Maize and Biodiversity: The Effects of Transgenic Maize in Mexico

Chapter 7
Assessment of Human Health Effects

for the Article 13 Initative on
Maize and Biodiversity

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Introduction

Plants have been grown as sources of human food ever since primitive hunter-gatherers first cultivated wild grasses over thousands of years ago. Maize is one of mankind's earliest innovations. It was domesticated approximately 5,000 years ago when humans, through primitive cross-pollination and selective breeding, turned a nondescript grass, teosinte, into productive modern maize (Gewin 2003). The domestication of teosintes in Mesoamerica provided a stable food source that was the basis for the development of the great civilizations in Mexico, Central and South America. Over the thousands of years that crops, such as maize, have been grown by human societies, changes have resulted in the development of different varieties through continued selective breeding. Seeds that tasted better, lasted longer, were more abundant or had other recognized beneficial properties were selected and propagated. More sophisticated breeding and selection methods continued to improve crops during the latter part of the 20th century, through hybridization with other varieties during the green revolution resulting in hybrid crop varieties that more than doubled crop production, particularly in developing countries (Wisniewski et al. 2002). These alterations in our food sources, including maize, have occurred in the absence of any regulatory or oversight environment into the mid to latter part of the twentieth century.

Most recently, molecular biology techniques have been used to alter plants to improve them as sources of human food. This process in general has been called genetic engineering although in terms of agricultural biotechnology, molecular breeding might be a more appropriate term, since this method can specifically identify and transfer one gene from one species organism to another and can cross species barriers. It has the advantage of being a much more rapid and precise method than traditional plant breeding. However, as with all new technologies, there are a number of concerns. A major concern has been unintended effect of products generated by this method on human health. Genetically modified crops have now been available for approximately eight years and larger numbers of the population in many countries have been exposed in increasing amounts to these foods over these periods of time. Based on this information, it can be stated and most would generally agree that biotechnology *per se* has no adverse effects on human health; individual products of biotechnology, such as individual products in conventional plant breeding, obviously could have unintended adverse effects. However, considering the period of time that genetically modified plants and products have been available and eaten by human populations, there is no evidence that such foods have any adverse effects on human health.
Regarding other GMOs used in the food industry, such as modified baking yeast or recombinant chymosin produced by GM microorganisms, an important difference should be stressed here: in these last examples, and in general transgenic microorganisms, GMOs are used as producers of industrial enzymes; therefore the transgenic organisms or parts of them are not necessarily present in the final product, opposed to what happens to transgenic maize which is consumed directly as whole grain. Having said this, certainly any new GMOs or GMO products should be tested for potential adverse effects on human health. The nutritional content of a particular plant product can be altered detrimentally, toxicity of a plant product may be affected by the toxicity of a novel protein introduced or the up-

regulation of a toxic component that is normally expressed at a low level. Since nutrition is an important area of any newly developed food, and allergenicity has been a potential problem frequently cited as a potential concern with these new products, this chapter will deal specifically with these issues. However, this is not to minimize other potential health problems with genetically modified foods such as toxicity and indeed most, if not all, new genetically modified products should be tested for them. The first section of this chapter will deal with nutritional issues of maize as they relate to genetically modified foods. The second section of this chapter will deal with the potential allergenicity of genetically modified maize. It has been a particular challenge to the authors and other individuals involved in this conference to address these issues with regard to Mexico since there may not always be a lot of information available about a particular subject in the Mexican literature (such as allergenicity of maize or genetically modified maize) so that one must extrapolate from the experiences of other countries where there has been considerable exposure to these new products and there is more information on inherent food allergy (such as North America and Europe) although consideration of differences in levels of exposure in different countries relative to Mexico must be considered.

The safety aspects of genetically modified foods of plant origin were considered following a joint Food and Agricultural Organization (FAO)/World Health Organization (WHO) expert consultation on foods derived from biotechnology held in Geneva, Switzerland in the year 2000. In that year, also Codex Alimentarius Commission along with FAO created as *ad hoc* task force that has been working since then on the “Safety of Foods derived from Modern Biotechnology”. Previous expert consultations convened by FAO/WHO and OECD recommended that substantial equivalence be an important component in the safety assessment of foods and food ingredients derived from genetically modified plants intended for human consumption (OECD, 1993; FAO, 1996). It utilizes a science-based approach in which comparison is made between the genetically modified food with its existing appropriate counterpart. There is no intent of this approach to establish absolute safety but rather to insure as best as possible that the food and any substances that have been introduced into it as a result of genetic modification is as safe as its traditional counterpart. The concept of substantial equivalence has been utilized by several countries as an important aspect of their safety evaluation of foods and ingredients derived from genetically modified organisms. Although this approach was found to be scientifically sound and practical, there has not been universal agreement on the application of this concept.

Several international organizations have begun to address issues associated with this novel food safety assessment in the present context of genetically modified plant and micro-organisms (WHO, 1991; OECD, 1993; WHO, 1995; FAO, 1996; ILSI, 1996; Commission of the European Communities, 1997). There is general agreement that such assessment requires an integrated and step-wise case-by-case approach and some authorities have developed decision processes to assist in determining the extent of testing required in specific cases (Scientist´s Working Group in Biosafety, 1998). This approach is generally useful in determining appropriate safety assessment strategies.

Achieving the objective of conferring a specific target trait (intended effect) to the host organism by insertion of defined DNA sequences, additional traits could, theoretically, be

acquired or existing traits lost (unintended effects). The assessment of genetically modified foods involves methods to detect such unintended effects and procedures to evaluate their biological relevance and impact on food safety. Unintended effects may be due to a number of events that result in disruption of existing genes. The resulting effects could alter enzyme levels and affect metabolism flux resulting in metabolic pattern changes.
The next generation of products, expressing nutraceuticals, enhanced nutrients, edible vaccines and non-edible industrial compounds, will be much more complex and the boundary between foods and therapeutics or industrial compounds will be blurred. For these products it will be much more difficult to find appropriate traditional counterparts and approaching the safety assessment using SE may not be effective (PEW Initiative on Food and Biotechnology, 2002; Agbios, 2001).

In addition, there are a number of nutrition-related issues as well as methodology for nutritional and safety evaluation; these will be considered in more detail later on in the chapter. Allergenicity, another trait that has received much attention, will be addressed in the second section of this chapter.

In conclusion, different consultations performed in the scientific community agreed that safety assessment of genetically modified foods requires an integrated and step-wise case-by-case approach. Presently there appear to be alternative strategies that would provide better assurance of food safety for genetically modified foods and the appropriate use of the concept of substantial equivalence. Any assessment of genetically modified foods requires methods to detect and evaluate the impact of unintended effects such as the acquisition of new traits or loss of existing traits. This could be particularly a problem when considering health effects on man. The issue of long-term effects from the consumption of genetically modified foods was considered; up to now the possibility of this occurring and being due to genetically modified foods has been considered highly unlikely.

The questions to be answered in this review are:
How is the nutrition of the Mexican population?
Which is the importance of maize in Mexican agriculture?
Which is the role of maize in Mexico for human consumption?
What is the need to have transgenic maize varieties or maize varieties with a modified composition?
Is there any potential hazard for human health in GM crops?
Is there any allergenic potential if the maize that has suffered introgression?
What are the risk issues to be resolved regarding potential health impacts due to the presence of GM proteins arising from introgression in maize landraces?

Effects on Nutrition

The purpose of this section is to examine the role of maize in the nutrition of Mexicans and to discuss the nutritional implications of gene flow from transgenic varieties to native landraces in Mexico. The subject is especially important for Mexicans since Mexico is a site of origin and genetic biodiversity of the plant and because of the enormous importance of maize in Mexican diet and culture**.** The notion of food is inevitably tied to the notion of nutrition; it is therefore pertinent to briefly review some aspects of nutrition.

Life depends on the supply of energy sources and of a series of substances necessary to maintain and reproduce the structures of the cells and for the control of metabolism; the origin of this supply is finally the environment. The organic and inorganic substances imported from the environment are called nutrients. Thus, nutrition is the series of coordinated processes involved in the obtention of the nutrients by the organism in the form of foods, and in the assimilation and metabolism of the nutrients by each cell (Bourges, 2003a).

The human diet contains several dozens of substances that may be considered nutrients; about 15 of them are inorganic and the rest are organic. The organism may synthesize some of the organic nutrients which presence in the diet is then dispensable, but about forty nutrients are strictly indispensable in the diet. Most nutrients are not usually found in nature in their free form; they are normally found as components of larger and more complex *compounds* such as starches, sucrose, fats and oils, proteins, dietary fiber and organic and inorganic salts from which nutrients are freed in the digestive tract.

The human requirement for organic nutrients results in the need to ingest the tissues or secretions of other organisms, which are called *foods*. Although potentially any of the nearly 2 million species already catalogued could serve, humans eat customarily only a few hundreds of species because a food should not only contain nutrient sources but also be innocuous, available, accessible and attractive to the senses and must be approved by the culture.

Currently, primary foods are not usually eaten alone or in their natural state, but are transformed into dishes or industrial products. The combination of foods, dishes and industrialized products eaten during the day is *the diet.* The quality of nutrition depends on the quality of the diet; it does not depend directly on foods, dishes or industrial products or on isolated nutrients. Therefore, it is diet that is the real *unit of nutrition* (Bourges, 1985).

As most biological phenomena, nutrition is a phenotype which is the result of the dynamic interaction between the information contained in the genome and the environmental history of every person The environmental history includes the dietetic history and the long-term relation of the individual with the physical, biological, psychological and social environments. Thus, inadequate nutrition may result from an incorrect diet but also from genetic factors and/or from adverse physical, biological, emotional and social conditions (Bourges, 2003b).

Besides providing nutrients to the body, meals provide pleasant stimuli for the senses and represent: a) means for esthetic expression and for communication with the group that strengthens social relations and the sense of identity, b) central elements in rites, ceremonies and celebrations and c) one of the main expressions of a given culture.

Eating is essentially a voluntary and conscious action, but it is finely regulated by biological mechanisms, especially by the sensations of hunger and satiety. Both mechanisms are highly precise to control the right amount of energy and food to be ingested in order to cover the requirements of every person.

Notwithstanding, there are many other factors that affect food intake and that may interfere with the physiological signals, giving place to an inadequate diet. Among them out stand: appetite, knowledge, either right or wrong, prejudices, preferences, likes and dislikes, recollections, mood, attitude, fears, values, traditions, habits and costumes, caprice and fashion.

No less important are the different historical, geographical, psychological, anthropological, sociological, commercial, economic, cultural and religious factors that determine the local availability of foods and the capacity of the people to access to the foods available (purchasing power, for example) and to prepare them (kitchen facilities, culinary ability, etc.). Because of the diversity and complexity of the above-mentioned factors, human nutrition is particularly susceptible to suffer qualitative and quantitative deviations.

In summary, nutrition must be recognized as a very complex bio-psycho-social phenomenon indispensable for biological, psycho-emotional and socio-cultural health. Thus, the cultural and psycho-emotional aspects of nutrition should not be overlooked since they are as important as its biological aspects. Certainly, nutrition is not only a biological process and should be analized from an integral perspective in which cultural aspects are essential.

Due to their paramount cultural importance, different foods have different cultural value. To date, humanity has diversified its diet and has access to a great deal of possible foods. Until not long ago, however, for most cultures there was, or there still is, certain food that clearly outstands in the daily diet and is called *staple* or *basic* food. In general, the staple food for most classical cultures is a cereal (Bennet, 1976).

De Garine and Vargas (De Garine. and Vargas, 1997) classify foods from the anthropological point of view in: a) staple, b) primary, c) secondary and d) peripheral. According to these authors, the characteristics of a staple food are:

a) They are considered to have divine origin or even to be a gift of the gods and frequently the food itself is deified
b) There is a long and close relationship of the food with the specific human group that usually results in full domestication.
c) The plant or animal has normally been the object of centuries of experimentation so it is usually well adapted to a variety of different climates and soils and has the capacity to resist many infectious and parasitic agents.
d) Basic plants or animals are exploited integrally with little if any waste and are frequently incorporated into crafts and artistic representations.
e) The culinary inventive of the group uses the food for a large variety of preparations, dishes and beverages.
f) It is consumed daily or almost daily and by most age groups, covering a significant part of the energy intake, and is particularly present during religious or festive celebrations.
g) The group has such a high esteem for the food that it is intolerant to its lack or scarcity.

“Primary” foods are those that have evolved biologically and culturally in parallel with the staple food, accompanying it in many dishes and often exhibiting nutritional complementation. “Secondary” foods are usually seasonal and consumed less often. Finally, “peripheral” foods are brought from abroad or are available for very short periods.
For millennia, maize has maintained a tight bi-directional relationship with Mexicans and it is clearly their staple food. The pre-Columbian mythology is rich in stories considering maize as a gift of gods (León Portilla 1980; Navarrete 2002). Maize was deified by the Aztecs in *Centeotl* and by Mayans in *Jun Ye Nal*. Mayans considered themselves as the “men of maize” and, for Aztecs, the condition of being human implied the consumption of maize. After domestication 5000 years ago, this plant shows a remarkable plasticity for cultivation in different terrains, climates and altitudes; specific varieties were developed for almost every imaginable use and conditions.

The maize plant is integrally used in Mesoamerica for both food and non-food purposes (fuel, forage, remedy, crafts, production of alcoholic beverages and wrapping). Several parts of the plant are used to decorate churches on special occasions; there are specific rules for using maize preparations in different holidays such as weddings, funerals, etc. In some tamales made in clearly distinct layers, Mayans represent their conception of the universe and *tesgüino*, an alcoholic beverage, is a must in religious ceremonies of some regions in the North of Mexico.

Mesoamericans found uses for maize in a remarkable variety of dishes and beverages. In a recent exhibit of the National Museum of Popular Cultures, 605 different recipes based on maize and 124 cooking methods were shown. In “Repertorio de tamales” (Perez San Vicente, 2000) a great number of different tamales are recorded. Maize accompanies Mesoamericans all through their life; it is consumed daily or almost daily and by most groups of age. *Atole* is the typical weaning preparation and is especially useful in the old age.

Maize tortilla; provides more energy (almost 60% of the total intake), protein (nearly 40% of the total intake), carbohydrate, dietary fiber, iron and many other nutrients than any other food in the average Mexican diet; its importance is even greater in the lower socioeconomic strata of the population providing up to 65% of the energy intake. Average per capita daily consumption of tortilla, which accounts for most of maize consumption in Mexico, was 370 g for the lowest income decile, and 330 g, 310 g and 170 g for the fourth, seventh and tenth income deciles. The typical average tortilla consumption in the rural diet is about 300g while the average for the urban population is around 180g. Maize tortilla is the most economically-efficient component in the Mexican diet since it offers more nutrients for a given price than any other food. In view of the above and of its unique cultural value, the consumption of maize tortilla deserves special protection in Mexico (Bourges, 2003a).

Settlements in Mesoamerica and agriculture probably started around 8000 to 6000 BC with cultivation of pumpkin, agave, common beans, chile peppers, manioc and sweet potatoes. Maize appeared later; there are some evidences of maize cultivation in Oaxaca by 3400 BC and clear signs of the use of hybrids, and barns to store them, by 2700 BC, but the precise date and site of its domestication is not clear yet. It is currently well accepted that maize resulted from hybridization and crossing of wild Teosinte (*Zea mexicana parviglumis*). According to McClung (2001), domestication could have started about 7 000 BC in the southern part of Mesoamerica. Whatever the date and site, it was a remarkable deed that changed the lives of Mesoamericans and made possible the development of the magnificent cultures that were to flourish in the region. Initially and for long time, maize grains were cooked and consumed directly, but around 900 BC a culinary and alimentary breakthrough occurred: *the development of nixtamal*.

N*ix*tamal is prepared by wet-grinding maize grains previously boiled for a couple of hours in ~5% lime water. The resulting *masa* is the base for several hundred different dishes. This fine culinary procedure gives place to a series of improvements: a) the pericarp softens; b) the zein content is reduced thus modifying the amino acid composition of the product and increasing its protein quality since the ratio Leu/Ile is improved; c) niacin, a vitamin which is not bioavailable in raw maize, becomes available; d) the use of lime water increases the content of bioavailable calcium in the product; e) an attractive flavor develops; and f) a consistency highly valued in Mexico is obtained that allows tortillas to fold without breaking when preparing a "taco." In a whole, nixtamalization might affect some amino acids, but overall the treatment is highly desirable, and the possibility of allergen reduction remains still unsubstantiated.

The *nixtamalization* process may have important reducing effects on fumonisin and aflatoxin content (Mendez-Albores, 2003), deoxynivalenol and zearalenone (Abbas, 1988).

For nearly two thousand years the main use of *nixta*mal was the preparation of tamale. Tortillas were a much later development that occurred at ~900 a. C. and became the principal form of *nixtamal* consumption and the center of the Mesoamerican diet.

The role of maize in human consumption in Mexico

Present forms of maize consumption in Mexico

As previously mentioned the National Museum of Popular Cultures recently presented more than 600 recipes that used maize and more than 100 preparation procedures, but this effort was far from exhaustive and maize based dishes are countless.

Briefly, both the green and the mature grains may be consumed directly or the mature grains may be converted into *nixtamal.* For all maize preparations, each region of Mexico has its own variants The ear as well as the separated grains may be boiled, roasted or toasted and then consumed as such or in a variety of soups, sweets, sherbets, cookies and cakes or added to rice, zucchini, squash flowers, chili peppers and many other ingredients. *Nixtamal* serves to prepare three large groups of derivatives: tortilla derivatives, tamal derivatives and beverages.

Tortilla is a dish by itself and also part of many dishes; additionally it serves as plate, as a spoon and as garnish. It may be eaten alone or accompanying meals. An apparently simple way to eat tortilla is as "taco" that is a tortilla folded to the shape of a cylinder or pipe with almost anything edible inside; the elasticity and flexibility obtained through the preparation of *nixtamal* are essential to allow tortillas to fold without breaking.

Fresh and dry tortillas (called tlayudas and totopos, etc.) are also the main ingredients of dozens of more complex dishes and there is a large group of tortilla-like pieces with different shapes. Each of these pieces originates a variety of preparations.

Tamales are steam-cooked *nixtama*l combined with different meats, sauces, fruits, vegetables or legume seeds and wrapped in leaves (mainly banana or corn leaves).

The number of fermented and not fermented maize based beverages is also very large. Especially interesting is the lactic fermentation of *atole* and *pozol* which results in amino acid enrichment of the product.

Last but not least it is necessary to mention *cuitlacoche* (corn smut), produced by the infection with *Ustilago maydis*, a fungus that grows on maize ears during cultivation and is a very appreciated delicatessen. The question arises whether the development of some new maize transgenic variety with anti-fungal properties may prevent production of this important product. Industrially prepared nixtamal flour appeared in the Mexican market about forty years ago; it is certainly a convenient and rather popular product, but its sensorial properties are not yet totally satisfactory.

The importance of maize in Mexican agriculture

Maize in Mexican agriculture

Maize is central in Mexican agriculture. Although the Mesoamerican agriculture developed many other important products, maize was particularly important and distinctive and the country is considered a site of origin and genetic diversity. Throughout 5000 years of manipulation, a great number of varieties have been developed for different purposes, environmental conditions and local food preferences; this treasure of biodiversity is considered a patrimony of mankind.

Although this subject is covered in other chapters, it is convenient to note that

a) Maize was the main crop in Mesoamerica, after the conquest and until today; there are more than 60 recorded varieties of maize in Mexico. Almost half of the arable land in the country is used to cultivate maize and the estimated annual average production in the last few years is about 19 million tons, that would amount to equivalent to 190 kg /person/ year or 500g /person / day. Additionally, nearly 5 million tons of corn are imported annually: mainly yellow corn from the United States and Canada. The official figures for 2003 were: 5,570,418 kg as grand total. Due to geographical, historical and social factors, Mexican agriculture has very special characteristics that drastically differ from Canadian and American agriculture. Most of the land is not appropriate for large-scale cultivation of a single extensively cultivated crop, and over ¾ of the area has no irrigation and depends on the eventual rain.
b) After the conquest, Mexican agriculture successfully adopted new products and techniques from the Old World. Simultaneously, a new social order developed imposing extreme differences between Spaniards and Indians. Although many Indians

were incorporated in this new order, many others escaped to mountains and jungles to maintain their traditional societies that still survive today. A way to manage in this new order was the conservation of the Mesoamerican *milpa* (Leon and Guzman-Gomez; 1999*)*, an ecologically sound concept that combines cultivation of many different plant products in a piece of land and allows people to survive on auto-consumption.

c) The sectors of society devoted to agriculture are usually the poorest and more marginated. During most of the 20th century and even today, the Mexican agriculture is clearly divided into a rather small sector that has access to irrigation, technology and finance support and produces commercially important goods with high yields, and a larger *campesino* sector that lacks those resources and practices subsistence agriculture. There are many variants of subsistence agriculture according to the region and other factors, but a common characteristic is the wise decision of *campesinos* to assure, in the first place, the maize supply for their families; the *milpa* continues to be a key way to survive for this sector, particularly for Indian communities.

d) In Mexico, maize agricultural systems are genetically open; the vast majority of farmers do not buy seed, they keep it from season to season. This practice has produced a large number of landraces over time, which makes the Mesoamerican region an important center of diversity. Landraces are continuously evolving and wild relatives grow in the proximity of maize fields. Mexico’s distinctive role as a center of genetic maize diversity suggests that the effects of gene flow could be extremely problematic; however no studies seem to exist about the possible side effects in plants that inherit transgenes season after season.

Mexican agriculture faces many problems arising from rural poverty, insufficient government support and other factors, however important, it is not the subject of this chapter.

Nutrition of the Mexican population

The nutrition of the Mexican population has been studied particularly during the second half of the 20th century, initially through local nutrition surveys, mainly in rural communities, and since 1979 through periodic national nutrition surveys. National surveys are done in representative samples of the population (in the order of 20 000 families) and include anthropometrical, dietary, clinical and socioeconomic evaluations, and often biochemical tests.

The diet

Today’s Mexicans are heirs of the enviable dietary tradition that resulted from the combination of two similarly rich, nutritionally sound and healthy diets: the Mesoamerican diet and the Spanish-Arabic diet of Mediterranean lineage.

The Mesoamerican diet (Bourges, 2002), with its many regional variations was most probably sufficient and healthy; otherwise, the magnificent Mesoamerican cultures could have never developed. It was based on maize, amaranth and common beans, but included many other seeds, vegetables and fruits, as well as insects (a highly appreciated delicatessen), algae and the products of hunting and fishing; turkeys and dogs were the only animals domesticated for alimentary purposes. According to the records of Spanish *conquistadores,* every day the Aztec emperor was offered about 300 different dishes for his choice; this was the table of the most powerful person in the empire and not of a normal citizen, but the point is that the culinary culture had the resources to offer such a rich menu.

Spaniards brought new products and culinary techniques that were easily incorporated and combined to create the New Spain cuisine, the immediate predecessor of today´ s Mexican culinary culture that has evolved during 500 years into one of the great culinary styles in the World. Certainly, not all Mexicans can enjoy this culinary treasure, mainly due to restraints on their physical and economic access to food. Today, in Mexico there are a wide variety of diets, partly because of the different regional traditions but also because of extreme economical differences.

Nutritional status

During the sixties, seventies and eighties child protein-energy malnutrition (PEM) appeared clearly as the main nutritional problem in Mexico. PEM concentrated in the lower socioeconomic strata and in rural areas, with greater prevalence in the South and Southeast, intermediate in the Center and lower in the North of the country; iron deficiency anemia was also highly prevalent while vitamin A and iodine deficiencies tended to decrease.

In contrast to previous studies, the most recent survey at the national level, the National Nutrition Survey (ENN, 1999), shows a decrease in the prevalence of PEM, but permanence of iron deficiency anemia and an increase of overweight and obesity. In surveys, PEM is estimated using anthropometrical measurements such as body weight and height from which several indexes are derived. In adults, Body Mass Index (BMI), is obtained dividing body weight (kg) by the square of height (m); it indicates fatness or leanness and normal values are 20 to 25. Briefly, the main results of ENN 1999 (Rivera-Dommarco et al., 2001) as compared with studies done 10 years before are:

1) PEM in preschool children has decreased during the last ten years from a national average of 14 to 7.5 % and severe forms from 6 to 2%.
2) Short stature in preschool children decreased from 23 to 18%, which is still high indicating that more efforts are needed in this regard.
3) Anemia attributable to iron deficiency is highly prevalent in preschool children, particularly during the second (50 %) and third (33%) years of life. There is concern about these figures since anemia may affect the development of the central nervous system for which the second year of life is critical.
4) In non-pregnant women in reproductive age (12 to 49 years of age), the prevalence of anemia was 20% and it was 26.4 % for pregnant women.
5) In women of reproductive age, the prevalence of overweight (BMI>25) was 52.5% and the prevalence of overt obesity (BMI>30) was 21.7 %. The survey did not include adult men, but other surveys suggest similar figures. Prevalence of overweight in preschool and school-age children was 5.4 % and 20%

The rise in the prevalence of excess body weight in comparison with previous studies is a major concern because it is a precipitating factor for dyslipidemia, type 2 diabetes, hypertension and some types of cancer, diseases accompanied of high mortality; according to national health statistics the prevalence of these diseases is increasing. The data in children indicates that now excess body weight is starting at an earlier age.

The figures showing a reduction in the prevalence of PEM are national averages. In a country with extreme social disparity as Mexico, averages hide what occurs in the extremes. The National Nutrition Survey (ENAL, 1996) (Avila, 1997), which concentrated in rural areas, illustrates this. In rural areas, the national prevalence of PEM was 43% with 4% of severe cases, clearly greater than the national averages shown by the ENN99. PEM in rural areas has a distinctive geographic distribution, with higher prevalence in the South and lower in the North of Mexico and, because of socioeconomic factors, is systematically higher in Indian (59%) than in non-Indian (38.5%) communities.

The State of Guerrero, south of Mexico City showed the worst situation with a PEM prevalence of 63% with 10% of severe cases, while the State of Sonora in the northwest of the country showed the best situation with a PEM prevalence of 13% and 0.2% of severe cases.

After the conquest, many Indians integrated to the new society mainly as slaves in the lowest socioeconomic strata and gradually lost their culture and identity; many others resisted this fate running away to remote mountains, deserts and jungles in order to keep their culture and identity, this last group comprising today about 9 million persons, is among the poorest in Mexican society; it is fractioned in many isolated ethnic subgroups that speak almost 100 different languages. Isolation, poverty, illiteracy and disease make them particularly vulnerable; they have the lowest income and the highest rates of PEM.

Causes of inadequate nutrition

Inadequate nutrition is a heavy load for any society. PEM, anemia and obesity are complex phenomena that result from the combination of many factors. Briefly, the most common factor for PEM is poverty but many other determinants intervene; it especially affects the lower socioeconomic strata of the population: rural communities, mainly in the southern states, and particularly Indian groups.

Good nutrition requires a good diet and a good diet requires foods. Thus, for the nutrition of individuals and families, foods must be available at home. The food available in a given home is the result of acquisition plus production, if it does exist. Acquisition of food depends on the purchasing capacity and, indirectly, on income.

The income and expense of homes surveys applied by the National Institute of Statistics, Geography and Informatics (Instituto Nacional de Estadística, Geografía e Informática, 2001) indicate a profound asymmetry in the income of Mexican homes. While the highest income decile takes 41% of the total income in the country and the upper fifth of homes take 69% of it, the lowest half gets only 16% and the two lower deciles 3.5%. What this means is that a high proportion of Mexicans are simply unable to acquire the necessary amounts of food in spite of devoting to it more than 40% of their total expense and in spite of them being more efficient buyers.

Beyond the purchasing capacity, acquisition of a given good depends on its local availability. While food availability is ample in cities and large towns, it is limited in small villages, especially if they are deficiently communicated. In Mexico there are tens of thousands of very small villages spread in abrupt territory that are not served by the commercial system due to their scarce demand and isolation

Even if food is available at home, it is not necessarily consumed in adequate form. PEM occurs mainly in children less than 3 years old; newborns and infants should ideally receive breast milk and this is what they receive in most rural areas though not in poor urban groups and is therefore exposed to malnutrition. A very delicate step in child feeding is weaning that should be done at the right age (around 6 months of age) and in the right form using the appropriate foods. In Mexican rural areas weaning is often done too late Incorporation of the child to the family diet should be done with especial caution to offer frequent meals with adequate nutrient density. Much too often, this is not correctly accomplished

The above combination of errors in child feeding practices is usually the main reason for PEM in Mexico. During the first and second years of life, especially in poor environments, infections are frequent becoming an aggravating factor.

The role of food production in the etiology of PEM

Food production is obviously a factor for adequate nutrition and in some countries of the world, insufficient production is the main determinant of famine and malnutrition. In many other countries it is not a highly relevant point. This is the case of Mexico where food production at the national, regional and often local levels does not sensibly affect food availability and where PEM is explained by many other factors. In Mexico, variations in food production are not normally associated with food intake or nutritional status; effective demand is a more important issue. Currently, the estimated Mexican production of maize is about 500 g/day/person, which is actually about twice its real consumption.

Benefits from increased food production cannot be disregarded provided that it may result in a better diet, higher income and higher level of life, but these are not automatic outcomes as it could apparently be the case. Some very poor Mexican *campesinos* living in extremely adverse environments would certainly benefit from higher yields, but the technologies to achieve them usually require conditions they do not have and inputs they cannot afford. In any case, improvement in child nutrition is not directly dependent on increased food production. There is no doubt that the nutrition of Mexicans faces many problems such as those derived from changes in eating habits and the introduction of exotic “fast foods” but this is not the subject of this chapter.

The purpose of this brief review of malnutrition and its main causes in Mexico is to give a picture of an important problem in the country. It should be clear that this problem has no relationship to the use of transgenic maize. On the other hand, from this review, it should also be clear that the future development of transgenic maize modified in its nutrient composition should not be expected to represent a solution, since it does not address the causes of malnutrition. In any case, transgenic maize varieties with improved amino acid balance, might de included as part of a varied and healthy diet in order to include them as one more factor to contribute to the good nutrition of the Mexican population.

Genetic engineering of maize

Crop breeding efforts to select specific attributes, improve yields, increase resistance to pests and diseases and to obtain better adaptation to specific environments, have a millenary history. Traditional breeding involves transformation of plants and animals, which represents a crude form of genetic manipulation that transfers hundreds of linked genes. The advances in molecular biology allowed the use of genetic engineering technology to accelerate and specifically target the crop improving efforts, due to the possibility to manipulate and clone DNA or genes from foreign species into new crops, which was not possible just by traditional plant breeding, opening many new potential applications.

As any technology, genetic engineering has advantages and limitations and certainly it should be used ethically, responsibly and with extreme care, avoiding unnecessary risks. Conner and Jacobs 1999, as well as other authors mention its advantages over traditional breeding:

a) It may transfer a single discrete gene instead of many unwanted and undefined linked genes, therefore offering greater confidence of achieving the desired outcome.
b) The transferred DNA fragment is usually very small and well understood, at least at the point when it is transferred, and can be controlled using cis-regulating sequences.
c) Promoters may be selected to allow either constitutive gene expression or to limit gene expression to specific cells or compartments or to specific environmental conditions.
d) It allows extension of the germplasm base to complement genes in declining gene pools as well as repeated transfer of new genes directly into existing cultivars or elite lines in traditional plant breeding programs that would otherwise require many generations cycles.
e) New gene formulations can be specifically designed for a variety of purposes.
f) Desired changes in nutrient composition can be achieved as it is the case of "golden rice" (YG Liu, 1995), canola oil with a "healthier" composition **(**KS Blundy, 1991), low-phytate content maize with a 49% higher iron absorption and many others. While positive impacts of these changes may be envisioned, substantial alteration of food nutrient profiles has potential ramifications that call for careful monitoring and public reporting (Reyes and Rozowski, 2003).

Although the production of transgenic crops has been claimed to be precise, specific and predictable, perhaps the main limitations of genetic engineered foods derived from the variability in expression of the transgene and its random integration in different sites of the plant chromatin, which seems to be highly unpredictable as it may vary among populations of plants independently transformed with a given gene. The transferred DNA may be one discrete copy of the transgene or it may be repeated in different regions and inserted in one or more sites (Jones et al. 1987; Jorgensen et al., 1987; Deroles and Gardner1988; Christey and Sinclair 1992). At a given site, a transferred gene may either be complete, truncated or rearranged; the ideal would be the insertion of one complete intact DNA copy in a single locus, in which case the behavior of the transgenic plant is as consistent as with natural genes. This is a usual outcome with the technique involving *Agrobacterium* but not with direct DNA transfer (Czernilofsky et al. 1986a; Czernilofsky, et al. 1986b). Although the intact nature of the transferred DNA can not be guaranteed, it can be determined by Southern blot or fluorescence *in situ* hybridization.

Potential hazards to human health from genetic engineering of crops

Biotechnology *per se* does not imply adverse effects on human health. In fact, during more than 8 years of global sale and use of the transgenic crops commercially available, there have not been reported cases of human or animal health damage due to their consumption. The general feeling is that, according to the available data, transgenic methods imply no danger to human health posed through food safety considerations. However the possibility of secondary effects of transgene expression will depend on the key regulatory points and rate limiting steps in biochemical pathways. Many of these are poorly understood in plants and can be expected to vary between crops as well as between cultivars and breeding lines within the same crop species. Expressed products of the transgenes can influence the expression of existing genes in plants, and therefore secondary effects may occur in tissues beyond those in which the transgenes is expressed. One could conceive that metabolites accumulating as a consequence of transgene-derived biochemical activity in one tissue are translocated within the plant to other tissues and organs such as the harvested food (Conner, 1993; Conner, 1999).

When genes are modified with foreign DNA there is a high degree of uncertainty about the site of insertion. If foreign DNA interrupts the sequence of an original gene, fusion proteins may appear besides the loss of function of the original gene**.** Metabolic routes may be altered in many different ways changing the production of essential substances or the nature and amount of metabolites potentially harmful. Dormant or previously non-functional genes may switch-on and others may switch-off.

Conner and Jacobs (Conner and Jacobs, 1999) have discussed the potential hazards arising specifically from the genetic engineering of crops. They include:

a) Protein products of the inserted genes. In most cases, the integrated gene would cause the expression of a trait and the products of the inserted genes are generally known and sensitive assays for them are usually available.
b) Secondary and pleiotropic effects of gene expression. Random insertion of DNA sequences into the plant genome, may disrupt or modify genes; active genes may be silenced or silent genes may be activated This may result in the formation of new metabolites or in alteration of levels of existing metabolites. Some of these unintended effects may be partially predictable although other effects are unpredictable due to the limited knowledge of gene regulation and gene-gene interaction**.**
c) A possible consequence of random integration of a transgene is insertional mutagenesis that may disrupt or modify expression of existing genes in the recipient plant. Some studies suggest that the possibility of insertional mutagenesis may be a relatively common event.

d) Inserted genes may encode for enzymes that are expressed at high activity levels resulting in alteration of the metabolic flow and an unanticipated increase or decrease of metabolites. The enzyme substrate may be depleted and end products may accumulate. Expression of new enzyme activity may divert metabolites from one pathway to another resulting in unpredictable effects in other metabolites and increase in secondary pathways. A special concern is the elimination of metabolites that play important roles in reducing human health risks such as antioxidants.

Regarding insertional mutagenesis, the most common event is inactivation of endogenous genes expected to be apparent in later generations in the homozygous state. Fusion proteins might also be detected at low frequency, in the order of 2%, and in most instances they are non-sense products of no biological significance that may be eliminated. Theoretically, silent genes may be activated and there is the possibility of appearance of a toxic compound in the edible organ; it is known for example that alpha solanine and alpha chaconine in domesticated potatoes remain high in the foliage and the possibility exists that could be expressed again in the tubercle of transgenic potatoes, but this is considered an exceedingly remote possibility (Van Gelder, 1991). Different experiments in which reporter genes were transferred without a promoter into a plant host, resulted in events that presented the activity of the transgene in high frequencies (30 to 70%) (Koncz et al., 1989, Kertbundit et al. 1991, Topping et al., 1991, Conner et al., 1993 and Christey et al., 1993). This suggests that the insertions into transcriptionally active DNA were randomly obtained under the regulation of endogenous sequences with promoter activity. About half of the insertions [44] involved low or single copies of DNA, which substantiates that insertional mutagenesis is a relatively common event. Random effects in plant genomes can also arise from naturally occurring chromosomal rearrangements, activity of transposable elements as well as genetic recombination (Fedoroff, 1989, Oosumi et al., 1995). The possible implications of these events should be taken into account, in view of transgenes introgression in Mexican landraces, in order to produce sound risk assessments.

In 1999, the British Medical Association published a statement on the potential health effects of transgenic foods, addressing the following areas:

a) Transfer of antibiotic resistance to gut flora
b) Toxicity due to expression of toxicants.
c) Allergenicity

No evidence exists so far for transfer of antibiotic resistance to human or animal gut flora. Toxic substances may exist in plants independently of the breeding technology; they could appear in transgenic plants, but are expected to be present in amounts not greater than those found in the non-transgenic variety. In most cases, toxic levels are actually lower.

There is also no evidence that the technology used for the production of transgenic foods poses an allergic threat *per se* compared to other methodologies widely accepted in the food industry. Theoretically allergic reactions may appear in sensitized individuals or de *novo* allergic sensitization from a newly expressed transgene could occur. Moreover, testing procedures in place can identify transfer and expression of a known allergen gene: an example of the first case could be the transference of a 2S albumen protein from Brazil nut into soybean (Nordlee et al 1996). The allergen was identified and product development stopped. Allergenicity is discussed in the second section of this chapter, but it is clear that transgenic foods should be individually assessed on the basis of their individual characteristics prior to introducing them into the market.

Another concern regarding transgenic foods is the possible loss or modification of nutritive value or the presence of anti nutrients above the levels found in non-transgenic varieties. The general feeling, since the commercial introduction of transgenic maize is that no increase in anti-nutrients have appeared due to genetic engineering and that no adverse reactions have been observed beyond those of the non-transgenic varieties. However new reported insect resistant transgenic maize has been transformed in order to express avidin, a well known antinutrient biotin fixing protein, generally found in eggs (Kramer et al., 2000). Although avidin is a thermolabile protein, the expression of a well known antinutrient for humans should not be the aim for a food crop.

Finally it should be stressed again that traditional breeding is not exempt from hazards as it is based in the introgression of chromosome regions from wild into domesticated species (Conner and Jacobs, 1999) with undetermined biochemical basis, and that many potential hazards as consequence of genetic engineering are expected to arise at a very low frequency not beyond the risk from traditional breeding**.**

Risk assessment

In order to assess the risk from genetically engineered foods, guidelines have been established since the first report of the Food Biotechnology Council in1990

In 1993 OECD established the concept of substantial *equivalence* in composition of key components, nutrients and natural toxicants. This does not constitute a safety assessment by itself, but it helps to identify similarities and potential differences between a transgenic and a non-transgenic variety. If substantial equivalence exists, no further testing is required. If it applies except for the inserted trait, testing for potential occurrence of unintended effect should be focused in that trait. In the case that substantial equivalence does not apply, a case-by-case analysis must be carried out.

Up to now, the most common concerns discussed here are: the ones arising from the inherent toxicity of the novel genes and their products, the potential of unintended effects resulting from alterations of the host metabolic pathways or even expression of inherently toxic or pharmacologically active substances, the potential for nutrient composition in the new food occur differing significantly from a conventional counterpart. But probably the most studied of all these concerns in the potential to express novel antigenic proteins or to alter the levels of existing protein allergens (T. Malarkey, 2003).

Regarding the inherent toxicity of a novel gene, the presence of transgenic DNA in foods represents less than 1/250,000 of the total amount of DNA consumed which amounts to a range going from 0.1 to 1.0 g per day. In view of the digestibility of dietary DNA (transgenic and non transgenic) the probabilities of gene transfer from genetically modified plants to microbial or mammalian cells is highly unlikely and extremely low (T. Malarkey, 2003). Even in the case where transgenic DNA could be uptaken by the gut flora or mammalian cells, these cells should be competent for the uptake, DNA would probably need to be as linear fragments, but to survive nucleases un the plant and in the gastrointestinal tract, and to compete with the rest of the amount of natural DNA from dietary origin. All these events together make the transference of DNA from foods to cells in the gut an extremely rare event under normal digestion conditions.

The potential toxicity of the transgenic protein expressed is considered on a case by case basis, and close attention is given to the transgenes that express a toxin, such is the case of the Bt toxins. Protein toxins are known to act via acute mechanisms and at low doses. Therefore when a protein demonstrates no-acute oral toxicity in high-dose testing with lab mammalian animals, it is considered as non-toxic to humans and other mammals, on a realistic scenario of exposure level, including long-term exposures. The acute toxicity assessment is an essential component of the risk evaluation of a novel transgenic food (T. Malarkey, 2003; Sjoblad et al. 1992).

Important guidelines have been established by a Joint FAO/WHO Expert Committee to assess the allergic risk of transgenic foods. A revision of the Joint FAO/WHO expert consultation 1995 establishes a step-wise assessment; the report points out that there is no single diagnostic test to assess the probability of transgenic food being allergenic. This is no easy task since 3D analysis reveals that allergens have no structurally defining features (G Lack 2002, Harry A Kuiper, 2001).

ILSI guidelines established the terms substantially equivalent, sufficiently similar and not sufficiently similar and included the examination of nutritional data, toxicological data and allergenic potential. A decision tree based on the level of allergenicity was also established.

FAO/WHO (FAO/WHO, 1996) dims animal studies necessary for all newly expressed proteins as it is done for food additives. If relevant changes in composition are found, the food should be tested on a case-by-case basis and a sub chronic study in animals (minimum 90 days or longer) should be done if adverse effects are observed. There are many confounding factors that make interpretation difficult so that post-marketing surveillance is recommended for monitoring adverse effects. The potential risk of transgenic foods to be allergenic should not be overlooked, especially in the case of the potential effects mentioned above in maize landraces that have suffered introgression and this should be addressed also in the risk assessment procedures for these varieties. From a battery of standardized tests a No Observed Adverse Effect Level (NOAEL) value may be established and an acceptable daily intake can be given (usually applying a x100 factor). The Monte Carlo model of distribution of exposure of individuals in a given population may be used, but requires collection of sufficiently sound input parameters for the population under investigation.

Allergenicity of Maize

Food Allergy: Facts, Risks and Myths

A number of types of adverse reactions to foods (defined as any aberrant reaction following the ingestion of a food or food additive) can occur ranging from toxic to nontoxic food reactions (Metcalfe, 2003). Nontoxic reactions depend on individual susceptibilities and may be the result of immune mechanisms (allergy or hypersensitivity) or nonimmune mechanisms (intolerance). IgE-mediated food allergies have been most clearly described; although non-IgE mediated immune reactions are being increasingly recognized. When considering food allergy or food hypersensitivity, we are referring to true IgE mediated food allergy. This is an immunological reaction and it is caused by sensitization of an individual to a particular protein allergen present in a food. Such sensitization is caused by more than one, and probably numerous, exposures to an allergic substance resulting in the production of IgE antibodies, which react to the stimulating allergen (Bischoff and Sellge 2003).

Food allergies occur generally in the United States in about 2% of the adult population (Metcalfe 2003), at least 5-6% of the pediatric population (Bock and Sampson 2003), and up to 8% of the children less than 3 years of age (Sampson 1999). Precise figures of the occurrence of food allergy in the Mexican population are not available but it is known that in industrialized countries, the public perception of the importance of allergic reactions to food ingredients substantially exceeds the prevalence of such reaction identified in clinical studies. According to Anderson's report, food allergies prevalence only amounts to 1-2% of the general population, although in some surveys one out of four atopic adults believed they had experienced an adverse reaction following the ingestion of a specific food (Anderson, 1996). In general allergies prevalence appear to be higher as compared to less industrialized countries (Kjellman 1977; Aberg 1995; Moneret-Vautrin 1998; Habbick et al. 1999). There are 8 foods or food groups that account for almost 90% of true food allergies; these include milk, eggs, fish, shellfish (which are primarily crustacea), wheat, peanuts, soybeans and tree nuts (Taylor 2002). Thus, maize is not recognized as a major food allergen, although allergic reactions can and do exist to maize. Management of food allergy to date can only be done through avoidance by elimination diet (Metcalfe 2003; Bock and Sampson 2003; Sampson 1999; Munoz-Furlong and Sampson 2003). Thus, a major aim of investigations of food allergy is to develop more specific methods of treatment.

Food allergies occur when an individual is sensitized by a food allergen or allergenic fragment crossing the mucosal membrane barrier. Following a series of immunological events, which include allergen processing, and stimulation of several types of lymphocytes, IgE antibodies are produced that react with the allergen which stimulated their production (Sampson 1999). IgE antibodies are unique in that although they are of very low concentration in human sera, they are very potent molecules. They have the ability to bind the surfaces of mast cells or basophiles, cells filled with preformed and newly generated mediators. Upon a subsequent exposure of an individual to that allergen, cross-linking two or more cell bound IgE antibodies triggers the release of preformed and newly generated mediators. These are potent pharmacological molecules that affect blood vessels, airways, smooth muscle contraction and cell migration and in effect result in the clinical symptoms that are observed during a food-induced allergic reaction (Bischoff and Sellge 2003). These can include gastrointestinal symptoms such as diarrhea, vomiting and nausea, respiratory symptoms including asthma and difficult breathing, skin symptoms including eczema and hives, as well as swelling of the lips, tongue and laringo edema (Metcalfe 2003; Bock and Sampson 2003; Sampson 1999). One or several of these symptoms can appear in food-allergic individuals. Other than atopic eczema, generally an adult individual is only allergic to one or a few foods. It is important to remember that a food allergen, in contrast to food toxin, needs several exposures to sensitize an individual to that particular food. Thus, the concept that one can have an immediate reaction upon first exposure to an allergic food is not true.

Recently it has been demonstrated that cross-reactivity between foods and among foods and inhalant allergens exists. That is, certain food and inhalant allergens may contain similar molecules or share parts of molecules called epitopes, to which IgE antibodies bind, thus these molecules cross-react. For example, a variety of fruits and vegetables contain allergens that have been shown to cross react with pollen allergens, a number of grains originating from the grass family such as maize or rice cross react with grass pollen allergens, and tropomyosin (an important muscle protein), the major shrimp allergen cross reacts with tropomyosins present in cockroaches and dust mites, two major inhalant indoor allergens (Bohle et al. 2003; Valenta and Kraft 1996; Kazemi-Shirazi et al. 2000; Hoffman-Sommergruber et al. 1999).

What are the risks of food allergy in general? Food allergies as mentioned earlier occur in approximately 2-8% of the population and most food allergies are caused by the eight major food groups. Foods are estimated to contain about 20,000 proteins, of which only a fraction (about ten to twenty) are allergenic (Taylor and Hefle 2001). Thus the chance of being exposed to a particular food allergen or developing a specific food allergy is low. A number of misconceptions with regard to the development of food-induced allergic reactions continue to exist. For example, the perceived prevalence of food allergies by the general population is about 25%; even parents' perceptions of their children with allergies are approximately one out of four of the children (Metcalfe 2003; Bock and Sampson 2003). A consumer survey regarding the prevalence of food induced allergic reactions indicated that 30% of the people interviewed felt that they or some family member had an allergy to a food product (Sloan and Powers 1986). This survey also found that 22% avoided particular foods on the mere possibility that the food may contain an allergen. Thus, public or family perceptions of food allergies are clearly much higher than the actual prevalence of food allergy. Exposure requirements to the development of sensitization to a particular food are not well understood by the public. As mentioned earlier, at least 2 and probably multiple exposures are required for sensitization to a particular food in contrast to a toxin in which only one exposure can induce the effect. Thus the risk of food allergy in the general population is considered low.

Maize Allergy in General and in Mexico: The Known and the Unknown

Maize, a major agricultural product grown throughout the world, has been a major source of food in Mexico for thousands of years (Goodman 1998). Unfortunately, in spite of the widespread use of maize and the suggestion of allergenicity of some maize-derived products, neither maize allergy nor maize allergens have been well investigated. The recent pressure for better allergy risk assessment of transgenic maize used in food products has added further urgency to the need for better-characterized maize allergens. In Mexico, little has been published about maize allergy or allergens. Dr. Ana Ma. Calderon, a physician who studied food allergy in infants under two years of age in Northwestern Mexico, did not identify maize allergy in this population, probably due to lactation and weaning habits in this area (Calderon, A.M. 1997). However, cow milk and soy proteins are common food allergens followed by eggs, fish and some fruits.

In general, maize allergy is seldom reported by the Mexican population. Possibly, this is due to the fact that maize is used in Mexico after the process called nixtamalization (alkaline cooking), a process described in an earlier section of this chapter which affects protein integrity, structure and conformation. Practically all the traditional Mexican dishes use maize prepared after this process. Apparently, the effects on allergenic properties of maize after nixtamalization have not been studied (Calderón, 2004).
In Europe, Pastorello and colleagues have reported observing several anaphylactic reactions to maize in a majority of patients who also reacted to the *Prunoideae* fruits (peach). Twenty-two patients reporting systemic symptoms after maize ingestion, and having positive skin tests and IgE antibody responses to maize were selected for study; unfortunately, the maize allergy of these patients was not objectively demonstrated by double-blind, placebo controlled challenges (DBPCFC), which is considered the gold standard of food allergy diagnosis. Nevertheless, sera from 86% of the 22 patients recognized a 9 kD protein (shown to be a lipid transfer protein – LTP). Pastorello and coworkers (Pastorello, 2000) concluded that this LTP is a major maize allergen, even though maize allergy was not definitively demonstrated by DBPCFC. In another study, Pasini and colleagues orally challenged 16 subjects with histories of positive reactions to maize flour; 6 patients demonstrated symptoms after ingestion of maize. A 16 kDa protein belonging to the corn-reduced soluble protein fraction was recognized by their serum IgE in all of the positive studies. Pasini concludes that this 16 kDa salt, unextractable protein was a potential allergen for food hypersensitivity in corn-allergic individuals because of its stability to cooking and digestion (Pasini et al. 2002).

Studies of maize allergy in the United States indicated that this response can be rather severe (Tanaka et al. 2004). In a double blind, placebo-controlled challenge study, a patient with a history of anaphylaxis demonstrated anaphylactic shock when exposed to maize in a double-blinded fashion. This indicates that although maize is a rare food allergen, when episodes occur, it can induce severe symptoms. In our patient population, IgE antibody reactivity was observed to a variety of molecules ranging from 9 kD to 90 kD in size. Generally, the most significant reactivity was observed at 9 kD, 16 kD, 21 kD, and 25 kD. However, a correlation of reactivity to these proteins with a maize-induced allergic response was not clearly demonstrated. IgE antibody reactivity to maize was not associated with a maize-allergic reaction in that there were subjects with elevated IgE antibody responses who did not react to maize under DBPCFC conditions. This may reflect reactivity of these individuals to other non-maize proteins, or variability of maize allergic reactions, which could be affected by other criteria (Aresery et al. 2002; Tanaka et al. 2002).

As mentioned earlier, cross-reactivity has been observed between grass pollens and grains derived from grasses such as rice and maize. Indeed, some individuals have developed reactivity to inhaled maize flour (an occupational reaction that can occur in bakers) as well as to maize pollens such as in farmers or growers of maize (Aresery et al., 2003). Because these IgE antibody induced reactions to pollens or other grains may cross-react with maize, it complicates the predictability of a maize-induced allergic reaction based solely on serological data.

Allergenic potential if maize that has suffered introgression

Assessment of Allergenicity of Genetically-Modified Foods

There are several potential possibilities when considering unintended affects on allergens in genetically-modified foods. First, the level of an endogenous protein within that food could be altered. For example, if a new protein is expressed in soybeans and that event affects the expression level of an endogenous soy protein, which is allergenic, this could be a problem for soy-allergic subjects. The second potential unintended affect is expression of known allergens in genetically-modified foods. This could occur, for example, if a peanut protein, expressed in rice was a known peanut allergen. This possibility is highly unlikely since candidate expression proteins can be easily tested and identified as known allergens and thus development of such a transgenic rice would be stopped as with the case of Brazil nut expressed in soybeans (Nordlee et al. 1996). The final possibility and one that is more difficult to assess, is expression of novel proteins that may be allergenic (Lehrer and Bannon 2004). For some proteins expressed in genetically modified foods (such as the pesticidal proteins from *Bacillus thuringiensis* (Bt)), there is little information of prolonged human exposure; thus the question of their allergicity has arisen (Kimber et al. 2003). The general approach to test these proteins for potential allergenicity is to assess their properties in comparison to those of known allergens such as stability to processing and enzymatic digestion and similarity of amino acid sequences to known allergens; this approach generally has been very useful over the years, but is not a definitive assay (Lehrer and Bannon 2004). Thus, there are a number of groups working to improve this assessment including development of animal models for allergenicity assessment of proteins and for food allergy in pigs, dogs and mice; such models may prove to be very useful in the future and should aid greatly in the assessment process (Kimber et al. 2003). An animal model, in my opinion, does not have to mimic the exposure of man to food allergens, but should react to known food allergens similarly as man.

The possibility that novel proteins are major food allergens is unlikely in that they are screened to exclude those molecules with properties of known food allergens, and also they are expressed at very low levels, which are generally much lower than that of most major food allergens. Thus, more definitive criteria are required and certainly such assessments should and will improve as our knowledge of food allergens increases (Lehrer and Bannon 2004; Astwood et al. 2003).

Post-Exposure Assessment: The StarLink Corn Saga-lessons learned

In the United States, an unintended exposure to genetically-modified corn occurred over a 2-3 year period. This corn, StarLink, had been modified to produce the insecticidal protein Cry 9C, which was a more stable protein compared to other Bt proteins expressed in crops approved for human consumption. StarLink® corn, produced by Aventis Corporation was approved by the FDA for sale only as an animal feed. In September of 2000, it was reported and confirmed that StarLink had contaminated the human food supply. The approval of StarLink only for animal use was based on the fact that its Bt protein, Cry 9C, appearing to be more stable as related to other Bt proteins could not be excluded as an allergen, although there was no evidence yet that Cry 9C was allergenic. Because of the importance of this exposure, the National Food Processors Association (NFPA) an organization that represents the food processing industry on issues of food safety as well as other food related topics, was asked by the FDA, the EPA, and the USDA Food Safety and Inspection Service to provide assistance in obtaining from its member companies information reasonably related to StarLink corn. The focus of this request was data addressing alleged allergenicity reactions that could be related to the presence of StarLink in processed foods.

Eleven food processing companies submitted data to the NFPA on consumer contacts associated with processed foods containing yellow corn and possibly StarLink. Additional information estimating production units, consumer contacts, and allergy/health contact/questions (1-800-number) were also provided. Four time periods, selected for review, were 1998 when 10,000 acres of StarLink corn was planted; 1999, when 250,000 acres of StarLink corn was planted, the year 2000 up to September 17th when 350,000 acres of StarLink corn was planted (prior to the reporting that StarLink had contaminated the human food supply), and the last period from September 18th through November 11th, the two-months in which numerous product recalls related to StarLink and thousands of media reports on the issue occurred. The NFPA studies demonstrate that there was no correlation between the amount of exposure to StarLink corn and the allergy/health contact/questions about yellow corn products. There did appear to be a positive association between the number of allergy complaints for processed food containing yellow corn and the number of StarLink related product recalls (Bernstein et al., 2002). Based on this, the CDC investigated if human illness was associated with potential exposure to Cry 9C in StarLink corn. Sera were obtained from 17 of the 28 subjects who had claimed to experience apparent allergic reactions, which they attributed to StarLink corn exposure as a part of the CDC analysis. Other sera were obtained from appropriate atopic control individuals, from sera tested prior to 1996, and sera against known allergens as positive controls. No IgE antibody reactivity to Cry 9C was demonstrated for the 17 sera selected. Although the results were clear-cut, there were concerns that since the Cry 9C tested was a recombinant protein produced in bacteria, it may differ from that protein expressed in plants to which consumers were exposed. Thus, the results to date suggest that Cry 9C does not have allergenicity in spite of the fact that there was such serious concern when it was found to be present in the human food. Recently one of the 28 patients from the CDC/FDA study who complained of at least three different allergic episodes to StarLink Corn products was evaluated for reactivity by DBPCFC. In this study, the patient was challenged in a blinded fashion to wild-type and StarLink Corn. Skin tests and challenge results were negative to both corn samples. The patient never developed any symptoms that had been reported in the past (Sutton et al., 2003). These results definitively established that this individual was not allergic to StarLink corn, and support the previous results that Cry 9C is not allergenic.

There are several general lessons learned from this saga of StarLink corn: First, it seems that the allergen assessment of novel proteins appears to be working reasonably well. A product that although not approved for human consumption, was never shown to be allergenic, and did not cause any demonstrable allergic reaction even when it unintentionally was present in the human food supply. In addition, the fact that there has been essentially no documented allergic reactions to other novel proteins present in genetically modified foods, suggest that the risk assessment policies for genetically modified foods are working well. Of course, this is not to say that these policies and regulations cannot be improved and certainly continued improvement and refinement in risk assessment is expected as our knowledge of food allergy and allergens increases. Second, from such a controversial and emotionally charged issue, the public may be unduly influenced by the press. The only way to determine allergenicity is the procedure in which this study was conducted by standard scientific methods that are well established and well proven in the assessment of food allergic responses.

Future Possible Effects of the Introgression

The evidence to date suggests that hybridization has occurred between maize crops that have been genetically engineered to express new proteins and related landraces of maize that grow throughout Mexico. The total effect of this introgression is not entirely clear. As an example of one health effect concern, the assessment of potential allergenicity of genetically modified crops, as well as the studies of corn allergens, has been reviewed. The evidence to date suggests that corn does not appear to be a major food allergen in different parts of the developed world where it has been investigated. In Mexico, although there is a paucity of published information, it appears that this is the case as well. Furthermore, genetic modification of maize has not appeared to enhance the allergenicity of maize proteins or added new allergens to maize. This is taken from the experience in the United States where genetically modified maize has been eaten for a number of years and also where an unapproved modified maize, StarLink corn, which was not released because of allergenic concerns, was shown not to be a major allergen problem.

As discussed above, maize appears to be a rather weak food allergen compared to other major food allergens. However, allergens have been identified in maize that can cause significant allergic reactivity in sensitized individuals. Thus, considering that the allergenic potential of maize is low, if gene transfer occurs, it is not expected that the potential for the native varieties would be any more allergenic than the current maize to which the Mexican population is exposed. Since maize apparently causes little allergy in Mexico, this is a relatively minor concern. Another concern would be with any new allergens if introduced from transgenic maize and transferred to the native maize. Clearly the chance of a known major food allergen being transferred is minimal, based on the experience with genetically modified corn in the United States. The chance that a novel protein will be a major food allergen has been reduced through testing and risk analysis as described above but is not absolute. In spite of the high levels of exposure in the United States, to date there has not been a definitive case of food allergy providing evidence that any of these novel proteins are causing allergic reactions. Clearly, more data is needed, but to date the evidence suggests that novel proteins in transgenic maize are not allergenic, although testing and risk assessment of any new products developed needs to be continued.

Having said that the possibility that introgression of the currently commercialized genetically modified maize does not appear to pose a serious health risk for the Mexican population, there are several concerns that still exist. Maize is increasingly being used as essentially a factory to produce the proteins for nonfood uses, such as pharmaceutical proteins and human proteins for treatment of different diseases. As gene flow occurs to the landraces present throughout Mexico, the possibility of hybridization between crops engineered to produce certain nonfood molecules, such as pharmaceuticals, which may be toxic, is of a great concern. Clearly the possibility of spreading such molecules, which could be potentially harmful to man through the landrace varieties by production of pharmaceutical substances, is an unacceptable risk. Thus, farming such a corn certainly would require multiple safety measures including production in remote areas, separate farm equipment, delayed planting to stop cross-pollination, etc. to avoid any potential contaminant of landraces (Gewin, 2003). However the opinion of the Mexican Government in this matter is different. According to an official declaration made by the National Delegation at the First Meeting of the Parties to the Cartagena Protocol on Biosafety, held in Malaysia on February, 2004, Mexico is opposed to the production of industrial products, vaccines, pharmaceuticals, and in general non edible compounds in maize, due to its nature as an open pollination species, as well as its strategic importance as the staple food in Mexico (Fragment of the Declaration of the Mexican Government, 2004[1]).

Risk issues to be solved regarding the potential effects on health of GM proteins in maize landraces

Although a number of health effects are of concern with any new food crop or product, allergy has been primarily addressed in this document since from the very beginning, allergenicity of genetically modified foods has been a real or suggested health issue by consumers, manufactures, regulators, farmers, and government agencies. However, although allergy had been addressed, it can serve as a paradigm for other potential or hypothetical unintended detrimental health effects of GM-crops.

Unfortunately, there have not been any studies in Mexico, the land where maize was first cultivated and grown thousands of years ago, and where it still is a major component of the diet for millions of Mexicans. Indeed, many of the ancestors of maize and numerous non-commercial maize varieties still survive and are considered an invaluable natural gene pool that cannot be replaced. It is estimated that in Mexico people eat between 285 to 400 g of tortilla per day (Quirasco et al., 2004) and thus their exposure to maize proteins is probably much higher *per capita* than that of American or Canadian populations as discussed previously.

To date the evidence that genetic modification (after the proper screening) may add new or elevated existing allergens is apparently highly unlikely, although the effects of multiple transgenes on endogenous maize allergens, the reuse of harvested maize seeds year after

[1] DECLARATION OF MEXICO ON TRANSGENIC MAIZE WITH PROPERTIES THAT LIMIT ITS CONSUMPTION AS FOOD (Fragment)

…Reasserting the importance of the conservation and sustainable use of this resource and understanding the strategic character that this crop has in the feeding of the Mexicans;

Manifests that has decided not to allow the liberation to the environment of genetically modified maize in which the modification impedes its use as food. That is to say, Mexico prohibits the experimentation as well as the liberation to the environment of transgenic maize modified for the obtention of drugs, vaccines, industrial oils, plastics or any modification that inhibits or affects its properties as a food .

We invite all member and non-member countries to reflection about the use of food crops, particularly in centers of origin, as factories for products that limit their properties as foods.

year, and the consequential increase in exposure through diet and over many years in families in rural communities which depend on the crops of these small Mexican farmers cannot be ignored. In addition, the possibilities that commingling of corn varieties non-intended for human use with those for human use (particularly through smuggling or unintentional release into the environment) or the possibility of gene exchange (via pollen and gene flow) are real concerns based in the StarLink and Prodigene incidents in the US. Therefore, it is important to differentiate between subsistence farmers who reuse seeds gathered each year from large commercial farms which purchase their seeds each year. Since most risk assessments are directed at situations that involve large farms that produce products consumed throughout the country/world, we clearly need to know much more about the effects of higher exposure over longer periods of time to maize proteins as well as to products of genetic modification. What are the differences in laboratory tests and field trials in regard to predicting risk for small farms as compared to large farms? Can such test accurately predict potentially adverse biological reactions in the Mexican population? This is an area that clearly requires more experimentation and study. Certainly, an improved (more extensive and rapid) exchange of information between governments will help to facilitate dealing with this problem—communications must be improved and increased.

Once transgenes are present, and under the control of local “campesino” farmers, the link between transgenes and their traits and their evolution over successive generations are difficult if not impossible to follow in comparison to control by breeders and companies in developed countries.

Development of biopharmaceutical and edible vaccines through production in genetically engineered plants may for a number of reasons be implemented in the near future. The activity of such biological products although well acknowledged for their beneficial properties in aiding human health and welfare are in most cases dose dependent for their desired effects. Thus, the presence of such products in a major source of food and the uncontrolled exposure of populations may present a potential hazard to populations of subsistence farmers if the genes coding for such biopharmaceuticals or vaccines transfer into land varieties of maize. Are the current tests employed sufficient to detect the presence of such genes and their products or do we need newer and better assessment methods? However, at this time because of the risk it is not recommended that such products produced in corn be grown in Mexico.

In essence assessment of GM-maize in Mexico is an extremely complicate issue that requires special consideration of the unique situation that Mexico presents. Since the chances for gene flow are greater in such farming situations performed in areas when maize is grown abundantly and in the wild, perhaps special vigilance is needed for genetically modified corn as compared to other countries where large farms with more restricted control exist with and maize varieties are not a major sources of food for the rural population, and whose landraces are essentially a national resource that must be preserved in effect as a gene repository for future generations. Conventional requirements for testing and screening new GM maize may need to be altered to reflect the special situation of maize and Mexico.

Transgenic maize Potential benefits

Transgenic technology offers very specific characteristics, so that the benefits largely depend on the kind of transgenic change, the place where transgenic maize is planted, and the people who plant it. First generation transgenic maize offered very interesting agricultural advantages reducing the need for pesticides and increasing yields under adverse environmental conditions. Besides, an extra advantage of insect resistant transgenic crops has been substantiated: there is evidence that mycotoxins (Munkvold, 2003) and deoxynivalenol (Schaafsma et al., 2002) content can be reduced in Bt maize. Fumonisin, in particular, is an interesting mycotoxin known to affect human health including potentially a high rate of neural tube birth defects it disrupts sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo. High fumonisin, content in tortillas has been reported **(**Dombrink-Kurtzman and Dvorak, 1999; Schaafsma et al., 2002). Prevention or reduction of fumonisin in maize derivatives would certainly be a benefit.

In Mexico, the agronomic advantages of transgenic maize are of especial interest to the sector engaged in high technology agriculture, which uses great amounts of pesticides and herbicides; those farmers are used to buy seeds and can afford it. However, according to the Secretary of Agriculture, this sector provides only about 14% of maize in Mexico. For the poor *campesinos* the benefit is not clear.

A second generation of transgenic maize is being developed to achieve compositional and nutrient changes. A low phytate variety of maize that enhances iron absorption may be useful in dealing with iron deficiency anemia and varieties with higher protein quality may help to fight children PEM; it may also add to the variety of choices and could facilitate the design of therapeutic regimes. In the past, non-transgenic varieties of maize with better amino acid composition, such as opaque 2 and HPQ maize, have been developed, but for many socioeconomic reasons have not resulted in better nutrition for sectors in which PEM is common.

It should be recognized that it is diet, and not particular foods and products, what determines nutrition, that nutrition is not only nutrients, and that diversity of the diet is particularly important. Diet may be adapted to any special nutritional condition using modified products, but also using regular foods and products.

A potential drawback of products, transgenic or not, that “provide all nutrients” is that promote dependence and dietary monotony. They may be very useful as a first on-hand strategy to fight malnutrition and in specific nutrition programs, but fail to solve the primary causes of the problem.

Need to have transgenic maize varieties with a modified composition in Mexico

Modified foods or products represent an additional choice for the consumer that adds to the variety of available resources. There will be many cases in which the specific nutrient modification is particularly useful or at least convenient. Nobody should complain about having a more ample offer or about having foods with particular characteristics; for example, low phytate maize may be very helpful in fighting iron deficiency anemia provided it is available and affordable, but it is clear that “magic bullets” do not exist, particularly for complex problems, and that many other measures have to be applied. On the other hand, the presence of phytates in the human diet may be beneficial from the point of view of chronic degenerative diseases such as dyslepidemia and cancer, so that solving a problem may cause other problems.

In the US and Canada a great deal of transformation events have been approved and they appear in Table 1. Some of these events are not commercially planted in certain seasons, but in theory, NAFTA imports for food feed and maize for processing would include a mix of these varieties. Their presence in Mexico amounts the official figures for 2003: 5,570,418 kg as grand total, from which 5% was white maize directed for processing (flour), 60% was yellow corn for feed and 35% for the starch and oil industry. The Mexican Government declared that Mexico was self sufficient regarding maize production for human consumption, therefore, no imported maize was directed to DICONSA silos that year (Written communication. Interministerial Commission on Biosafety. February 18, 2004).

For the case of agronomical useful traits, there are not manipulated landraces that really address a particular Mexican problem. Mexican biotech developments regarding drought resistance or tolerance to alkaline and high aluminium soils have not been escalated, mainly due to the *de facto* moratorium for the planting of transgenic maize in Mexico, but they show interesting traits for Mexico. Their usefulness will have to be proved and risk evaluations remain still to be done.

In reference to other traits like herbicide resistance, transgenic maize resistant to herbicides allows the use of these chemicals in commercial agriculture. It could be a problem regarding the destruction of *quelites, verdolagas, quintoniles* and *amaranhtus* which are wild vegetables that are traditional since ancient times and have an important role in the diet of *campesinos* as sources of carotenes and phytonutrients in subsistence *milpas.* Other cultivated species in the *milpa* are zucchini or squash, squash flowers and beans. The possible transference of this trait into landraces in *milpas* seems not to pose an impact to the nature of these varieties.

In view of the above comments transgenic maize with interesting agronomical traits might be useful, and foods modified in nutrient composition are more than welcome but they cannot be considered strictly essential; in other words they do not represent “a need.” This is not surprising since, contrary to what applies to some specific nutrients, no food may be labelled as indispensable.

Transgenic maize. Potential risks in Mexico

The issue of potential health risks of transgenic foods has been a topic of considerable concern and interest. In the case of first generation transgenic maize varieties, no harmful effect has been observed in studies made in different countries, although few peer-reviewed published reports of experimental studies in this area have been published (Domingo, 2000).

Mexican agriculture and the role of maize in the diet of Mexicans are substantially different from those in the US and Canada. Studies specifically regarding the Mexican population are strongly needed but lacking up to date.

The maize agricultural systems in Mexico have a somewhat open nature and are less structured, compared to those of Canada and the US. In Mexico, many farmers collect the seed from their harvest to use for the subsequent planting period. This practice has produced a large number of landraces over time, which makes the Mesoamerican region an important center of diversity, but opens the door for uncontrolled diffusion by transgenes, which could be interacting, with the different genetic background of landraces. In a theoretical case in which transgenic Mexican landraces (See Chapter 10 Transgenic maize in its center of origin: The Mexican case by Ariel Alvarez and John Komen) were developed and released in the environment, the evolution of transgenes would be in the hands of farmers. However, there is not sufficient knowledge about the possible expression or consequences of gene stacking in the conditions of *campesino* farmers. There are so many variables interacting together and thus so many scenarios can be imagined that the outcome is uncertain and possibly unpredictable.

Transgenic varieties have been designed for extremely structured agricultural systems. Certainly the companies developing transgenic seeds do not contemplate the idea to have the transgenes released into the environment, and to let them evolve under the particular site of release, notwithstanding intellectual property rights for these seeds. The firms that produce these varieties have not generated information or knowledge about the behavior of such varieties apart from what is normally expected in structured agricultural systems (Chauvet and Galvez 2004).

Another important point in this discussion deals with the disparity between the permits and authorizations given in the US and Canadian system (see **Table 1**) versus the authorizations granted in Mexico for maize imports (see **Table 2**, containing data from the Mexican Health Ministry and CONABIO). Under the Cartagena Protocol, maize imports for feed and processing seem to be excessive, and a part of it ends up in silos of the National storage system commingled with Mexican white maize for tortilla making. Thus an unexpected presence of several GM maize varieties could be found in Mexican imports due to the disparity in permits issuing. Unrecognized transgenic maize may easily enter the open Mexican agricultural system since nearly five million tons of maize are imported every year whose use cannot be adequately controlled.

Table 1. GMOs in International Commerce

Organism	Trade Mark	Event	Promoter gene	Terminator gene	Markers
Zea mays L.		MON 832	goxv247:CamV35S; EPSPS: E35S;	EPSPS: nos; goxv247: ND	neo: neomycin phosphotransferase II
Zea mays L.	NaturGard™ KnockOut™	Event 176	Cry1Ab: PEPC+CaMV35S y CDPK+CaMV35S; bar:CaMV35S	Cry1Ab: CaMV35S; bar:CaMV35S	bla: beta-lactamase
Zea mays L.	Libertylink	T14	CaMV35S	CaMV35S	bla: beta-lactamase
Zea mays L.	Libertylink	T25	CaMV35S	CaMV35S	bla: beta-lactamase
Zea mays L.	Yieldgard®	MON810	CaMV35S enhanced	nos	NA
Zea mays L.		MON809	Cry1Ab:E35S; CP4EPSPS:E35S	Cry1Ab:nos; CP4EPSPS: nos	goxv247:glyphosate oxidoreductase; neo: Kanamicin resistance
Zea mays L.	Roundup Ready®	GA21	Rice actin I	nos	NA
Zea mays L.		MON802	EPSPS:E35S; Cry1Ab:E35S; goxv247: ND	cry1Ab:nos; EPSPS: nos; goxv247: nos	neo: Kanamicin resistance
Zea mays L.		DLL25(B16)	CaMV35S	Tr7	bla: beta-lactamase
Zea mays L.		Bt11, (X47334CBR)	pat: CaMV35S; cry1Ab: CaMV35S	pat, cry1Ab: nos	bla: beta-lactamase (not incorporated)
Zea mays L.	InVigor™	MS3	bar:CaMV35S; barnase:pTa29 de *N. tabacum*	bar: nos; barnase: ND	bla: beta lactamase
Zea mays L.		676	pat: CamV35S; dam:512del, maize anther specific promoter	dam:pin II terminator from protease inhibitor II from *Solanum tuberosum*; pat: ND	Same pat
Zea mays L.		678	pat: CamV35S; dam:512del, maize anther specific promoter	dam:pin II terminator from protease inhibitor II from *Solanum tuberosum*; pat: ND	Same pat
Zea mays L.		680	pat: CamV35S; dam:512del, maize anther specific promoter	dam:pin II terminator from protease inhibitor II from *Solanum tuberosum*; pat: ND	Same pat
Zea mays L.	StarLink™	CBH-351	bar: CamV35S; cry9c: CamV35S	bar: nos; cry9c: polyA from CamV35S	bla: beta lactamase
Zea mays L.	InVigor™	MS6	bar: CamV35S; barnase:pTa29 specific promoter for *N. tabacum* pollen	bar: nos; barnase: ND	bla: beta lactamase
Zea mays L.	Roundup Ready®	NK603	EPSPS: P-ract1/ract1 rice actin 1promoter; EPSPS: E35S	The two genes EPSPS:nos	Same EPSPS

Zea mays L.		MON863	4AS1	3' NTr from tahsp17 wheat	neo: neomycin phosphotransferase II
Zea mays L.	Bt Xtra™	DBT418	bar: CaMV35S; Cry1Ac: CaMV35S +octopine sinthase;pinII: CaMV35S	bar: Tr7; Cry1Ac: pinII; pinII: native	bla: beta lactamase
Zea mays L.		Bt 11, (X4334CBR)	pat: CaMV35S; cry1Ab: CaMV35S	pat, cry1Ab: nos.	bla: beta lactamase (not incorporated

Source: Consultation to the Information System of Living Modified Organisms (under construction). GEF-CONABIO Project

Table 2. Biotechnological Products Approved in Mexico

VARIETY	ORGANISM	INTRODUCED GENE	COMMERCIAL EVENT	UI OCDE
		1995		
Delayed ripening tomato	*Lycopersicon esculentum Mill.*	Antisense tomato poligalacturonase gene in	B, Da, F (Zeneca Seeds) FLAVR SAVR (Calgene Inc.)	
		1996		
Colorado beetle resistant potato	*Solanum tuberosum L.*	*cry3A* gene from *Bacillus thuringiensis* subsp. tenebrionis	ATBT04-6, ATBT04-27, ATBT04-30, ATBT04-31, ATBT04-36, SPBT02-5, SPBT02-7, BT6, BT10, BT12, BT16, BT17, BT18, BT23 (Monsanto)	NMK-89367-8 NMK-89613-2 NMK-8917Ø-9 NMK-89279-1 NMK-89576-1 NMK-89175-5 NMK-89167-6, NMK-89593-9 NMK-89675-1
Lepidopteran insect resistant cotton	*Gossypium hirsutum L.*	*cry1ac* gene from *Bacillus thuringiensis* subsp. kurstaki	MON531/757/1076 (Monsanto)	MON-ØØ531-6 MON-ØØ757-7 MON-89383-1
Glyphosate herbicide tolerant canola	*Brassica napus L.*	5-enolpiruvilshinkimato-3-phosphate sintetase gene from *Agrobacterium* subsp 4 strain	GT200, GT73, RT73 (Monsanto)	MON-89249-2, MON-ØØØ73-7
Delayed ripening tomato	*Lycopersicon esculentum Mill.*	Tomato poligalacturonase gene with reduced activity	B, Da, F (Zeneca Seeds) FLAVR SAVR (Calgene Inc.)	CGN-89564-2
Glyphosate herbicide tolerant Soybean	*Glycine max* (L.) Merr.	5-enolpiruvilshikimate-3-phosphate sinthase gene from *Agrobacterium* subsp 4 strain	GTS 40-3-2	MON-Ø4Ø32-6
		1998		
Delayed ripening tomato	*Lycopersicon esculentum L.*	Fragment of Aminociclopropane carboxílic acid sintetasa´s gene from tomato	8338 (Monsanto)	CGN-89322-3

		1999		
Ammonium gluphosinate tolerant canola	*Brassica napus L*	*Streptomyces viridochromo*genes fosfinotricine acetyl transferase gene	HCN10, HCN92, T45(HCN28) (AgrEvo)	ACS-BNØØ7-1 ACS-BNØØ8-2
		2000		
Glyphosate herbicide tolerant cotton	*Gossypium hirsutum L.*	EPSPS gene from *Agrobacterium* spp CP4 strain	MON1445/1698 (Monsanto)	MON-Ø1445-2 MON-89383-1
		2001		
Gluphosinate ammonium tolerant canola	*Brassica napus L.*	fosfinotricine acetyl transferase gene from *Streptomyces viridochromogenes*	HCN10, HCN92, T45(HCN28) (AgrEvo)	ACS-BNØØ7-1 ACS-BNØØ8-2
Colorado beetle and potato leafroll virus (PLRV) resistant potato	*Solanum tuberosum L.*	*cry3A* gene from *Bacillus thuringiensis* subsp. tenebrionis and PLRV virus replicate gene	RBMT21-129, RBMT21-350, RBMT22-82 (Monsanto)	NMK-89185-6 NMK-89896-6
Colorado beetle and potato virus resistance in potato	*Solanum tuberosum* L.	*cry3A* gene from *Bacillus thuringiensis* subsp. tenebrionis and potato virus Y (PVY) coat protein gene	RBMT15-101, SEMT15-02, SEMT15-15 (Monsanto)	NMK-89653-6 NMK-89935-9
		2002		
Bromoxinil herbicide tolerant cotton	*Gossypium hirsutum L.*	Bxn gene from *Klebsiella ozaneae* that codes for a nitrilase	BXN (Calgene Inc.)	
Lepidopteran insect and glyphosate tolerant cotton	*Gossypium hirsutum L.*	*cry1Ac gene from Bacillus thuringiensis* subsp. kurstaki HD-73 and CP4 EPSPS gene from *Agrobacterium sp.* CP4 strain	MON531 X MON14457 (Monsanto)	MON-89383-1
Glyphosate tolerant maize	*Zea mays L.*	EPSPS gene from maize	NK603 (Monsanto) GA21 (Monsanto) MON832 (Monsanto)	MON-ØØØ21-9
Glyphosate tolerant maize	*Zea mays L.*	CP4 EPSPS and CP4 EPSPS L214P genes from *Agrobacterium sp*. CP4 strain	MON802	
Lepidopteran resistant maize	*Zea mays L.*	*cry1Ab* gene from *Bacillus*	BT11 (X4334CBR,	SYN-BTØ11-1

		thuringiensis subsp. kurstaki	X4734CBR) (Syngenta Seeds, Inc.) DBT418 (Dekalb Genetics Corporation)	
		2003		
Lepidopteran insect resistant cotton	*Gossypium hirsutum L.*	*cry1Ac* gene from *Bacillus thuringiensis* subsp. kurstaki and *cry2Ab2* gene from *Bacillus thuringiensis.*	MON-15985-7	MON-15985-7
Lepidopteran resistance and ammonium gluphosinate tolerant maize	*Zea mays L.*	*cry1F* gene from *Bacillus thuringiensis* var. Aizawai PS811 strain and PAT (fosfinotricin acetyl transferase) gene from *Streptomyces viridochromogenes*	TC1507 (Mycogen; Pioneer)	DAS-Ø15Ø7-1
Root worm (*Diabrotica spp.*) resistant maize	*Zea mays L.*	*cry3Bb1* gene from *Bacillus thuringiensis* subsp. kumatoensis	MON-00863-5 (Monsanto)	MON-ØØ863-5
Ammonium gluphosinate tolerant Soybean	(*Glycine max* (L.) Merr.	PAT gene from *Streptomyces viridochromogenes* Tü 494 strain	A5547-127, A2704-12, A2704-21, A5547-35 (AgrEvo)	ACS-GMØØ5-3
		2004		
European corn borer and other lepidopteran resistant and Roundup Ready (Glyphosate) tolerant maize	(*Zea mays L.)*	*cry1Ab* gene from *Bacillus thuringiensis* subsp. kurstaki and CPGEPSPS gene from *Agrobacterium sp.*	MON-810x HK-603 (Monsanto)	

Sources: Consultation to the Living Modified Organisms Information System (in construction). GEF-CONABIO Project
http://www.cofepris.gob.mx/pyp/biotec/biotec.htm
http://www.agbios.com/dbase.php
http://www1.oecd.org/scripts/biotech/frameset.asp

By the current trilateral arrangement, permitted under the Cartagena Protocol and signed by the USA, Canada and Mexico, corn shipments with as much as five percent of genetically-modified organisms are allowed into the country with a label that says only that it "may contain" GMOs. By contrast, the latest European Union proposal would set a maximum of 0.3 to 0.7 % GMO. Contamination of corn shipments that occurred "accidentally" below these thresholds would not trigger any labeling requirement, and any label would only be seen by distributors, not by consumers. The identity of the possible fortuitous varieties present in the imports should be analyzed and the Mexican Health Sector should exert a close oversight.

No studies have been conducted about the possible side effects in plants that inherit transgenes season after season. In this respect, chapter 8 (“A framework by which potential benefits and risks can be judged.” M.R. Bellon, G. Tzozos and P. Thompson) includes a detailed discussion of points not adequately covered by traditional risk assessment systems. Emphasis is made on:

- Possible gene stacking. Maize populations may end harboring multiple transgenes that have never been tested together, eventually including transgenes that should not enter the human food chain (those used in biofarming, for example)
- Introduction of transgenes into the new genetic background of the local maize populations. Since the expression of a gene depends on the genetic background in which it exists, the expression (or lack) of the transgenes may be very different from the expression in the original phenotype.
- Possible indirect effects of changes in maize agricultural practice on health and environment

The level of exposure of Mexicans to eventual heterologous proteins in maize is higher than in the US or Canadian populations since per capita maize consumption is 285-400 g per day and it is eaten directly. Furthermore, there are no adequate tools such as 2-D maps for proteins of Mexican gene varieties available, necessary as a comparative base to detect and study transgenic proteins or any other protein newly expressed in maize landraces that might have suffered introgression.

The second generation of transgenic maize varieties should apparently impose no risk using the substantially equivalent concept. However, an unexpected presence of compounds such as lutein and zeaxanthin was observed in golden rice (Moneret-Vautrin 2003). There is a need for caution.

A third generation of transgenic maize, not yet commercialized, is oriented to the production of vaccines, industrial oils and compounds, as well as new proteins that are not supposed to enter the human food chain and to be managed under strict control and in confinement to experimental facilities. Although these varieties are clearly not intended for human consumption and are supposedly well controlled, there are evidences of irresponsible management and poor control (USDA and FDA Press Releases) and thus reasons for concern about their eventual cultivation in Mexico that would allow gene flow into local landraces and teosintes.

Chauvet and Gálvez. (Chauvet and Galvez, 2004) express their concern about:

- The possibilities to have commingled in the corn grains some varieties of corn that are not intended for human consumption. The basis for this preoccupation is the couple of incidents with Prodigene, as well as the Starlink incident. Use of a variety that is open-pollinated as a reactor for the production of pharmaceuticals and industrial substances may be considered not appropriate at all.
- The possibility of gene exchange (via pollen) of these varieties non- intended for human consumption in the US or in Mexico (via smuggling of seeds or by and unintentional release in the environment via the use of grains as seeds which is illustrated by the Prodigene incident)

In the event of contamination of Mexican maize hybrids with such non-food properties, the results would be serious since maize is massively consumed as such by most Mexicans and especially by the poorest sectors such as Indian communities that are particularly unaware of possible risk. Cooking may not be protective enough. Recently, Galvez' team (Quirasco et al., 2004) immunologically identified transgenic proteins or fragments of them in different maize preparations—indicating that the traditional cooking methods, including preparation of nixtamal, are unable to completely destroy transgenic derivatives.

For a population so intimately and so strongly tied to maize in many aspects of its life, the event of contamination of Mexican hybrids would affect its well-being and its cultural and nutritional rights.

There is need of examining hazard identification and risk evaluation in light of the cultural and nutritional importance of maize, especially for small-scale, subsistence-oriented farmers in Mexico.

CONCLUSIONS AND RECOMMENDATIONS

The following is a set of conclusions and recommendations drawn from the research used to write this chapter, from information obtained through interviews as well as information provided by the Mexican government through the Inter-Ministerial Commission on Biosafety and information available in the websites of the Mexican Ministry of Agriculture and The National Commission on Biodiversity (CONABIO).

> Maize has a unique importance to Mexico, which differs from other countries such as the US and Canada.
>
> One of the most important differences deals with the ways maize is grown in Mexico compared to more structured agricultural systems used in other countries. The main consequence is that most of Mexican farmers keep seed from their harvest for use in the subsequent planting period. This has generated the large variety of landraces in the country. The unique value of the landraces obtained over thousands of years in Mexico, is not only due to its intrinsic value as a part of Nature's

biodiversity, but also as a dynamic biodiversity with economic value for the Mexican agriculture, and a repository of maize genes of world value.

The role of maize in the Mexican diet is not only cultural, but also of paramount importance as the main source of energy and nutrients for the most vulnerable segments of the population, as compared to the role of this cereal in societies of the US and Canada where the main use of maize is for feed, and the production of starch and oil. Thus exposure to any expressed novel proteins (in maize) is much higher in subsistence farmers and those consumers in rural communities who are dependent on them, since maize is their main source of protein/food and they will use seeds harvested to plant the next years crops insuring a continuity of exposure for years.

Monitoring Mexican maize population to search the levels of introgression would help to assess this. There is also a need for compliance standards regarding this assessment.

Through recurring gene flow and recombination, maize populations may eventually contain multiple transgenes. The effects of such combinations have not been tested previously in the laboratory, and could have deleterious effects on the Mexican food supply.

In order to examine more closely the effects of gene manipulation in maize, there are many techniques available whose usefulness should be evaluated in a multidisciplinary decision making process to decide which is the best approach to this problem. Gene expression analysis using DNA micro-array technology, proteomics, chemical fingerprinting, assessment of marker genes for horizontal gene transfer, etc. are techniques that should be systematically applied to greatly contribute to exposure assessment of maize landraces that have suffered introgression. Such studies should join resources of molecular biology, toxicology, nutrition and genetics, and performed by public institutions or with public funds in order to produce unbiased results.

In the absence of a solid reassurance that they can be contained, prohibit the growth of corn varieties that have been altered for non-food use (such as vaccines).

Funding actual and future repositories to store seeds of important landraces in Mexico.

The road ahead seems long. Time has come for better long-term safety studies in humans done by independent scientists according to the highest standards of study design, methodology and interpretation. There is no urgency for the large-scale use of transgenic foods, at least for the nutrition of Mexicans, because no commercial transgenic variety addresses the problems specific to Mexico such as cultivation in arid areas. While enough knowledge is obtained to solve the many existing questions, better controls on experimentation, cultivation and trade of transgenic maize, presently insufficient, should be established. This does not mean inhibiting research or limiting human creativity, but protecting them from premature use that may harm people and affect the credibility and prestige of biotechnology.

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