



Revista de la Universidad Industrial de Santander. Salud

ISSN: 0121-0807

ISSN: 2145-8464

saluduis1@uis.edu.co

Universidad Industrial de Santander

Colombia

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Revista de la Universidad Industrial de Santander. Salud,
vol. 52, núm. 4, 2020, Octubre-Diciembre, pp. 440-449

Universidad Industrial de Santander
Bucaramanga, Colombia

DOI: <https://doi.org/10.18273/revsal.v52n4-2020011>

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Evidence of Food Safety in Relation to *Cry* Proteins in Genetically Modified Foods

Evidencias sobre seguridad alimentaria con relación a *Cry* proteínas en alimentos transgénicos

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Suggested citation: Gomez-Camponovo M. Evidence of Food Safety in relation to *Cry* proteins in genetically modified foods. Salud UIS. 2020; 440-449. doi: <https://doi.org/10.18273/revsal.v52n4-2020011> 

Resumen

El desarrollo de la biotecnología aplicada a los cultivos ha generado fuertes debates éticos y sociales sobre su uso. El presente estudio tuvo por objetivo revisar las evidencias epidemiológicas existentes relacionando el consumo de alimentos genéticamente modificados, en particular aquellos provenientes de cultivos con resistencia a algunos insectos plagas en los que se han introducido proteínas *Cry* aisladas de *Bacillus thuringiensis* con probables daños o trastornos en la salud de las personas. Se realizó una revisión en profundidad en el periodo 2007 a 2019, en bases de datos. Se excluyeron aquellos artículos que no hacían referencia a salud humana. Se obtuvieron 1 350 y finalmente se revisaron 118. La revisión permitió concluir que la mayoría de los estudios existentes se centran en información respecto a la composición química y ensayos in vitro o en laboratorio con animales. Igualmente, que el principio rector de equivalencia sustancial hoy utilizado en forma generalizada para la evaluación de potenciales efectos en salud, no debería sustituir la necesidad de una evaluación rigurosa de los productos incluyendo ensayos nutricionales, inmunológicos y toxicológicos. Por último se comprueba también que la evidencia epidemiológica incluida es insuficiente por lo que no es posible concluir a partir de ella, sobre la inocuidad de estos alimentos.

Palabras clave: Alimentos transgénicos; Organismos genéticamente modificados; OGM; Seguridad Alimentaria; *Bt* toxinas.

Abstract

Developments in applying biotechnology to crops have generated strong ethical and social debates about its use. This study was aimed at reviewing epidemiological evidence regarding the consumption of genetically modified foods and the possible effects on human health, particularly certain insect-resistant crops in which isolated *Bacillus thuringiensis* *Cry* protein has been introduced. An in-depth review of databases was conducted for 2007-2019. Articles not referring to human health were excluded. In total, 1,350 were obtained and 118 were reviewed. As a result, it can be concluded that most studies have focused on chemical composition and *in vitro* or laboratory animal

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trials. Furthermore, the guiding principle of substantial equivalency, generally used today to evaluate potential health effects, should not replace rigorously evaluating products with nutritional, immunological, and toxicological trials. Lastly, this review demonstrates a lack of epidemiological evidence, and therefore, the safety of these foods cannot be conclusively determined based on evidence.

Keywords: Transgenic food; Genetically modified organisms; GMO; Food safety; *Bt* toxins.

Introduction

In Uruguay, a large proportion of agriculture uses genetically modified (GM) varieties. Soybean is the primary extensive crop in Uruguay and has been since 2004. It covers over 1 million hectares, nearly all of which is planted with GM crops. A large percentage of corn in the country is also grown with GM seeds.

Although no official data exist, 2016 and 2017 annual reports by the ISAAA (International Service for the Acquisition of Agri-biotech Applications) indicate the magnitude to which this technology has been adopted for both crops, as well as its continued growth. The percentages of soy and corn crops that have been planted with GM seeds were 97.6% and 85.7 % in 2016¹, and 98% and 100% in 2017², respectively.

The adoption of this agricultural production profile is similar to other Latin American countries (Argentina, Brazil, Paraguay, and Bolivia). Moreover, several sources have reported that the greatest expansion of GM crops has occurred in the Southern Cone of Latin American.

The introduction of genetically modified organisms (GMOs) in Uruguay began early. The planting of this type of crop was first approved in 1996 with the Monsanto company's release of the use of soybean event GTS 40-3-4, commercially known as RR (Roundup Ready) soybean, which is tolerant to glyphosate herbicide. At that time, soy was an insignificant crop in the country, with under 10,000 hectares³, and there was no regulatory framework for the introduction of GM vegetables. The approval was the responsibility of the Department of Agricultural Protection Services, of the General Directorate of Agriculture Services at the Ministry of Agriculture, Livestock, and Fisheries (MGAP in Spanish). The Committee for Risk Analysis of Genetically Modified Materials was responsible for the risk assessment, made up of representatives from the National Institute for Food Research (INIA in Spanish), the National Seeds Institute (INASE in Spanish), and the General Directorate of Agricultural Services (DGSA in Spanish).

Next to be released for commercial use, in 2003 and 2004, were GM corn events, specifically, MON810 by the Monsanto company and *Bt11* by Syngenta. MON810 is resistant to lepidopteran insects and tolerant to glyphosate and *Bt11* is lepidopteran resistant and tolerant to glufosinate-ammonium herbicide.

The Commission for Risk Assessment of Genetically Modified Vegetables (CERV in Spanish) recommended the release of these two new corn events. Created in August 2000, this commission was tasked with advising the MGAP and the Ministry of the Economy and Finances (MEF in Spanish) on the evaluation, management, and communication of risks.

In the midst of strong controversy around the procedures for these releases, the National Directorate of the Environment (DINAMA in Spanish) implemented a project in 2005 to develop a National Biosafety Framework that would bring the country in line with the commitments it assumed under the Cartagena Protocol on Biosafety to the Convention on Biological Diversity. These actions led to the creation of the National Project Coordinating Committee, responsible for defining the various participating entities.

In 2007, having understood that it was impossible to simultaneously debate the creation of the National Biosafety Framework while also approving new GM events, an 18-month moratorium was established on new requests for authorization to introduce GM events for vegetables.

After a series of actions, a presidential executive order for a moratorium was issued in 2008 and a new institutional biosafety structure was created, placing the National Biosafety Cabinet (GNBio in Spanish) in charge of decision-making and resulting in the regulatory framework that is still in effect today.

This cabinet includes the MGAP, which presides, as well as the ministries of: Public Health (MSP in Spanish); Economy and Finances (MEF in Spanish); Housing, Land Planning, and the Environment (MVOTMA in Spanish); Industry, Energy, and Mining

(MIEM in Spanish); and Foreign Relations (MRE in Spanish). This marked the inception of the political entity that authorizes new applications for vegetables. The Commission for Risk Management (CGR in Spanish) advises this cabinet and handles operations, and is made up of a delegate from each of the six ministries in the GNBio. In turn, the CGR is advised by another technical-scientific entity, the Commission for Biosafety Risk Assessment (ERB in Spanish), which is composed of members proposed by the CGR and designated by the GNBio, and is responsible for developing risk assessment reports.

Another group that supports the risk assessment process is made up of representatives from governmental organizations, research institutes, and the University of the Republic. This group is called the Inter-Institutional Development Committee (CAI in Spanish), and acts as a case-by-base advisor upon request by the ERB.

The executive order mentioned also provides for the participation of social organizations through two non-binding entities: the Consulting Committee on Biosafety (CCB in Spanish) and a consulting group on the release of new events.

With this new regulatory framework in force, the release of several new events has been authorized. In 2011, five corn events were released (two tolerant to glyphosate herbicide, one glyphosate tolerant and lepidopteran resistant, one tolerant to glufosinate-ammonium herbicide and lepidopteran resistant, and one that is lepidopteran resistant and tolerant to glyphosate and glufosinate-ammonium). In 2012, there were the approvals of three soy events (two tolerant to glufosinate-ammonium and one glyphosate tolerant and lepidopteran resistant) and three corn events (all lepidopteran resistant and tolerant to glyphosate and glufosinate).

Another soybean was released in 2014, which was tolerant to imidazolinone herbicides. And in December of 2017, four events were authorized, about which there were very opposing views at the core of the CGR, and which in a certain way reflected highly disparate positions among the members of this committee virtually since its outset.

The four events that were released were done so without the approval of the MSP or the MVOTMA. A lack of strong evidence regarding the safety of these foods played a role in this absence of an official statement by the country's health authorities.

This has been a presentation of how decision-making regarding the release of GM foods has been managed in Uruguay. In this light, a question arises as to why some ministries in the country have not reached a common position on the approval of new events. To this end, it is important to mention the studies and contributions on this topic by various authors.

According to Landrigan⁴, GM crops can potentially produce previously unknown allergens or toxins, which could alter the nutritional quality of the food.

This conviction has led to intense debate between the supporters and opponents of the use of GM foods, in the absence of the evidence needed to characterize their risks and benefits with certainty^{5,6}. The strong ethical and social debates revolve around their use and their ability to affect the ecosystem and human health⁷. With regard to human health, Hilbeck⁸ states that no epidemiological studies have been conducted that enable asserting that there are no risks to human health⁹, and that those who make that assertion do not have a scientific basis for it. For others¹⁰, it seems that GM crops do not pose a greater risk to the population's health than traditional crops.

The principal genetic modifications that have been used in food crops have resulted from the introduction of tolerance to herbicides and resistance to insect infestations.

The present work is primarily focused on reviewing the effects of using crops in which resistance to insect infestations has been introduced, particularly *Cry* proteins.

Genetically modifying crops to be resistant to insect infestations involves the introduction of *Cry* protein-coding genes. *Cry* proteins are highly toxic and are the primary ones to have been widely demonstrated to have insecticidal power on *Bacillus thuringiensis* (*Bt*).

The insecticidal power of this bacteria was discovered early in Japan, in 1901, and was rediscovered in Germany in 1911¹¹. This is related with the production of a group of proteins known as δ -endotoxins¹². Of these, *Cry* proteins have the greatest virulence against several economically important insect infestations, including Lepidoptera, Coleoptera, Diptera, and Nematodes^{13,14}.

In addition to their high specificity against various types of insects, their persistence in the environment is low. These supposed benefits drove numerous efforts that led

to the isolation of several genes coding for the synthesis of these proteins with insecticidal properties, which are being inserted into various vegetables in order to keep crops healthy.¹¹ This type of compound has led to most of the existing evidence as well as most of the concerns about the possibility of health disorders in humans.

This is why an exhaustive study is required before introducing a new food to the market^{15,16}. Thus, the objective of this work was to review and describe the epidemiological evidence related to the consumption of insect-resistant GM foods and its possible association with harm to health or health disorders in humans.

Methodology

A search was performed in the databases PUBMED, SCIELO, TOXNET, and LILACS for the period 2007-2019, with keywords “Genetically modified food,” “Genetically modified crops,” “Human Health,” “Epidemiological Research,” “Allergenicity,” “Safety,” “Health Risks,” “*Bt* proteins,” “*Bt* toxins,” and “Human cells.” Boolean operators were used. The languages included Spanish, Portuguese, and English. In order to cover the largest possible amount of articles, a search was also conducted in Google Scholar, and a snowball search was performed with the references of the articles selected.

A total of 1,350 articles were obtained (**Figure 1**). Repeated articles were excluded, as well as those that were not related to population health based on an epidemiological approach. A total of 118 were analyzed.

PUBMED	854
TOXNET	247
LILACS	111
GOOGLE SCHOLAR	71
SCIELO	58
REFERENCED ARTICLES	9

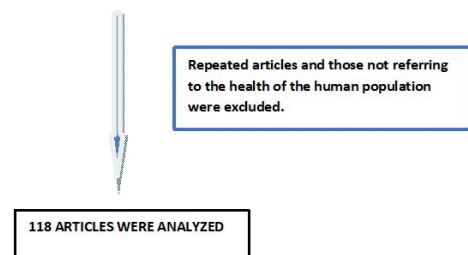


Figure 1. Number of articles found and selected from the databases consulted.

Results

Types of Actions by *Cry* proteins

Cry proteins are active on various orders of insects and nematodes¹¹.

These proteins take effect when they are ingested and solubilized in the alkaline medium of the middle intestine and bind to specific receptors in the cellular epithelial of the gastrointestinal tract, inserting themselves into the membrane and generally resulting in the destruction of the intestinal epithelial¹⁷.

Different models have been proposed to explain the lethal activity of *Cry* proteins when binding to specific receptors. The most well-known are Bravo, Zhang, and Jurat-Fuentes.¹² These three models come from studies performed primarily with Lepidoptera, in which *Cry* proteins are particularly active, although the physiological and/or biochemical characteristics in the middle intestine of different types of insects are known to alter the process under which the *Cry* protein carries out its activity¹⁸.

According to Bravo et al.¹², BL2 is the enzyme that facilitates the binding of *Cry* proteins to aminopeptidase N (APN), alkaline phosphatase (ALP), and glycolipid receptors. The absence of the BL2 enzyme in mammals is considered to be the reason why it is not toxic to them^{19,11}.

Nevertheless, it is not clear that the binding of *Cry* proteins to receptors is indispensable for its developing lethal action^{20,21}. That some *Cry* proteins can be toxic through other mechanisms is even accepted.

Additionally, and equally important, *Cry* proteins that are consumed when eating food produced with GM crops are not identical to those that naturally occur in *B.thuringensis*.

An important distinction is the lower specificity and greater toxicity of the gene-encoded *Cry* proteins that are introduced in certain crops. A good deal of research has been conducted on the mechanism of action of *Cry* proteins, which has generated diverse types of knowledge that is used to develop strategies to modify recombinant *Cry* proteins in order to broaden the spectrum of activity and/or increase lethality. By combining some of these strategies, the toxicity has increased 36 times over the original toxin²², and the specificity has decreased, expanding control over a larger number of species.

Health Effects

This could negatively affect health, and those who question introducing foods that are produced with GM crops into the diet assert that the health effects have not been adequately studied and should be exhaustively researched before they enter the market, including the possibility of allergic reactions^{15,23} and antibiotic resistance¹⁵.

For example, an event that has been authorized for animal consumption, such as the case of the company that created a GM corn called Star Link²⁴, was modified with an additional gene to produce insect resistance. Information from *Bacillus thuringiensis* was transferred, resulting in the expression of the *Cry9c* protein, which demonstrated significant allergenic activity. This is why its use in the United States was approved only for animal feed²⁴. Nevertheless, it was later found in food destined for human consumption, for example in “tacos,”^{15,25} a traditional food in Mexico, as well as in other corn-based products²⁵.

Currently, when an event is approved in the United States, it is either approved for all types of consumption or it is not approved²⁶.

The allergic reaction is generated due to a protein that is synthesized from the inserted gene²⁷. Only one allergy has been reported, which occurred in a worker in a plant using *Bacillus thuringiensis* microbials¹¹.

The *Cry1Ab* protein has been detected in events such as *Bt11* corn¹⁹ and Mon810²⁸, and was isolated in the gastrointestinal tract of calves fed with GM foods. Nevertheless, it was not detected in their blood¹⁹, nor were IgE antibodies detected in people who consumed this food.

And in the particular case of *Bt* MON810, no changes in the immune response have been found²⁶.

It is worth noting that while many proteins are digested through the action of gastric juices,²⁶ certain health conditions can cause people to have a higher pH, which could enable these allergens to act²⁶.

Bioequivalence

Bioequivalence studies have been developed that involve measurements to determine whether a new product will act in a way that is similar to the original product²⁹. Authors such as Garcia³⁰ consider this model to be flexible, malleable, and open to interpretation.

Bioequivalence tests are recognized as valuable for determining the safety of an event to be introduced. This means demonstrating that no significant differences exist between the use of the food from the new event and the use of the original food under the same conditions and dose^{31,32}.

Nutritional equivalence can be inferred when a comparative evaluation of the compositions does not find any significant differences between the GM plant and its appropriately-selected counterpart (the closest or most isogenic but not genetically modified). Nevertheless, when this cannot be shown, then there is a need to conduct more in-depth and adequately-designed studies³³. Not all authors consider this equivalence test to be a guarantee for safety^{7,34,35}.

The set of accumulative tests that is needed for this evaluation involves comparing the similarity of amino acid sequences (bioinformatics search), which is recognized as substantial equivalence^{24,27,36,37}.

These tests are used to study the amount of proteins or RNA, or the total number of changes in individual transcripts or proteins that would be produced in the GMO versus its conventional counterpart³⁸. Even with these more complex techniques, a failure to detect differences does not prove the absence of differences, and by extension, does not prove safety³⁸. All methods have detection limits, which may limit the ability to detect and analyze the possible harm that may actually exist³⁸.

See Heinemann (2011)³⁸ for details about molecular profiling and what is known as Omic's Technologies³⁹.

In the European Union, the safety of a GM crop is partly determined using an equivalence test approach, as reported previously⁴⁰. Under this paradigm, no phenotypic differences or differences in composition should exist between the GM crop and its isogenic⁴⁰. These comparisons are made by cultivating the two varieties under the same conditions in the same field trial⁴⁰.

This requires randomly selecting the sites where the different genotypes will be planted.⁴⁰ The aim is to show that they are not different than their isogenic referent and are equivalent to others that have been used and have been proven to be safe⁴⁰.

The use of new mathematical models has been proposed to measure the effect of the experiment while controlling the background⁴¹, which has to do with the natural genetic variability of crops as well as the conditions under which the experiment is performed.

These are not the only tests. Serum specific³⁶, pepsin-resistance³⁶, and *in vitro* digestibility tests can also be performed³⁹.

IgE-Mediated Responses

IgE-mediated responses can occur in human and animal models without an adverse clinical reaction. Therefore, systemic reactions need to be more thoroughly understood so as to better understand and refine approaches to evaluating safety in the future⁴². *In vitro* trials have not identified an IgE-mediated response²⁴.

Current guidelines also include evaluating non-IgE-mediated adverse reactions (enteropathies that are not IgE-mediated, e.g. allergic eosinophilic gastroenteropathy) on a case-by-case basis⁴³. As the number of events begin to increase, evaluations are needed to determine that allergic reactions in human and animal populations are not also increasing, on a case-by-base basis, given that addition, synergism, or antagonism could occur⁴³.

Toxicological Evaluation

The EFSA has published guidelines for toxicological evaluations, which recommend animal feeding trials in order to characterize the risks of using GMOs and to detect possible toxicological effects of test diets compared to control diets. It has also established a protocol to follow when testing foods using 90-day repeated dose toxicity studies in rodents⁴³, considered reliable animals for detecting undesired health effects⁴⁴. Other authors^{30,37} have indicated the limits of these studies as well as the need for case-by-case evaluations^{35,37}. Those who promote and conduct risk assessment studies, also suggest the need for case-by-case monitoring¹⁶.

Lastly, limitations have been highlighted regarding the ability to conclude whether or not these foods are safe, given that most of the evidence has primarily been based on *in vitro* and experimental animal studies. And since the latter have generally been conducted over short time periods, they cannot detect long-term effects^{8,26}.

Epidemiological Evidence

In the absence of long-term studies, the specific hypotheses about GM foods have been analyzed by comparing epidemiological data from Canada and the United States with data from the United Kingdom

and the Western European Union²⁶, populations that are recognized as comparable. GM foods have been consumed in the United States and Canada since the mid 1990s, while they have not been as widely consumed in the United Kingdom and the Western European Union²⁶.

The changing patterns in the incidence of cancer in the United States and Canada are very similar to those in the United Kingdom and the Western European Union.²⁶ In addition, the available data do not support the hypothesis that the consumption of GM foods has increased the rates of obesity or type 2 diabetes, or the prevalence of chronic kidney disease in the United States²⁶.

The detection of an increase in Celiac disease began in the United States before GM crops were introduced, and this increase seems to be similar in the United Kingdom where GM foods are not typically consumed²⁶.

The increasing pattern in autism spectrum disorder in children in the United States and the United Kingdom does not support the hypothesis that consuming GM foods is associated with the prevalence of this disorder²⁶.

Therefore, based on a detailed examination of short- and long-term toxicity tests in laboratory animals and the available epidemiological data, it has not been possible to demonstrate a greater risk to human health from GM foods than from conventional foods²⁶. Given the type of evidence available, many uncertainties exist, making it difficult to extrapolate the findings to the human population.

Monitoring System

There is a need for a well-designed, long-term, post-market monitoring system with sufficient resources^{4,16}.

As part of this proposed monitoring system, databases on the composition of foods should be improved and maintained so that they are kept up-to-date on changes that may be identified by post-market studies^{24,44}. Adverse health effects, such as a type of allergy, also need to be registered even if serious clinical manifestations are not⁴⁴.

Based on what has already been stated, post-market monitoring systems should be case-by-case, and preferably designed before products enter the market in order to facilitate prospective observation.

Consumer Information

When mentioning the advantages of using GM foods, it is important to remember that the molecules (*Cry* proteins) that have been introduced act as toxins⁴⁵.

One of the measures that is being promoted is food labels for products that are developed from genetic modifications. Nevertheless, companies have shown a degree of fear about the effect of labeling on their business.

Labeling is essential for monitoring, and, is important considering the possible emergence of new food allergies. This would respect the wishes of a growing number of consumers who insist on the right to know what kind of food they are buying^{4,7,46}. Some even believe that labeling should be mandatory.⁴⁷

Discussion

Most of the existing information is related with studies of chemical composition, *in vitro* trials, or laboratory animal trials.

Substantial equivalence is the guiding principle, which indicates equivalence in all senses. This assumes that undesired negative side effects can be detected just by performing a chemical analysis. Actually, this concept should not substitute the need to rigorously evaluate products through nutritional, immunological, and toxicological trials²⁷, supplemented by *in vivo* studies⁴⁸. The lack of specific receptors in mammals cannot be one of the main arguments²³ for a lack of toxicity to human health. *Cry* proteins are known to be found in soluble form, which can damage cellular membranes⁴⁸ as well as bioaccumulate⁴⁸.

Complex studies are needed that reliably evaluate the effects of consuming foods produced through genetic engineering⁴⁴, regardless of the idea that people are accustomed to eating processed foods and that such a diet would denaturalize these proteins, making them safer to consume⁴⁹.

Another argument is that they are consumed in small quantities, but that does not take into account their possible bioaccumulation in tissues, given that they have been detected in the blood of pregnant women⁴⁸, according to a study in Canada⁵⁰. Although this was later refuted due to a discrepancy in the technique used to measure *Cry* proteins^{51,52}. An additional step in generating scientific evidence is needed, namely,

conducting epidemiological studies that can measure individual consumption^{44,53}. This would generate the greatest amount of evidence⁵⁴.

In order to determine whether or not conventional or GM foods can cause specific health and food safety problems, it is also important to better understand the impact of foods on the immune system and their interaction with it⁵⁵.

Therefore, this review has relied on short- and long-term toxicity tests in animals and on the available epidemiological information. Even with all this, it has not been possible to demonstrate that GM foods present a greater health risk than conventional foods^{26,56}.

Nevertheless, the insertion of genes is known to impact the metabolites of the plant itself²⁶, which makes it very difficult to evaluate the safety of a food product.

One limitation of this review is its focus on a search of epidemiological studies and on evaluating the level of evidence⁵⁷. This review demonstrates the existence of descriptions of isolated cases and ecological studies. No studies are available that enable being conclusive about the reported findings.

Given this uncertainty, effective communications with the population should be a priority so that consumers can choose between conventional and unconventional foods⁴.

Based on the studies analyzed, it is not possible to be conclusive about the safety of these foods.

Conflict of interests

The authors declare that they have no conflict of interests.

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