Food and Chemical Toxicology 86 (2015) 132–143

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Review

Safety assessment of foods from genetically modified crops in countries with developing economies

Bryan Delaney

Global Industry Affairs and Regulatory, DuPont Pioneer, 7100 NW 62nd Avenue, P.O. Box 1004, Johnston, IA 50131, United States

ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form 2 October 2015
Accepted 5 October 2015
Available online 9 October 2015

Keywords:
Biotechnology
Safety assessment
Agriculture

ABSTRACT

Population growth particularly in countries with developing economies will result in a need to increase food production by 70% by the year 2050. Biotechnology has been utilized to produce genetically modified (GM) crops for insect and weed control with benefits including increased crop yield and will also be used in emerging countries. A multicomponent safety assessment paradigm has been applied to individual GM crops to determine whether they as safe as foods from non-GM crops. This paper reviews methods to assess the safety of foods from GM crops for safe consumption from the first generation of GM crops. The methods can readily be applied to new products developed within country and this paper will emphasize the concept of data portability; that safety data produced in one geographic location is suitable for safety assessment regardless of where it is utilized.

Contents

E-mail address: bryan.delaney@pioneer.com

http://dx.doi.org/10.1016/j.fct.2015.10.001

1. Introduction

Environmental factors including damage from insects and competition with weeds for resources necessary to support growth contribute to decreased yield from field crops. Historically, synthetic insecticides have been used for protection of plants from insect damage and herbicides have been used to control weeds. While they have been effective, more recent developments have applied the tools of biotechnology to produce genetically modified (GM) crops for insect and weed control. Early generation GM crops including insect resistant maize and herbicide tolerant soybeans express proteins from foreign sources that endow them with these particular phenotypes. They have been cultivated in the United States for nearly 20 years with significant benefits including decreased use of pesticides and herbicides, yield increases, decreased labor costs and improvements in quality (Brookes and Barfoot, 2009; Klümper and Qaim, 2014).

During the course of developing early generation GM field crops a multicomponent safety assessment paradigm was also developed based on recommendations and guidelines from scientific and regulatory authorities that is referred to as substantial equivalence (World Health Organization (WHO, 1991). The goal of the safety assessment for foods from GM crops was to produce laboratory data for individual GM crops to demonstrate that they were *as safe as* foods from non-GM crops. This paradigm and the ensuing methods to demonstrate substantial equivalence has proven quite effective as foods from GM crops have a well-established history of safe consumption in the US and in other countries that have imported foods from GM crops and cultivated GM crops.

It has been estimated that food production will need to increase by 70% by the year 2050 based on population growth particularly in countries with developing economies (Food and Agriculture Organization of the United Nations (FAO, 2009)). The number of countries and the populations within them far exceed those countries with more advanced economies as they include China, India, and other populous Asian and African countries.

Countries with developing economies stand to benefit from biotechnology for the same reasons as countries with more advanced economies. Whether imported or cultivated from other countries or developed independently, foods from GM crops should be as safe as those from non-GM plants regardless of where they originate. This chapter reviews methods established to assess the safety of foods from GM crops for safe consumption in early generation products. Insect resistance and herbicide tolerance are emphasized for two reasons. First, there is a tremendous amount of experience with variants of GM crops with these two phenotypes. The methods used to assess the safety of these early generation GM crops are relevant for safety assessment of any type of GM plant – not just soybeans and maize. Second, both the insect resistance and herbicide tolerance phenotypes will be used regardless of geography even if the range of plants to be transformed with them is more diverse than common US field crops such as soybeans and corn.

Understanding the methods of safety assessment will benefit crop developers, regulators and other scientists as it will be shown that they not only demonstrate the safety of currently available GM products but that the methods can readily be applied to newly developed GM crops as well.

1.1. Agricultural biotechnology

Biotechnology is a process whereby specific genes are transferred from one species into another. This introduction of foreign DNA is typically via engineered plasmids (i.e., vectors) or by physical processes such as particle bombardment that deliver the DNA to the nucleus of the recipient plant. In many cases these genes express proteins that endow the plant with a targeted phenotype or trait by design. The most commonly expressed proteins in GM crops are those with herbicide tolerance and insect resistance activities though other GM crops products express proteins that express disease resistance, stress tolerance or other phenotypes (http://www.isaaa.org/). It should also be noted that not all GM crops express proteins as some modifications are attributable to gene regulation by RNA (Petrick et al., 2013). Regardless of the intent, the introduced gene or genes become permanently incorporated into the genome of the recipient. The process of biotechnology permits the transfer of genes across species so that compatibility between the source and the recipient of any particular gene is not a limiting factor in transfer as it typically is using traditional breeding methods.

Within approximately the last 20 years, biotechnology has been applied to agriculture with a focus on two factors that significantly impact yield; damage from insects and competition with weeds. Earlier generation GM crops express traits that increase yield, improve quality and reduce the costs of production as these benefits have long been sought by traditional breeders as well (Organization for Economic Co-operation and Development OECD, 1993). It is also not surprising that these traits were targeted since they were developed by seed companies.

Insect resistant and herbicide tolerant phenotypes have been produced in numerous field crops from the transfer of genes that express proteins with insecticidal or herbicide tolerance activities, respectively. Extensive research has and will continue to focus on identification and characterization of proteins that are capable of imparting these types of traits. It should also be noted that GM crops have also been developed using proteins with other biological activities and with RNAi technology to silence specific genes rather than introduce new ones. For information related to the methods of safety assessment for these technologies the reader is referred elsewhere (Parrott et al., 2010; Petrick et al., 2013; Bushey et al., 2014).

1.1.1. Insect resistance

Every year, insects cause damage to field crops that result in loss of yield. In the case of maize, Lepidoptera species including the European corn borer (*Ostrinia nubilalis*) and corn earworm (*Helicoverpa* zea) and Coleopteran species such as the corn rootworm (*Diabrotica virgifera virgifera*) are particularly damaging predatory insects owing to the feeding patterns of the larvae from these species.

Synthetic insecticides including organophosphates, pyrethroids, and carbamates have been used to control damage from these and other types of insects. In organic farming where pesticide use is much more restricted, the soil bacteria *Bacillus thuringiensis* (*Bt*) has been used as an insecticide for more than 50 years (United States Environmental Protection Agency US-EPA, 1998a, b). The insecticidal activity of certain strains of *Bt* bacteria is attributable to the expression of crystalline (Cry) proteins that form pores in the uniquely alkaline gut of the sensitive insects when consumed (Chambers et al., 1991; Kullik et al., 2011; Schnepf et al., 1998; Tan et al., 2011). Insects including the lepidopteran and coleopteran species cited above also express receptors that specifically bind the Bt proteins which greatly restricts their selectivity (Pigott and Ellar, 2007).

Biotechnology has been used to produce insect resistant maize plants by transforming plants to express Cry proteins from different strains of *Bt* bacteria. To date, many different varieties of GM maize and other crops have been commercialized that express different Cry proteins from different strains of *Bt* bacteria and they have been successful in controlling numerous yield damaging insects (http://www.isaaa.org). During the course of developing these

products much has been learned about the safety of insecticidal proteins that will be discussed in subsequent sections of this chapter.

1.1.2. Herbicide tolerance

Another early generation GM crop that will be discussed in this chapter is herbicide tolerant crops (Barry et al., 1992). As an example, consider glyphosate tolerant soybeans. All plants, bacteria, and fungi express 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), an enzyme that is critical for biosynthesis of aromatic amino acids (Padgette et al., 1991, 1996). The native form of this enzyme expressed in field crops is sensitive to inhibition by glyphosate so that phytotoxicity results following *in planta* application of glyphosate. However, the EPSPS variant expressed in *Agrobacterium tumefaciens* strain CP4 is not sensitive to inhibition from glyphosate (Padgette et al., 1995; Funke et al., 2006). Glyphosate tolerant soybeans were produced using biotechnology to express the CP4 variant of the EPSPS enzyme and therefore tolerate application of glyphosate.

Numerous other proteins have been used to confer tolerance to other herbicides including dicamba, 2,4-D, sulfonylurea, and glufosinate in variety of different field crops and all of them are attributable to expression of an exogenous protein (http://www.isaaa.org).

2. Early safety assessment guidelines and recommendations

Crops produced with biotechnology have been referred to as genetically modified or GM crops. Correspondingly, the whole foods and edible fractions from these plants are often considered "GM foods" but it is more accurate to refer to them as foods obtained from GM crops. Perhaps because the methods by which they have been produced differ from traditional breeding or possibly because the genes responsible for the imparted traits came from sources unrelated to the crop in which they are expressed, there were questions about whether foods obtained from them were safe to eat.

Among the first organizations to propose strategies to assess the safety of foods obtained from biotechnology was that of WHO (1991). The document this group authored noted that the application of biotechnology to food production is really not particularly novel as it can be traced to more than 8000 years ago so that the technology itself is not inherently hazardous. Shortly after the publication of these recommendations, other scientific and regulatory agencies published similar documents that rather than specifically indicating which tests or what types of data should be considered, these recommendations did allow for flexibility with regard to data requirements in the safety assessment of individual GM crops (Codex Alimentarius Commission [Codex], 2001, 2003a, 2003b, 2007; European Commission [EC], 1997, 2003a, 2003b, 2004; European Food Safety Authority EFSA, 2006a, 2006b; FAO, 1996; FAO/WHO, 2000; International Life Sciences Institute ILSI, 1996, 1997, 2003, 2004; OECD, 1993, 1997, 2003; WHO, 1995).

Recognizing that most of the early generation GM crops were developed to express proteins, these documents highlighted that proteins are an integral component of the diet. As a general class of dietary ingredients, proteins do not present an obvious risk to humans since they are readily digested into amino acids that are absorbed for nutritive purposes. They did, however, acknowledge that there are some proteins that exist in nature that can present hazard in the form of potential for allergenicity or toxicity. Accordingly, a weight of evidence approach was developed based on what was known about allergenic and toxic proteins to compare candidate proteins with known allergenic and toxic proteins before commercialization.

In addition to testing the proteins to be expressed in GM crops, the strategy discussed in the documents cited above with regard to safety testing of whole foods obtained from them was that of "substantial equivalence." The central tenet of this concept was the definition of food safety as "based on the concept that there should be a reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption (OECD, 1993)."

The concept of substantial equivalence refers primarily to the composition of the newly developed GM crop in relation to the composition of non-GM crops. Because GM crops have been developed specifically to have a particular phenotype that otherwise would not be present in the non-GM crop they cannot be expected to be *absolutely* equivalent. Rather, that compositional data is necessary to demonstrate that the process of transformation did not result in the introduction of unintended changes that could result in a product that was not safe. The OECD stated "If a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety. No additional safety concerns would be expected (OECD, 1993)." This prescient concept remains the basis of safety assessment of foods from GM crops today.

While the documents cited above highlighted concepts, they did not mandate specific studies or data that would be absolutely required in every instance for newly developed GM crops. Nevertheless, a data driven paradigm has been developed to assess the safety of foods from GM crops. Details about the individual components in these types of assessments is discussed below but what should be noted here with particular regard to countries with developing economies is that the methods used to assess the safety of proteins expressed in GM crops and in foods obtained from them are well established and new methods should not be required. Some of the individual steps require very little with regard to resources and training whereas others can be particularly resource intensive.

3. Safety assessment of proteins used in GM crops

Candidate proteins to be expressed in GM crops are compared and contrasted with proteins that are allergenic or toxic using a weight of evidence approach consisting of individual and independent studies. None of the individual studies or data are necessarily more or less important than the others when considered in the context of weight of evidence but typically numerous studies are conducted.

3.1. Allergenicity

In the US, it has been estimated that 6–8% of children and 1–2% of adults are allergic to one or more foods (Sampson and Metcalfe, 1992; Metcalfe et al., 1996). The percentage decreases with age as many people outgrow the allergy. The incidence of food allergy outside of the US is unknown. Based on the US population, it is also known that the majority of food allergies are attributable to a relatively small number of foods that include peanuts, soybeans, cow's milk, eggs, fish, shellfish, wheat and tree nuts. Many other foods have also been reported to cause allergic reactions though the frequency of these sensitivities is much lower (Hefle et al., 1996). Persons that are allergic to particular foods possess antibodies to certain proteins present within those foods and the primary method of treating food allergies is for the allergic person to simply avoid consuming them.

Much is known about the particular proteins in foods to which persons are sensitive and they are often referred to as allergenic proteins. This is not necessarily a technically accurate appellation since it implies that these proteins present some risk of allergic

reactions in anyone when in fact they only present a risk in persons that are sensitive to them. Nevertheless, key learnings about these proteins have been applied to the safety assessment of foods from GM crops. In particular, safety assessments are designed to ensure that the developer did not select a protein to which a sensitive person could be exposed to unknowingly. Potential for allergenicity is assessed for proteins to ensure that they are not similar enough to cross react with the antibodies present in persons with food allergies.

One well documented example illustrating the potential to unintentionally transfer an allergenic protein is the case where a GM soybean was transformed to express a protein obtained from Brazil nuts to increase the concentration of the amino acid methionine. The protein that was selected, 2S albumin, happened to be the primary allergenic protein in persons allergic to Brazil nuts (Nordlee et al., 1996). Development of this product was halted before commercialization and it never went to market. If it had, persons that were allergic to Brazil nuts could have been unintentionally and unknowingly exposed to this protein upon consumption of GM soybeans in which this protein was expressed. Since it was never commercialized no allergic reactions ever occurred.

The source of the proteins is an important criterion in selection of candidate proteins. This is one component of the safety assessment for individual proteins called History of Safe Use (HOSU; Constable et al., 2007). However, each situation needs to be considered on a case-by-case basis. For example, peanuts may not be the best source for proteins to be used in GM crops like maize or soybeans simply because peanuts are known to possess more than one allergenic protein (De Jong et al., 1998). Regardless of the identity of the individual protein there would likely be concerns that any protein from peanuts could possibly be allergenic. Alternatively, if a protein from peanuts were to be selected to be expressed in a GM peanut plant then no new hazard has been introduced with regard to potential allergenicity.

In the case of Cry proteins from different strains of *Bt* bacteria or EPSPS from *Agrobacterium* strain CP4, the source organisms simply do not produce proteins that are known to be allergenic in humans. Therefore, GM crops expressing proteins from these sources do not present an obvious risk for introduction of an allergenic or toxic protein. These examples illustrate the concept that the sourcing of proteins from non-allergenic sources is prudent.

Another key component in the allergenicity assessment is a bioinformatics comparison of the amino acid sequence of the protein with the sequences of known allergenic proteins for similarity using computational methods. The identity and amino acid sequence of all known allergenic proteins is available on line and updated annually (www.allergenonline.org). While this database does not contain every allergenic protein in existence it does contain every *known* allergenic protein. Importantly, of the millions or greater number of proteins that exist in nature, only 1706 were actually considered allergens as of the latest update (January 20, 2014).

The Codex Alimentarius Commission reported that proteins in GM crops could present a risk for cross reactivity if they have 35% amino acid sequence identity over any 80 amino acid sequence or any identical 8 amino acid matches with known allergens (Codex, 2003a, b, c). More recent data has demonstrated that the latter criterion is prone toward giving false positives (Ladics et al., 2011). What it important for countries with developing economies is that this database and the bioinformatics software programs necessary to make these types of comparison (e.g., FASTA) are freely available online.

A physical property shared by numerous, but not all, allergenic proteins is resistance to degradation in the presence of digestive enzymes (Astwood et al., 1996). Standardized *in vitro* methods have been developed to evaluate the sensitivity of proteins to degradation in the presence of digestive enzymes (pepsin and pancreatin; Thomas et al., 2004). Numerous publications have reported that insecticidal and herbicide tolerance proteins are rapidly degraded in the presence of digestive enzymes (Harrison et al., 1996; Privalle, 2002; Herman et al., 2003; Sinagawa-Garcia et al., 2004; Herouet et al., 2005; Hileman et al., 2006; Singh et al., 2006; Delaney et al., 2008a, b; Hérouet-Guicheney et al., 2009; Mathesius et al., 2009; Xu et al., 2009; Cao et al., 2010, 2012). This property is not absolute as some non-allergenic proteins are also insensitive to degradation by digestive enzymes. Nevertheless, this data is another component of the overall weight of evidence in the assessment of whether proteins to be used in GM crops are likely to be allergenic.

These methods have been very effective at identifying potentially allergenic proteins, however, if questions still remain following these analyses further studies can be considered. For example, if bioinformatics results suggest marginal sequence similarities between the protein to be expressed in GM crops and a known allergen, serum obtained from individuals sensitive to that protein can be used to determine whether cross reactivity is possible. Results from these types of studies have not revealed evidence of cross reactivity though they are not commonly conducted (Ladics et al., 2006). It has also been suggested that clinical trials in sensitive individuals could be conducted though to date none have been reported.

Collectively, these studies have successfully demonstrated that the methods used to assess the allergenicity of proteins expressed in GM crops are effective. Nearly 20 years following commercialization of the first GM crop there have been no reports of allergic reactions that are attributable to exposure to foods obtained from them.

3.2. Toxicity

In addition to allergenicity, some proteins are known to exist in nature that are capable of causing adverse effects when consumed. Though many are found in venomous snakes and insects or are produced by pathogenic bacteria, there are some that are found in plants such as kidney bean lectin and ricin (Rossi et al., 1984; Lafont et al., 1988; Weinman et al., 1989; Ishiguro et al., 1992a and 1992b; Cook et al., 2006). Accordingly, proteins used in GM crops have also been assessed for potential to cause adverse effects if for no other reason that they too are proteins.

There are obvious overlaps in the methods used to assess the potential toxicity and allergenicity of proteins. Specifically, consideration of history of safe use of the source of the protein, bioinformatics comparison to known protein toxins, and *in vitro resistance* to digestive enzymes (Delaney et al., 2007). The primary basis of these considerations being that proteins selected from sources that are not known to produce toxic proteins, are not similar in sequence to known protein toxins, and are readily degraded in the presence of digestive enzymes are unlikely to be toxins.

There are differences in the bioinformatics analysis compared with the allergenicity assessment to note. First, there are no predefined criteria that identify a “match” between two proteins such as the 35% identity over 80 amino acid sequences for allergenic proteins. Second, there is currently no annotated and updated database in which the sequence of protein toxins is maintained. Rather, what is commonly conducted is a comparison to all known protein sequences in the NCBI BLAST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) followed by manual inspection to determine if sequence similarities are present. Consideration of these data provides strong evidence for whether the protein intended for use in a GM crop is likely to introduce a hazard.

In numerous cases, acute toxicology studies have been conducted in laboratory mice using proteins isolated from heterologous expression systems. The acute toxicology study is conducted

because proteins that are toxic are believed to act through acute mechanisms of action (Sjoblad et al., 1992). Some acute toxicology studies have applied the OECD limit oral dose of 2000 mg/kg of body weight while others have used other doses that are orders of magnitude in excess of possible human exposure (Delaney et al., 2008b; Mathesius et al., 2009). When compared with the concentrations at which they are expressed *in planta* it is possible to dose with much smaller quantities of protein but still be well in excess of margins of possible exposure that would normally be used for this component of the risk assessment. For that reason, more recent studies have been conducted by estimating the dietary exposure to the proteins from the edible part of the GM crop and dosing at approximately 1000× higher amounts which again has demonstrated that very high doses of these proteins does not result in adverse effects (Juberg et al., 2009; Stagg et al., 2012). This approach can substantially decrease the quantity of protein required for conducting these types of studies which is particularly important for intractable proteins – proteins with physical properties that will not allow them to be isolated in the quantities necessary to conduct these studies (Bushey et al., 2014). None of the studies has demonstrated evidence of adverse effects regardless of dosing regimen and, collectively, these studies have been effective in establishing the safety of individual proteins expressed in GM crops.

Though regulatory testing requirements are not the focus of this chapter, it should be noted that one regulatory agency (European Food Safety Authority) does require a 28 day rodent toxicology study conducted in accordance with OECD 407 guidelines with purified protein (OECD, 1995). The guidelines allow for flexibility with regard to administration of the test substance with regard to whether the test substance is administered in the diet or by oral gavage and at which doses it is to be administered. As with the acute toxicology study, the OECD has established a limit dose for repeated dose toxicology studies which is 1000 mg/kg of body weight per day. They also provide details related to the conduct of these types of studies that include the number of animals (minimum of 5/sex/dose), housing conditions, and variables to be monitored. As would be expected, the variables include primary indicators of health such as body weights, food consumption, clinical chemistry, hematology and organ weights and histopathology. The guidelines are written for conducting the studies in rodents which leaves open the option of the study sponsor as to whether the studies are to be conducted in rats or mice. In most cases, the studies have been conducted with mice because they require smaller quantities of the test substance than if they were to be conducted with rats. A number of repeated dose studies have been conducted with herbicide tolerance and Cry proteins but to date there has been no evidence of adverse effects (Delaney et al., 2008a, b; Juberg et al., 2009; Mathesius et al., 2009; Stagg et al., 2012). These observations support that the previous data (HOSU, MOA, digestibility, and even acute toxicology) were predictive of the outcome in the repeated dose study and therefore brings into question whether they are necessary for every protein to be considered in a GM plant.

4. Safety assessment of whole foods from GM crops

The testing paradigm in the safety assessment of the foods from GM crops can be considered in two different overall objectives. The first objective is to assess the safety of the intended change which for purposes of this chapter, has been imparted by the expression a protein as discussed above. The second objective is to determine if transformation of the plant to express the protein(s) results in unintended changes. Early recommendations and guidelines concluded that it is not possible to demonstrate that any food is *absolutely* safe but that there are many foods with a long history of safe consumption (WHO, 1991). The standard for safety of foods from GM crops is therefore to determine whether they are *substantially equivalent* with foods obtained from closely related non-GM crops. The foods from GM crops should be essentially identical with the same food from a non-GM crop with the obvious exception of the protein and its intended effect on phenotype.

Characterization of the insertion using molecular biology has been conducted for individual GM events. This is done to confirm the integrity of the DNA insert and copy number, that the insert did not disrupt any genes or create new open reading frames, and that no backbone DNA was inserted (Codex, 2003b). These elements relate to the safety of the product because unintended effects could occur from transformation.

4.1. Composition analysis

An important component of the data produced in the comparative safety assessment is a detailed compositional analysis of the key nutrients, antinutrients, secondary metabolites and toxins of a GM crop and a non-GM comparator (WHO, 1991; OECD, 1993; ILSI, 1996). Typically, these are conducted with whole foods from controlled field trials in which the GM crop, its closely related non-GM control (i.e., near isoline), and several unrelated commercially available non-GM varieties are grown together. For more information about the design and conduct of these field trials the reader is encouraged to look elsewhere as that topic falls outside of the scope of this chapter (Paoletti and Germini, 2012).

While some foods are manufactured using “pure” ingredients such as sugar and salt that can easily be viewed as chemicals, foods from GM crops are also a combination of individual chemicals. As such, whole foods from field crops can be analyzed chemically to determine their chemical composition. In the case of maize, soybeans, canola, rice and cotton, the concentrations of the components are available at a publicly available web site that is managed by the International Life Sciences Institute (https://www.cropcomposition.org/query/index.html). This information is particularly valuable in these types of comparison trials because simply comparing any individual component of GM and a non-GM crop could identify statistical differences that are not biologically relevant. Information about the range of concentrations for the individual components in non-GM crops is particularly important in assessing their concentrations as there would be expected to be some statistical differences between GM and non-GM crops since so many different components are measured.

To date, many compositional studies have been conducted with whole grains, seed, forage and in some cased processed fractions from insect resistant and herbicide tolerant GM maize and GM soybean plants and no unintentional compositional changes have been observed (For example see Padgette et al., 1996; Ridley et al., 2002, 2011; George et al., 2004; Herman et al., 2004, 2007; Obert et al., 2004; McCann et al., 2005, 2007; Oberdoerfer et al., 2005; Harrigan et al., 2007a, 2007b, 2009, 2010; Drury et al., 2008; Lundry et al., 2008, 2013; Berman et al., 2009, 2010, 2011; Zhou et al., 2011; Venkatesh et al., 2014). This indicates that the process of transformation and expression of proteins in GM crops does not present a clear risk in unintended compositional changes and supports that they are substantially equivalent to their non-GM comparators with a history of safe use.

4.2. Feeding studies

When the compositional analysis between foods from GM and non-GM crops is equivalent, there is not an obvious scientific reason to conduct animal feeding trials as stated in a number of guidelines and recommendations. However, “If the assessment based on molecular, biological, and chemical data does not allow

an informed judgment on safety, conventional assessment procedures based on animal studies will be needed (WHO, 1991)." Toxicology studies are not recommended in a check the box approach but rather on a case-by-case basis based on the findings from the compositional analysis (ILSI, 1996).

There are elements attributable to the food portion of crops that cannot be assessed by compositional analysis (i.e., palatability). Perhaps also because of the "newness" of the methods that were used to produce the early generation GM crops, a number of feeding studies have been conducted in support of their safety. It has been acknowledged that toxicologic assessments of whole foods is difficult using traditional laboratory toxicology methods (WHO, 1991). Nevertheless, laboratory methods have been adapted to assess the safety of foods from GM crops using a comparative approach.

One often conducted safety study with foods from GM crops is the 90 day feeding study in laboratory rats. In most cases, laboratory toxicology studies have been conducted in accordance with OECD 408 Guidelines (OECD, 1998). Historically, 90 day toxicology studies have been conducted with pure chemicals that were administered at low, middle, and high doses. Over the course of the in-life phase of the study, body weights and feed consumption and behavioral and clinical observations are measured at regular intervals. At the end of the study, a comprehensive battery of variables including clinical chemistry, hematology and coagulation, urinalysis, organ weights and histopathology are evaluated in order to determine the highest dose that the animals could tolerate without evidence of adverse effect (i.e., the No Observed Adverse Effect Level [NOAEL]).

One design element that is often included in 90 day rat feeding studies with whole foods from GM crops is the multiple reference groups that are fed diets containing similar concentrations of either the whole grain or processed feed fraction obtained from unrelated, commercially available, non-GM plants. Because the primary statistical comparison in these studies is between the GM and the non-GM group and because there are large number of comparisons it is expected that statistical difference between these two groups will occur. Data obtained from the reference groups has been useful in instances such as this to establish within study ranges for the individual variables to determine whether they were treatment related and biologically relevant. As an example, in one recent 90 day feeding study, a lower serum sodium concentration was observed in female rats consuming grain from an insect resistant GM maize plant compared with rats consuming maize grain from the control non-GM group (Appenzeller et al., 2009a). While the difference was slight in magnitude it was statistically significant. In this case the serum concentrations of sodium from the reference groups was helpful in establishing that the values from the GM group were within the range of control values from within this study.

Using another example, the concentration of serum alkaline phosphatase (ALKP) was lower in male rats consuming diets containing grain from and insect resistant crop relative to rats consuming maize grain from the near isogenic control crop. Though lower ALKP concentrations are not typically considered an adverse effect, the inclusion of reference groups was helpful in determining that the values observed in the 1507 group were well within those expected to be observed in animals consuming diets containing non-GM maize grain (MacKenzie et al., 2007).

Because the test substance administered in feeding studies with foods from GM crops are complex mixtures it is not possible to administer multiple doses that are orders of magnitude in excess of the next closest dose. Furthermore, in the case where the food from the GM crop was obtained from soybeans or maize grain, these substances are already significant components of the grain-based diets which laboratory animals are usually fed. What is important to note from the feeding studies conducted with foods from GM crops is that they have been conducted for comparative purposes. In other words, they are conducted with foods from GM crops that presumably have been demonstrated to be compositionally equivalent using analytical methods and that they express proteins that are not allergenic or toxic using methods described earlier in this chapter.

Of the published 90 day feeding studies that have been conducted with GM maize grain (Hammond et al., 1996, 2004, 2006a, 2006b; Dryzga et al., 2007; MacKenzie et al., 2007; Malley et al., 2007; Healy et al., 2008; Appenzeller et al., 2009a, 2009b; He et al., 2009; Liu et al., 2012; Delaney et al., 2013; Zhu et al., 2013), processed soybean fractions (Appenzeller et al., 2008; Delaney et al., 2008a; Chukwudebe et al., 2012; Qi et al., 2012), rice (Song et al., 2014), and canola (Delaney et al., 2014) and no evidence of adverse effects have been observed. Additional studies have been conducted to assess the effects of longer term dietary exposure to foods from GM crops and others to assess the effects on reproductive and developmental effects and again no evidence of adverse effects was observed (Brake and Evenson, 2004).

In addition to these studies, numerous feeding studies have been conducted in livestock species. The most frequently conducted study is the 6 week feeding study conducted in broiler chickens. This species was selected for evaluating the nutritional quality of foods, or more specifically maize grain and processed soybean fractions, for two reasons. First, the broiler chicken is very sensitive to nutritional imbalances in the diet. Over the interval of a 6 week feeding interval, these animals grow from approximately 50 g to nearly 2 kg. Second, the early generation GM crops were soybeans and corn which compose a large fraction of the diets normally consumed by broiler chickens. In fact, maize grain and soybean meal combined can constitute nearly 90% of the diet consumed by broiler chickens. Much of the maize grain and soybean meal that is produced annually is specifically intended for commercial livestock feeding.

These poultry would be classified nutritional performance or comparison studies rather than safety studies as they compare variables including body weight, weight gain, and carcass traits between animals consuming diets that contained whole grains or processed fractions from GM and non-GM crops. A large number of broiler feeding studies indicate no evidence of nutritional inferiority has been observed for whole foods from GM crops (Taylor et al., 2004, 2007; McNaughton et al., 2007a, 2007b, 2008a, 2008b, 2011a, 2011b, 2014; Herman et al., 2011).

Many feeding studies with whole foods from GM crops have also been conducted in other livestock species including laying hens, swine, dairy and beef cattle, and a number of other species as well (http://www.fass.org/page.asp?pageID=52&autotry=true&ULnotkn=true). While historically these studies have not included as many in-life variables as were assessed in the rat studies, they were based on commercial practice. It would not be accurate to characterize them as safety studies but more in support of nutritional comparison. As with laboratory rats and broiler chickens, no evidence of adverse effects has been observed in these studies in animals consuming whole foods and processed feed fractions from GM crops.

In finding no evidence of adverse effects these feeding studies support the substantial equivalence of foods from GM crops.

5. Countries with developing economies

Though the benefits and safety of biotechnology have been well documented they have typically been developed by large, multinational companies and required significant resources and time. Countries with developing economies certainly stand to benefit from crops developed with biotechnology whether importing grain

or seeds from foreign countries or developing their own GM crops. However, access to the same resources may not be available in countries with developing economies.

There are several scenarios that apply to consideration of the safety of foods from GM crops as related to countries with developing economies that will be discussed in the final section of this chapter. The first is considering importation of foods from GM crops that were developed in another country. The second relates to import of seeds from GM crops developed in other countries for cultivation. Third is the consideration of safety assessment for products developed within country. Finally, the possibility of exportation of foods or seeds from GM crops developed within country.

Regardless of the possible scenario to consider, the assessment of safety should be based on demonstrating that the foods from any particular GM crop are as safe as the appropriate comparator from non-GM crops.

5.1. Import of foods from GM crops

The methods discussed above demonstrate how the safety of foods from GM crops has been assessed primarily for GM maize and soybeans that express insecticidal or herbicide tolerance proteins. When considering the import of maize grain or soybeans from GM crops into countries with developing economies it would be expected that high quality data generated to demonstrate the safety of any individual GM crop would be suitable regardless of the country in which it is submitted. This is referred to as data transportability – the application of data produced in one geographic location to support the safety assessment of that same product in another.

Regulators, scientists, and to whatever extent possible the public at large, in countries with developing economies should expect the same level of confidence about the safety of imported foods from GM crops. The methodology described in the prior sections emphasizes the universality of the data to demonstrate that when produced in accordance with the appropriate guidelines. Safety data for proteins expressed in GM crops and from composition and feeding studies conducted with foods from GM crops is suitable to support the safety assessment regardless of the geography toward which it is applied. From the scientific perspective, it is not necessary to reproduce safety assessment data in countries with developing economies to improve the confidence in the safety of GM crops though it is likely that some regulatory agencies will require repetition of some studies.

5.2. Importation of seeds for cultivation of GM crops

The primary factor that differentiates importation of foods from seeds is environmental exposure. For example, many countries import soybeans from the US for use in animal feed. Because nearly all of the soybeans grown in the US are GM, it is very likely that the imported soybeans will therefore be GM. However, they will not be imported for cultivation as the safety assessment for cultivation would almost certainly necessitate a consideration for environmental impact. Recommendations and guidelines for these types of assessments are available but will not be discussed here as they fall outside of the scope of this chapter (US EPA, 1998b and 2001; Garcia-Alonso et al., 2006; Raybould, 2006; Rose, 2007; Romeis et al., 2008, 2011; Carstens et al., 2012, 2014).

5.3. In country development

Recognizing the benefits of biotechnology, countries with developing economies are also produce GM crops for domestic use. It is also anticipated that these nascent GM crops will include agriculturally beneficial traits such as insect resistance and herbicide tolerance which was shy those traits were extensively discussed in this chapter. It is also anticipated that these crops will include many foods intended for human consumption like papaya, squash, tomato, pepper and potatoes rather than soybeans and maize grain – much of which is used as animal feed. In fact, it has been estimated that as much as 90% of the GM crop biomass is currently used as livestock feed (Van Eenennaam and Young, 2014). What is important to remember is that the safety assessment processes and the tools that have been developed to assess the safety of soybeans and maize from GM crops apply regardless of the plant.

It is possible and perhaps even likely that GM crops produced in countries with developing economies will be modified to express proteins that have already been evaluated for safety (i.e., allergenicity and toxicity). In this case, there is also likely to be significant relevant data for those proteins that should be considered. Most importantly, if such data are available then the developers would not need to reproduce the data but rather to simply access it and possibly update bioinformatics comparisons if necessary.

In the case where novel proteins are being considered, safety data and new studies will be required. Some of the information in these assessments can be obtained with minimal resources and training and should be considered very early in the development process. For example, the source of the particular protein and whether that source is known to produce allergenic or proteins can be done largely by analysis of published literature. Bioinformatics comparison tools are also readily available but will require some amount of training for the user.

In vitro digestion studies with purified proteins are the first point in the discussion of this topic where the data requirements would necessitate significant resources. Though the digestion assays themselves are reproducible and can be conducted inexpensively using commonly available laboratory equipment and reagents, it is the production and characterization of the protein itself that could present a significant barrier in countries with developing economies. The amounts of proteins typically used in these assays are relatively small (several milligrams) and the resources necessary to isolate them may not be an issue, what does become an issue is their characterization. Testing guidelines and recommendations state that the proteins used in these studies should be biochemically equivalent to those expressed *in planta* in GM crops. However, in most instances, the proteins are expressed *in planta* at very low concentrations so it is not practical to isolate the quantity of protein necessary to conduct these studies from the plants in which they are expressed. Accordingly, heterologous expression systems have been utilized to express and isolate the proteins that are used in digestibility studies and in studies to be discussed later in this chapter. The isolation of proteins from heterologous expression systems can be difficult and time consuming and in some cases such as with transmembrane proteins may not even be possible (Bushey et al., 2014).

What may prove to be more challenging in countries with developing economies is the analytical instrumentation often necessary to establish “identity” of the proteins when expressed *in planta* and *in vitro*. Biochemical equivalence includes laboratory data about the purity of the protein in the preparation but often also includes comparison of the size and immunoreactivity, peptide mapping, N-terminal sequencing, glycosylation, and biological activity may be necessary.

Additional challenges will be encountered when and if acute and repeated dose toxicology studies are to be conducted. In the case of proteins that have already been evaluated for safety and this information already exists, again, there is no obvious need to reproduce it. If these types of studies are to be conducted with novel proteins then it will likely be necessary to isolate and char-

acterize multiple gram quantities of pure protein which can be extremely time consuming and resource intensive. To date, of the proteins used in GM crops that have been evaluated for acute and repeated dose toxicity, no evidence of adverse effects have been observed. This supports the concept that the information necessary to evaluate protein safety can be obtained without these types of studies (Delaney et al., 2007) however, regulatory agencies may require them.

Composition analysis of whole foods from GM crops should be considered as a component of the safety testing. As noted above, the identities and concentration ranges of key components from a number of field crops (maize, soybeans, canola, rice and cotton) is publicly available and maintained (https://www.cropcomposition.org/query/index.html). If a newly developed GM crop is among these products then that information is available then access to that type of information will be very helpful establishing substantial equivalence. What will also need to be considered is the design of the field studies to produce the substances that will be evaluated compositionally.

If the new GM crop is not among these (and many will not be) or for which historical composition data is available then establishing substantial equivalence may prove more challenging. Composition data may need to be considered on a food-by-food basis is which components to test and the normal range of their concentrations in non-GM plants. It may be necessary to obtain detailed information about the individual components of the food from non-GM crops obtained over the course of multiple growing seasons. In the absence of this information, statistical comparisons of the concentrations of individual components are likely to be difficult to interpret.

Finally, there is the issue of animal feeding studies using whole foods from GM crops. Early recommendations for the safety assessment of whole foods from GM crops stated that they should be considered if indicated from consideration of other data as they could help to support substantial equivalence for individual GM crops. Over the past nearly 20 years many such studies have been completed in rats, broiler chickens, and other livestock species as noted earlier in this chapter. In none of these cases was there any evidence of treatment related adverse effects. From these observations it could be concluded that feeding studies with whole foods from GM crops should not necessarily be required for every new product.

Nevertheless, if for whatever reason, it is determined that a feeding study is to be conducted with a whole food from a GM crop in a country with a developing economy, there is much to be learned from the studies that have been conducted. The studies conducted to date have focused on comparative assessment of the food from the GM crop with a corresponding control from an appropriate, closely related, non-GM crop. In these types of studies, the test substance should be incorporated into the diet at a significant proportion of the diet, fed to the animals for a reasonably long period of time and statistical comparisons should be made for predetermined variables that are relevant to assess the overall health and nutritional performance of the diets.

Studies with processed soybean fractions and maize grain from GM crops have consistently demonstrated the importance of additional control data. Should these types of studies be conducted, comparison of a large number of variables between a GM and non-GM control group will be expected to identify statistical differences between them. However, the purpose of conducting these studies is not to identify statistical differences, but to identify treatment-related adverse effects. As discussed above, previous studies have wisely included additional “reference” groups that consumed diets containing the same concentration of whole foods from unrelated, non-GM, commercially available crops to assist in establishing within-study ranges for individual variables that was necessary to interpret the data from the studies. Similarly, data from historical control animals has also been useful in interpretation of the results from these types of studies when statistical differences were observed between animals consuming whole foods from GM and non-GM crops. The data from reference control groups and historical control groups has aided in determining whether statistical differences between the primary control and experimental groups simply do not automatically serve to identify an adverse effect.

With regard to conducting animal feeding studies with whole foods from GM crops in countries with developing economies for the purposes of safety assessment, the first consideration should be whether or not they should be conducted. They are resource intensive and prone to misinterpretation if not properly controlled or conducted in a laboratory that does not have an established history of conducting these types of studies. Should an animal feeding study be conducted, it will be critical that it be properly controlled with a predesigned protocol in a quality laboratory with the facilities necessary to interpret the results. In some cases, animal feeding studies have been conducted in the country in which they were developed. In situations like this there is either little or nothing to gain from repeating them in other locations.

6. Conclusions

It has been estimated that hunger affects an estimated 1 billion people many of whom live in countries with developing economies. Population growth and decreased availability of arable land will continue to confound this issue. Modern biotechnology has the potential to be a significant tool in fighting hunger as it has been well established to address agricultural problems such yield loss from insect infestation, completion with weeds, and even drought.

Biotechnology has been used to improve the quality and yield of field crops in many parts of the world for more than 20 years. It is more specific and relatively fast in development compared with traditional breeding techniques. A comprehensive safety assessment has been conducted on GM crops before the products are commercialized that includes evaluation of proteins used in these crops for potential allergenicity and toxicity as well as analysis of the composition of the crop and often feeding studies in rodents and livestock species in support of demonstrating substantial equivalence. The studies to do so have required significant resources, equipment and training and to date they have collectively demonstrated that biotechnology as a tool in agriculture is not inherently hazardous as no evidence of adverse effects has been observed.

These learnings apply to countries with developing economies for various reasons. To begin, they highlight the methods that have been used to assess the safety of foods from GM crops for more than 20 years. The methods have proven very effective and the concepts will readily apply to field crops regardless of the geography in which they are developed. Regulators and scientists in countries with developing economies have the opportunity to benefit from these findings with regard to the methods so that they can consider the safety studies submitted from outside their country for the purposes of import and possibly for cultivation (i.e., portability). These learning can also serve to minimize or eliminate within country study repeat of laboratory studies that is unnecessary and consumes resources that could be better applied elsewhere.

Countries in which GM crops have been developed have benefited from agricultural biotechnology and countries with developing economies will also benefit from application of this technology. The standards followed for safety assessment of foods from GM crops are comprehensive and have proven very effective. Countries with developing economies will also benefit from key learn-

ings with early generation GM crops and applying them to a different range of field crops for products developed in their own countries.

Presuming that countries with developing economies will have less access to funding and technical resources they will need to determine if that alone will prevent them from developing GM crops. Alternatively, that key learnings be taken from the GM products currently on the market and determine what will be necessary to develop them on their own. The lack of resources should not negatively impact the beneficial effects and improved qualities of GM plants and the standards for safety should not be any different than in more developed countries.

Conflicts of interest

Bryan Delaney is an employee of DuPont Pioneer, USA, which is a producer of genetically modified organisms.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2015.10.001.

References

Appenzeller, L.M., Malley, L., Mackenzie, S.A., Hoban, D., Delaney, B., 2009a. Subchronic feeding study with genetically modified stacked trait lepidopteran and coleopteran resistant (DAS-Ø15Ø7-1xDAS-59122-7) maize grain in Sprague-Dawley rats. Food Chem. Toxicol. 47, 1512–1520.

Appenzeller, L.M., Munley, S.M., Hoban, D., Sykes, G.P., Malley, L.A., Delaney, B., 2009b. Subchronic feeding study of grain from herbicide-tolerant maize DP-Ø9814Ø-6 in Sprague-Dawley rats. Food Chem. Toxicol. 47, 2269–2280.

Appenzeller, L.M., Munley, S.M., Hoban, D., Sykes, G.P., Malley, L.A., Delaney, B., 2008. Subchronic feeding study of herbicide-tolerant soybean DP-356Ø43-5 in Sprague-Dawley rats. Food Chem. Toxicol. 46, 2201–2213.

Astwood, J.D., Leach, J.N., Fuchs, R.L., 1996. Stability of food allergens to digestion in vitro. Nat. Biotechnol. 14, 1269–1273.

Barry, G., Kishore, G., Padgette, S.R., Taylor, M.L., Kolacz, K., Weldon, M., Re, D.B., Eichholtz, D.A., Fincher, K., Hallas, L., 1992. Inhibitors of amino acid biosynthesis: strategies for imparting glyphosate tolerance to crop plants. In: Singh, B.K., Flores, H.E., Shannon, J.C. (Eds.), Biosynthesis and Molecular Regulation of Amino Acids in Plants. American Society of Plant Physiologists, Madison, WI, pp. 139–145.

Berman, K.H., Harrigan, G.G., Nemeth, M.A., Oliveira, W.S., Berger, G.U., Tagliaferro, F.S., 2011. Compositional equivalence of insect-protected glyphosate-tolerant soybean MON 87701 × MON 89788 to conventional soybean extends across different world regions and multiple growing seasons. J. Agric. Food Chem. 59, 11643–11651.

Berman, K.H., Harrigan, G.G., Riordan, S.G., Nemeth, M.A., Hanson, C., Smith, M., Sorbet, R., Zhu, E., Ridley, W.P., 2009. Compositions of seed, forage, and processed fractions from insect-protected soybean MON 87701 are equivalent to those of conventional soybean. J. Agric. Food Chem. 57, 11360–11369.

Berman, K.H., Harrigan, G.G., Riordan, S.G., Nemeth, M.A., Hanson, C., Smith, M., Sorbet, R., Zhu, E., Ridley, W.P., 2010. Compositions of forage and seed from second-generation glyphosate-tolerant soybean MON 89788 and insect-protected soybean MON 87701 from Brazil are equivalent to those of conventional soybean (*Glycine max*). J. Agric. Food Chem. 58, 6270–6276.

Brake, D.G., Evenson, D.P., 2004. A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. Food Chem. Toxicol. 42, 29–36.

Brookes, G., Barfoot, P., 2009. Global impact of biotech crops: Income and production effects, 1996–2007. AgBioForum 12, 184–208.

Bushey, D.F., Bannon, G.A., Delaney, B.F., Graser, G., Hefford, M., Jiang, X., Lee, T.C., Madduri, K.M., Pariza, M., Privalle, L.S., Ranjan, R., Saab-Rincon, G., Schafer, B.W., Thelen, J.J., Zhang, J.X., Harper, M.S., 2014. Characteristics and safety assessment of intractable proteins in genetically modified crops. Regul. Toxicol. Pharmacol. 69, 154–170.

Cao, B., He, X., Luo, Y., Ma, L., Liu, P., Cao, S., Liu, Y., Zou, S., Xu, W., Huang, K., 2012. Safety assessment of dehydration-responsive element-binding (DREB) 4 protein expressed in *E. coli*. Food Chem. Toxicol. 50, 4077–4084.

Cao, S., He, X., Xu, W., Ran, W., Liang, L., Luo, Y., Yuan, Y., Zhang, N., Zhou, X., Huang, K., 2010. Safety assessment of Cry 1C protein from genetically modified rice according to the national standards of PR China for a new food resource. Regul. Pharmacol. Toxicol. 58, 474–481.

Carstens, K.L., Anderson, J., Bachman, P., De Schrijver, A., Dively, G., Federici, B., Hamer, M., Gielkins, M., Jensen, P., Lamp, W., Rauschen, S., Ridley, G., Romeis, J., Waggoner, A., 2012. Genetically modified crops and aquatic ecosystems: considerations for environmental risk assessment and non-target organism testing. Transgenic Res. 21, 813–842.

Carstens, K., Cayabyab, B., De Schrijver, A., Gadaleta, P.G., Hellmich, R.L., Romeis, J., Storer, N., Valicente, F.H., Wach, M., 2014. Surrogate species selection for assessing potential adverse environmental impacts of genetically engineered insect-resistant plants on non-target organisms. GM Crops Food Biotechnol. Agric. Food Chain 5, 1–5.

Chambers, J.A., Jelen, A., Gilbert, M.P., Jany, C.S., Johnson, T.B., Gawron-Burke, C., 1991. Isolation and characterization of a novel insecticidal crystal protein gene from *Bacillus thuringiensis* subsp. *aizawai*. J. Bacteriol. 173, 3966–3976.

Chukwudebe, A., Privalle, L., Reed, A., Wandelt, C., Contri, D., Dammann, M., Groeters, S., Kaspers, U., Strauss, V., van Ravenzwaay, B., 2012. Health and nutritional status of Wistar rats following subchronic exposure to CV127 soybeans. Food Chem. Toxicol. 50, 956–971.

Codex Alimentarius Commission, 2001. Principles for the Risk Analysis of Foods Derived from Modern Biotechnology CAC/GL 44-2003.

Codex Alimentarius Commission, 2003a. Joint FAO/WHO Food Standards Programme. Codex Alimentarius Commission. Report of the Fourth Session of the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology.

Codex Alimentarius Commission, 2003b. Guideline for the Conduct of Food Safety Assessments of Foods Derived from Recombinant-DNA Plants. CAC/GL 45–2003. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission. Food and Agriculture Organisation, Rome, Italy Available from: http://www.biosafety.gov.cn/image20010518/5074.pdf.

Codex Alimentarius Commission, 2003c. Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-fifth Session, Rome, Italy 30 June–5 July, 2003 (Appendix III. Draft guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants, and Appendix IV, Proposed draft annex on the assessment of possible allergenicity).

Codex Alimentarius Commission, 2007. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission. Report of the Sixth Session of the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology, Alinorm 07/30/34. Food and Agriculture Organisation, Rome, Italy Available from: http://www.codexalimentarius.net/web/archives.jsp?year=07.

Constable, A., Jonas, D., Cockburn, A., Davi, A., Edwards, G., Hepburn, P., Herouet-Guicheney, C., Knowles, M., Moseley, B., Oberdörfer, R., Samuels, F., 2007. History of safe use as applied to the safety assessment of novel foods derived from genetically modified organisms. Food Chem. Toxicol. 45, 2513–2525.

Cook, D.L., David, J., Griffiths, G.D., 2006. Retrospective identification of ricin in animal tissues following administration by pulmonary and oral routes. Toxicology 223, 61–70.

De Jong, E.C., van Zijverden, M., Spanhaak, S., Koppelman, S.J., Pellegrom, H., Penninks, A.H., 1998. Identification and partial characterization of multiple major allergens in peanut proteins. Clin. Exp. Allergy 28, 743–751.

Delaney, B., Appenzeller, L.M., Munley, S.M., Hoban, D., Sykes, G.P., Malley, L.A., Sanders, C., 2008a. Subchronic feeding study of high oleic acid soybeans (Event DP-3Ø5423-1) in Sprague-Dawley rats. Food Chem. Toxicol. 46, 3808–3817.

Delaney, B., Appenzeller, L.M., Roper, J.M., Mukerji, P., Hoban, D., Sykes, G.P., 2014. Thirteen week rodent feeding study with processed fractions from herbicide tolerant (DP-Ø73496-4) canola. Food Chem. Toxicol. 66, 173–184.

Delaney, B., Astwood, J.D., Cunny, H., Eichen-Conn, R., Hereout, C., Levine, M., MacIntosh, S., Meyer, L., Privalle, L., Gao, Y., Mattsson, J., 2007. Evaluation of protein safety in the context of agricultural biotechnology. Prepared by the ILSI International food biotechnology committee task force on protein Safety. Food Chem. Toxicol. 46 (Suppl. I), S71–S97.

Delaney, B., Karaman, S., Roper, J., Hoban, D., Sykes, G., Mukerji, P., Frame, S.R., 2013. Thirteen week rodent feeding study with grain from molecular stacked trait lepidopteran and coleopteran protected (DP-ØØ4114-3) maize. Food Chem. Toxicol. 53, 417–427.

Delaney, B., Zhang, J., Carlson, G., Schmidt, J., Stagg, B., Comstock, B., Babb, A., Finlay, C., Cressman, R.F., Ladics, G., Cogburn, A., Siehl, D., Bardina, L., Sampson, H., Han, Y., 2008b. A gene-shuffled glyphosate acetyltransferase protein from *Bacillus licheniformis* (GAT4601) shows no evidence of allergenicity or toxicity. Toxicol. Sci. 102, 425–432.

Drury, S.M., Reynolds, T.L., Ridley, W.P., Bogdanova, N., Riordan, S., Nemeth, M.A., Sorbet, R., Trujillo, W.A., Breeze, M.L., 2008. Composition of forage and grain from second-generation insect-protected corn MON 89034 is equivalent to that of conventional corn (*Zea mays* L.). J. Agric. Food Chem. 56, 4623–4630.

Dryzga, M.D., Yano, B.L., Andrus, A.K., Mattsson, J.L., 2007. Evaluation of the safety and nutritional equivalence of a genetically modified cottonseed meal in a 90-day dietary toxicity study in rats. Food Chem. Toxicol. 45, 1994–2004.

EC, 1997. Commission recommendation of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under regulation (EC) No. 258/97 of the European parliament and of the Council (97/618/EC). Off. J. Eur. Union L253, 1–36.

EC., 2003a. Regulation (EC) 1829/2003 of the European parliament and of the Council of 22 September 2003 on genetically modified food and feed. Off. J. Eur. Union L268, 1–23. Available from: http://europa.eu.int/eur-Lex/pri/en/oi/dat/2003/1_268/1_26820031018en00010023.pdf.

EC, 2003b. Guidance Document for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed. The Joint Working Group on Novel Foods and GMOs, European Commission document. European Commission, Brussels, Belgium Available from: http://ec.europa.eu/food/fs/sc/ssc/out327_en.pdf.

EC, 2004. Genetically Modified Crops in the EU: Food Safety Assessment, Regulation, and Public Concerns – Overarching Report, ENTRANSFOOD. European Communities, Luxembourg, Belgium, p. 99 Available from: http://www.agbios.com/docroot/articles/05-268-002.pdf.

EFSA, 2006a. Risk assessment of genetically modified plants and derived food and feed. Guidance document of the scientific panel on genetically modified organisms of the European food safety authority (EFSA). EFSA J. 99, 1–100. Available from: http://www.efsa.eu.int/science/gmo/gmo_guidance/660_en.html.

EFSA, 2006b. Safety and Nutritional Assessment of GM Plant Derived Foods/feed: the Role of Animal Feeding Trials. Draft Report of the Scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA) Available from: http://www.efsa.europa.eu/en/science/gmo/gmo_consultations/gmo_AnimalFeedingTrials.html.

FAO, 1996. Biotechnology and Food Safety. Report of a Joint FAO/WHO Consultation, 30 September–4 October, 1996. FAO Food and Nutrition Paper 61, Food and Agriculture Organisation of the United Nations, Rome.

FAO, 2009. How to Feed the World in 2050. Discussion Paper, High-level Expert Forum. The Food and Agriculture Organization, Rome, Italy http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf.

FAO/WHO, 2000. Safety Aspects of Genetically Modified Foods of Plant Origin. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, May 29–June 2, 1996, Rome, Italy.

Funke, T., Han, H., Healy-Fried, M.L., Fischer, M., Schönbrunn, E., 2006. Molecular basis for the herbicide resistance of roundup ready crops. Proc. Natl. Acad. Sci. U. S. A. 103, 13010–13015.

Garcia-Alonso, M., Jacobs, E., Raybould, A., Nickson, T.E., Sowig, P., Willekens, H., Van Der Kouwe, P., Layton, R., Amijee, F., Fuentes, A.M., Tencalla, F., 2006. A tiered system for assessing the risk of genetically modified plants to non-target organisms. Environ. Biosaf. Res. 5, 57–65.

George, C., Ridley, W.P., Obert, J.C., Nemeth, M.A., Breeze, M.L., Astwood, J.D., 2004. Composition of grain and forage from corn rootworm-protected corn event MON 863 is equivalent to that of conventional corn (*Zea mays* l.). J. Agric. Food Chem. 52, 4149–4158.

Hammond, B., Dudek, R., Lemen, J., Nemeth, M., 2004. Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. Food Chem. Toxicol. 42, 1003–1014.

Hammond, B., Dudek, R., Lemen, J., Nemeth, M., 2006a. Results of a 90 day safety assurance study with rats fed grain from corn borer-protected corn. Food Chem. Toxicol. 44, 1092–1099.

Hammond, B., Lemen, J., Dudek, R., Ward, D., Jiang, C., Nemeth, M., Burns, J., 2006b. Results of a 90 day safety assurance study with rats fed grain from corn rootworm-protected corn. Food Chem. Toxicol. 44, 147–160.

Hammond, B.G., Vicini, J.L., Hartnell, G.F., Naylor, M.W., Knight, C.D., Robinson, E.H., Fuchs, R.L., Padgette, S.R., 1996. The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by genetic incorporation of glyphosate tolerance. J. Nutr. 126, 717–727.

Harrigan, G.G., Glenn, K.C., Ridley, W.P., 2010a. Assessing the natural variability in crop composition. Regul. Toxicol. Pharmacol. 58 (3 Suppl. l), S13–S20.

Harrigan, G.G., Lundry, D., Drury, S., Berman, K., Riordan, S.G., Nemeth, M.A., Ridley, W.P., Glenn, K.C., 2010b. Natural variation in crop composition and the impact of transgenesis. Nat. Biotechnol. 28, 402–404.

Harrigan, G.G., Ridley, W.P., Miller, K.D., Sorbet, R., Riordan, S.G., Nemeth, M.A., Reeves, W., Pester, T.A., 2009. The forage and grain of MON 87460, a drought-tolerant corn hybrid, are compositionally equivalent to that of conventional corn. J. Agric. Food Chem. 57, 9754–9763.

Harrigan, G.G., Ridley, W.P., Riordan, S.G., Nemeth, M.A., Sorbet, R., Trujillo, W.A., Breeze, M.L., Schneider, R.W., 2007a. Chemical composition of glyphosate-tolerant soybean 40-3-2 grown in Europe remains equivalent with that of conventional soybean (*Glycine max* L.). J. Agric. Food Chem. 55, 6160–6168.

Harrigan, G.G., Stork, L.G., Riordan, S.G., Reynolds, T.L., Ridley, W.P., Masucci, J.D., Macisaac, S., Halls, S.C., Orth, R., Smith, R.G., Wen, L., Brown, W.E., Welsch, M., Riley, R., McFarland, D., Pandravada, A., Glenn, K.C., 2007b. Impact of genetics and environment on nutritional and metabolite components of maize grain. J. Agric. Food Chem. 55, 6177–6185.

Harrison, L.A., Bailey, M.R., Naylor, M.W., Ream, J.E., Hammond, B.G., Nida, D.L., Burnette, B.L., Nickson, T.E., Mitsky, T.A., Taylor, M.L., Fuchs, R.L., Padgette, S.R., 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. Strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. J. Nutr. 126, 728–740.

He, X.Y., Tang, M.Z., Luo, Y.B., Li, X., Cao, S.S., Yu, J.J., Delaney, B., Huang, K.L., 2009. A 90-day toxicology study of transgenic lysine-rich maize grain (Y642) in Sprague-Dawley rats. Food Chem. Toxicol. 47, 425–432.

Healy, C., Hammond, B., Kirkpatrick, J., 2008. Results of a 13-week safety assurance study with rats fed grain from corn rootworm-protected, glyphosate-tolerant MON 88017 corn. Food Chem. Toxicol. 46, 2517–2524.

Hefle, S.L., Nordlee, J.A., Taylor, S.L., 1996. Allergenic foods. CRC Crit. Rev. Food Sci. Nutr. 36S, S69–S89.

Herman, R.A., Dunville, C.M., Juberg, D.R., Fletcher, D.W., Cromwell, G.L., 2011. Performance of broiler chickens fed diets containing DAS-68416-4 soybean meal. GM Crops 2, 169–175.

Herman, R.A., Phillips, A.M., Collins, R.A., Tagliani, L.A., Claussen, F.A., Graham, C.D., Bickers, B.L., Harris, T.A., Prochaska, L.M., 2004. Compositional equivalency of Cry1F corn event TC6275 and conventional corn (*Zea mays* L.). J. Agric. Food Chem. 52, 2726–2734.

Herman, R.A., Schafer, B.W., Korjagin, V.A., Ernest, A.D., 2003. Rapid digestion of Cry34Ab1 and Cry35Ab1 in simulated gastric fluid. J. Agric. Food Chem. 51, 6823–6827.

Herman, R.A., Storer, N.P., Phillips, A.M., Prochaska, L.M., Windels, P., 2007. Compositional assessment of event DAS-59122-7 maize using substantial equivalence. Regul. Toxicol. Pharmacol. 47, 37–47.

Herouet, C., Esdaile, D.J., Mallyon, B.A., Debruyne, E., Schulz, A., Currier, T., Hendrickx, K., van der Klis, R.-J., Rouan, D., 2005. Safety evaluation of the phosphinothricin acetyltransferase proteins encoded by the *pat* and *bar* sequences that confer tolerance to glufosinate ammonium herbicide in transgenic plants. Regul. Pharmacol. Toxicol. 41, 134–149.

Herouet-Guicheney, C., Rouquié, D., Freyssinet, M., Currier, T., Martone, A., Zhou, J., Bates, E.E.M., Ferullo, J.-M., Hendrickx, K., Rouan, D., 2009. Safety evaluation of the double mutant 5-enol pyruvylshikimate-3-phosphate synthase (2mEPSPS) from maize that confers tolerance to glyphosate herbicide in transgenic plants. Regul. Toxicol. Pharmacol. 54, 143–153.

Hileman, R.E., Bonner, H.K.S., Kaempfe, T.A., Hammond, B.G., Glenn, K.C., 2006. Safety assessment of Cre recombinase. J. Agric. Food Chem. 54, 8640–8647.

ILSI., 1996. The safety assessment of novel foods. Guidelines prepared by ILSI Europe novel food task force. Food Chem. Toxicol. 34, 931–940.

ILSI., 1997. ILSI Europe novel task foods force: the safety assessment of novel foods. Food Chem. Toxicol. 34, 931–940.

ILSI, 2003. The Safety Assessment of Novel Foods and Concepts to Determine Their Safety in Use. Expert Group Report, ILSI Europe Novel Food Task Force. International Life Sciences Institute, Brussels, Belgium Available from: http://europe.ilsi.org/publications/Articles/novelfoodsafety.htm.

ILSI, 2004. Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology. Comp. Rev. Food Sci. Food Saf. 3, 35–104. Available from: http://www.ift.org/pdfs/crfsfs/crfsfsv3n2p0035-0104ms20040106.pdf.

Ishiguro, M., Matori, Y., Tanabe, S., Kawase, Y., Sekine, I., Sakakibara, R., 1992a. Biochemical studies on oral toxicity of ricin. V. The role of lectin activity in the intestinal absorption of ricin. Chem. Pharm. Bull. 40, 1216–1220.

Ishiguro, M., Tanabe, S., Matori, Y., Sakakibara, R., 1992b. Biochemical studies on oral toxicity of ricin. IV. A fate of orally administered ricin in rats. J. Pharmacobiodyn. 15, 147–156.

Juberg, D.R., Herman, R.A., Thomas, T., Brooks, K.J., Delaney, B., 2009. Acute and repeated dose (28 day) mouse oral toxicology studies with Cry34Ab1 and Cry35Ab1 Bt proteins used in Coleopteran resistant DAS-59122-7 corn. Regul. Pharmacol. Toxicol. 54, 154–163.

Klümper, W., Qaim, M., 2014. A meta-analysis of the impacts of genetically modified crops. PLoS One 9, e111629.

Kullik, S.A., Sears, M.K., Schaafsma, A.W., 2011. Sublethal effects of Cry 1F Bt corn and clothianidin on black cutworm (Lepidoptera: Noctuidae) larval development. J. Econ. Entomol 104, 484–493.

Ladics, G.S., Bardina, L., Cressman, R.F., Mattsson, J.L., Sampson, H.A., 2006. Lack of cross-reactivity between the Bacillus thuringiensis derived protein Cry1F in maize grain and dust mite Der p7 protein with human sera positive for Der p7-IgE. Regul. Toxicol. Pharmacol. 44, 136–143.

Ladics, G.S., Cressman, R., Herouet-Guicheney, C., Herman, R., Privalle, L., McClain, S., 2011. Bioinformatics and the allergy assessment of agricultural biotechnology products: industry practices and recommendations. Regul. Toxicol. Pharmacol. 60, 46–53.

Lafont, J., Rouanet, J.M., Gabrion, J., Assouad, J.L., Zambonino Infante, J.L., Besancon, P., 1988. Duodenal toxicity of dietary Phaseolus vulgaris lectins in the rat: an integrative assay. Digestion 41, 83–93.

Liu, P., He, X., Chen, D., Luo, Y., Cao, S., Song, H., Liu, T., Huang, K., Xu, W., 2012. A 90-day subchronic feeding study of genetically modified maize expressing Cry1Ac-M protein in Sprague-Dawley rats. Food Chem. Toxicol. 50, 3215–3221.

Lundry, D.R., Burns, J.A., Nemeth, M.A., Riordan, S.G., 2013. Composition of grain and forage from insect-protected and herbicide-tolerant corn, MON 89034 × TC1507 × MON 88017 × DAS-59122-7 (SmartStax™), is equivalent to that of conventional corn (*Zea mays* L.). J. Agric. Food Chem. 61, 1991–1998.

Lundry, D.R., Ridley, W.P., Meyer, J.J., Riordan, S.G., Nemeth, M.A., Trujillo, W.A., Breeze, M.L., Sorbet, R., 2008. Composition of grain, forage, and processed fractions from second-generation glyphosate-tolerant soybean, MON 89788, is equivalent to that of conventional soybean (*Glycine max* L.). J. Agric. Food Chem. 56, 4611–4622.

MacKenzie, S.A., Lamb, I., Schmidt, J., Deege, L., Morrisey, M.J., Harper, M., Layton, R.J., Prochaska, L.M., Sanders, C., Locke, M., Mattsson, J.L., Fuentes, A., Delaney, B., 2007. Thirteen week feeding study with transgenic maize grain containing event DAS-Ø15Ø7-1 in Sprague-Dawley rats. Food Chem. Toxicol. 45, 551–562.

Malley, L.A., Everds, N.E., Reynolds, J., Mann, P.C., Lamb, I., Schmidt, J., Layton, R.J., Prochaska, L.M., Hinds, M., Locke, M., Chui, C.-F., Claussen, F., Mattsson, J.L., Delaney, B., 2007. Subchronic feeding study of DAS-59122-7 transgenicmaize grain in Sprague-Dawley rats. Food Chem. Toxicol. 45, 1277–1292.

Mathesius, C.A., Barnett Jr., J.F., Cressman, R.F., et al., 2009. Safety assessment of a modified acetolactate synthase protein (GM-HRA) used as a selectable marker in genetically modified soybeans. Regul. Toxicol. Pharmacol. 55, 309–320.

McCann, M.C., Liu, K., Trujillo, W.A., Dobert, R.C., 2005. Glyphosate-tolerant soybeans remain compositionally equivalent to conventional soybeans (*Glycine max* L.) during three years of field testing. J. Agric. Food Chem. 53, 5331–5335.

McCann, M.C., Trujillo, W.A., Riordan, S.G., Sorbet, R., Bogdanova, N.N., Sidhu, R.S., 2007. Comparison of the forage and grain composition from insect-protected and glyphosate-tolerant MON 88017 corn to conventional corn (*Zea mays* L.). J. Agric. Food Chem. 55, 4034–4042.

McNaughton, J., Roberts, M., Rice, D., Smith, B., Hinds, M., Delaney, B., Iiams, C., Sauber, T., 2011b. Nutritional equivalency evaluation of transgenic maize grain from event DP-Ø9814Ø-6 and transgenic soybeans containing event DP-356Ø43-5: laying hen performance and egg quality measures. Poult. Sci. 90, 377–389.

McNaughton, J., Roberts, M., Smith, B., Rice, D., Hinds, M., Rood, T., Layton, R., Lamb, I., Delaney, B., 2008b. Comparison of broiler performance and carcass yields when fed diets containing transgenic maize grains from event DP-Ø9814Ø-6 (Optimum™ GAT™), near-isogenic control maize grain, or commercial reference maize grains. Poult. Sci. 87, 2562–2572.

McNaughton, J., Roberts, M., Smith, B., Rice, D., Hinds, M., Sanders, C., Layton, R., Lamb, I., Delaney, B., 2008a. Comparison of broiler performance when fed diets containing event DP-3Ø5423-1, nontransgenic near-isoline control, or commercial reference soybean meal, hulls, and oil. Poult. Sci. 87, 2549–2561.

McNaughton, J., Roberts, M., Rice, D., Smith, B., Hinds, M., Delaney, B., Iiams, C., Sauber, T., 2011a. Comparison of broiler performance and carcass yields when fed transgenic maize grain containing event DP-Ø9814Ø-6 and processed fractions from transgenic soybeans containing event DP-356O43-5. Poult. Sci. 90, 1701–1711.

McNaughton, J.L., Roberts, M., Rice, D., Smith, B., Hinds, M., Schmidt, J., Locke, M., Bryant, A., Rood, T., Layton, R., Lamb, I., Delaney, B., 2007a. Feeding performance in broiler chickens fed diets containing DAS-59122-7 maize grain compared to diets containing non-transgenic maize grain. Anim. Feed Sci. Technol. 132, 227–239.

McNaughton, J., Roberts, M., Rice, D., Smith, B., Hong, B., Delaney, B., Iiams, C., 2014. Comparison of broiler performance and carcass yields when fed diets containing genetically modified canola meal from event DP-Ø73496-4, near-isogenic canola meal, or commercial canola meals. Poult. Sci. 93, 1713–1723.

McNaughton, J., Roberts, M., Smith, B., Rice, D., Hinds, M., Schmidt, J., Locke, M., Brink, K., Bryant, A., Rood, T., Layton, R., Lamb, I., Delaney, B., 2007b. Comparison of broiler performance when fed diets containing event DP-356Ø43-5 (Optimum™ GAT™), nontransgenic near-isoline control, or commercial reference soybean meal, hulls, and oil. Poult. Sci. 86, 2569–2581.

Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L., Fuchs, R.L., 1996. Assessment of the allergenic potential of foods from genetically engineered crop plants. CRC Crit. Rev. Food Sci. Nutr. 36, 165–186.

Nordlee, J.A., Taylor, S.L., Townsend, J.A., Thomas, L.A., Bush, R.K., 1996. Identification of a Brazil-nut allergen in transgenic soybeans. New Eng. J. Med. 344, 688–692.

Oberdoerfer, R.B., Shillito, R.D., de Beuckeleer, M., Mitten, D.H., 2005. Rice (*Oryza sativa* L.) containing the bar gene is compositionally equivalent to the nontransgenic counterpart. J. Agric. Food Chem. 53, 1457–1465.

Obert, J.C., Ridley, W.P., Schneider, R.W., Riordan, S.G., Nemeth, M.A., Trujillo, W.A., Breeze, M.L., Sorbet, R., Astwood, J.D., 2004. The composition of grain and forage from glyphosate tolerant wheat MON 71800 is equivalent to that of conventional wheat (*Triticum aestivum* L.). J. Agric. Food Chem. 52, 1375–1384.

OECD, 1993. Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles. Organisation for Economic Co-operation and Development (OECD), Paris.

OECD, 1995. Guideline for the Testing of Chemicals. Repeated Dose 28-day Oral Toxicity Study in Rodents, vol. 407 www.oecd.ord.dataoecd/50/41/37477972.pdf.

OECD, 1997. OECD Documents: Report of the OECD Workshop on the Toxicological and Nutritional Testing of Novel Foods, SG/ICGB(1998)1. Organisation of Economic Co-operation and Development, Paris, France.

OECD, 1998. OECD Guidelines for Testing of Chemicals No. 408, Repeated Dose 90-day Oral Toxicity Study in Rodents Paris, France.

OECD, 2003. Considerations for the Safety Assessment of Animal Feedstuffs Derived from Genetically Modified Plants. Organization for Economic Co-operation and Development ENV/JM/MONO(2003)10, Paris, France.

Padgette, S.R., Kolacz, K.H., Delannay, X., Re, D.B., LaValee, B.J., Tinius, C.N., Rhodes, W.K., Otero, Y.I., Barry, G.F., Eichholtz, D.A., Peschke, V.M., Nida, D.L., Taylor, N.B., Kishore, G.M., 1995. Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Sci. 35, 1451–1461.

Padgette, S.R., Re, D.B., Gasser, C.S., Eichholtz, D.A., Frazier, R.B., Hironaka, C.M., Levine, E.B., Shah, D.M., Fraley, T.R., Kishore, G.M., 1991. Site-directed mutagenesis of a conserved region of the 5-enoylpyruvylshikimate-3-phosphate synthase active site. J. Biol. Chem. 266, 22364–22369.

Padgette, S.R., Taylor, N.B., Nida, D.L., Bailey, M.R., MacDonald, J., Holden, L.R., Fuchs, R.L., 1996. The composition of glyphosate-tolerant soybean seeds is equivalent to that of conventional soybeans. J. Nutr. 126, 702–716.

Paoletti, C., Germini, A., 2012. Compositional analysis: field trial design and statistical analysis. EFSA Technical Meeting with Applicants on GMOs; Parma, Italy.

Parrott, W., Chassy, B., Ligon, J., Meyer, L., Petrick, J., Zhou, J., Herman, R., Delaney, B., Levine, M., 2010. Application of food and feed safety assessment principles to evaluate transgenic approaches to gene modulation in crops. Food Chem. Toxicol. 48, 1773–1790.

Petrick, J.S., Brower-Toland, B., Jackson, A.L., Kier, L.D., 2013. Safety assessment of food and feed from biotechnology-derived crops employing RNA-mediated gene regulation to achieve desired traits: a scientific review. Regul. Pharmacol. Toxicol. 66, 167–176.

Pigott, C.R., Ellar, D.J., 2007. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol. Mol. Biol. Rev. 71, 255–281.

Privalle, L.S., 2002. Phosphomannose isomerase, a novel plant selection system. Potential allergenicity assessment. Ann. N. Y. Acad. Sci. 964, 129–138.

Qi, X., He, X., Luo, Y., Li, S., Zou, S., Cao, S., Tang, M., Delaney, B., Xu, W., Huang, K., 2012. Subchronic feeding study of stacked trait genetically-modified soybean (3Ø5423 × 40-3-2) in Sprague-Dawley rats. Food Chem. Toxicol. 50, 3256–3263.

Raybould, A., 2006. Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. Environ. Biosaf. Res. 5, 119–125.

Ridley, W.P., Harrigan, G.G., Breeze, M.L., Nemeth, M.A., Sidhu, R.S., Glenn, K.C., 2011. Evaluation of compositional equivalence for multitrait biotechnology crops. J. Agric. Food Chem. 59, 5865–5876.

Ridley, W.P., Sidhu, R.S., Pyla, P.D., Nemeth, M.A., Breeze, M.L., Astwood, J.D., 2002. Comparison of the nutritional profile of glyphosate-tolerant corn event NK603 with that of conventional corn (*Zea mays* L.). J. Agric. Food Chem. 50, 7235–7243.

Romeis, J., Bartsch, D., Bigler, F., Candolfi, M.P., Gielkens, M.M.C., Hartley, S.E., Hellmich, R.L., Huesing, J.E., Jepson, P.C., Layton, R., Quemada, H., Raybould, A., Rose, R.I., Schiemann, J., Sears, M.K., Shelton, A.M., Sweet, J., Vaituzis, Z., Wolt, J.D., 2008. Assessment of risk of insect-resistant transgenic crops to non-target arthropods. Nat. Biotechnol. 26, 203–208.

Romeis, J., Hellmich, R., Candolfi, M., Carstens, K., De Schrijver, A., Gatehouse, A., Herman, R., Huesing, J., McLean, M., Raybould, A., Shelton, A., Waggoner, A., 2011. Recommendations for the design of laboratory studies on non-target arthropods for risk assessment of genetically engineered plants. Transgenic Res. 20, 1–22.

Rose, R.I., 2007. White Paper on Tier-based Testing for the Effects of Proteinaceous Insecticidal Plant-incorporated Protectants on Non-target Arthropods for Regulatory Risk Assessments. USDA-APHIS and US Environmental Protection Agency http://www.epa.gov/pesticides/biopesticides/pips/non-target-arthropods.pdf.

Rossi, M.A., Mancini Filho, J., Lajolo, F.M., 1984. Jejunal ultrastructural changes induced by kidney bean (Phaseolus vulgaris) lectins in rats Br. J. Exp. Pathol. 65, 117–123.

Sampson, H.A., Metcalfe, D.D., 1992. Food allergies J. Am. Med. Assoc. 268, 2840–2844.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R., Dean, D.H., 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 62, 775–806.

Sinagawa-Garcia, S.R., Rascón-Cruz, Q., Valdez-Ortiz, A., Medina-Godoy, S., Escobar-Gutiérrez, A., Paredes-López, O., 2004. Safety assessment by in vitro digestibility and allergenicity of genetically modified maize with an amaranth 11S globulin. J. Agric. Food Chem. 52, 2709–2714.

Singh, A.K., Mehta, A.K., Sridhara, A., Gaur, S.N., Singh, B.P., Sarma, P.U., Arora, N., 2006. Allergenicity assessment of transgenic mustard (*Brassica juncea*) expressing bacterial *codA* gene. Allergy 61, 491–497.

Sjoblad, R.D., McClintock, J.T., Engler, R., 1992. Toxicological considerations for protein components of biological pesticide products. Regul. Pharmacol. Toxicol. 15, 3–9.

Song, H., He, X., Zou, S., Zhang, T., Luo, Y., Huang, K., Zhu, Z., Xu, W., 2014 Nov 1. A 90-day subchronic feeding study of genetically modified rice expressing Cry1Ab protein in Sprague-Dawley rats. Transgenic Res. [Epub ahead of print] PubMed PMID: 25367203.

Stagg, N.J., Thomas, J., Herman, R.A., Juberg, D.R., 2012. Acute and 28-day repeated dose toxicology studies in mice with aryloxyalkanoate dioxygenase (AAD-1) protein expressed in 2,4-D tolerant DAS-40278-9 maize. Regul. Toxicol. Pharmacol. 62, 363–370.

Tan, S.Y., Cayabyab, B.F., Alcantara, E.P., Ibrahim, Y.B., Huang, F., Blankenship, E.E., Siegfried, B.D., 2011. Comparative susceptibility of *Ostrinia furnacalis, Ostrinia nubilalis* and *Diatraea saccharalis* (*Lepidoptera: Crambidae*) to *Bacillus thuringiensis* Cry1 toxins. Crop Prot. 30, 1184–1189.

Taylor, M., Hartnell, G., Lucas, D., Davis, S., Nemeth, M., 2007. Comparison of broiler performance and carcass parameters when fed diets containing soybean meal produced from glyphosate-tolerant (MON 89788), control, or conventional reference soybeans. Poult. Sci. 86, 2608–2614.

Taylor, M.L., Stanisiewski, E.P., Riordan, S.G., Nemeth, M.A., George, B., Hartnell, G.F., 2004. Comparison of broiler performance when fed diets containing roundup ready (event RT73), nontransgenic control, or commercial canola meal. Poult. Sci. 83, 456–461.

Thomas, K., Aalbers, M., Bannon, G.A., Bartels, M., Dearman, R.J., Esdaile, D.J., Fu, T.J., Glatt, C.M., Hadfield, N., Hatzos, C., Hefle, S.L., Heylings, J.R., Goodman, R.E., Henry, B., Herouet, C., Holsapple, M., Ladics, G.S., Landry, T.D., MacIntosh, S.C., Rice, E.A., Privalle, L.S., Steiner, H.Y., Teshima, R., van Ree, R., Woolhiser, M., Zawodny, J., 2004. A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. Regul. Toxicol. Pharmacol. 39, 87–98.

US-EPA, 1998a. Reregistration Eligibility Decision (RED): Bacillus Thuringiensis. United States Environmental Protection Agency EPA738-R-98-004, http://www.epa.gov/oppsrrd1/REDs/0247.pdf .

US-EPA, 1998b. Guideline for Ecological Risk Assessment. United States Environmental Protection Agency EPA/630/R-95/002F.

US-EPA, 2001. Environmental Assessment, in Biopesticides Registration Action Document: Bt Plant-incorporated Protectants. United States Environmental Protection Agency, pp. IIC1–IIC109. http://www.epa.gov/oppbppd1/biopesticides/pips/bt_brad2/3-ecological.pdf.

Van Eenennaam, A.L., Young, A.E., 2014. Prevalence and Impacts of Genetically Engineered Feedstuffs on Livestock Populations http://www.journalofanimalscience.org/content/early/2014/08/27/jas.2014-8124.

Venkatesh, T.V., Breeze, M.L., Liu, K., Harrigan, G.G., Culler, A.H., 2014. Compositional analysis of grain and forage from MON 87427, an inducible male sterile and tissue selective glyphosate-tolerant maize product for hybrid seed production. J. Agric. Food Chem. 62, 1964–1973.

Weinman, M.D., Allan, C.H., Trier, J.S., Hagen, S.J., 1989. Repair of microvilli in the rat small intestine after damage with lectins contained in the red kidney bean. Gastroenterology 97, 1193–1204.

WHO, 1991. Strategies for Assessing the Safety of Foods Produced by Biotechnology. Report of a Joint FAO/WHO Consultation. World Health Organization, Geneva, Switzerland.

WHO., 1995. Application of the Principles of Substantial Equivalence to the Safety Evaluation of Foods and Food Components from Plants Derived from Modern Biotechnology. Report of WHO Workshop WHO/FNU/FOS/95.1. World Health Organization, Geneva, Switzerland.

Xu, W., Cao, S., He, X., Luo, Y., Guo, X., Yuan, Y., Huang, K., 2009. Safety assessment of Cry1Ab/Ac fusion protein. Food Chem. Toxicol. 47, 1459–1465.

Zhou, J., Harrigan, G.G., Berman, K.H., Webb, E.G., Klusmeyer, T.H., Nemeth, M.A., 2011. Stability in the composition equivalence of grain from insect-protected maize and seed from glyphosate-tolerant soybean to conventional counterparts over multiple seasons, locations, and breeding germplasms. J. Agric. Food Chem. 59, 8822–8828.

Zhu, Y., He, X., Luo, Y., Zou, S., Zhou, X., Huang, K., Xu, W., 2013. A 90-day feeding study of glyphosate-tolerant maize with the G2-aroA gene in Sprague-Dawley rats. Food Chem. Toxicol. 51, 280–287.