitro*;

(4) stability to heating;

(5) glycosylation status;

(6) abundance of the novel protein in the article of commerce; and

(7) immunological methods when necessary.

Animal models, while useful for research purposes, need further evaluation, and none, whether rodent or non-rodent, have been validated or are widely accepted. Therefore, animal models should not be currently included in the regulatory safety assessment.

To evaluate the transfer of an existing allergen or cross-reactive protein into another crop, *in silico* bioinformatics analysis is conducted. The allergen database employed to conduct the bioinformatics analysis by the agricultural biotechnology industry is AllergenOnline (www.allergenonline.org). This is an industry sponsored, peer-reviewed database housed at the University of Nebraska in the US. The database is peer-reviewed by clinical and research allergists from around the world. The inclusion of protein allergens is based on available data in the public literature. The database is updated once a year and is available free to the general public. The novel protein sequence is compared to sequences in the allergen database by using local

alignment programs such as BLAST or FASTA (Pearson and Lipman, 1988) [20]. IgE cross-reactivity between a novel protein and a known allergen is considered a possibility at a very conservative level when there is more than 35% identity over a segment of 80 or greater amino acids (FAO/WHO, 2001) [21]. Additionally, ≥ 8 contiguous identical amino acid segment searches are also performed to identify sequences that may represent potential (theoretical) linear IgE binding epitopes. The contiguous identical amino acid search, however, has been reported to produce too many false positive findings. Therefore, the > 35% identity over an 80 or greater amino acid window is considered to be the more relevant bioinformatics criteria for identifying alignments with allergens (Ladics et al., 2011) [14].

For novel proteins originating from an allergenic source or having significant identity to a known allergen, immunological methods (i.e., specific IgE sera screening studies) are conducted to support commercial development of a crop with a protein possessing putative allergy risk. The availability of well-characterized human sera from a sufficient number of patients is an issue of critical importance when conducting IgE sera screening studies in order to decrease the potential for false positive or equivocal results, as well as to support conclusions of safety where no IgE binding to the novel protein is observed. The clinical history of subjects should be well characterized, and should be double-blind, placebo-controlled, and food-challenged to ensure they are clinically allergic to the food in question.

To evaluate the second potential allergy risk associated with agricultural biotechnology, the creation of food allergens *de novo*, biochemical and physical properties of the protein are evaluated. These endpoints include stability to pepsin and trypsin digestion *in vitro*, glycosylation status, and stability to heating. A standardized protocol for evaluating the *in vitro* pepsin resistance to proteins has been established (Thomas et al., 2004) and the assay provides a loose correlation for major food allergens, many of which are stable in the assay.
The third potential allergenicity concern (and least important in regard to identified risks) involves the comparison of the endogenous allergen levels in the GM versus non-GM comparators(Herman and Ladics, 2011) [23]. This concern is evaluated using various analytical methods such as IgE sera screening or mass

spectrometry studies to show that endogenous levels of known allergens are not altered by the new genetic material in the GM varieties.

A consistent assessment process has been in place across the globe for many years to support the safety of GM crops. To date, there is no scientific evidence that a novel GM protein or a GM crop has increased the allergenic risk to the susceptible public or produced new allergens.

TOXICITY ASSESSMENT OF GM CROPS

Data Sources for Safety Assessment

A GM food toxicity assessment does not necessarily require animal toxicity studies. The first and most pivotal step is a consideration of the potential for toxicity. Agreat dealis known about maize for example, in terms of its safety as a human food and animal feed. At the level of DNA, maize naturally has multiple transposons (i.e., jumping genes) and millions of single nucleotide polymorphisms (SNPs), and the resulting genetic variation among maize varieties is as great asthat between humans and chimpanzees (Buckler et al., 2006) [24]. Yet, toxic maize has never been observed in either conventionally bredor in the multiple GM hybrids or varietiesdeveloped using multiple transgenes from multiple sources. There is no plausible mechanism for the *de novo* generation of toxicity in maize through insertion of a transgene; consequently, the probability of producing a toxic maize, unrelated to the protein expressed by the transgene itself, solely through the method of insertion of a transgene, is essentially zero.Therefore, there is no science-based requirement or value in routine toxicity studies on GM maize.

For a less well characterized crop variety, the key considerations are the phenotypic and biophysical characteristics of the parent plant and the transgene, as well asthe source of that transgene. If any variety of either organism, or a related species, produces toxic proteins or toxic secondary metabolites, then the presence and level of those substances should be determined using analytical chemistry. Other key information supporting a safety assessment are the number of backcrosses involved (every backcross to the parent line reduces the genetic material from the initial hybrid) and the consistency of the agronomic properties with that

of the parent line plus the transgene. This assessment supports a conclusion of anabsence of unintended genetic effects.

Historical Precedent

Safety concerns around reduced nutrition and production of unintended and unknown toxic radiolytic products in irradiated food echo those expressed for GM crops, and have resulted in extensive chemical analysis and toxicology studies. Adoption of irradiated food technology was consequently delayed for nearly half a century and remains controversial.

In a review of food irradiation, a joint FAO/WHO/IAEA expert committee (WHO, 1999) [25] "recognised the value of chemical studies as a basis for evaluating the wholesomeness of irradiated foods," and concluded that "the determination of wholesomeness for a representative food could be extrapolated to other foods of similar composition on the basis of available chemical data" (i.e., without animal testing). The expert committee also concluded that although "several different chemical bonds in the constituents are broken or formed, leading to either desired or undesired effects... it is through a consideration of the radiation chemistry of food that these chemical differences and their implications for wholesomeness and product quality can be understood."

Despite compositional analysis providing the most robust basis for safety assessment, tens of thousands of experimental animals were sacrificed in whole food animal studies over a 50-year period, including rats, mice, dogs, primates, chickens, and quail. No results were obtained that were not predictable from a knowledge of radiation chemistry. Whole food animal testing was criticized by Elias (1980) who observed that these studies suffered from a number of inherent limitations, including "…the impossibility of physically or chemically identifying what was being tested; the inability to incorporate sufficient irradiated food into the animal diet without seriously disturbing the nutrition of the test animals giving rise to secondary toxicological findings totally unrelated to irradiation effects, and the obvious impossibility of using sufficiently large numbers of animals in each experimental group to permit ascribing with an acceptable degree of statistical confidence any observed variations to the effect of radiolytic products present in minute amounts."Elias concluded that "…It is

more convincing to be able to state that certain likely effects have been searched for and found absent than to admit that one did not know quite what to look for – but found it absent nevertheless[26]." These conclusions remain valid for GM crop safety assessment today (Bartholomaeus et al., 2013) [27].

Predictable outcomes of toxicity studies

With there being no hazards identified for GM crops, it is not surprising and remains unique in the field of toxicology that risk analysis of GM crops based on agronomic and compositional analysis always reflects the no-observed-adverse-effects findings when experimental animals are exposed to diets containing the GM crops. This outcome reflects both the negligible potential for accidental generation of toxic substances through gene insertion and the high limit of detectionforbioassays when used to identifyputative but undefined adverse effects.

Role of toxicology studies in GM risk assessment

Animal toxicology studies conducted on purified or at least substantially enriched test substances (i.e., the transgenic GM protein) may occasionally have value in GM risk assessment. Their value is limited to cases where there is a scientifically plausible hypothesis ofa novel secondary metabolite(s), when a novel protein has been introduced at significant levels, or there is a need to characterize herbicide or pesticide metabolites unique to the transgenic variety.

Classical toxicology studies have little role in GM crop risk assessment, and whole food studies are generally scientifically invalid, uninterpretable, unethical, and unnecessary. The key determinants of GM crop safety are:

(1) the known characteristics of the parent crop species;

(2) chemical analysis for any endogenoustoxins in the parent crop (e.g., toxic alkaloids in species of the Solanaceae - potatoes, tomatoes – or cyanogenic glycosides in cassava);

(3) characterization of the transgene;

(4) consideration of novel herbicide (or pesticide) residual expressed protein residues from herbicide tolerance or detoxifying genes;

(5) chemical analysis of chemical herbicide residues left over from treatment of herbicide tolerant varieties; and

(6) evaluation of the crop development process (backcrossing, proven agronomic characteristics consistent with the parent crop and transgene).

ECOLOGICAL RISK ASSESSMENT

Ecological risk assessments estimate the probability and seriousness of harmful effects from changes in biodiversity following an activity. Ecological risk assessments may be carried out on GM crops to help make decisions about whether to allow field trials, import, or cultivation(Wolt et al., 2010) [28].

To begin an ecological risk assessment, the risk assessor must identify the potential ecological harm that may be caused by use of the GM crop. For regulatory risk assessments, definitions of ecological harm must be sought in legislation, regulations, or instruments of public policy (Evans et al., 2006; Raybould, 2012) [29-30]. Exact definitions of harm vary among countries, but usually include reduction in crop yield or quality, loss of ecological functions such as pollination or pest control, and reduced abundance of species that have cultural significance (Sanvido et al., 2012) [31].

Once definitions of harm are agreed upon, the next stage of the ecological risk assessment involves elucidating plausible pathways by which use of the GM crop may cause harm. The plausibility of a pathway depends on the scale of the release of the crop: a small-scale, confined field trial is far less likely to lead to ecological harm than is an unrestricted release of the crop for cultivation (Wolt et al., 2010) [28].

Two pathways to harm are featured in most ecological risk assessments for cultivation of GM crops: 1) The crop may reduce valuable biodiversity because it is toxic to non-pest organisms, and 2) the crop may reduce valuable biodiversity because it becomes a serious weed of agricultural or non-agricultural land.

The main part of an ecological risk assessment is a test of the hypothesis that use of the GM crop will not cause ecological harm. Usually, the GM crop is derived from a non-GM crop that is not ecologically harmful if used properly. Therefore, the hypothesis under test may be that use of the GM crop is no more likely to cause harm than a similar use of the non-GM crop.

Usually two sets of experiments are conducted to test the hypothesis that the GM crop is no more likely than the non-GM crop to cause harm through toxicity. The first set of experiments tests for potentially harmful changes in the nutritional quality of the crop as a result of unintended effects of transformation. There is a generally accepted standard set of data requirements among many countries to assess risks from unintended effects: molecular analysis of the inserted DNA, including its sequence and copy number; and a comparison of the GM crop and a suitable non-GM comparator for differences in composition, such as amounts of protein, minerals, vitamins, fatty acids, and antinutrients (see Raybould et al., 2010, for details) [32]. In some circumstances, animal feeding trials with GM grain or silage may be used to assess the potential for harmful unintended effects of transformation.

The second set of experiments tests whether the intended effect of the genetic modification has harmful side effects on biodiversity. For insect-resistant crops producing crystal, delta-endotoxin proteins from *Bacillus thuringiensis* (Cry proteins), experiments expose representative organisms to high concentrations of Cry proteins in the laboratory (Romeis et al., 2008) [33]. If the proteins produce no adverse effects at concentrations greater than those likely in the field, cultivation of the crop is considered unlikely to lead to ecological harm via toxicity.

A comparison of the phenotypes of the crops in field trials in several locations is the usual test for the hypothesis that the GM crop is no more likely than the non-GM crop to become a serious weed. If there are no significant differences in the growth and reproduction of the crops, the GM crop is unlikely to be a more serious weed than is the non-GM crop (Raybould et al., 2010) [32]. Depending on the crop and location, it may also be necessary to assess whether hybrids between the GM crop and wild plants are likely to cause ecological

harm through their being invasive of agricultural or non-agricultural land (e.g., see Hokanson et al., 2010) [34]. Negligible ecological risk may be shown if the crop is unlikely to hybridize with a wild species, or if the genetic modification is unlikely to increase the abundance of a wild species (Raybould and Cooper, 2005) [35].

FOOD ALLERGEN DETECTION METHODS

Preventative medical treatment is not yet available for individuals suffering from food allergies. Strict avoidance of the allergy-causing food is the only means of avoiding reactions. In some populations for particular allergens, most cases of severe allergic reactions are caused by hidden or undeclared allergens (Añíbarro et al., 2007) [36]. Allergenic proteins can be introduced unintentionally into or hidden in food due to labeling errors, cross-contact during or after processing, and incomplete cleaning of food production equipment (Jackson et al., 2008) [37]. In order to comply with legislation and to check end products for label compliance, as well as to validate the effectiveness of allergen control programs, analytical methods have been developed to detect and quantify the presence of allergens in foods, ingredients, and in the food processing environment. While no food allergen detection methods were available before 1990, they have evolved significantly over the past ten years resulting in new strategies such as liquid chromatography coupled to mass spectrometry (LC-MS) (Fæste et al., 2011) [38].

The selection of a suitable method depends on various factors such as identifying therequired level of detection (i.e., detection limits)and clarifying whether allergens would need to be quantified in a finished food product or only detected as part of the sanitation process using “swap” samples. The technical capability of the laboratory can also play a role in selecting a particular allergen detection technology. For example,polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISA), or even mass spectrometry(MS) represent different platforms that can all be used, depending on the technological demand. Currently used analytical methods target either the allergen itself or markers that represent the offending food containing the allergen.Target molecules are usually specific proteins, peptides or DNA fragments (Kirsch et al., 2009) [39]. The perfect analytical method would have the following characteristics:

(1) applicable to all food commodities whether they are processed or not,

(2) highly specific,

(3) quantitative to allow for a health risk assessment,

(4) highly sensitive because thresholds have not been established,

(5) validated, and

(6) internationally recognized.

Recommendations on how to validate methods for food allergen testing are given, for example, in Abbott et al. (2010) [40] and Johnson et al. (2011) [41].

General protein tests can be used to control the effectiveness of sanitation programs. These tests usually target any protein or adenosine triphosphate (ATP) as a marker for any biological source, but they are not specific to an allergen. They are rather quick and can be easily performed on-site. However, the result may not correlate with the amount of allergen present on the production line.

DNA-based methods are mainly used to detect the presence of allergenic food commodities or ingredients in cases where immunochemical or mass spectrometric methods are not yet available, or where the use of these methods is limited due the ability to detect the parental molecule (e.g.,reduced capacity to detect a protein due to modification during food processing). DNA-based methods are, in most cases, highly specific and rapid (Poms et al., 2004) [42]. However, the detection of DNA does not necessarily mean that the allergenic proteins are present in quantities to elicit allergic reactions. After an extraction of the DNA from the samples,the DNA initially present in a sample is doubled during each amplification step during PCR. This amplification is very sensitive and allows detectionof DNA from only very smallamounts of the initial DNA in the sample (10 molecules). The detection andquantification of the DNA present can be performed in different ways depending on the needs and laboratory resources. Following PCR, the amplification product can be detected by electrophoresis using agarose gels or by PCR-ELISA. Real-time PCR, also called quantitative real-time PCR,canamplify whilesimultaneously quantifying the targeted DNA molecules.

Today, the favored methods for detecting and quantifying food allergens or marker proteins are ELISA and lateral flow devices (LFDs) (dipsticks). These methodscan specifically detect and/or quantify individual allergenic food commodities with detection limits in the low mg/kg (ppm) range. Like general protein tests, LFDs can be easily performed on-site and return rapid results, but they also would require confirmatory methods since they are not typically quantitative. Even though immunochemical detection (ELISA) is generally considered to be specific, accurate, sensitive, and relatively simple to use, the interaction between target protein or peptide and antibodies can be affected by any changes of the binding properties. These can occur during food processing but also during sample extraction. Hydrolysis, thermal, or chemical denaturation, as well as exposure to oxidizing conditions, can change the immunoreactivity and therefore the analytical result. Manufacturers of immunochemical methods are now offering more assaysthat can detect both native and processed foods.

In the last couple of years, strategies for the detection and quantification of food allergens using mass spectrometry in combination with liquid chromatography have profited from the technological evolutions in the field of mass spectrometry driven by the demands in proteomics research. LC-MS/MS allows a direct determination of food allergen proteins using selected peptides and their characteristic masses (m/z). This approach is a direct measurement and not dependent on antibodies like in ELISA or LFD assays. Proteins that have been modified by thermal processing, hydrolysis, or oxidation can still be detected through LC-MS/MS by selecting suitable marker peptides. Furthermore, multiple peptides of one or more proteins can be analyzed simultaneously. This allows for multiple proteins/allergens analyses in one single experiment and can reduce time-to-results and analysis costs if one sample has to be analyzed for the presence of several allergens. MS also offers a stoichiometric cross-verification of results as determined peptide amounts of the sample protein should be equimolar. This makes the approach a powerful method for confirming results after themore rapid immunochemical and DNA-based tests have been performed or where these methods have limited capabilities to detect the allergen.

Today, threshold or action levels are not set for all regulated food allergens. However, such levels would help industry to know exactly how to perform allergen management and would lead to a harmonization of warning labels for consumers. The capability of MS technology to be further refined and validated in orderto provide confirmatory methods for food allergen quantification has been demonstrated in recent publications (Lutter et al., 2011; Monaci et al., 2011; Resta et al., 2012; Azarnia et al., 2013; Heick et al., 2011)[43-47]. Efforts on setting guidance documents and validation protocols at the international level will help future standardizations to ensure a maximum recognition of methods for food allergen detection.

AUSTRALIAN REGULATORY REQUIREMENTS FOR GM CROPS

Australian regulatory environment

Australia's regulatory environment is guided by general principles that apply to all areas of regulation. In the context of food regulation, these principles can be summarised as follows:

(1) Ethical regulation is proportionate to risk:regulatory requirements are evidence-based and founded on good science.Regulatory burden (and cost) is commensurate with the risk to be managed and the value of the expected benefit.

(2) Value of information (VOI):data requirements address and inform viable risk management options.Data is necessary only where it has a material influence on the outcome (risk management strategies).

(3) A "precautionary approach" is not necessarily: precautionary and may be risk-generating if the broader consequences of regulation are not adequately considered.

(4) The regulatory objective is balance, proportionality, pragmatism, cost effectiveness, impartiality, and, most importantly, scientific integrity.

These principles acknowledge that government intervention is sometimes necessary to protect human health and safety, the environment, and/or to maintain public confidence. However,if this intervention is excessive and disproportionate in the marketplace,it may undermine the competitiveness of Australian industry and reduce national prosperity. To ensure agencies with the responsibility for introducing new regulations adequately consider the broader economic consequences of proposed regulation, there is a requirement for a

Regulatory Impact Statement (RIS) to be produced before a regulation is considered for adoption. The RIS must weigh the economic costs of the regulation against the economic gain and is assessed for accuracy and completeness by the Office of Best Practice Review (OBPR). This process applies a discipline on regulatory agencies that supports the objective of regulatory balance, proportionality, pragmatism, and cost effectiveness.

Food Standards Australia New Zealand (FSANZ): regulatory requirements for GM crops

The FSANZ data requirements for GM crops can be found in the "Application Handbook." Approval is required for any new GM food crop event to be sold in Australia for human consumption, but no approval is required for stacked events where each of the crop varieties used in developing the stack has previously been approved [48]. This arrangement recognises that there is no evidence or scientifically valid theoretical concern supporting additional risk arising from the conventional crossing of GM crops in comparison to conventionally developed crops.

Toxicity

For a new GM event, FSANZ requires information supporting a consideration of the safety ofthe novel protein(s), altered pesticide residue profile, altered nutritional profile, or any other significant alteration to the composition of the crop as consumed. Acute or short-term oral toxicity studies in animals on novel proteins are not required unless bioinformatic comparisons and biochemical studies indicate either a relationship with known protein toxins/anti-nutrients or resistance to proteolysis. Similarly, if novel substances are identified, then animal toxicity studies on the isolated purified substances are required. Toxicity studies on whole foods are recognised as being scientifically invalid and are explicitly not required. Where they have been conducted to meet the regulatory requirements of other jurisdictions, FSANZ requires the submission of these studies to ensure it has a complete dataset.

Allergenicity

Information must be provided that enables FSANZ to consider whether a newly expressed protein is potentially allergenic, including the source of the introduced protein, any significant similarity between its

amino acid sequence and that of known allergens, its structural properties, susceptibility to enzymatic degradation, heat and/or acid stability, and specific serum screening where a newly expressed protein is from a source known to be allergenic or has sequence homology with a known allergen. Analysis of the levels of an endogenous allergen are not required as this information has no bearing on the risk management of an allergenic food, and natural variability in the levels of allergens generally exceeds that which might result from the insertion of a transgene unrelated to the pathway of formation of the allergen.

Compositional analysis

FSANZ requires compositional analysis to support approval of new GM crop varieties. However, the scientific basis for this continued requirementis now questionable. Compositional analysis is expensive with no evidence that it adds to public health and safety. To date, there is no instance of compositional data revealing risks for commercial GM crops not predictable from knowledge of the parent line and source of the transgene. On the other hand, there is clear and extensive evidence that considerable variation due to seasonal and environmental variability generally exceeds genetic influences which limits the applicability of interpreting the outcomes of these studies. A requirement for compositional analysis of GM crops but not of “conventionally” bred crops, which often have greater genetic alteration, is irrational, logically inconsistent, and discriminatory.

Labelling

Food derived from GM crops must be labelled where they contain novel protein or DNA or they are substantially different from the unmodified crop. Purified oils that do not contain protein and DNA do not require labelling unless they are compositionally altered (e.g., high SDA soybean oil).

PROGRESS OF SAFETY REGULATIONS AND EVALUATIONS OF AGRICULTURAL GM ORGANISMS IN CHINA

There are regulations for safety evaluation,label management, production approval, business permit management and import/export safety approval for GM organisms in China. These are described in “Regulations on Administration of Agricultural Genetically Modified Organisms Safety”(State Council, 2001)-

[49]. All administrative departments of agriculture, quarantine inspection, industry and commerce,and quality supervision are involved in planting, producing, marketing, and importing/exporting of GM products. Safety evaluation, supervision, systematic construction, standard setting, import approval, and label management of GM products are performed by a joint ministry board which consists of 11 ministries, the administrative leader group for GM product safety management of the China Ministry of Agriculture (MOA), as well as the department of biosafety and intellectual property of MOA. Agricultural administrative departments above county level and other working bodies are responsible for reviewing GM products. The fourth GM product safety committeeis in charge of GM product safety evaluation and technical consultation. This committee consists of GM product experts for research, production, processing, inspection and quarantine, health effects, and environmental protection.

MOA references Codex guidelines on "Principles for the Risk Analysis of Foods Derived from Modern Biotechnology," "Guideline for Safety Assessment of Food Derived from Modern Biotechnological Crops" (CAC, 2003)[1], and summarized practical work experience of safety assessment on GM products in China. MOA issued "Guideline for Safety Evaluation of GM Plants,""Guideline for Food Safety of GM Plants and Production,""Guideline for Safety Evaluation of GM Microorganism for Animals," and related food safety testing standards (Ministry of Agriculture, 2002)[50]. All of these guidelines present specific requirements for key aspects, review procedures, and guidance for the methods employedduring safety assessment of GM crops and related products. The "Guideline for Safety Evaluation of GM Microorganism for Animals" is a new technical support document which includes definitions, procedures, general requirements,and requirements for safety evaluation on GM organisms for different kind of animals. As for safety assessment of GM crops with stacked traits, specific regulations were made considering safety assessment of the stacked trait obtained from conventional breeding.For a stacked trait GM crop where the producerplans to import the crop as raw material for processing, it could apply for a safety certification directly when the transformant used during breeding already has a GM safety certification. Otherwise,the producer should apply for production testing certification if imported GM will be considered for aproduction application. The safety assessment would focus on whether there is gene interaction. In this scenario, the approval procedure would be optimized and simplified. To

strengthen breed management, a breed production and business certification process has been developed. All research units or corporations involved in GM seed/animal/aquatic product production could operate only after GM product biosafety certification and GM seed/animal/aquatic product production certification are issued by MOA. Any GM product included in the identification index of agricultural GM products must be labeled under regulation if marketed in China; otherwise, importing or marketing would be prohibited. The first batch of GM product identification index includes: soybean seed, soybean, soybean flour, soybean oil, soybean pulp, corn seed, corn, corn oil, corn flour, rape seed, rapeseed, rapeseed oil, rapeseed pulp, cotton seed, tomato seed, tomato, and tomato sauce. The "Management Regulation for GM Identification" explicitly stipulates procedures of application/re-application and cancellation, and also gives concrete suggestions on method/pattern/literalness of labeling.

SAFETY EVALUATION IN ADMINISTRATIVE PERMISSION – NECESSITY AND REALITY OF GLP

Chemical products may have effects on environment and human health. Therefore, administrative permission should be given before they are put on the market. Those products are as follows: pesticides, veterinary medicines, feed additives, common chemicals (new chemicals), drugs, medical instruments, cosmetics, health foods, food additives, as well as biotechnology products such as biotech drugs and transgenic organisms and products. In China, these products are under the administration of different government authorities, i.e.,MOA, Ministry of Environmental Protection, CFDA, and Ministry of Health. The process of administrative permission includes the following stages: application, acceptance, technical review, and administrative approval. The result of the technical review supports the administrative approval.

The technical review consists mainly of biological safety tests (mainly referring to toxicology tests). The process of finishing the safety tests is known as safety evaluation. One of the important principles of the safety evaluation is Good Laboratory Practice (GLP).

GLP, which first appeared in the drug registration process as normative documents, now has developed as an essential requirement for safety evaluation of chemical products and as regulatory documents in developed

countries to regulate corresponding areas listed above. The purpose of GLP is to guarantee the quality of the non-clinical safety evaluation studies and the authenticity, integrity, and credibility of the registration application documents.

The regulatory documents of drug GLP have been promulgated one by one since 1991. SFDA (State Food and Drug Administration) did not begin to inspect the first batch of drug safety evaluation facilities as a pilot until 2002. Until now, the first GLP of China (drug GLP, Good Laboratory Practice for Pre-Clinical Laboratory Studies, SFDA administration order No.2, promulgated in August 2003) has been followed for nearly 10 years. More than 50 GLP agencies have been accredited by SFDA (changed to CFDA in 2013). Drug GLP of China is law, as is FDA GLP and drug GLP of Japan.

Pesticide GLP, Chemical GLP, and CNCA GLP have been issued in the form of an announcement or notice by MOA or Ministry of Environmental Protection since 2006, and they still have not become laws. CNCA (Committee of National Certification and Accreditation) GLP is more like a guidance document, similar to the recommendations byOECD for GLP compliance. It has no force of law because of no specific product being targeted within the guidance.

The level of the drug non-clinical safety evaluation study has been greatly improved since the implementation of drug GLP in China. A competitive GLP-compliant industry has also been established in China. Study data of some facilities have been proved to be acceptable by the US and EU. Researchers understand GLP very well and produce highly professional studies when employing their skills under GLP. However, areas other than drugs have not started the accreditation process of approving GLP accredited facilities. The quality of the safety testing performed at these facilities is still a problem. As far as transgenic products are concerned, the quality of the current safety evaluation tests and studies receive much attention when considering their quality. The tests and studies might be standardized by reference to the drug GLP management pattern.

SUMMARY

The benefit of biotechnology-based crops to the world's food supply is shown by ever increasing uptake into the global market. Toxicology, allergy, and environmental safety of the crops and their transgenic traits are the cornerstones of registering them for use around the world. Protein and molecular assessments of the genetic traits provide a thorough description that helps identify any safety concerns. Environmental assessments are hypothesis based which directs the design of studies to identify concerns for NTOs (for insecticidal traits) and any impact to the surrounding environment during and after crop harvest. Australia provides an example of how to apply practical considerations in performing only those studies that are purposeful and pertinent to identifying safety of GM crops. Some studies have become routine, although they provide little safety value in that they do not necessarily provide greater certainty of safety. All of the characterizations constantly undergo technical evaluations for potential improvements in the ability to measure crop molecules.This is particularly evident for the measurement of crop allergens and other components in compositional analyses, although it should be recognized that little value is gained where there is no hypothesis-based concern for potential increases in allergen content. To maintain the quality of these analyses across facilities, quality standards such as GLP are considered useful. Ultimately, the characterization studies and safety testing undergo regulatory reviews which are accounted for in China by a group of highly organized agencies that ensure food and feed safety for consumers.

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